NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF CHLORAL HYDRATE

(CAS NO. 302-17-0)

IN B6C3F$_1$ MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

February 2002

NTP TR 502

NIH Publication No. 02-4436

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control and Prevention. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

Details about ongoing and completed NTP studies are available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov. Abstracts of all NTP Technical Reports and full versions of the most recent reports and other publications are available from the NIEHS' Environmental Health Information Service (EHIS) http://ehis.niehs.nih.gov (800-315-3010 or 919-541-3841). In addition, printed copies of these reports are available from EHIS as supplies last. A listing of all the NTP Reports printed since 1982 appears on the inside back cover.

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The studies on chloral hydrate were conducted at the FDA's National Center for Toxicological Research under an interagency agreement between the FDA and the NIEHS. The studies were designed and monitored by a Toxicology Study Selection and Review Committee, composed of representatives from the NCTR and other FDA product centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers information for hazard identification and risk assessment.

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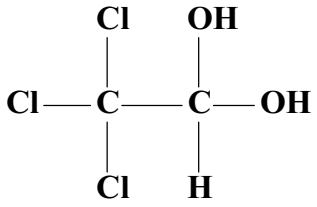
ABSTRACT



CHLORAL HYDRATE

CAS No. 302-17-0

Chemical Formula: $C_2H_3Cl_3O_2$ Molecular Weight: 165.42

Synonyms: Trichloracetaldehyde monohydrate; 1,1,1-trichloro-2,2-ethanediol; 2,2,2-trichloro-1,1-ethanediol
Trade names: Aquachloral Supprettes; Noctec; Somnos

Chloral hydrate is used medically as a sedative or hypnotic and as a rubefacient in topical preparations, and it is often given to children as a sedative during dental and other medical procedures. Chloral hydrate is used as a central nervous system depressant and sedative in veterinary medicine and as a general anesthetic in cattle and horses. It is a byproduct of the chlorination of water and has been detected in plant effluent after the bleaching of softwood pulp. Chloral, the anhydrous form of chloral hydrate, is used as a synthetic intermediate in the production of insecticides and herbicides. Chloral hydrate was nominated for study by the Food and Drug Administration based upon widespread human exposure and its potential hepatotoxicity and the toxicity of related chemicals. One goal of the study was to assess the effect of the animal's age and the duration of dosing on the tumorigenicity of chloral hydrate. Beginning on postnatal day 28, female B6C3F$_1$ mice received chloral hydrate (99.5% pure) in water by gavage for 3, 6, or 12 months, 2 years, or as a single dose; on postnatal day 15, male and female B6C3F$_1$ mice received a single dose by gavage. Tumorigenicity was assessed for 2 years after the initial dose. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, and mouse bone marrow cells.

2-Year Study

Groups of female mice (regimens A, B, C, and D) and groups of male mice (regimen E) received chloral hydrate in distilled water by gavage; control groups received distilled water only. In regimen A, groups of 48 female mice received 0, 25, 50, or 100 mg chloral hydrate/kg body weight 5 days per week for 104 weeks beginning when they were 28 days old. In regimen B, 24 female mice received 0 mg/kg and three groups of 48 female mice received 100 mg/kg 5 days per week beginning when they were 28 days old. Eight mice from the 0 and 100 mg/kg groups were killed at 3, 6, or 12 months. The remaining mice were held without dosing for the duration of the 2-year study. In regimen C, groups of 48 female mice received a single dose of 0, 10, 25, or 50 mg/kg when they were 28 days old and were held for 104 weeks. In regimens D and E,

groups of 48 female and 48 male mice, respectively, received a single dose of 0, 10, 25 or 50 mg/kg when they were 15 days old and were held for 104 weeks. Additional groups of four mice from regimens C, D, and E and mice killed at 3 or 6 months from regimen B (eight mice per group) were designated for hepatic cell proliferation analyses; mice killed at 3 or 6 months in regimen B were also designated for apoptosis analyses.

Survival and Body Weights

Survival of all dosed mice in all regimens was similar to that of the vehicle control groups. Mean body weights of 100 mg/kg female mice in regimen B dosed for 3 or 6 months were generally greater than those of the vehicle controls during the second year of the study. Mean body weights of 25 mg/kg male mice in regimen E were generally less than those of the controls beginning at week 19; mean body weights of 10 and 50 mg/kg mice were generally less than those of the vehicle controls beginning at week 80.

Pathology Findings

A dose-related and significant increase in the incidence of pars distalis adenoma occurred in regimen A 100 mg/kg females. There was also a time-related increase in the incidence of adenoma in female mice administered 100 mg/kg for up to 24 months in regimen B, and the increase in the incidence of this neoplasm at 24 months was significant. There was a significant increase in the severity of pars distalis hyperplasia in regimen A 100 mg/kg female mice.

GENETIC TOXICOLOGY

Chloral hydrate was mutagenic *in vitro* and *in vivo*. It induced mutations in *Salmonella typhimurium* strain TA100, with and without liver S9 activation; an equivocal response was obtained in *S. typhimurium* strain TA98 in the absence of S9, and no mutagenicity was detected with strain TA1535 or TA1537, with or without S9. Chloral hydrate was shown to produce chromosomal damage in mammalian cells. It induced significant increases in sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, with and without S9. Results of sex-linked recessive lethal (SLRL) tests in *Drosophila melanogaster* were inconclusive. Chloral hydrate, administered by feeding, produced an inconclusive increase in SLRL mutations in the germ cells of male flies. Results of an *in vivo* mouse bone marrow micronucleus test with chloral hydrate were positive.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *equivocal evidence of carcinogenic activity* of chloral hydrate in female $B6C3F_1$ mice treated continuously for two years based on increased incidences of pituitary gland pars distalis adenomas. No increased incidences of neoplasms were seen in female $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 or 28 days of age or in male $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 days of age. No hepatocarcinogenicity was seen under any dosing condition.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Study of Chloral Hydrate

	Regimen A (Female Mice)	Regimen B[a] (Female Mice)
Doses in distilled water by gavage	Vehicle control, 25, 50, or 100 mg/kg for 2 years	Vehicle control or 100 mg/kg for 3, 6, 12, or 24 months; remaining mice held without dosing for the duration of the study
Body weights	Dosed groups similar to the vehicle control group	Mice dosed for 3 or 6 months greater than the vehicle controls; miced dosed for 12 months similar to vehicle controls
Survival rates	37/48, 39/48, 43/48, 36/48	37/48, 34/48, 31/48, 33/48, 36/48
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Equivocal effects	Pituitary gland (pars distalis): adenoma (0/45, 2/44, 0/47, 5/41)	Pituitary gland (pars distalis): adenoma (0/45, 3/36, 1/36, 1/33, 5/41)
Level of evidence of carcinogenic activity for regimens A and B	Equivocal evidence	
Genetic toxicology		
Salmonella typhimurium gene mutations:	Positive in strain TA100 with and without S9; negative in strains TA1535 and TA1537 with and without S9; equivocal in strain TA98 without S9	
Sister chromatid exchanges		
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9	
Chromosomal aberrations		
Cultured Chinese hamster ovary cells *in vitro*:	Positive with and without S9	
Sex-linked recessive lethal mutations		
Drosophila melanogaster:	Inconclusive when administered in feed Negative when administered as intraperitoneal injection	
Micronucleated erythrocytes		
Mouse bone marrow *in vivo*:	Positive	

[a] Includes regimen A vehicle control group and regimen A 100 mg/kg group dosed for 2 years

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Study of Chloral Hydrate

	Regimen C (Female Mice)	Regimen D (Female Mice)	Regimen E (Male Mice)
Doses in distilled water by gavage	Single dose of 0, 10, 25, or 50 mg/kg at 28 days of age	Single dose of 0, 10, 25, or 50 mg/kg at 15 days of age	Single dose of 0, 10, 25, or 50 mg/kg at 15 days of age
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups generally less than the vehicle control group
Survival rates	42/48, 40/48, 45/48, 40/48	36/48, 41/48, 39/48, 40/48	45/48, 41/48, 46/48, 40/48
Nonneoplastic effects	None	None	None
Neoplastic effects	None	None	None

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on chloral hydrate on 18 May 2000 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 18 May 2000, the draft Technical Report on the toxicology and carcinogenesis studies of chloral hydrate received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. F.A. Beland, National Center for Toxicological Research, introduced the toxicology and carcinogenesis studies of chloral hydrate by discussing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in male and female mice. The proposed conclusions for the 2-year studies were *some evidence of carcarcinogenic activity* in female $B6C3F_1$ mice.

Dr. Beland noted that the major concern driving initiation of these studies for the FDA was its use as a pediatric sedative. Animal cancer bioassays by other investigators resulted in high incidences (71%-75%) of liver tumors in male $B6C3F_1$ mice exposed orally to chloral hydrate compared to those of the vehicle control mice (10%-15%). Dr. Beland stated that based on chloral hydrate's use in children and on the tumorigenic effects in mice, the objective of the current 2-year study was to assess the effect of age and duration of exposure on the possible tumorigenicity of choral hydrate. He said that the study included mechanistic studies and a bioassay, and he described findings from studies of metabolism and of DNA adduct formation.

Dr. Chatman, a principal reviewer, agreed with the proposed conclusions, noting concerns whether dose concentrations were robust enough to achieve an optimum tumor response and whether females were the right sex to study. She cited a reference indicating that female mice had a more rapid metabolism and excretion of trichloroacetic acid than males. Dr. J.K. Haseman, NIEHS, commented that most chemicals that cause cancer in mice do so in both sexes, but for those chemicals that cause cancer in only one sex, females outnumber males about 2 to 1. Finally, Dr. Chatman suggested that since there was no dose response for adenomas and the incidence even at the highest dose was similar to the high end of the historical control range, the appropriate conclusion might be *equivocal evidence of carcinogenic activity*.

Dr. Drinkwater, the second principal reviewer, agreed with the proposed conclusions but also had concerns about the rationale for choosing females for the chronic studies and with the discussion regarding the lack of liver tumor development in this study compared to the Daniel *et al.* (1992a) study. He said further discussion of the discrepancy would be appropriate. Dr. Beland agreed to add additional information related to these issues.

Dr. McCarver, Medical College of Wisconsin, Expert Consultant, the third principal reviewer, agreed in principle with the proposed conclusions, adding that the dosing regimens need to be more specifically stated in the conclusion statement and suggested adding to the end of the first sentence the phrase, "in animals treated for two years at the maximum dose of 100 mg/kg." Dr. McCarver offered some perspective on the clinical value of chloral hydrate in thousands of children in the United States daily and the potential negative impact of labeling chloral hydrate as carcinogenic, particularly when there are so much negative data regarding the carcinogenicity of chloral hydrate.

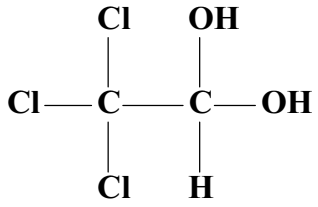
There was some discussion about whether or not there were real differences in severity grades of pituitary gland hyperplasia between control and dosed mice. Dr. Chatman stated that severity grading is subjective. Dr. J.R. Bucher, NIEHS, commented that emphasis should be given to the overall incidence of tumors within the context of the corresponding historical control data and to focus less on the severity grade differences for hyperplasia. Dr. Beland agreed that the hyperplasia findings should not be a primary factor in determining the level of evidence but rather should be viewed along with the incidences of adenomas.

Dr. Chatman moved that the conclusions for the findings in regimens A and B be changed from *some*

evidence of carcinogenic activity to *equivocal evidence of carcinogenic activity* based on a lack of a dose response for the incidences of pituitary gland pars distalis adenomas, and because the incidence in the 100 mg/kg group was similar to the historical control range. She also moved that a statement be added that no hepatocarcinogenicity or chloral hydrate-induced liver lesions were seen, and that the last paragraph discussing the severity of hyperplasia of the pituitary gland pars distalis be deleted. Dr. Bus seconded the motion. Dr. Cullen moved to amend the motion by retaining the last sentence pertaining to hyperplasia. Dr. Medinsky seconded the motion. Dr. Chatman asked whether the sentence could include incidence as well as severity of hyperplasia, and Dr. Cullen agreed. Dr. McCarver suggested removing 'chloral hydrate lesions' from the sentence: "No hepatocarcinogenicity or chloral hydrate-induced lesions were seen" since such lesions have not been defined. Drs. Cullen and Medinsky agreed. Dr. Cullen's revised amendment was accepted with five yes votes to one no vote (Dr. Chatman).

Moving to the rest of Dr. Chatman's original motion, Dr. Drinkwater noted that the only significant increase in the incidence of pituitary gland neoplasms was in the high dose group of regimen A. Dr. Chatman proposed adding the specific dose level (100 mg/kg). Dr. Bucher indicated that this would set a precedent with regards to the bioassay reports in that when a positive response is seen at any dose, it is indicated that it is a positive response in the study. Dr. Medinsky suggested returning to the original first sentence of the conclusion without regimens A or B, only with *equivocal* rather than *some evidence*. Dr. Beland suggested adding "in female mice treated for two years." There was agreement by the Members. Dr. M.S. Wolfe, NIEHS, read the revised conclusion regarding carcinogenicity: "Under the conditions of this 2-year gavage study, there was *equivocal evidence of carcinogenic activity* of chloral hydrate in female $B6C3F_1$ mice treated continuously for two years based on increased incidences of pituitary gland pars distalis adenomas. No increased incidences of neoplasms were seen in female $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 or 28 days of age or in male $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 days of age. No hepatocarcinogenicity was seen under any dosing condition." The motion was accepted unanimously with six yes votes. In view of the change in level of evidence, Dr. Cullen moved to change his position on retaining the last sentence on hyperplasia, and Dr. Medinsky seconded the motion to delete the sentence. The motion was accepted unanimously with six votes.

INTRODUCTION



CHLORAL HYDRATE

CAS No. 302-17-0

Chemical Formula: $C_2H_3Cl_3O_2$ Molecular Weight: 165.42

Synonyms: Trichloracetaldehyde monohydrate; 1,1,1-trichloro-2,2-ethanediol; 2,2,2-trichloro-1,1-ethanediol
Trade names: Aquachloral Supprettes; Noctec; Somnos

PHYSICAL PROPERTIES, USE, AND EXPOSURE

Chloral hydrate is produced by the addition of water to trichloracetaldehyde (*Merck Index*, 1989). It is soluble in water, acetone, and methyl ethyl ketone and slightly soluble in turpentine, petroleum ether, carbon tetrachloride, benzene, and toluene. Chloral hydrate has an aromatic, penetrating, and slightly acrid odor and a slightly bitter, caustic taste. It is used medically as a sedative or hypnotic and as a rubefacient in topical preparations, and it is often given to children as a sedative during dental and other medical procedures at doses of approximately 50 mg/kg body weight; when given to adults, doses of 7 to 14 mg/kg are used (Blacow, 1972; AHFS, 1993). Chloral hydrate is used as a central nervous system depressant and sedative in veterinary medicine and as a general anesthetic in cattle and horses (Rossoff, 1974). Chloral hydrate is a byproduct of the chlorination of water, and concentrations in drinking water in the United States may reach 28 µg/L (IARC, 1995). It has also been detected in plant effluent after the bleaching of softwood pulp (IARC, 1995). Chloral, the anhydrous form of chloral hydrate, is used as a synthetic intermediate in the production of insecticides and herbicides, including DDT, methoxychlor, naled, trichlorfon, dichlorvos, and trichloroacetic acid (IARC, 1995).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

The absorption and systemic distribution of chloral hydrate are rapid. In $B6C3F_1$ mice, peak plasma concentrations were observed within 15 minutes of oral dosing, and the plasma half-life ($t_{½}$) was 2.7 minutes (Beland *et al*., 1998). After intravenous dosing, the $t_{½}$ of chloral hydrate in plasma was 5 to 24 minutes in $B6C3F_1$ mice (Abbas *et al*., 1996); likewise, the $t_{½}$ value for chloral hydrate in Swiss-Webster mice treated intraperitoneally was 12 minutes (Cabana and Gessner, 1970). In F344 rats treated orally, the $t_{½}$ in plasma was 3.8 minutes (Beland *et al*., 1998). A biphasic elimination profile has been observed in F344 rats after intravenous administration, with $t_{½}$ values of 5.4 and 45 minutes (Merdink *et al*., 1999). A similar rapid

clearance of chloral hydrate has been reported in dogs ($t_{½}$ = 4 minutes, Breimer *et al*., 1974; Hobara *et al*., 1987).

Chloral hydrate is oxidized to trichloroacetic acid and reduced to trichloroethanol; the latter compound can be conjugated to give trichloroethanol glucuronide (Figure 1). Cofactors for these conversions are NAD^+ (NADH) and $NADP^+$ (NADPH), which suggest the involvement of alcohol dehydrogenase, aldehyde reductase, aldehyde dehydrogenase, and cytochrome P450 (Ikeda *et al*., 1980; Hara *et al*., 1991; Lipscomb *et al*., 1996; Ni *et al*., 1996). In $B6C3F_1$ mice and F344 rats given chloral hydrate orally, peak plasma concentrations of trichloroethanol occurred in 15 minutes and the plasma $t_{½}$ was 4.5 and 8.0 minutes, respectively (Beland *et al*., 1998). Following intravenous dosing, $t_{½}$ was 15.6 to 21.6 minutes in $B6C3F_1$ mice (Abbas *et al*., 1996) and 43 minutes in F344 rats (Merdink *et al*., 1999).

Trichloroethanol is rapidly conjugated to trichloro-ethanol glucuronide, and very little free trichloro-ethanol is excreted (Hobara *et al*., 1987; Abbas *et al*., 1996). In $B6C3F_1$ mice treated orally with chloral hydrate, the plasma $t_{½}$ for trichloroethanol glucuronide was 7.3 minutes (Beland *et al*., 1998). In $B6C3F_1$ mice treated intravenously, the plasma $t_{½}$ for trichloroethanol glucuronide was 12.6 to 43.2 minutes (Abbas *et al*., 1996). In Swiss-Webster mice, the $t_{½}$ for combined trichloroethanol and trichloroethanol glucuronide was 211 minutes (Cabana and Gessner, 1970). The $t_{½}$ of trichloroethanol glucuronide was 24 minutes in F344 rats (Beland *et al*., 1998) and 52 minutes in dogs (Breimer *et al*., 1974).

Trichloroacetic acid is the most persistent metabolite detected in $B6C3F_1$ mice (Abbas *et al*., 1996), with a plasma $t_{½}$ of 8.5 hours after oral administration (Beland *et al*., 1998); this value decreased after repeated dosing. Trichloroacetic acid was also the most persistent metabolite in F344 rats treated orally with chloral hydrate, with a plasma $t_{½}$ of 11.2 hours (Beland *et al*., 1998). Trichloroacetic acid also persists in dogs; a plasma $t_{½}$ of 5.5 days has been reported (Breimer *et al*., 1974). Similar conclusions regarding the persistence of trichloroacetic acid in dogs have been reported in other studies (Marshall and Owens, 1954; Owens and Marshall, 1955; Hobara *et al*., 1987).

Abbas *et al*. (1996) reported another metabolite of chloral hydrate, dichloroacetic acid, at concentrations approaching those of trichloroacetic acid in $B6C3F_1$ mice administered chloral hydrate intravenously. However, other investigators have not found dichloroacetic acid in $B6C3F_1$ mice or F344 rats treated orally (Beland *et al*., 1998) or intravenously (Merdink *et al*., 1998, 1999). Other reports suggest that earlier evidence of dichloroacetic acid may have been the result of an experimental artifact (Ketcha *et al*., 1996; Fisher, 1997).

Chloral hydrate is an early but rapidly metabolized intermediate in the metabolism of trichloroethylene in $B6C3F_1$ mice and F344 rats, with subsequent metabolites being trichloroethanol and trichloroacetic acid (Green and Prout, 1985; Prout *et al*., 1985; Abbas and Fisher, 1997; Stenner *et al*., 1997, 1998; Greenberg *et al*., 1999). The metabolism of trichloro-ethanol derived from trichloroethylene is very similar in rats and mice; however, higher concentrations of trichloroacetic acid occurred in mice, which suggests a faster metabolism of chloral hydrate in mice than in rats (Prout *et al*., 1985). This is consistent with the observation that $B6C3F_1$ mice metabolize inhaled trichloroethylene to a greater extent than Osborne-Mendel rats (Stott *et al*., 1982). Furthermore, the metabolism of trichloroethylene administered orally in $B6C3F_1$ mice was linear over the range of 10 to 2,000 mg/kg, while in Osborne-Mendel rats, metabolism became constant and independent of dose (saturated) at 1,000 mg/kg or greater (Prout *et al.*, 1985). When trichloroethylene was administered orally to female Wistar rats or NMRI mice at doses of 2, 20, or 200 mg/kg, there was no evidence of saturation of trichloroethylene metabolism in the mice, but saturation was apparent at 200 mg/kg in the rats (Dekant *et al.*, 1986). A pronounced sex difference in the elimination of trichloroacetic acid has been observed in $B6C3F_1$ mice administered trichloroethylene; the $t_{½}$ in females (2.2 to 11.2 hours) was half that in males (5.6 to 24.8 hours) (Fisher *et al*., 1991; Fisher and Allen, 1993).

Humans

In humans, chloral hydrate is metabolized to trichloroethanol and trichloroacetic acid and is excreted in the urine as trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid (Marshall and Owens, 1954; Owens and Marshall, 1955; Blacow,

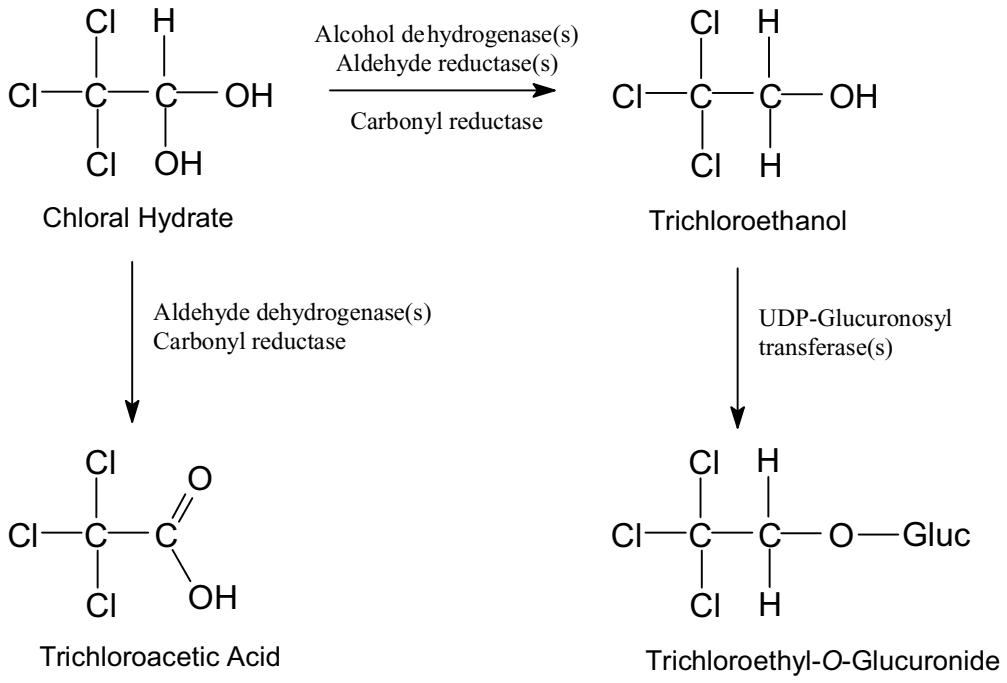


FIGURE 1
Metabolism of Chloral Hydrate

1972). When chloral hydrate was administered orally (15 mg/kg), a minimal amount of the parent compound was found in the blood, while the metabolites trichloroethanol, trichloroethanol glucuronide, and trichloroacetic acid rose to peak concentrations within an hour of administration (Breimer *et al.*, 1974). The estimated plasma half-lives of trichloroethanol and trichloroethanol glucuronide were about 7 hours, while the $t_{½}$ of trichloroacetic acid was 4 to 5 days. In later work (Breimer, 1977), chloral hydrate (15 mg/kg) was not detected and the peak concentrations of trichloroethanol and trichloroethanol glucuronide were observed after 20 to 60 minutes. The $t_{½}$ for trichloroethanol ranged from 7 to 9.5 hours (mean = 8.0 hours), the $t_{½}$ for trichloroethanol glucuronide ranged from 6.0 to 8.0 hours (mean = 6.7 hours), and the $t_{½}$ for trichloroacetic acid was approximately 4 days.

Zimmermann *et al*. (1998) conducted a similar study and obtained similar values; i.e., chloral hydrate could only be detected 8 to 60 minutes after dosing, the peak plasma concentration of trichloroethanol occurred at 40 minutes, and the peak trichloroacetic acid concentration was found at 32 hours. In addition, the $t_{½}$ for trichloroethanol was 9.3 to 10.2 hours and the $t_{½}$ for trichloroacetic acid was 89 to 94 hours. The same metabolites were identified in the blood of males administered 11 mg/kg (adults) or 44 mg/kg (infants) chloral hydrate orally (Gorecki *et al.*, 1990). In this study, trichloroacetic acid was the predominant metabolite in the adult, rising to peak concentrations 50 hours after administration. The predominant metabolite in the infant during the first 100 hours after administration was trichloroethanol. The half-lives of trichloroethanol and trichloroethanol glucuronide were greatest in newborn infants and decreased with time, which is probably a reflection of the decreased capacity for glucuronidation in neonates (Reimche *et al*., 1989; Gorecki *et al*., 1990; Hindmarsh *et al*., 1991; Mayers *et al*., 1991).

Dichloroacetic acid has been reported as a metabolite of chloral hydrate in humans (Henderson *et al*., 1997); however, as noted previously, this may have been due to an experimental artifact. Chloral hydrate has also been shown to cross the placenta and to be secreted in breast milk (Bernstine *et al*., 1954, 1956; MSDS, 1991).

TOXICITY

Experimental Animals

The oral LD_{50} of chloral hydrate in Sprague-Dawley rats following an acute dose is 480 mg/kg (Goldenthal, 1971). In a short-term study, eight male and eight female F344/N rats were administered 0, 50, 100, 200, 400, or 800 mg chloral hydrate/kg body weight in water by gavage 5 days per week for 17 days for a total of 12 doses (NTP, 1999). One male and two females dosed with 800 mg/kg died. The final mean body weight of 800 mg/kg males and the mean body weight gains of 400 and 800 mg/kg males were significantly less than those of the vehicle controls. The only clinical finding attributed to chloral hydrate treatment was light sedation in the 400 mg/kg groups and heavy sedation in the 800 mg/kg groups; sedation subsided within 30 minutes and 3 hours, respectively. No chemical-related histopathologic lesions were observed.

In male Sprague-Dawley rats administered daily doses of 24, 48, 96, or 168 mg/kg chloral hydrate in drinking water for 13 weeks, mean body weight, feed consumption, water consumption, and thymus weight were significantly decreased in the 168 mg/kg group (Daniel *et al.*, 1992b). Blood activities of lactate dehydrogenase and alanine aminotransferase were increased in all exposed groups, particularly in the 168 mg/kg group; aspartate aminotransferase activities were significantly increased in all exposed groups. Hepatocellular necrosis was observed in all groups except the 48 mg/kg group. No effects were observed in females receiving up to 288 mg/kg per day (Daniel *et al.*, 1992b).

The LD_{50} of chloral hydrate in CD-1 mice following a single acute dose was reported to be 1,442 mg/kg in males and 1,265 mg/kg in females (Sanders *et al*., 1982). In a 14-day gavage study in male CD-1 mice, increased liver weights, decreased spleen weights, and decreased blood lactate dehydrogenase concentrations were observed in mice administered 144 mg/kg chloral hydrate per day (Sanders *et al.*, 1982). In the NTP (1999) study, eight male and eight female $B6C3F_1$ mice were administered 0, 50, 100, 200, 400, or 800 mg chloral hydrate/kg body weight in water by gavage 5 days per week for 16 days for a total of 12 doses. One male in each group except the 400 mg/kg group died, and two females in the 800 mg/kg group died. The mean

body weight gains of all groups of dosed males were significantly greater than those of the vehicle control group. The only clinical finding attributed to chloral hydrate treatment was light sedation in the 400 mg/kg groups and heavy sedation in the 800 mg/kg groups; sedation subsided in these groups within 30 minutes and 3 hours, respectively. Liver weights of 400 mg/kg males and 800 mg/kg males and females were significantly greater than those of the vehicle control groups. No chemical-related histopathologic lesions were observed.

In CD-1 mice given 0, 70, or 700 ppm chloral hydrate (0, 17, or 170 mg/kg per day) in drinking water for 13 weeks, increases in body weight gain and relative liver weights were observed in males but not females (Sanders *et al.*, 1982). Body temperatures were somewhat depressed in males in each exposed group. Dose-related increases in the activities of the liver microsomal enzymes cytochrome b5, aniline hydroxylase, and aminopyrine-N-demethylase were observed in exposed males. Increased aniline hydroxylase activity was also observed in 700 ppm females. Serum lactate dehydrogenase and aspartate aminotransferase activities were also increased in males administered 700 ppm, while blood urea nitrogen concentrations decreased with increasing dose.

DNA strand breaks by chloral hydrate have been induced in rats and mice, although the metabolite responsible has not been established (Nelson and Bull, 1988). In this study, direct administration of trichloroethanol and trichloroacetic acid were also shown to induce strand breaks in DNA. In a subsequent study, chloral hydrate did not induce DNA strand breaks (Chang *et al.*, 1992).

Several studies have demonstrated covalent binding of trichloroethylene to DNA when incubated *in vitro* in the presence of a microsomal fraction; however, covalent binding has not been demonstrated convincingly following *in vivo* administration of trichloroethylene (Uehleke and Poplawski-Tabarelli, 1977; Stott *et al.*, 1982; Bergman, 1983; Crebelli and Carere, 1989). Trichloroethylene has been shown to induce unscheduled DNA synthesis in isolated rat hepatocytes (Costa and Ivanetich, 1984) and in human lymphocytes (Perocco and Prodi, 1981), but, again, the metabolite responsible for this induction has not been established.

Microsomal metabolism of chloral hydrate, trichloroethanol, or trichloroacetic acid results in lipid peroxidation (Ni *et al.*, 1994, 1996) and the resultant formation of a DNA adduct [3-(2-deoxy-β-D-erythro-pentofuranosyl)pyrimido[1,2α]purin-10(3H)-one] from the reaction of malondialdehyde with deoxyguanosine (Ni *et al.*, 1995). It is not known if treatment with chloral hydrate, trichloroethanol, or trichloroacetic acid will result in the formation of this adduct *in vivo*.

Elcombe *et al.* (1987) observed that trichloroethylene induced liver peroxisomes in mice but not in rats, while trichloroacetic acid induced peroxisomes in both species. More recently, DeAngelo *et al.* (1989) showed that trichloroacetic acid was a less effective peroxisome proliferator in rats than in mice. Because there is a more rapid conversion of trichloroethylene to trichloroacetic acid in mice than rats (Prout *et al.*, 1985; Elcombe *et al.*, 1987), the difference in peroxisome induction by trichloroethylene appears to be due to the decreased rate of formation of trichloroacetic acid in rats and/or the resistance of rats to peroxisome induction by trichloroacetic acid. Perchloroethylene, a mouse liver carcinogen that is metabolized much like trichloroethylene, was found to induce peroxisomes more readily in mice than rats (Odum *et al.*, 1988). This result correlated with much higher levels of circulating trichloroacetic acid in mice than in rats and led to the suggestion that peroxisome induction by trichloroacetic acid has a causal role in liver carcinogenesis in rodents.

Humans

In humans, chloral hydrate is corrosive to skin and mucous membranes. Therapeutic doses (50 mg/kg) may cause gastritis, with nausea and vomiting, and, occasionally, allergic skin reactions. Chronic exposure may result in symptoms similar to those of chronic alcoholism (Blacow, 1972). Hepatic damage with jaundice, renal damage with albuminuria, or heart damage may occur. The lethal human dose is estimated to be 5 to 10 g (MSDS, 1991); however, there have been reports of survival after a 30-g dose and death following a 4-g dose (IARC, 1995).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Kallman *et al.* (1984) exposed female CD-1 mice to 21 or 205 mg/kg chloral hydrate per day in drinking water starting 3 weeks before gestation and continuing through pregnancy and lactation. A chemical-related increase in gestational weight gain was observed, but no effects on gestation length, litter size, pup weight, or pup mortality were noted. No gross malformations were noted in the offspring, nor were there any effects on development of neurobehavioral reflexes or motor control.

The effects of chloral hydrate on sperm morphology and motility were examined in F344 rats administered 0, 55, or 188 mg/kg in drinking water for 52 weeks (Klinefelter *et al*., 1995). The highest exposure concentration caused a decrease in the percentage of motile sperm but did not affect epididymal or testicular histology.

Johnson *et al*. (1998) administered chloral hydrate in drinking water at 151 mg/kg to female Sprague-Dawley rats from gestation days 1 to 22. There was no evidence of maternal toxicity or changes in the number of implantation or resorption sites, number of live or dead fetuses, placental or fetal weight, crown-rump length, or incidence of morphological changes. In similar experiments, Johnson *et al.* (1998) did not observe developmental effects with trichloroethanol (153 mg/kg); however, trichloroacetic acid (291 mg/kg) caused increased numbers of resorptions, implantations, and cardiac anomalies. In earlier studies with trichloroethanol administered orally or by inhalation to Swiss-Webster mice, Sprague-Dawley rats, or Long-Evans rats during pregnancy, no clear signs of embryotoxicity or teratogenicity occurred; however, developmental delay did occur (reviewed by Crebelli and Carere, 1989). Adverse developmental effects of trichloroacetic acid have also been reported by Smith *et al.* (1989), who started dosing Long-Evans rats by gavage on day 6 of pregnancy and continued for 10 days. In this study, the trichloroacetic acid caused soft tissue malformations at incidences ranging from 9% at 330 mg/kg to 97% at 1,800 mg/kg. These malformations were primarily in the cardiovascular system.

The developmental toxicity of chloral hydrate has also been investigated *in vitro* using a rat whole embryo culture system (Saillenfait *et al*., 1995). No adverse effects were observed in Sprague-Dawley rat embryos explanted on gestation day 10 and exposed to 0.5 mM chloral hydrate for 46 hours; however, 1 to 2 mM chloral hydrate caused dose-related increases in numbers of malformations, and 2.5 mM was lethal.

Humans

No reproductive or developmental studies of chloral hydrate exposure in humans were found in the literature. Maternal exposure to trichloroethylene in contaminated drinking water has been associated with congenital defects such as eye and ear disorders, central nervous system abnormalities, chromosomal aberrations, and oral/cleft anomalies and with increases in perinatal deaths (Lagakos *et al.,* 1986; Goldberg *et al.,* 1990).

IMMUNOTOXICITY

Experimental Animals

No alteration in humoral or cell-mediated immunity was observed in male CD-1 mice administered 14.4 or 144 mg chloral hydrate/kg body weight by gavage for 14 days or in male mice exposed to 0.07 or 0.7 mg/mL (70 or 700 ppm) in drinking water for 90 days; however, female CD-1 mice exposed to 0.07 or 0.7 mg/mL for 90 days exhibited depressed humoral, but not cell-mediated, immune function (Kauffmann *et al.*, 1982). Immune function was evaluated by exposing spleen cells from treated mice to sheep erythrocytes and assessing antibody production. The number of antibody-forming cells per spleen was significantly reduced in both exposed groups of females, but the number of antibody-forming cells per million cells was significantly reduced only in the 0.7 mg/mL group.

Both cell-mediated and humoral immunity were depressed by trichloroethylene in CD-1 mice, particularly in females (reviewed by Davidson and Beliles, 1991).

Humans

In humans, sensitization may occur from repeated topical application of chloral hydrate (MSDS, 1991).

NEUROBEHAVIORAL TOXICITY

Experimental Animals

Male CD-1 mice administered chloral hydrate by gavage for 14 days or in drinking water for 90 days exhibited no behavioral responses besides those attributed to the acute sedative effects of chemical exposure (Kallman *et al.,* 1984). Pups of pregnant CD-1 mice exposed to 205 mg/kg chloral hydrate in drinking water through gestation and weaning demonstrated a depressed retention of passive avoidance learning shortly after weaning; pups of dams exposed to 21 mg/kg were not affected (Kallman *et al.*, (1984). Clinical signs of central nervous system toxicity, including ataxia, lethargy, convulsions, and hindlimb paralysis, have been reported in male and female ACI, August, Marshall, and Osborne-Mendel rats given up to 1,000 mg trichloroethylene/kg body weight by gavage for 2 years (NTP, 1988); ICR/HA-Swiss mice showed a period of excitement followed by a 15 to 30 minute subanesthetic state (Henschler *et al*., 1984).

Humans

In a review of hospital records, Miller and Greenblatt (1979) found depression to be the most common adverse central nervous system effect of choral hydrate exposure, with an incidence of 1.1%. Central nervous system excitement occurred at a lower frequency (0.22%). Evidence suggests neurotoxicity may result from chronic exposure of humans to trichloroethylene (Juntunen, 1986; Feldman *et al.*, 1988; Davidson and Beliles, 1991).

CARCINOGENICITY

Experimental Animals

In a skin paint study in "S" strain albino mice, the incidence of skin neoplasms was not significantly increased in mice treated with 12 or 15 mg/kg chloral hydrate once weekly for 2 or 15 weeks, respectively, followed by repeated applications of croton oil (Roe and Salaman, 1955). Rijhsinghani *et al.* (1986) reported that oral administration of a single dose of chloral hydrate (10 mg/kg) to eight 15-day-old male B6C3F$_1$ mice caused a significant increase in the incidence of hepatic neoplasms in a 92-week study; three adenomas and three carcinomas were observed in dosed mice compared to two carcinomas in 19 control mice.

Daniel *et al.* (1992a) reported that 1,000 ppm chloral hydrate (166 mg/kg per day) was hepatocarcinogenic when administered to male B6C3F$_1$ mice in drinking water for 104 weeks. The incidence of hepatocellular adenoma or carcinoma (combined) was 17 of 24 (71%) in treated mice versus 3 of 20 (15%) in control mice; the incidences of carcinoma were 2 of 20 (controls) and 11 of 24 (1,000 ppm group). In a recent study, male B6C3F$_1$ mice were exposed to 0, 13.5, 65, or 146.6 mg/kg chloral hydrate daily in drinking water for 104 weeks (George *et al.,* 2000). The incidence of hepatocellular carcinoma in the 146.6 mg/kg males was significantly greater (84.4%) than that in the controls (54.8%). In the 65 (79.5%) and 146.6 (90.6%) mg/kg males, the incidences of hepatocellular adenoma or carcinoma (combined) were significantly greater than that in the controls (64.3%). Neoplasms also occurred in the kidney, spleen, and testes, but were not exposure-related.

Male and female Sprague-Dawley rats were administered up to 135 mg chloral hydrate/kg body weight per day in drinking water for 124 or 128 weeks, respectively; the highest dose resulted in a statistically significant increase in the incidence of hepatocellular hypertrophy, but there was no evidence of neoplasia in any organ (Leuschner and Beuscher, 1998). A similar lack of neoplasia was reported for male F344 rats administered up to 162.6 mg/kg chloral hydrate daily in drinking water for 2 years (George *et al.,* 2000).

In B6C3F$_1$ mice, trichloroethylene (males and females) (NCI, 1976; NTP, 1983) and trichloroacetic acid (males) (Herren-Freund *et al.*, 1987) have been shown to be hepatocellular carcinogens. Forestomach papillomas and carcinomas were noted in Ha:ICR mice given epoxide-stabilized trichloroethylene by gavage (Henschler *et al.*, 1984), and a significant increase in the incidence of malignant lymphoma was observed in female NMRI mice exposed to epoxide-free trichloroethylene by inhalation (Henschler *et al.*, 1980). In other mouse inhalation studies, the incidences of lung adenocarcinoma were increased in exposed male and female ICR and Swiss mice and in female B6C3F$_1$ mice, and hepatocellular carcinoma was induced in male Swiss mice and male and female B6C3F$_1$ mice (reviewed by Davidson and Beliles, 1991). Trichloroethylene was not carcinogenic when administered by gavage to Osborne-Mendel, Sprague-Dawley, or Wistar rats

(reviewed by Crebelli and Carere, 1989). Male F344/N rats (NTP, 1990) exposed to trichloroethylene by gavage had a significantly increased incidence of renal adeno-carcinoma; however, the study was considered inadequate due to high mortality. Increased incidences of renal adenocarcinoma and Leydig cell tumors were observed in Sprague-Dawley rats exposed to trichloroethylene by inhalation (reviewed by Crebelli and Carere, 1989). Trichloroethanol did not induce tumors in male $B6C3F_1$ mice treated intraperitoneally with 1,000 nmol (15 mg/kg) or 2,000 nmol (30 mg/kg), with one-third of the dose given on day 8 and two-thirds on day 15 (Von Tungeln *et al.*, 1997; Von Tungeln, personal communication, 2000).

Humans

No epidemiologic studies of chloral hydrate were found in a review of the literature.

GENETIC TOXICITY

Chloral hydrate has been tested for mutagenicity *in vitro* and *in vivo* in a variety of assays. Positive responses were obtained in many assays, particularly those that induced chromosomal damage in the form of aneuploidy. A thorough review of genetic toxicology data for chloral hydrate is available (IARC, 1995).

Chloral hydrate gave positive results in the *Salmonella typhimurium* gene mutation assay in strains TA98 and TA100, with and without S9 activation (Waskell, 1978; Bruce and Heddle, 1979; Bignami *et al.*, 1980; Haworth *et al.*, 1983); no mutagenic activity was detected in these strains in other studies (e.g., Leuschner and Leuschner, 1991). Mutation induction has also been reported in *S. typhimurium* TA104 (Ni *et al.*, 1994).

Positive results have been seen with chloral hydrate in several genotoxicity assays in yeast and molds. In *Saccharomyces cerevisiae*, chloral hydrate induced chromosomal malsegregation (Albertini, 1990), aneuploidy (Parry and James, 1988), disomy and diploidy (Sora and Agostini Carbone, 1987), and increased mitotic gene conversion in the presence of metabolic activation in the D7 diploid strain (Bronzetti *et al.*, 1984). In a diploid strain of *Aspergillus nidulans*, chloral hydrate exposure resulted in increased numbers of nondisjunction diploids and haploids, and hyperploidy was seen in haploid strain 35 after treatment with chloral hydrate (Crebelli *et al.*, 1991). Aneuploidy was also observed in *A. nidulans* after chloral hydrate exposure (Crebelli and Carere, 1987).

In *Drosophila melanogaster*, a small increase in the frequency of sex-linked recessive lethal mutations was induced in germ cells of male flies fed chloral hydrate (5,500 ppm in 5% sucrose); chloral hydrate administered by abdominal injection (10,000 ppm in saline) did not induce germ cell mutations (Yoon *et al.*, 1985).

In mammalian cells treated with chloral hydrate *in vitro*, the observed genotoxic effects included aneuploidy in human lymphocytes (Vagnarelli *et al.*, 1990; Sbrana *et al.*, 1993) and Chinese hamster embryo cells (Furnus *et al.*, 1990; Natarajan *et al.*, 1993) and increased frequencies of kinetochore-positive micronucleated Cl-1 hamster cells (Degrassi and Tanzarella, 1988). However, no induction of DNA single-strand breaks was noted in rat or mouse hepatocytes or in human CCRF-CEM cells (Chang *et al.*, 1992) treated with chloral hydrate, and chloral hydrate failed to produce DNA-protein cross-links when incubated with isolated rat liver nuclei (Keller and Heck, 1988). Chloral hydrate has been shown to be weakly clastogenic in L5178Y mouse lymphoma cells (Harrington-Brock *et al.*, 1998).

In vivo, several studies have provided evidence of chloral hydrate-induced aneuploidy in spermatocytes of mice (Russo *et al.*, 1984; Liang and Pacchierotti, 1988; Miller and Adler, 1992), but not in oocytes (Mailhes *et al.*, 1988, 1993). Chloral hydrate exposure of premeiotic spermatocytes (Russo and Levis, 1992) and spermatogonial stem cells (Allen *et al.*, 1994; Nutley *et al.*, 1996) resulted in increased numbers of micronuclei in spermatids. In one experiment, however, kinetochore labeling of induced spermatid micronuclei did not indicate the presence of centromere-containing whole chromosomes, which would have been expected if aneuploidy had been induced (Allen *et al.*, 1994). In a study by Nutley *et al.* (1996), two methods of aneuploidy assessment were used (anti-kinetochore antibody staining and fluorescence *in situ* hybridization with centromeric DNA probes), and both showed increased numbers of micronuclei with centromeric

labels. Nutley *et al.* (1996) suggested that chloral hydrate induced structural chromosomal damage in treated spermatogonial stem cells. Another study reported induction of single-strand breaks in hepatic cell DNA of rats and mice treated with chloral hydrate (Nelson and Bull, 1988), but a similar study failed to replicate these results (Chang *et al.*, 1992). Some somatic cell studies in rats and mice showed induction of micronuclei or chromosomal aberrations (Leopardi *et al.*, 1993), while others did not (Xu and Adler, 1990; Adler *et al.*, 1991).

STUDY RATIONALE

The Food and Drug Administration (FDA) nominated chloral hydrate for study based upon widespread human exposure and its potential hepatotoxicity and the toxicity of related chemicals (Smith, 1990). The NCTR conducted studies on chloral hydrate as part of an interagency agreement with the NIEHS to conduct comprehensive toxicologic assessments of FDA priority chemicals nominated to the NTP. Data from the studies will be used to augment the regulatory decision process in terms of accurately assessing human health risk.

The goal of the study was to assess the effect of the animal's age and the duration of dosing on the tumorigenicity of chloral hydrate. The study was conducted in B6C3F$_1$ mice and consisted of five regimens: regimen A, female B6C3F$_1$ mice dosed for 2 years beginning on postnatal day 28; regimen B, female B6C3F$_1$ mice dosed for 3, 6, or 12 months beginning on postnatal day 28; regimen C, female B6C3F$_1$ mice given a single dose on postnatal day 28; regimen D, female B6C3F$_1$ mice given a single dose on postnatal day 15; and regimen E, male B6C3F$_1$ mice given a single dose on postnatal day 15.

As noted previously, chloral hydrate was tumorigenic in male B6C3F$_1$ mice exposed to 1,000 ppm in drinking water, which was equivalent to an average daily dose of 166 mg/kg per day (Daniel *et al.,* 1992a). An increase in the multiplicity of hepatocellular carcinoma was also reported in male B6C3F$_1$ mice exposed to 800 ppm (87 mg/kg per day) or 1,400 ppm (165 mg/kg per day) (DeAngelo and George, 1995). Based on the reported neoplasm data, regimen A mice were dosed for 2 years with 0, 25, 50, or 100 mg/kg chloral hydrate by gavage. The 100 mg/kg dose approximated the dose reported to induce a high hepatic neoplasm incidence in male B6C3F$_1$ mice, while 25 mg/kg approximated the dose of chloral hydrate used in pediatric medicine. These doses would provide fundamental dose response information on the tumorigenicity of chloral hydrate. In addition, a comparison of these data with the results from the U.S. Environmental Protection Agency studies would indicate if sex differences exist in the response to chloral hydrate. Furthermore, in a companion study using male B6C3F$_1$ mice (NTP, 2002), the doses were identical to regimen A in the current study, providing additional data for possible sex-related differences.

Although chloral hydrate was reported to be tumorigenic during continuous lifetime administration to male B6C3F$_1$ mice (Daniel *et al.*, 1992a), the tumorigenicity was unknown when administration was of shorter duration. This was important to establish because the clinical usage of chloral hydrate typically does not involve lifetime exposures but rather involves short-term treatments to children and the elderly. To establish the effect of treatment duration on neoplasm incidence, regimen B mice were administered the highest dose (100 mg/kg) used in regimen A for 3, 6, or 12 months; at these times, dosing was discontinued, selected mice were killed, and the remaining mice were held for the duration of the study. A comparison of regimen A to regimen B provided the data needed to determine the length of time necessary for full expression of the tumorigenicity of chloral hydrate.

As noted previously, a single administration of 10 mg/kg chloral hydrate to male B6C3F$_1$ mice at 15 days of age induced a dose-dependent increase in hepatic neoplasms when assessed at 2 years (Rijhsinghani *et al.*, 1986). While the results from this experiment suggested that preweanling mice may be unusually sensitive to the tumorigenic effects of chloral hydrate, the study was compromised due to the small number of animals in each group. Therefore, to confirm and expand these data, regimens C and D female mice were given a single dose of 0, 10, 25, or 50 mg/kg chloral hydrate on postnatal days 28 and 15, respectively. A comparison of regimen C to regimen D would indicate whether preweanling mice were inordinately sensitive to the tumorigenic effects of chloral hydrate, while a comparison of regimen C to regimen B would indicate whether a single dose of chloral hydrate in juvenile mice was sufficient to increase the neoplasm incidence.

In lifetime dosing studies, female B6C3F$_1$ mice appear to be more sensitive to chemically induced neoplasms than male B6C3F$_1$ mice (Haseman *et al.*, 1984).

However, male mice appear to be more responsive than females when the treatment phase is restricted to infancy (Wislocki *et al.*, 1986; Fujii, 1991; Flammang *et al.*, 1997; Fu *et al.*, 1998, 2000). Therefore, regimen E male B6C3F$_1$ mice were given a single dose of 0, 10, 25, or 50 mg/kg chloral hydrate on postnatal day 15. A comparison of sex differences between regimen E and regimen D would possibly indicate a more sensitive neoplasm response when the mice are dosed as infants.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF CHLORAL HYDRATE

Chloral hydrate was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (12H0289), which was used during the 2-year study. Identity, purity, and stability analyses were conducted by the study laboratory (Appendix H).

Lot 12H0289, a white, crystalline solid, was identified as chloral hydrate by gas chromatography/mass spectrometry and proton nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of chloral hydrate; the mass spectra were also consistent with the literature spectra of chloral hydrate.

The purity of the lot 12H0289 was determined by gas chromatography. Samples from each of the two bottles of lot 12H0289 received from the manufacturer were analyzed. One impurity peak was detected for each sample. For one sample, the impurity peak had an area of approximately 0.5% relative to the major peak area; the relative area of the impurity peak for the second sample was too small to be quantified. The results indicated a purity of approximately 99.5% or greater.

The bulk chemical was stored in the original amber glass bottles at room temperature, protected from light, in a container of dry indicating silica gel. Stability was monitored during the 2-year study with gas chromatography and with gas chromatography/mass spectrometry. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing chloral hydrate with distilled deionized water to give the required concentrations (Table H2). The dose formulations were prepared monthly and stored for up to 4 weeks at room temperature in amber glass bottles with Teflon®-lined caps, protected from light.

Stability studies of the 0.85, 1.4, and 2.5 mg/mL dose formulations were performed by the study laboratory using gas chromatography. Stability was confirmed for 24 days for the 0.85 mg/mL formulation, 78 days for the 2.5 mg/mL formulation, and 7 months for the 1.4 mg/mL formulation when stored in amber glass vials at room temperature.

Periodic analyses of the dose formulations were conducted by the study laboratory with gas chromatography. During the 2-year study, the dose formulations were analyzed approximately every 3 months, and animal room samples were analyzed every 6 months (Table H3). All of the dose formulations analyzed were within 10% of the target concentrations, with no value greater than 109% of the target concentration. Of the animal room samples analyzed, 69 of 78 were within 10% of the target concentrations. Samples outside the 10% range were those obtained from the first sample collection (Appendix H). By the third collection, all samples were within the required specification. Animals were dosed after a minimum of three samples had been drawn through the dosing machine.

STUDY DESIGN

Groups of female mice from four regimens and groups of male mice from one regimen received chloral hydrate in distilled water by gavage; vehicle control groups received distilled water only (Table 1). In regimen A, groups of 48 female mice received 0, 25, 50, or 100 mg chloral hydrate/kg body weight 5 days per week for 104 weeks beginning when they were 28 days old. In regimen B, 24 female mice received 0 mg/kg and three groups of 48 female mice received 100 mg/kg 5 days per week for 3, 6, or 12 months beginning when they were 28 days old. Eight mice from the 0 and 100 mg/kg groups were killed at 3, 6, or 12 months. The remaining mice were held without dosing for the duration of the

2-year study. In regimen C, groups of 48 female mice received a single dose of 0, 10, 25, or 50 mg/kg when they were 28 days old and were held for 104 weeks. In regimens D and E, groups of 48 female and 48 male mice, respectively, received a single dose of 0, 10, 25 or 50 mg/kg when they were 15 days old and were held for 104 weeks. Dosing volumes were 100 µL for 15-day-old mice, 200 µL for mice 28 to 41 days old, and 500 µL for mice older than 41 days.

Additional groups of four mice from regimens C, D, and E and mice killed at 3 or 6 months from regimen B (eight mice per group) were designated for hepatic cell proliferation analyses; mice killed at 3 or 6 months in regimen B were also designated for apoptosis analyses.

Source and Specification of Animals

Male and female B6C3F$_1$/Nctr BR (C57BL/6N × C3H/HeN MTV$^-$) mice were obtained from the study laboratory's breeding colony. Before the beginning of the study, mice were acclimated for 7 days in regimens A, B, and C. The health of the animals was monitored during the studies according to the protocols of the study laboratory's Sentinel Animal Program (Appendix J).

Animal Maintenance

Mice were housed four per cage. Feed and water were available *ad libitum*. Cages were changed once per week. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations

All animals were observed twice daily. Clinical findings were recorded weekly. Body weights were recorded weekly and at the end of the study.

Hepatic Cell Proliferation and Apoptosis Analyses

Special study mice included in regimens C, D, and E were sacrificed 24 hours after dosing for hepatic cell proliferation analyses. Liver slices (approximately 5 mm thick) from the median lobe were fixed in 10% neutral buffered formalin for 24 hours, processed for 8 hours on a Shandon Pathcenter Tissue Processor (Shandon, Inc., Pittsburgh, PA), embedded in paraffin, sectioned to a thickness of 4 µm, and mounted on positive-charged slides. Cell proliferation indices were determined by immunohistochemical localization of proliferating cell nuclear antigen (PCNA), slightly modified from Foley *et al.* (1991). Liver sections were deparaffinized in xylene and rehydrated with decreasing concentrations of ethanol into phosphate-buffered saline. Endogenous peroxidase was quenched with 3% H_2O_2 containing 0.1% sodium azide. The sections were placed in an antigen-retrieval solution consisting of 1% zinc sulfate in deionized water and heated for 7.5 minutes in a 700-watt microwave oven set to full power. A routine streptavidin procedure was performed, beginning with application of 0.5% casein to block nonspecific binding of subsequent antibody and sequential incubation of sections in a mouse monoclonal anti-PCNA antibody (clone PC10, Dako Corp., Carpinteria, CA), biotinylated goat anti-mouse IgG (Boehringer-Mannheim, Indianapolis, IN), and streptavidin-conjugated horseradish peroxidase (Jackson Immunoresearch Laboratories, West Grove, PA). The PCNA-positive cells were visualized by incubating the sections in 3,3′-diaminobenzidine hydrochloride chromogen followed by counterstaining with Mayer's hematoxylin. The stained slides were analyzed with the point counting feature of an image analysis system (Optimas Corporation, Bothell, WA). The number of cells analyzed per liver to determine the percentage of cells in the G0, G1, G2, S, and M phases of the cell cycle was 2,045 ± 30 cells (mean ± standard deviation) for mice in regimen C, 2,043 ± 32 cells for mice in regimen D, and 2,069 ± 60 cells for mice in regimen E.

For mice sacrificed at 3 or 6 months in regimen B, the PCNA analysis was used to measure hepatic cell proliferation with 2,033 ± 21 cells being analyzed per liver. Apoptotic cell indices were determined with an Apoptag detection system (Oncor, Gaithersburg, MD), which measures *in situ* end-labeling of 3′-hydroxy DNA strand breaks localized in apoptotic bodies (Gavrieli *et al*., 1992). Permeabilized tissue sections were enzymatically labeled with digoxigenin-nucleotide via terminal deoxynucleotidyl transferase and subsequently exposed to horseradish peroxidase-conjugated anti-digoxigenin antibody. Staining was developed with 3,3′-diaminobenzidine, and sections were counterstained with methyl green. The apoptosis assay was conducted on 2,100 ± 178 cells per liver.

Tumor Mutation Analyses

DNA was isolated from 17 formalin-fixed, paraffin-embedded, sectioned mouse liver tumors (approximately 2-4 mm × 10-12 mm × 5 µm) from female mice in regimens A and B. The DNA samples were further purified on QIAQuick Separation columns (Qiagen Inc., Valencia, CA). For each DNA sample, K-*ras* exons 1 and 2, H-*ras* exons 1 and 2, *p53* exons 5, 6, 7, and 8, and β-catenin exon 2 were amplified by polymerase chain reaction (PCR). A 50 µL reaction mixture contained 200 µM each of dATP, dCTP, dGTP, and dTTP; 250 nM of appropriate primers (Table 2); 1.5 U Ampli Taq GOLD DNA polymerase (Perkin Elmer, Applied Biosystems Division, Foster City, CA); and 1× Ampli Taq buffer [50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, and 10 mM Tris-HCl (pH 8.3)]. A 40-base-pair GC clamp was attached to the 5′-end of all forward primers; for H-*ras* exons 1 and 2, K-*ras* exon 2, and β-catenin exon 2, a round of PCR amplification was conducted before adding the GC clamp. Samples were preheated to 94° C for 10 minutes and cycled for 35 rounds of 1 minute at 94° C, 1 minute at 62° C (58° C for K-*ras*), and 1 minute at 72° C. A final extension period of 7 minutes at 72° C was added at the end of the reaction. All amplified fragments were prescreened by denaturing gradient gel electrophoresis (Mittelstaedt *et al*., 1999). The acrylamide gels (9.6%) were run overnight at 90 volts and 64° C, stained with ethidium bromide, and photographed under ultraviolet illumination. H-*ras* exon 2, K-*ras* exon 1, and *p53* exons 6 and 7 were run on 30% to 55% gradient gels; H-*ras* exon 1 on 40% to 55% gels; K-*ras* exon 2 on 20% to 45% gels; *p53* exons 5 and 8 on 40% to 65% gels; and β-catenin exon 2 on 25% to 50% gels.

Pathology

Complete necropsies were performed on all mice. Microscopic examinations were performed on all vehicle control mice, on all mice from the highest dose group in each regimen, and on all mice that died during the study. The liver was weighed, and all organs and tissues were examined for grossly visible lesions. All major tissues were fixed and preserved in 10% neutral buffered formalin. Tissues from the vehicle control groups, highest dose groups, and animals removed before the scheduled terminal sacrifice were processed and trimmed, embedded in Tissue-Prep II, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the study laboratory's Micropath Data Collection System. The slides, paraffin blocks, and residual wet tissues were sent to the study laboratory's Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment group. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year study, a quality assessment pathologist evaluated slides from all tumors and all potential target organs. This included the liver, pituitary gland, and lung from mice in regimen A, the lung from mice in regimen B, and the pituitary gland and lung from vehicle control mice in regimens C and D. In addition, all previously diagnosed tumors in all organs from all animals were reexamined.

Differences of opinion were reconciled between the study and quality assessment pathologists. The quality assessment pathologist served as the Pathology Working Group (PWG) chairperson and presented histopathology slides containing the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist, the study pathologist, and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory

pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al*. (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al*. (1986).

TABLE 1
Experimental Design and Materials and Methods in the Gavage Study of Chloral Hydrate

Study Laboratory
National Center for Toxicological Research (NCTR) (Jefferson, AR)

Strain and Species
B6C3F$_1$/Nctr BR (C57BL/6N × C3H/HeN MTV$^-$) mice

Animal Source
NCTR breeding colony (Jefferson, AR)

Time Held Before Studies
Regimen A: 7 days
Regimen B: 7 days
Regimen C: 7 days
Regimen D: 15 days
Regimen E: 15 days

Age When Studies Began
Regimen A: 28 days
Regimen B: 28 days
Regimen C: 28 days
Regimen D: 15 days
Regimen E: 15 days

Date of First Dose
Regimen A: 15 May-4 July 1995
Regimen B: 15 May-4 July 1995
Regimen C: 16 May-3 July 1995
Regimen D: 2 May-19 June 1995
Regimen E: 2 May-19 June 1995

Duration of Dosing
Regimen A: 5 days/week for 104 weeks
Regimen B: 5 days/week for 3, 6, or 12 months
Regimen C: single dose
Regimen D: single dose
Regimen E: single dose

Date of Last Dose
Regimen A: 12 May-1 July 1997
Regimen B: 14 August 1995-2 July 1996
Regimen C: 16 May-3 July 1995
Regimen D: 2 May-19 June 1995
Regimen E: 2 May-19 June 1995

Necropsy Dates
Regimen A: 13 May-2 July 1997
Regimen B: 15 August 1995-2 July 1997
Regimen C: 14 May-1 July 1997
Regimen D: 20 April-17 June 1997
Regimen E: 29 April-17 June 1997

Average Age at Necropsy
Regimen A: 109 weeks
Regimen B: 109 weeks; interim sacrifice, 17, 30, or 56 weeks
Regimen C: 109 weeks
Regimen D: 107 weeks
Regimen E: 107 weeks

TABLE 1
Experimental Design and Materials and Methods in the Gavage Study of Chloral Hydrate

Size of Study Groups
Regimens A, C, and D: 48 females
Regimen B: 24 (0 mg/kg) or 48 (100 mg/kg) females
Regimen E: 48 males
Special study
Regimens C, D, and E: 4 males or 4 females

Method of Distribution
Animals were distributed randomly into groups of approximately equal initial mean body weights.

Animals per Cage
4

Method of Animal Identification
Ear clip

Diet
NIH-31 autoclaved pelleted diet (Purina Mills, Richmond, IN), available *ad libitum*

Water
Millipore-filtered water (Jefferson municipal supply) via water bottle, available *ad libitum*

Cages
Polycarbonate (Lab Products, Seaford, DE), changed once weekly

Bedding
Hardwood chips (Northeastern Products, Warrensburg, NY), changed once weekly

Racks
Stainless steel (Allentown Caging Equipment Co., Inc., Allentown, NJ), changed once monthly

Animal Room Environment
Temperature: 72° ± 3° F
Relative humidity: 50% ± 15%
Room fluorescent light: 12 hours/day
Room air changes: 10/hour

Doses
Regimen A: 0, 25, 50, or 100 mg/kg (dosing volume 200 µL until 41 days of age, then 500 µL)
Regimen B: 0 or 100 mg/kg (dosing volume 200 µL until 41 days of age, then 500 µL)
Regimen C: 0, 10, 25, or 50 mg/kg (dosing volume 200 µL)
Regimen D: 0, 10, 25, or 50 mg/kg (dosing volume 100 µL)
Regimen E: 0, 10, 25, or 50 mg/kg (dosing volume 100 µL)

Type and Frequency of Observation
Observed twice daily; clinical findings were recorded weekly; animals were weighed once weekly and at the end of the study.

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsy was performed on all animals. The liver was weighed.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Study of Chloral Hydrate

Histopathology
Complete histopathology was performed on 0 and 100 mg/kg mice in regimens A and B, on 0 and 50 mg/kg mice in regimens C, D, and E, and on all mice dying during the study. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, coagulating gland, esophagus, eye, gallbladder, harderian gland, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, lacrimal gland, larynx, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland (females), muscle, nose, ovary, pancreas, pancreatic islets, parathyroid gland, peripheral nerve, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spinal cord, spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland. The liver, lung, and pituitary gland were also examined in all remaining groups.

Special Study
Hepatic Cell Proliferation and Apoptosis Analyses
Four mice per group in regimens C, D, and E were sacrificed 24 hours after dose administration and liver samples were collected for hepatic cell proliferation analyses. Liver samples were also collected from eight mice per group killed at 3 or 6 months in regimen B for hepatic cell proliferation and apoptosis analyses.

TABLE 2
Polymerase Chain Reaction Primers

Primer	Sequence
H-*ras* Primers	
Exon 1	
Forward	5′-GGC CTT GGC TAA GTG TGC TT-3′
Reverse	5′-CCT GGG CTG TTT GGT CAT TT-3′
Exon 2	
Forward	5′-GGC TGG TTC TGT GGA TTC TC-3′
Reverse	5′-CAC GGG CTA GCC ATA GGT-3′
K-*ras* Primers	
Exon 1	
Forward	5′-GCC TGC TGA AAA TGA CTG A-3′
Reverse	5′-GCA GCG TTA CCT CTA TCG TA-3′
Exon 2	
Forward	5′-TTC TCA GGA CTC CTA CAG GAA A-3′
Reverse	5′-CCC ACC TAT AAT GGT GAA TAT C-3′
***p53* Primers**	
Exon 5	
Forward	5′-TCC CCG ACC TCC GTT CTG-3′
Reverse	5′-CCC ACA GGC GGT GTT GAG-3′
Exon 6	
Forward	5′-CAT CTC CCG GCT TCT GAC-3′
Reverse	5′-CAG GAG GGT GAG GCA AAC-3′
Exon 7	
Forward	5′-TGT GCC GAA CAG GTG GAA-3′
Reverse	5′-CGG GAC TCG TGG AAC AGA-3′
Exon 8	
Forward	5′-GGC TTC TCG GGG TTC CTG-3′
Reverse	5′-CTC CTC CGC CTC CTT GGT-3′
β-Catenin Primers[a]	
Exon 2	
Forward	5′-TAG AGG TAG CAT TTT CAG TTC AC-3′
Reverse	5′-TAG CTT CCA AAC ACA AAT GC-3′

[a] de La Coste *et al*. (1998)

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are one sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B3, C1, C3, D1, D3, E1, and E3 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, D2, and E2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. Tables A2, B2, C2, D2, and E2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, to animals that do not reach terminal sacrifice.

Analysis of Neoplasm Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of neoplasm incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a neoplasm prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and $B6C3F_1$ mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require neoplasm lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of neoplasm incidence, and reported P values are one sided. Positive trends are reported with right-tailed P values. Negative trends are reported with left-tailed P values, with the letter N added to indicate a lower incidence as dose increases.

Analysis of Nonneoplastic Lesion Incidences

The relationship between chloral hydrate dose and nonneoplastic lesion severity was analyzed by the Jonckheere-Terpstra test (Jonckheere, 1954). This test looks for monotonic trends. Unlike the Poly-3 test, it is not age adjusted, and animals that died early without nonneoplastic lesions or with less severe lesions were counted as though they had full opportunity to develop a more severe nonneoplastic lesion. The P values are one tailed (i.e., only increasing severity with dose will be significant). Pairwise comparisons were conducted using Williams' modification (Williams, 1986) of Shirley's test (Shirley, 1977). As with the Jonckheere-Terpstra test, this analysis detects monotonic differences and is not age adjusted. Because the test is monotonic, a mid-dose comparison cannot be significant unless all of the doses greater than it are also significant.

Analysis of Continuous Variables

Body weight comparisons were made at 6-month intervals. Body weights were analyzed separately for each regimen using repeated measures for mixed models. At each time interval, tests were conducted for linear dose trends. Dunnett's test (Dunnett, 1955) was used to compare the dosed group means to the vehicle control means at each time interval. The mice sacrificed at interim evaluations in regimen B were excluded from the analysis. Mice in regimen B were compared to the control mice in regimen A. One-way analysis of variance (ANOVA) was used to analyze liver weight, terminal body weight, and the ratio of liver weight to the terminal body weight. Only terminal sacrifice animals were included in this analysis. Dunnett's test was used to compare the dosed group means to the vehicle control means.

Student's t-test was used to compare cell proliferation and apoptosis parameters between vehicle control mice and mice dosed with 100 mg/kg chloral hydrate for 3 or 6 months in regimen B. One-way ANOVA was used to analyze cell proliferation parameters in mice from regimens C, D, and E; Dunnett's method was used to compare individual dose groups to the vehicle control groups.

Quality Assurance Methods

The study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of the National Center for Toxicological Research performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at the NCTR. The audit findings were reviewed and assessed by the NCTR staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of chloral hydrate was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, sex-linked recessive lethal mutations in *Drosophila melanogaster*, and micronucleated erythrocytes in mouse bone marrow. The protocols for these studies and the results are given in Appendix F.

The genetic toxicity studies of chloral hydrate are part of a larger effort by the NTP to develop a comprehensive database that would permit a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous con-siderations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). These short-term genetic toxicity tests were originally developed to clarify mechanisms of chemical-induced DNA damage growing out of the earlier electrophilicity/mutagenicity relationship proposed by Miller and Miller (1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). Therefore, the information obtained from these tests applies only to mutagenic carcinogens.

For mutagenic carcinogens, the combination of DNA reactivity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in multiple species and genders of rodents and at multiple tissue sites (Ashby and Tennant, 1991). Data from NTP studies show that a positive response in *Salmonella* is the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) and that there is no com-plementarity among the *in vitro* genetic toxicity tests (Tennant *et al*., 1987; Zeiger *et al*., 1990). That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. Although other *in vitro* genetic toxicity tests correlate less well with rodent carcinogenicity compared with the *Salmonella* test, these other tests can provide useful information on the types of DNA and chromosomal effects induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in the acute *in vivo* bone marrow chromosome aberration test or micronucleus test appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests are associated with high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

Regimen A mice were dosed for 2 years with 0, 25, 50, or 100 mg/kg chloral hydrate by gavage. The 100 mg/kg dose approximated the dose reported to induce a high hepatic neoplasm incidence in male B6C3F$_1$ mice, while 25 mg/kg approximated the dose of chloral hydrate used in pediatric medicine. To establish the effect of treatment duration on neoplasm incidence, regimen B mice were administered the highest dose (100 mg/kg) used in regimen A, but the dosing was discontinued in regimen B after 3, 6, or 12 months; the animals were then held for the duration of the study. Regimens C and D female mice were given a single dose of 0, 10, 25, or 50 mg/kg chloral hydrate on postnatal days 28 and 15, respectively, in order to assess the sensitivity of preweanling and juvenile mice to the tumorigenic effects of chloral hydrate. Regimen E male B6C3F$_1$ mice were given a single dose of 0, 10, 25, or 50 mg/kg chloral hydrate on postnatal day 15 to determine if males were more sensitive to exposure during infancy than females.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for regimens A, B, and C are shown in Tables 3, 4, and 5, respectively, and in the Kaplan Meier survival curves (Figures 2 and 3). Estimates of 2-year survival probabilities for regimens D and E are shown in Table 6 and in the Kaplan Meier survival curves (Figure 4). Survival of all dosed groups in all regimens was similar to that of the vehicle control groups.

TABLE 3
Survival of Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Animals initially in study	48	48	48	48
Accidental deaths[a]	0	0	1	1
Moribund	2	4	3	2
Natural deaths	9	5	1	9
Animals surviving to study termination	37	39[e]	43	36
Percent probability of survival at end of study[b]	77	81	92	77
Mean survival (days)[c]	676	699	720	681
Survival analysis[d]	P=0.498	P=0.299N	P=0.026N	P=0.490

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.
[e] Includes one mouse that died after removal from the animal room but before the terminal sacrifice

TABLE 4
Survival of Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

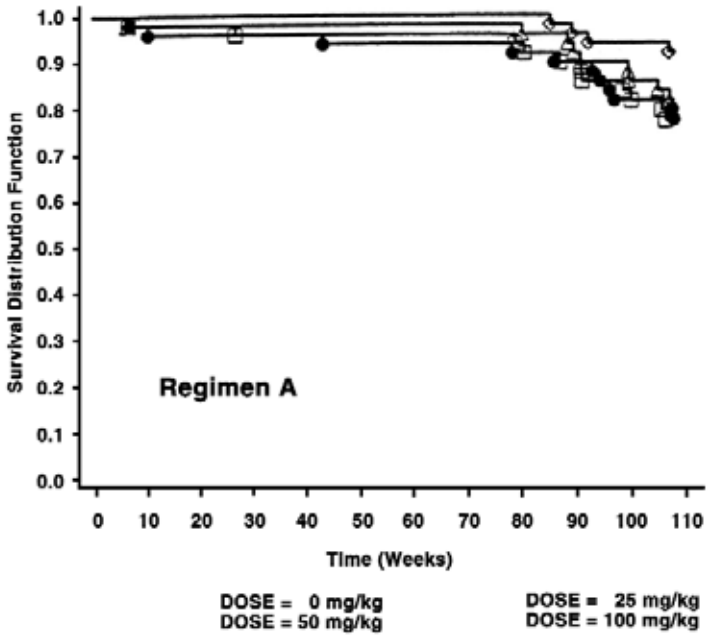
	Vehicle Control[a]	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)	24 Months[b]
Animals initially in 2-year study	72	48	48	48	48
3-month interim evaluation[c]	8	8	0	0	0
6-month interim evaluation[c]	8	0	8	0	0
12-month interim evaluation[c]	8	0	0	8	0
Accidental deaths[c]	0	0	0	0	1
Moribund	2	2	3	0	2
Natural deaths	9	4	6	7	9
Animals surviving to study termination	37	34	31	33	36
Percent probability of survival at end of study[d]	77	85	78	83	77
Mean survival (days)[e]	676	699	691	710	681
Survival analysis[f]	P=0.390	P=0.179N	P=0.458N	P=0.248N	P=0.490

[a] Forty-eight mice served as vehicle controls for regimens A and B; the remaining 24 mice were designated for regimen B interim evaluations.
[b] Mice from regimen A
[c] Censored from survival analyses
[d] Kaplan-Meier determinations
[e] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[f] The result of the life table trend test is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.

TABLE 5
Survival of Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Animals initially in study	48	48	48	48
Moribund	3	4	0	2
Natural deaths	3	4	3	6
Animals surviving to study termination	42	40	45	40
Percent probability of survival at end of study[a]	88	83	94	83
Mean survival (days)[b]	715	716	725	705
Survival analysis[c]	P=0.383	P=0.299	P=0.140N	P=0.277

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A lower mortality in a dose group is indicated by **N**.



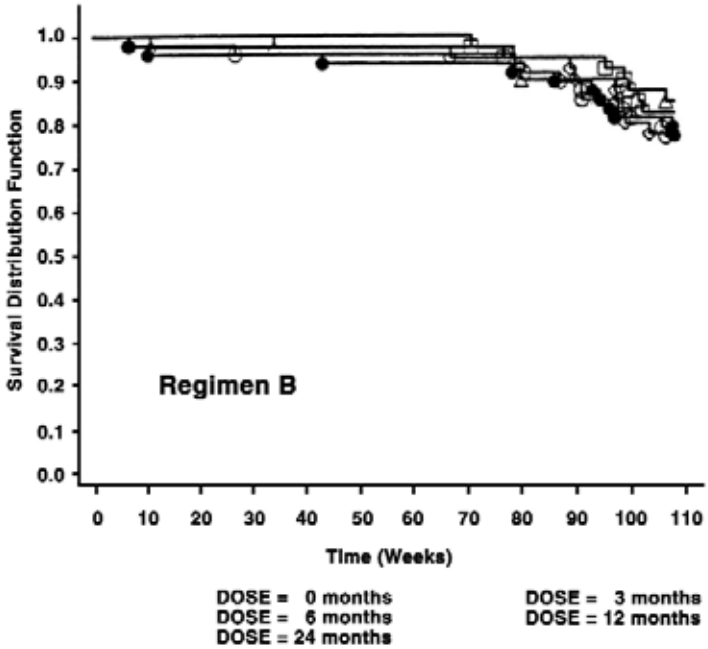


FIGURE 2
Kaplan-Meier Survival Curves for Female Mice Administered a Single Dose of Chloral Hydrate by Gavage for 2 Years (Regimen A) or 3, 6, or 12 months (Regimen B)

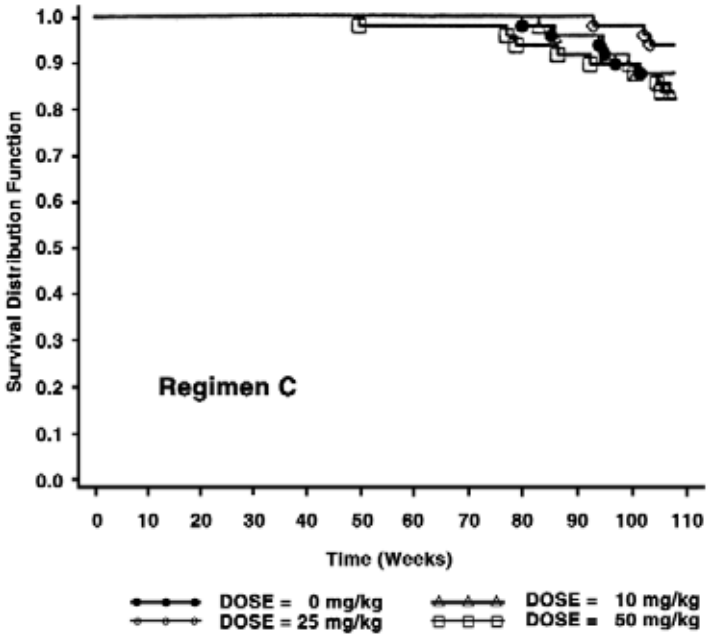


FIGURE 3
Kaplan-Meier Survival Curves for Regimen C Female Mice Administered a Single Dose of Chloral Hydrate by Gavage

TABLE 6
Survival of Regimen D Female Mice and Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Animals initially in study	48	48	48	48
Regimen D				
Accidental deaths[a]	2	0	0	0
Moribund	4	1	6	4
Natural deaths	6	6	3	4
Animals surviving to study termination	36	41[e]	39[e]	40
Percent probability of survival at end of study[b]	79	85	81	83
Mean survival (days)[c]	687	707	708	707
Survival analysis[d]	P=0.351N	P=0.208N	P=0.351N	P=0.246N
Regimen E				
Moribund	1	1	0	3
Natural deaths	2	6	2	5
Animals surviving to study termination	45	41	46	40[e]
Percent probability of survival at end of study	94	85	96	83
Mean survival (days)	724	697	726	720
Survival analysis	P=0.129	P=0.089	P=0.324N	P=0.059

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[e] Includes one mouse that died after removal from the animal room but before the terminal sacrifice

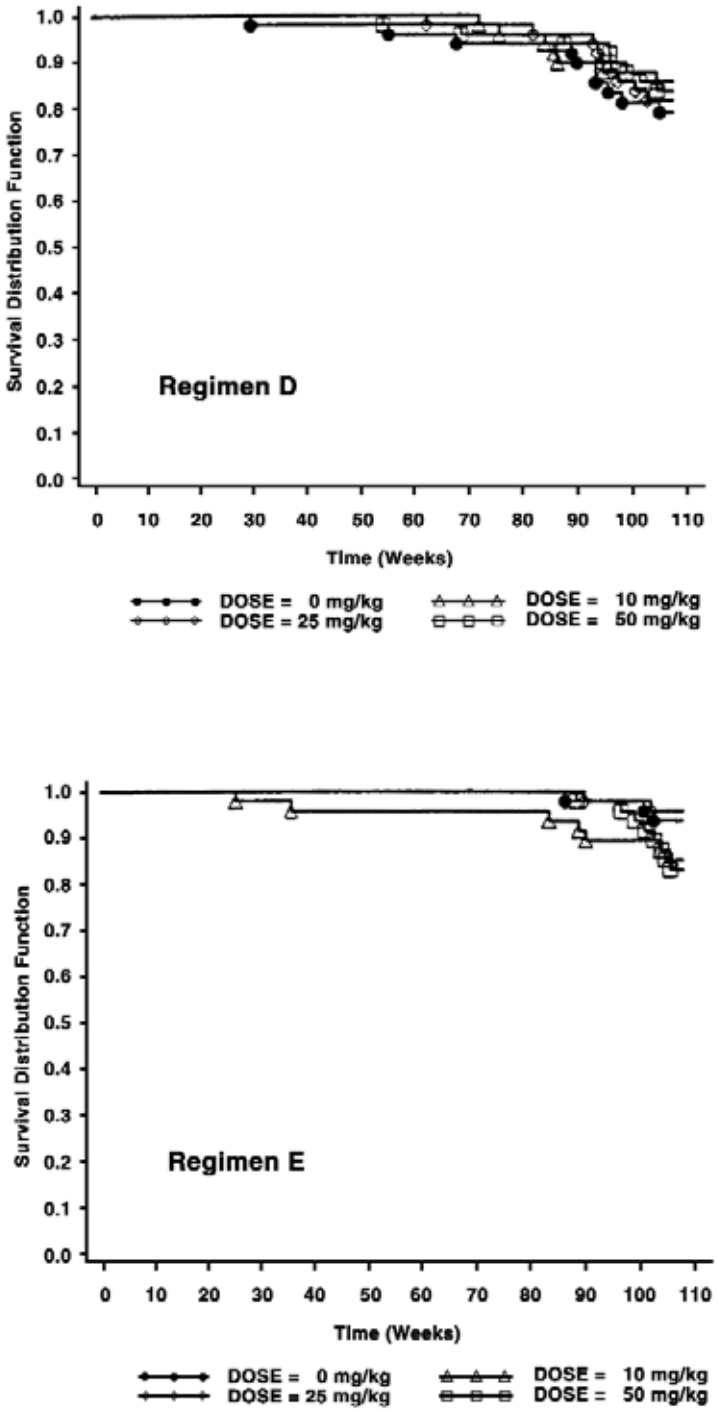


FIGURE 4
Kaplan-Meier Survival Curves for Regimen D Female Mice and Regimen E Male Mice Administered a Single Dose of Chloral Hydrate by Gavage

Body Weights and Clinical Findings

Mean body weights of female mice in regimen A were similar to those of the vehicle controls (Table 7 and Figure 5). Mean body weights of female mice given 100 mg/kg chloral hydrate in regimen B for 3 or 6 months (Table 8 and Figure 5) were generally greater than those of the vehicle controls during the second year of the study; mean body weights of mice given 100 mg/kg for 12 months were generally similar to those of the vehicle controls throughout the study. Mean body weights of female mice in regimen C were similar to those of the vehicle controls (Figure 6 and Table 9). Mean body weights of female mice in regimen D were generally similar to those of the vehicle controls throughout the study (Table 10 and Figure 7). Mean body weights of 25 mg/kg male mice in regimen E were generally less than those of the vehicle controls beginning at week 19 (Table 11 and Figure 7); mean body weights of 10 and 50 mg/kg mice were generally less beginning at week 80. There were no clinical findings related to chloral hydrate administration.

TABLE 7
Mean Body Weights and Survival of Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	11.7	48	11.5	98	48	11.3	97	48	11.0	94	48
2	15.0	48	15.0	100	48	14.4	96	48	14.2	95	48
3	16.9	48	17.0	101	48	16.9	100	48	16.6	98	48
4	18.0	47	18.2	101	47	17.8	99	48	17.6	98	47
5	19.1	47	19.1	100	47	18.6	97	48	18.4	96	47
6	19.8	47	20.1	102	47	19.5	98	48	19.4	98	47
7	20.5	47	20.7	101	47	20.3	99	48	20.1	98	47
8	21.2	46	21.4	101	47	20.9	99	48	20.8	98	47
9	21.8	46	22.0	101	47	21.4	98	48	21.4	98	47
10	22.3	46	22.4	100	47	22.0	99	48	22.0	99	47
11	22.9	46	22.9	100	47	22.4	98	48	22.4	98	47
12	23.4	46	23.6	101	47	22.9	98	48	22.9	98	47
13	23.5	46	24.1	103	47	23.3	99	48	23.1	98	47
14	23.9	46	24.1	101	47	23.5	98	48	23.5	98	47
15	24.1	46	24.2	100	47	23.7	98	48	23.6	98	47
16	24.4	46	24.6	101	47	24.0	98	48	23.9	98	47
17	24.6	46	24.9	101	47	24.1	98	48	24.1	98	47
18	24.8	46	24.9	100	47	24.2	98	48	24.3	98	47
19	25.1	46	25.6	102	47	24.4	97	48	24.6	98	47
20	25.5	46	25.6	100	47	24.7	97	48	24.7	97	47
21	25.6	46	26.0	102	47	25.1	98	48	25.2	98	47
22	26.0	46	26.7	103	47	25.4	98	48	25.6	98	47
23	26.1	46	26.6	102	47	25.8	99	48	25.6	98	47
24	26.7	46	27.3	102	47	25.8	97	48	25.9	97	46
25	26.9	46	27.0	100	47	26.1	97	48	26.5	99	46
26	26.9	46	27.6	103	47	26.4	98	48	26.5	99	46
27	27.0	46	27.3	101	47	26.4	98	48	26.5	98	46
28	27.2	46	27.6	101	47	26.7	98	48	26.9	99	46
29	27.6	46	27.5	100	47	26.6	96	48	27.0	98	46
30	27.4	46	27.8	101	47	26.8	98	48	27.0	99	46
31	27.4	46	28.0	102	47	26.9	98	48	26.9	98	46
32	27.4	46	28.1	103	47	26.9	98	48	27.2	99	46
33	27.8	46	27.8	100	47	27.0	97	48	27.3	98	46
34	27.9	46	28.6	103	47	27.1	97	48	27.3	98	46
35	28.1	46	28.5	101	47	27.4	98	48	28.0	100	46
36	28.2	46	28.8	102	47	27.5	98	48	27.9	99	46
37	28.7	46	28.5	99	47	28.0	98	48	28.1	98	46
38	28.6	46	28.9	101	47	27.6	97	48	28.2	99	46
39	28.9	46	29.2	101	47	28.0	97	48	28.7	99	46
40	29.0	46	29.5	102	47	28.3	98	48	28.8	99	46
41	29.1	45	29.6	102	47	28.5	98	48	28.8	99	46
42	29.6	45	29.7	100	47	28.5	96	48	29.0	98	46
43	29.7	45	29.8	100	47	28.8	97	48	29.4	99	46
44	29.6	45	29.8	101	47	28.9	98	48	29.6	100	46
45	29.7	45	30.3	102	47	28.9	97	48	29.2	98	46
46	29.9	45	30.2	101	47	29.0	97	48	29.4	98	46
47	30.2	45	30.3	100	47	29.3	97	48	29.6	98	46
48	30.1	45	30.5	101	47	29.1	97	48	30.2	100	46
49	30.1	45	30.7	102	47	29.3	97	48	29.8	99	46
50	30.5	45	30.8	101	47	29.5	97	48	30.2	99	46
51	30.8	45	31.2	101	47	29.7	96	48	30.3	98	46
52	30.6	45	31.2	102	47	29.8	97	48	30.6	100	46
53	31.2	45	31.5	101	47	30.4	97	48	30.4	97	46
54	31.3	45	31.9	102	47	30.1	96	48	30.9	99	46

TABLE 7
Mean Body Weights and Survival of Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
55	31.7	45	32.6	103	47	30.4	96	48	30.8	97	46
56	31.5	45	32.7	104	47	30.8	98	48	31.0	98	46
57	31.9	45	32.7	103	47	30.9	97	48	31.5	99	46
58	31.7	45	32.9	104	47	31.0	98	48	31.7	100	46
59	32.3	45	33.2	103	47	31.1	96	48	31.8	98	46
60	32.2	45	33.3	103	47	31.5	98	48	31.9	99	46
61	32.2	45	33.1	103	47	31.8	99	48	31.7	98	46
62	32.5	45	33.5	103	47	31.6	97	48	31.9	98	46
63	32.5	45	33.2	102	47	31.8	98	48	32.1	99	46
64	32.8	45	33.4	102	47	31.9	97	48	32.3	98	46
65	32.8	45	33.6	102	47	31.9	97	48	32.4	99	46
66	32.9	45	33.9	103	47	32.0	97	48	32.4	98	46
67	33.0	45	34.3	104	47	32.1	97	48	32.8	99	46
68	32.9	45	34.4	105	47	32.3	98	48	33.1	101	46
69	33.2	45	33.9	102	47	32.7	98	48	32.9	99	46
70	33.3	45	34.5	104	47	32.5	98	48	33.3	100	46
71	33.1	45	34.4	104	47	32.7	99	48	32.7	99	46
72	33.4	45	34.8	104	47	32.9	99	48	33.2	99	46
73	33.5	45	34.7	104	47	33.0	99	48	33.5	100	46
74	33.7	45	34.8	103	47	33.0	98	48	33.4	99	46
75	33.6	45	35.1	104	47	32.9	98	48	33.3	99	46
76	33.7	44	35.3	105	47	33.1	98	48	33.7	100	46
77	33.8	44	35.0	104	47	33.6	99	48	33.9	100	45
78	34.2	44	35.4	104	46	33.6	98	48	34.0	99	44
79	34.3	44	35.3	103	46	33.6	98	48	33.9	99	44
80	34.2	44	35.6	104	46	33.7	99	48	33.9	99	44
81	34.4	44	35.6	103	46	33.7	98	48	33.9	99	44
82	34.4	44	35.9	104	46	33.7	98	48	34.1	99	44
83	34.9	43	36.2	104	46	34.4	99	47	34.3	98	44
84	35.2	43	35.8	102	46	34.2	97	47	34.4	98	44
85	34.9	43	36.2	104	46	34.6	99	47	34.5	99	43
86	34.9	43	36.6	105	45	34.6	99	47	34.3	98	43
87	35.1	43	36.4	104	44	34.9	99	46	34.7	99	43
88	35.2	43	35.9	102	43	34.9	99	46	34.6	98	43
89	35.1	43	36.3	103	43	35.2	100	46	34.4	98	41
90	35.2	42	36.2	103	43	35.2	100	45	34.4	98	40
91	35.4	42	36.1	102	43	35.1	99	45	33.9	96	40
92	35.6	41	35.8	101	43	35.2	99	45	34.0	96	40
93	35.8	41	36.0	101	43	35.3	99	45	34.2	96	40
94	35.5	39	36.2	102	43	35.5	100	45	34.6	97	40
95	35.5	39	35.9	101	43	35.4	100	45	34.8	98	40
96	35.7	39	36.3	102	43	35.6	100	45	34.9	98	39
97	35.7	39	36.9	103	41	35.5	99	45	34.9	98	39
98	35.7	39	36.5	102	41	35.3	99	45	34.8	97	38
99	35.8	39	36.7	103	41	35.3	99	45	34.8	97	38
100	36.0	39	36.9	102	41	35.4	98	44	35.1	98	38
101	35.8	39	36.6	102	41	35.3	99	44	35.0	98	38
102	35.8	39	36.2	101	40	35.3	99	44	34.9	97	38
103	36.2	39	36.0	99	40	35.0	97	44	34.6	96	37
104	36.3	39	36.1	99	40	34.8	96	44	34.6	95	36
Mean for weeks											
1-13	19.7		19.9	101		19.5	99		19.3	98	
14-52	27.7		28.1	101		27.0	97		27.3	99	
53-104	34.0		35.0	103		33.4	98		33.4	98	

TABLE 8
Mean Body Weights and Survival of Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		3 Months (Stop-Exposure)			6 Months (Stop-Exposure)			12 Months (Stop-Exposure)		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	11.7	48	11.9	102	48	11.9	102	48	11.7	100	48
2	15.0	48	14.9	99	48	14.9	99	48	14.6	97	48
3	16.9	48	16.9	100	48	17.1	101	48	16.6	98	48
4	18.0	47	18.0	100	48	18.2	101	48	17.9	99	48
5	19.1	47	18.8	98	48	19.1	100	48	18.7	98	48
6	19.8	47	19.7	99	48	20.0	101	48	19.6	99	48
7	20.5	47	20.4	100	48	20.8	101	48	20.3	99	48
8	21.2	46	20.9	99	48	21.4	101	47	21.0	99	48
9	21.8	46	21.6	99	48	21.7	100	47	21.6	99	48
10	22.3	46	22.3	100	48	22.3	100	47	22.3	100	48
11	22.9	46	22.3	97	48	22.8	100	47	22.4	98	48
12	23.4	46	23.1	99	48	23.3	100	47	23.1	99	48
13	23.5	46	23.7	101	48	23.8	101	47	23.6	100	48
14	23.9	46	23.4	98	40	24.2	101	47	23.8	100	48
15	24.1	46	23.5	98	40	24.2	100	47	24.1	100	48
16	24.4	46	24.1	99	40	24.4	100	47	24.3	100	48
17	24.6	46	24.1	98	40	24.8	101	47	24.5	100	48
18	24.8	46	24.5	99	40	25.2	102	47	24.8	100	48
19	25.1	46	24.9	99	40	25.4	101	47	25.0	100	48
20	25.5	46	25.5	100	40	25.6	100	47	25.2	99	48
21	25.6	46	25.5	100	40	26.2	102	47	25.6	100	48
22	26.0	46	25.9	100	40	26.4	102	47	25.9	100	48
23	26.1	46	26.0	100	40	26.6	102	47	26.0	100	48
24	26.7	46	26.5	99	40	26.8	100	47	26.4	99	48
25	26.9	46	26.4	98	40	27.2	101	47	26.6	99	48
26	26.9	46	27.1	101	40	27.4	102	47	27.0	100	48
27	27.0	46	27.0	100	40	27.1	100	39	26.9	100	48
28	27.2	46	27.2	100	40	26.6	98	39	27.2	100	48
29	27.6	46	27.8	101	40	27.4	99	39	27.2	99	48
30	27.4	46	27.7	101	40	27.2	99	39	27.5	100	48
31	27.4	46	27.5	100	40	27.6	101	39	27.8	101	48
32	27.4	46	27.6	101	39	27.8	101	39	27.7	101	48
33	27.8	46	28.2	101	39	28.1	101	39	27.7	100	48
34	27.9	46	28.4	102	39	28.3	101	39	28.2	101	48
35	28.1	46	28.5	101	39	28.8	102	39	28.3	101	48
36	28.2	46	28.9	102	39	29.2	104	39	28.5	101	48
37	28.7	46	29.0	101	39	29.3	102	39	28.7	100	48
38	28.6	46	29.5	103	39	29.7	104	39	28.8	101	48
39	28.9	46	29.7	103	39	30.1	104	39	29.2	101	48
40	29.0	46	30.3	104	39	30.1	104	39	29.4	101	48
41	29.1	45	30.1	103	39	30.4	104	39	29.8	102	48
42	29.6	45	30.3	102	39	30.7	104	39	29.7	100	48
43	29.7	45	30.6	103	39	31.0	104	39	30.1	101	48
44	29.6	45	30.5	103	39	31.2	105	39	29.9	101	48
45	29.7	45	30.9	104	39	31.3	105	39	30.3	102	48
46	29.9	45	30.7	103	39	31.7	106	39	30.2	101	48
47	30.2	45	31.1	103	39	32.0	106	39	30.4	101	48
48	30.1	45	31.7	105	39	32.4	108	39	30.8	102	48
49	30.1	45	31.9	106	39	32.5	108	39	30.6	102	48
50	30.5	45	31.9	105	39	32.6	107	39	30.8	101	48
51	30.8	45	32.5	106	39	32.5	106	39	31.2	101	48
52	30.6	45	32.4	106	39	32.8	107	39	31.3	102	48
53	31.2	45	32.9	105	39	33.0	106	39	31.2	100	40
54	31.3	45	33.2	106	39	33.2	106	39	31.1	99	40

TABLE 8
Mean Body Weights and Survival of Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		3 Months (Stop-Exposure)			6 Months (Stop-Exposure)			12 Months (Stop-Exposure)		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
55	31.7	45	33.7	106	39	33.5	106	39	31.8	100	40
56	31.5	45	33.6	107	39	33.6	107	39	31.7	101	40
57	31.9	45	33.8	106	39	33.7	106	39	32.1	101	40
58	31.7	45	34.3	108	39	33.8	107	39	32.2	102	40
59	32.3	45	34.1	106	39	34.0	105	39	32.6	101	40
60	32.2	45	34.2	106	39	34.0	106	39	32.7	102	40
61	32.2	45	34.1	106	39	34.4	107	39	33.1	103	40
62	32.5	45	34.3	106	39	34.9	107	39	32.9	101	40
63	32.5	45	34.7	107	39	34.9	107	39	33.4	103	40
64	32.8	45	34.7	106	39	35.0	107	39	33.7	103	40
65	32.8	45	35.0	107	39	34.7	106	38	33.4	102	40
66	32.9	45	35.2	107	39	35.4	108	38	33.4	102	40
67	33.0	45	35.0	106	39	35.5	108	38	33.5	102	40
68	32.9	45	35.7	109	39	35.8	109	38	34.3	104	39
69	33.2	45	35.7	108	39	35.7	108	38	34.0	102	39
70	33.3	45	35.9	108	39	35.9	108	38	34.5	104	39
71	33.1	45	35.9	108	39	36.0	109	38	34.2	103	39
72	33.4	45	36.4	109	39	36.5	109	38	34.9	104	39
73	33.5	45	35.9	107	39	36.3	108	38	34.7	104	39
74	33.7	45	36.1	107	39	36.7	109	38	34.9	104	38
75	33.6	45	36.6	109	39	36.9	110	38	34.8	104	38
76	33.7	44	36.1	107	38	36.7	109	38	35.1	104	38
77	33.8	44	36.0	107	37	36.8	109	38	35.2	104	38
78	34.2	44	36.4	106	36	36.7	107	38	35.1	103	38
79	34.3	44	36.3	106	36	37.8	110	38	35.3	103	38
80	34.2	44	36.0	105	36	37.6	110	38	35.6	104	38
81	34.4	44	36.8	107	36	37.7	110	38	36.0	105	38
82	34.4	44	37.1	108	36	37.6	109	38	36.4	106	38
83	34.9	43	36.8	105	36	37.8	108	38	36.4	104	38
84	35.2	43	37.1	105	36	37.9	108	38	36.7	104	38
85	34.9	43	37.4	107	36	38.1	109	38	36.7	105	38
86	34.9	43	37.2	107	36	38.3	110	38	37.1	106	38
87	35.1	43	37.7	107	36	38.9	111	37	37.6	107	38
88	35.2	43	37.2	106	36	38.6	110	36	37.2	106	38
89	35.1	43	37.1	106	36	38.6	110	36	37.4	107	38
90	35.2	42	36.9	105	36	38.4	109	36	37.8	107	38
91	35.4	42	37.3	105	36	38.6	109	36	38.4	108	38
92	35.6	41	37.2	104	36	38.2	107	36	38.1	107	38
93	35.8	41	37.2	104	36	38.2	107	36	36.2	101	37
94	35.5	39	38.1	107	36	38.5	108	36	36.4	103	37
95	35.5	39	37.5	106	36	38.0	107	33	36.5	103	37
96	35.7	39	37.7	106	36	38.2	107	33	36.8	103	36
97	35.7	39	37.7	106	35	37.9	106	32	37.1	104	35
98	35.7	39	37.5	105	35	37.5	105	32	37.2	104	35
99	35.8	39	37.6	105	35	38.1	106	32	36.9	103	34
100	36.0	39	37.8	105	35	38.1	106	32	37.1	103	33
101	35.8	39	37.9	106	35	38.0	106	31	37.4	104	33
102	35.8	39	38.0	106	35	38.1	106	31	37.3	104	33
103	36.2	39	37.9	105	35	37.6	104	31	37.3	103	33
104	36.3	39	38.1	105	34	37.8	104	31	37.7	104	33
Mean for weeks											
1-13	19.7		19.5	99		19.6	99		19.5	99	
14-52	27.7		28.2	102		28.5	103		27.9	101	
53-104	34.0		36.2	106		36.6	108		35.2	104	

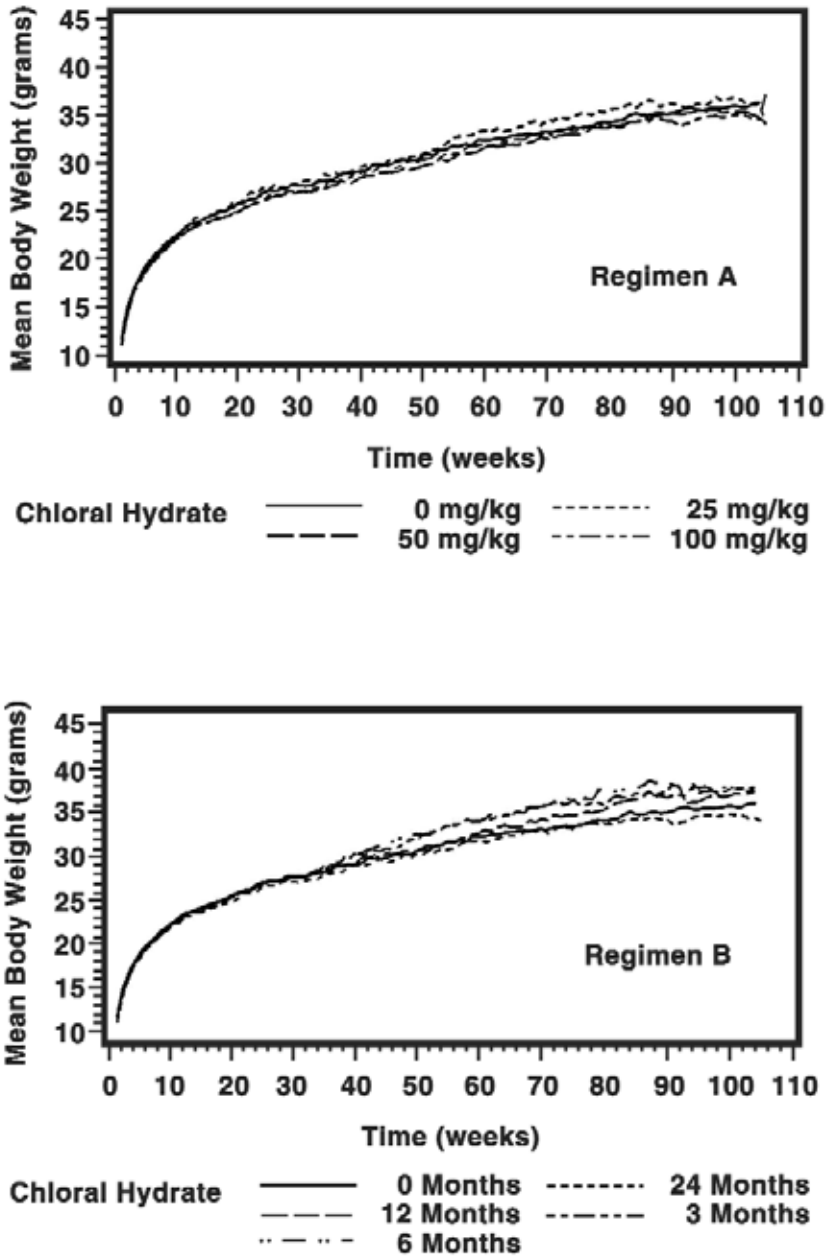


FIGURE 5
Growth Curves for Female Mice Administered Chloral Hydrate by Gavage for 2 Years (Regimen A) or 3, 6, or 12 Months (Regimen B)

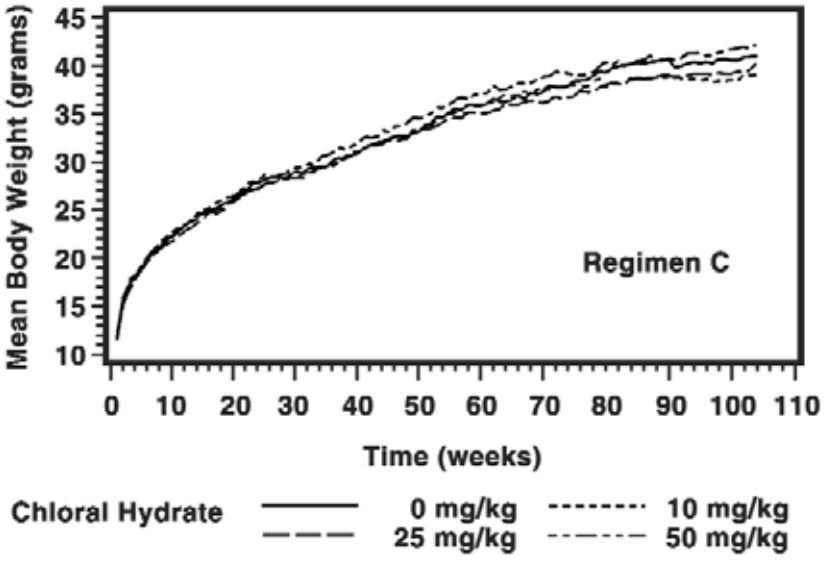


FIGURE 6
Growth Curves for Regimen C Female Mice Administered a Single Dose of Chloral Hydrate by Gavage

TABLE 9
Mean Body Weights and Survival of Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	11.7	48	12.0	103	48	11.4	97	48	12.3	105	48
2	15.7	48	15.7	100	48	15.2	97	48	16.0	102	48
3	17.3	48	17.5	101	48	16.9	98	48	17.8	103	48
4	18.1	48	18.1	100	48	17.9	99	48	18.4	102	48
5	19.0	48	18.9	99	48	18.8	99	48	19.3	102	48
6	19.9	48	19.8	99	48	19.6	98	48	20.2	102	48
7	20.6	48	20.7	100	48	20.3	99	48	20.9	101	48
8	21.1	48	21.3	101	48	20.9	99	48	21.6	102	48
9	21.7	48	21.8	100	48	21.4	99	48	22.1	102	48
10	22.3	48	22.1	99	48	21.7	97	48	22.7	102	48
11	22.8	48	22.6	99	48	22.3	98	48	23.2	102	48
12	23.3	48	23.4	100	48	22.8	98	48	23.5	101	48
13	23.8	48	23.7	100	48	23.2	97	48	24.0	101	48
14	24.4	48	24.1	99	48	23.7	97	48	24.7	101	48
15	24.5	48	24.7	101	48	24.1	98	48	25.0	102	48
16	24.7	48	25.0	101	48	24.8	100	48	25.2	102	48
17	25.2	48	24.9	99	48	24.5	97	48	25.7	102	48
18	25.5	48	25.3	99	48	24.9	98	48	26.1	102	48
19	25.7	48	25.6	100	48	25.6	100	48	26.3	102	48
20	26.2	48	26.2	100	48	25.8	98	48	26.5	101	48
21	26.7	48	26.5	99	48	26.4	99	48	27.0	101	48
22	27.0	48	26.9	100	48	26.7	99	48	27.6	102	48
23	27.6	48	27.0	98	48	27.2	99	48	28.0	101	48
24	27.7	48	27.4	99	48	27.1	98	48	28.2	102	48
25	28.1	48	27.5	98	48	27.7	99	48	28.6	102	48
26	28.3	48	27.7	98	48	27.7	98	48	28.3	100	48
27	28.3	48	28.0	99	48	28.0	99	48	28.5	101	48
28	28.1	48	28.0	100	48	28.2	100	48	28.6	102	48
29	28.6	48	28.2	99	48	28.2	99	48	29.1	102	48
30	28.9	48	28.4	98	48	28.3	98	48	29.3	101	48
31	28.6	48	28.2	99	48	28.6	100	48	29.4	103	48
32	29.2	48	28.8	99	48	28.8	99	48	29.6	101	48
33	29.2	48	28.8	99	48	29.0	99	48	29.6	101	48
34	29.4	48	29.2	99	48	29.3	100	48	30.4	103	48
35	29.4	48	29.5	100	48	29.4	100	48	30.6	104	48
36	30.1	48	29.4	98	48	29.8	99	48	30.9	103	48
37	30.3	48	30.0	99	48	29.9	99	48	31.4	104	48
38	30.2	48	30.5	101	48	30.2	100	48	31.1	103	48
39	30.5	48	30.9	101	48	30.5	100	48	31.5	103	48
40	30.7	48	30.9	101	48	30.8	100	48	31.8	104	48
41	31.3	48	31.2	100	48	31.3	100	48	32.4	104	48
42	31.6	48	31.6	100	48	31.5	100	48	32.4	103	48
43	31.9	48	31.8	100	48	31.6	99	48	32.5	102	48
44	32.0	48	31.9	100	48	31.7	99	48	33.1	103	48
45	32.3	48	32.1	99	48	32.2	100	48	33.0	102	48
46	32.5	48	32.4	100	48	32.2	99	48	33.5	103	48
47	32.6	48	32.7	100	48	32.1	98	48	33.7	103	47
48	32.2	48	32.6	101	48	32.5	101	48	33.9	105	47
49	33.0	48	33.3	101	48	32.8	99	48	34.5	105	47
50	33.0	48	33.0	100	48	33.1	100	48	34.4	104	47
51	33.6	48	33.9	101	48	33.3	99	48	34.6	103	47
52	33.9	48	33.7	99	48	33.4	99	48	35.0	103	47
53	34.6	48	34.2	99	48	33.7	97	48	35.6	103	47
54	34.5	48	34.0	99	48	34.0	99	48	35.6	103	47

TABLE 9
Mean Body Weights and Survival of Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
55	35.0	48	34.6	99	48	34.5	99	48	35.9	103	47
56	35.5	48	35.1	99	48	34.5	97	48	36.3	102	47
57	35.2	48	34.9	99	48	34.4	98	48	36.7	104	47
58	35.9	48	35.1	98	48	35.1	98	48	36.4	101	47
59	35.8	48	35.7	100	48	35.1	98	48	37.0	103	47
60	35.7	48	35.9	101	48	35.0	98	48	37.0	104	47
61	35.9	48	35.9	100	48	35.1	98	48	37.1	103	47
62	36.5	48	36.1	99	48	35.3	97	48	37.9	104	47
63	36.2	48	36.6	101	48	35.4	98	48	37.5	104	47
64	36.1	48	37.0	102	48	36.1	100	48	37.5	104	47
65	36.4	48	36.8	101	48	35.9	99	48	37.7	104	47
66	36.9	48	37.0	100	48	35.9	97	48	38.1	103	47
67	36.4	48	37.3	102	48	36.2	99	48	38.1	105	47
68	37.2	48	37.0	99	48	36.4	98	48	38.2	103	47
69	36.9	48	37.5	102	48	36.2	98	48	38.5	104	47
70	37.2	48	37.8	102	48	36.1	97	48	38.9	105	47
71	37.9	48	37.5	99	48	36.3	96	48	38.8	102	47
72	37.8	48	37.6	99	48	36.8	97	48	39.3	104	47
73	37.7	48	37.8	100	48	36.6	97	48	39.3	104	47
74	37.8	48	37.9	100	48	36.7	97	48	39.3	104	47
75	38.4	48	38.1	99	48	36.8	96	48	39.1	102	46
76	38.4	48	37.8	98	48	37.5	98	48	39.0	102	46
77	38.5	47	37.9	98	48	37.3	97	48	39.1	102	45
78	39.0	47	38.4	98	48	37.4	96	48	39.4	101	45
79	39.5	47	38.7	98	48	37.8	96	48	40.0	101	45
80	39.3	47	37.8	96	47	38.1	97	48	40.5	103	45
81	39.8	47	38.2	96	47	38.3	96	48	40.1	101	45
82	39.9	47	38.1	95	47	38.2	96	48	40.6	102	45
83	40.1	46	38.1	95	47	38.2	95	48	39.9	100	45
84	40.2	46	38.9	97	46	38.6	96	48	40.3	100	44
85	40.3	46	38.6	96	46	38.9	97	48	40.6	101	44
86	40.2	46	38.7	96	46	38.7	96	48	40.6	101	44
87	40.4	46	38.7	96	46	38.8	96	48	41.1	102	44
88	40.5	46	38.8	96	46	38.9	96	48	40.5	100	44
89	40.5	46	38.8	96	46	39.0	96	48	40.8	101	44
90	40.7	46	38.9	96	46	39.1	96	47	40.9	100	43
91	39.8	46	38.9	98	46	38.8	97	47	40.6	102	43
92	40.0	45	38.7	97	45	39.0	98	47	40.8	102	43
93	40.2	44	38.6	96	44	39.0	97	47	41.3	103	43
94	40.4	44	38.7	96	44	39.2	97	47	41.3	102	43
95	40.2	43	38.5	96	44	39.2	98	47	41.3	103	43
96	40.4	43	38.6	96	44	39.2	97	47	41.0	101	43
97	40.2	43	38.3	95	43	39.1	97	47	41.2	102	43
98	40.7	43	38.4	94	43	39.3	97	47	41.4	102	42
99	40.7	42	38.4	94	42	39.2	96	47	41.6	102	42
100	40.5	42	38.6	95	42	39.2	97	46	41.7	103	42
101	40.5	42	38.4	95	42	39.4	97	45	41.8	103	42
102	40.8	42	39.0	96	42	39.3	96	45	42.0	103	41
103	40.9	42	39.0	95	41	39.5	97	45	41.8	102	40
104	41.0	42	39.1	95	41	40.2	98	45	42.2	103	40
Mean for weeks											
1-13	19.8		19.9	101		19.5	98		20.2	102	
14-52	29.3		29.2	100		29.0	99		30.1	103	
53-104	38.5		37.6	98		37.4	97		39.4	102	

TABLE 10
Mean Body Weights and Survival of Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	12.4	48	12.8	103	48	12.4	100	48	12.7	102	48
2	15.7	48	16.1	103	48	15.5	99	48	15.9	101	48
3	16.8	48	17.3	103	48	16.9	101	48	17.3	103	48
4	17.6	48	18.3	104	48	17.9	102	48	18.1	103	48
5	18.5	48	19.1	103	48	19.0	103	48	19.1	103	48
6	19.3	48	19.8	103	48	19.8	103	48	19.8	103	48
7	20.0	48	20.5	102	48	20.6	103	48	20.5	102	48
8	20.7	48	21.3	103	48	21.2	102	48	21.2	102	48
9	21.1	48	21.5	102	48	21.6	102	48	21.6	102	48
10	21.5	48	21.9	102	48	22.2	103	48	22.2	103	48
11	22.1	48	22.6	102	48	22.7	103	48	22.7	103	48
12	22.5	48	22.8	101	48	23.1	103	48	23.2	103	48
13	22.7	48	23.4	103	48	23.5	104	48	23.2	102	48
14	23.1	48	23.8	103	48	23.9	103	48	23.8	103	48
15	23.5	48	24.2	103	48	24.3	103	48	23.9	102	48
16	23.5	48	24.4	104	48	24.3	103	48	24.2	103	48
17	23.9	48	24.4	102	48	24.7	103	48	24.4	102	48
18	23.9	48	24.5	103	48	25.2	105	48	24.7	103	48
19	24.1	48	25.1	104	48	25.0	104	48	24.7	102	48
20	24.4	48	25.4	104	48	25.4	104	48	25.0	102	48
21	24.7	48	25.6	104	48	25.9	105	48	25.5	103	48
22	25.2	48	26.4	105	48	26.4	105	48	25.8	102	48
23	25.4	48	26.2	103	48	26.5	104	48	26.0	102	48
24	25.6	48	26.5	104	48	26.4	103	48	26.1	102	48
25	25.6	48	26.4	103	48	26.9	105	48	26.1	102	48
26	25.8	48	26.6	103	48	27.0	105	48	26.3	102	48
27	26.2	47	26.8	102	48	27.1	103	48	26.7	102	48
28	26.3	47	27.1	103	48	27.4	104	48	26.4	100	48
29	26.4	47	27.2	103	48	27.4	104	48	27.1	103	48
30	26.8	47	27.7	103	48	27.8	104	48	27.1	101	48
31	26.7	47	27.7	104	48	27.9	104	48	27.1	101	48
32	26.8	47	27.5	103	48	28.4	106	48	27.3	102	48
33	26.9	47	27.8	103	48	28.0	104	48	27.4	102	48
34	27.1	47	28.4	105	48	28.8	106	48	27.7	102	48
35	27.3	47	28.2	103	48	28.7	105	48	27.5	101	48
36	27.0	47	28.4	105	48	29.0	107	48	27.7	103	48
37	27.2	47	28.5	105	48	29.0	107	48	27.9	103	48
38	27.6	47	28.8	104	48	29.1	105	48	28.3	103	48
39	27.8	47	29.1	105	48	29.4	106	48	28.3	102	48
40	28.0	47	29.0	104	48	29.7	106	48	28.9	103	48
41	28.3	47	29.5	104	48	29.7	105	48	29.1	103	48
42	28.5	47	29.8	105	48	29.8	105	48	29.3	103	48
43	28.4	47	30.0	106	48	30.5	107	48	29.3	103	48
44	28.6	47	30.1	105	48	30.6	107	48	29.7	104	48
45	28.7	47	30.5	106	48	30.3	106	48	29.7	103	48
46	29.0	47	30.7	106	48	30.9	107	48	29.8	103	48
47	29.1	47	30.5	105	48	31.0	107	48	30.2	104	48
48	29.3	47	30.7	105	48	30.9	105	48	30.0	102	48
49	29.3	47	31.3	107	48	31.3	107	48	30.3	103	48
50	29.5	47	31.0	105	48	31.2	106	48	30.5	103	48
51	29.6	47	31.5	106	48	31.4	106	48	30.9	104	48
52	29.6	47	31.5	106	48	31.9	108	48	31.0	105	47
53	30.1	46	31.5	105	48	32.1	107	48	31.2	104	47
54	30.5	46	31.9	105	48	32.0	105	48	31.3	103	47

TABLE 10
Mean Body Weights and Survival of Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

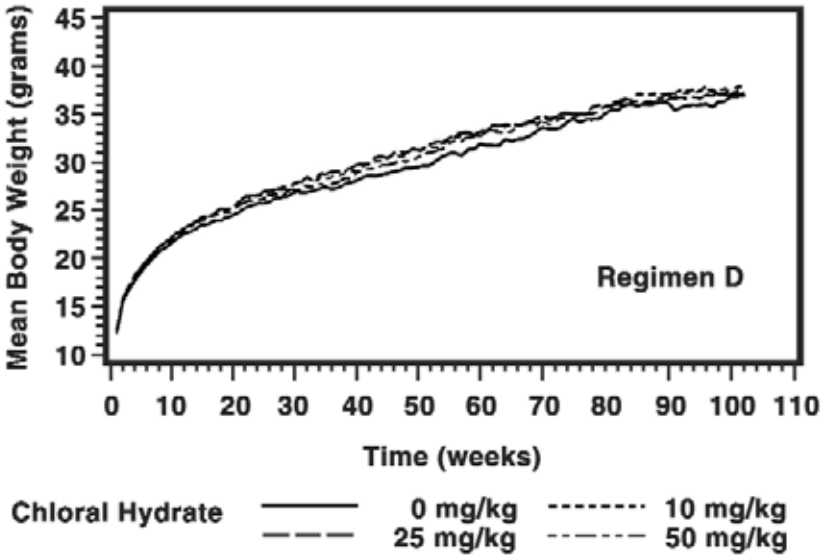
Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
55	30.8	46	32.1	104	48	32.5	106	48	31.9	104	47
56	30.9	46	32.6	106	48	32.9	106	48	32.2	104	47
57	30.6	46	32.7	107	48	33.0	108	48	32.1	105	47
58	31.3	46	32.5	104	48	33.1	106	48	32.8	105	47
59	31.3	46	32.6	104	48	32.9	105	48	32.8	105	47
60	31.9	46	33.2	104	48	33.2	104	47	32.7	103	47
61	31.7	46	33.3	105	48	33.3	105	47	33.3	105	47
62	31.7	46	33.3	105	48	33.9	107	47	33.2	105	47
63	31.7	46	33.4	105	48	33.8	107	47	33.1	104	47
64	32.4	46	33.7	104	48	34.0	105	47	33.2	102	47
65	32.1	46	34.2	107	48	33.8	105	47	33.2	103	47
66	32.4	45	33.8	104	48	33.8	104	47	33.8	104	46
67	32.4	45	33.8	104	48	34.1	105	47	34.0	105	46
68	32.8	45	34.3	105	48	34.3	105	47	34.2	104	46
69	33.2	45	33.8	102	48	34.6	104	47	34.1	103	46
70	33.7	45	34.6	103	47	34.9	104	47	34.3	102	46
71	33.5	45	34.4	103	47	34.6	103	47	34.0	101	46
72	33.4	45	34.4	103	47	34.7	104	47	34.3	103	46
73	33.6	45	34.6	103	46	35.1	104	47	34.7	103	46
74	34.2	45	34.8	102	46	35.2	103	47	35.2	103	46
75	34.6	45	35.1	101	46	34.7	100	47	35.0	101	46
76	34.1	45	35.0	103	46	34.9	102	47	35.1	103	46
77	34.5	45	35.0	101	46	35.0	101	47	35.1	102	46
78	34.5	45	35.7	103	46	35.1	102	47	35.8	104	46
79	34.6	45	36.1	104	46	35.4	102	47	35.7	103	46
80	35.1	45	35.9	102	46	35.9	102	46	35.8	102	46
81	35.5	45	35.8	101	46	36.0	101	46	36.3	102	46
82	35.4	45	36.2	102	45	36.1	102	46	36.4	103	46
83	36.0	45	36.4	101	45	35.9	100	46	36.7	102	46
84	36.1	45	36.3	101	43	36.0	100	46	36.5	101	46
85	35.8	45	36.4	102	43	36.0	101	46	37.2	104	45
86	36.1	45	36.4	101	43	36.2	100	46	37.1	103	45
87	36.0	44	36.4	101	43	36.4	101	46	37.1	103	45
88	36.1	43	36.6	101	43	36.3	101	46	37.3	103	45
89	36.3	43	36.7	101	43	36.2	100	46	37.1	102	45
90	36.2	43	36.8	102	43	36.7	101	46	37.2	103	45
91	35.4	39	36.9	104	43	36.7	104	45	37.3	105	45
92	35.7	39	37.2	104	42	36.8	103	43	37.6	105	45
93	35.9	38	37.2	104	42	36.6	102	43	37.5	104	45
94	36.0	38	37.2	103	42	36.9	102	42	37.6	104	43
95	35.9	38	37.4	104	42	36.7	102	41	37.5	104	43
96	36.1	37	37.6	104	42	37.0	102	41	37.9	105	43
97	35.7	37	37.3	104	42	36.9	103	41	37.6	105	42
98	36.3	37	37.5	103	42	37.2	102	40	37.1	102	42
99	36.4	37	37.7	104	42	36.9	101	40	37.3	102	41
100	36.8	37	37.3	101	42	37.1	101	40	37.6	102	41
101	36.8	37	37.5	102	42	37.1	101	39	37.9	103	41
102	36.9	37	37.4	101	41	37.2	101	39	38.1	103	40
Mean for weeks											
1-13	19.3		19.8	103		19.8	103		19.8	103	
14-52	26.8		27.9	104		28.2	105		27.5	103	
53-102	34.1		35.2	103		35.2	103		35.3	104	

TABLE 11
Mean Body Weights and Survival of Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	15.7	48	14.8	94	48	14.5	92	48	14.9	95	48
2	20.3	48	19.6	97	48	19.2	95	48	19.6	97	48
3	22.1	48	21.4	97	48	21.0	95	48	21.4	97	48
4	23.8	48	23.1	97	48	22.7	95	48	23.2	97	48
5	25.1	48	24.5	98	48	24.2	96	48	24.6	98	48
6	26.3	48	25.6	97	48	25.3	96	48	25.8	98	48
7	27.3	48	26.4	97	48	26.1	96	48	26.6	97	48
8	28.1	48	27.3	97	48	27.0	96	48	27.5	98	48
9	28.7	48	28.0	98	48	27.5	96	48	28.1	98	48
10	29.7	48	28.7	97	48	28.4	96	48	28.9	97	48
11	30.2	48	29.2	97	48	29.0	96	48	29.4	97	48
12	30.9	48	29.8	96	48	29.5	95	48	30.1	97	48
13	31.1	48	30.1	97	48	29.8	96	48	30.4	98	48
14	31.7	48	30.7	97	48	30.2	95	48	30.9	97	48
15	32.1	48	30.9	96	48	30.6	95	48	31.4	98	48
16	32.4	48	31.4	97	48	31.0	96	48	31.8	98	48
17	32.7	48	31.6	97	48	31.3	96	48	32.2	98	48
18	33.1	48	31.8	96	48	31.6	95	48	32.5	98	48
19	33.6	48	32.1	96	48	31.7	94	48	32.8	98	48
20	34.0	48	32.5	96	48	32.2	95	48	33.4	98	48
21	34.6	48	33.1	96	48	32.6	94	48	33.9	98	48
22	35.1	48	33.9	97	48	33.0	94	48	34.1	97	48
23	35.2	48	34.0	97	47	33.3	95	48	34.2	97	48
24	35.4	48	34.0	96	47	33.5	95	48	34.5	97	48
25	35.6	48	34.4	97	47	33.6	94	48	34.8	98	48
26	35.1	48	34.3	98	47	33.4	95	48	34.8	99	48
27	35.5	48	34.5	97	47	33.6	95	48	35.2	99	48
28	35.8	48	34.7	97	47	33.9	95	48	35.1	98	48
29	36.1	48	35.2	98	47	34.1	94	48	35.3	98	48
30	36.3	48	35.3	97	47	34.2	94	48	35.6	98	48
31	36.6	48	35.4	97	47	34.7	95	48	36.1	99	48
32	36.9	48	35.4	96	47	34.7	94	48	36.2	98	48
33	36.8	48	35.9	98	46	35.0	95	48	36.3	99	48
34	37.6	48	36.3	97	46	35.5	94	48	36.7	98	48
35	37.9	48	36.5	96	46	35.6	94	48	36.9	97	48
36	38.2	48	37.0	97	46	35.6	93	48	37.3	98	48
37	38.3	48	37.3	97	46	35.7	93	48	37.6	98	48
38	38.7	48	37.5	97	46	36.0	93	48	37.7	97	48
39	39.3	48	37.9	96	46	36.6	93	48	38.3	97	48
40	39.8	48	38.4	96	46	36.8	92	48	38.4	96	48
41	40.4	48	38.6	96	46	37.1	92	48	38.7	96	48
42	40.5	48	38.6	95	46	38.1	94	48	39.0	96	48
43	40.2	48	38.8	97	46	37.3	93	48	39.1	97	48
44	40.3	48	39.1	97	46	37.7	94	48	39.1	97	48
45	40.3	48	39.2	97	46	37.8	94	48	39.2	97	48
46	40.5	48	39.4	97	46	38.1	94	48	39.5	98	48
47	40.7	48	39.5	97	46	38.1	94	48	39.3	97	48
48	40.9	48	39.3	96	46	38.3	94	48	39.5	97	48
49	41.0	48	39.6	97	46	38.6	94	48	39.4	96	48
50	41.2	48	39.9	97	46	38.6	94	48	39.5	96	48
51	41.8	48	40.3	96	46	38.6	92	48	39.9	95	48
52	41.8	48	40.4	97	46	38.7	93	48	40.3	96	48
53	42.1	48	40.5	96	46	39.0	93	48	40.6	96	48
54	42.5	48	40.6	96	46	39.4	93	48	40.7	96	48

TABLE 11
Mean Body Weights and Survival of Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

Weeks on Study	0 mg/kg		10 mg/kg			25 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
55	42.7	48	40.7	95	46	39.6	93	48	40.7	95	48
56	42.6	48	40.6	95	46	39.6	93	48	40.8	96	48
57	42.7	48	40.5	95	46	39.5	93	48	40.9	96	48
58	42.6	48	40.6	95	46	39.7	93	48	41.0	96	48
59	42.7	48	40.8	96	46	39.5	93	48	41.1	96	48
60	43.2	48	41.1	95	46	40.2	93	48	41.4	96	48
61	43.3	48	41.1	95	46	40.0	92	48	41.4	96	48
62	43.3	48	41.4	96	46	40.1	93	48	41.6	96	48
63	43.3	48	41.3	95	46	40.0	92	48	41.4	96	48
64	43.0	48	41.3	96	46	39.9	93	48	41.2	96	48
65	43.2	48	41.4	96	46	39.9	92	48	41.2	95	48
66	43.3	48	41.4	96	46	40.1	93	48	41.0	95	48
67	43.2	48	41.5	96	46	40.3	93	48	41.2	95	48
68	43.4	48	41.7	96	46	40.1	92	48	41.5	96	48
69	43.4	48	41.7	96	46	40.1	92	48	41.3	95	48
70	43.5	48	41.7	96	46	40.2	92	48	41.6	96	48
71	43.3	48	41.5	96	46	40.2	93	48	41.3	95	48
72	43.3	48	41.1	95	46	40.2	93	48	41.5	96	48
73	43.1	48	41.3	96	46	40.1	93	48	41.6	97	48
74	43.2	48	40.9	95	46	40.3	93	48	41.4	96	48
75	43.1	48	40.8	95	46	40.1	93	48	41.3	96	48
76	43.1	48	41.0	95	46	40.0	93	48	41.3	96	48
77	43.0	48	41.1	96	46	40.1	93	48	41.4	96	48
78	43.2	48	41.1	95	46	40.5	94	48	41.3	96	48
79	43.5	48	41.4	95	46	40.7	94	48	41.5	95	48
80	43.6	48	40.8	94	46	40.6	93	48	41.1	94	48
81	43.4	48	41.0	94	45	40.4	93	48	41.3	95	48
82	43.6	44	40.9	94	45	40.3	92	48	41.2	94	48
83	43.6	48	40.9	94	45	40.3	92	48	41.0	94	48
84	43.5	47	40.2	92	45	40.3	93	48	40.9	94	48
85	43.1	47	40.4	94	45	40.4	94	48	40.9	95	48
86	42.9	47	40.4	94	44	40.2	94	48	40.7	95	47
87	43.0	47	40.6	94	44	40.1	93	47	40.6	94	47
88	43.2	47	40.5	94	43	39.9	92	47	40.6	94	47
89	43.2	47	40.6	94	43	40.0	93	47	40.3	93	47
90	43.0	47	40.3	94	43	39.7	92	47	40.3	94	47
91	42.7	47	40.1	94	43	39.6	93	47	40.0	94	47
92	42.9	47	40.1	93	43	39.7	93	47	39.7	93	47
93	42.8	47	40.0	93	43	39.4	92	47	39.8	93	47
94	42.3	47	39.9	94	43	39.3	93	47	39.6	94	46
95	41.9	47	39.8	95	43	39.0	93	47	39.2	94	46
96	42.1	47	39.6	94	43	38.8	92	47	39.4	94	46
97	41.8	47	39.6	95	43	38.8	93	47	39.5	94	45
98	41.9	46	39.3	94	43	38.9	93	47	39.4	94	44
99	42.0	46	39.5	94	43	39.0	93	47	39.4	94	44
100	41.8	45	39.5	94	43	38.8	93	46	39.0	93	43
101	41.8	45	39.6	95	42	38.9	93	46	39.2	94	42
102	41.5	45	39.8	96	42	38.7	93	46	39.3	95	41
Mean for weeks											
1-13	26.2		25.3	97		25.0	95		25.5	97	
14-52	37.3		36.0	97		35.0	94		36.3	97	
53-102	42.9		40.7	95		39.8	93		40.7	95	



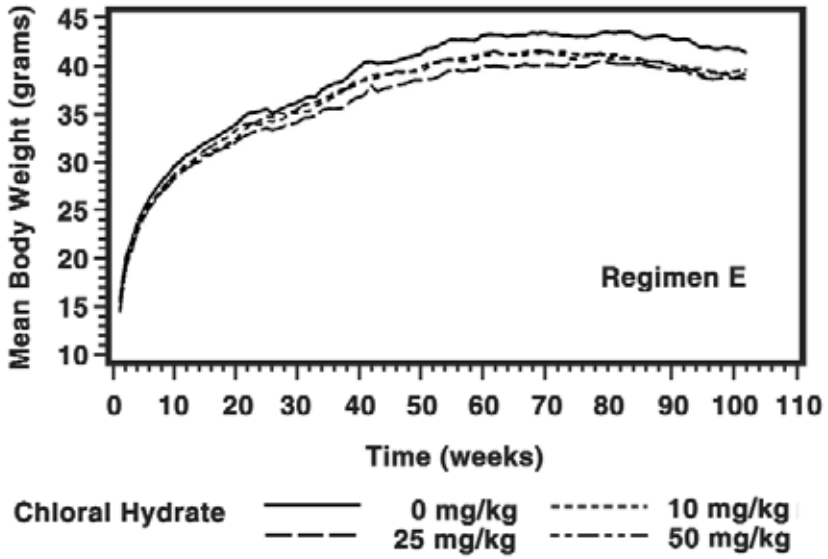


FIGURE 7
Growth Curves for Regimen D Female Mice and Regimen E Male Mice Administered a Single Dose of Chloral Hydrate by Gavage

Hepatic Cell Proliferation and Apoptosis Analyses

Results of hepatic cell proliferation analyses in regimens B, C, D, and E are given in Table 12. Female mice given 100 mg/kg chloral hydrate for 3 months in regimen B showed a significant increase in G0 phase and concomitant decreases in G1, G2, S, and M phases of the cell cycle compared to the vehicle controls (Figure 8); the decreases were significant in the G1 and S phases. These changes were not present in mice given 100 mg/kg for 6 months (Figure 8).

A single dose of 10, 25, or 50 mg/kg in regimen C 28-day-old female mice did not affect the cell cycle parameters (Table 12). A similar result was obtained when all dosed groups were combined and compared to the vehicle control group (Figure 9).

There was a significant decrease in G0 phase and an increase in G1 and M phases in regimen D females administered a single dose of 10 or 50 mg/kg at 15 days of age. A comparison of the combined regimen D dosed groups to the vehicle controls indicated a significant decrease in the percentage of G0-phase cells and increases in the percentage of cells in G1 and S phases (Figure 9).

A comparison of combined regimen E dosed groups to the vehicle controls indicated a significant increase in the percentage of cells in G0 phase and a decrease in the percentage of cells in G1 phase (Figure 9).

Hepatic cell apoptosis analyses were evaluated in regimen B female mice given 100 mg/kg for 3 or 6 months. There were no time-related effects related to chloral hydrate administration (Figure 10).

TABLE 12
Cell Cycle Distribution in the Liver of Regimens B, C, and D Female Mice and Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

Dose (mg/kg)	G0 Phase	G1 Phase	G2 Phase	S Phase	M Phase
Regimen B (2 years)					
3-month interim evaluation					
0	99.01 ± 0.345	0.43 ± 0.191	0.12 ± 0.126	0.40 ± 0.192	0.04 ± 0.041
100	99.64 ± 0.241*	0.17 ± 0.146*	0.07 ± 0.099	0.10 ± 0.067*	0.018 ± 0.037
6-month interim evaluation					
0	99.88 ± 0.143	0.05 ± 0.084	0.05 ± 0.088	0.018 ± 0.025	0.0
100	99.76 ± 0.185	0.04 ± 0.035	0.12 ± 0.121	0.09 ± 0.168	0.0
Regimen C (single dose on postnatal day 28)					
0	98.92 ± 0.529	0.85 ± 0.494	0.0	0.23 ± 0.188	0.0
10	99.36 ± 0.084	0.62 ± 0.055	0.016 ± 0.028	0.0	0.0
25	98.87 ± 0.439	0.88 ± 0.297	0.06 ± 0.120	0.20 ± 0.113	0.0
50	99.16 ± 0.543	0.68 ± 0.426	0.0	0.16 ± 0.146	0.0
Regimen D (single dose on postnatal day 15)					
0	86.67 ± 4.796	9.89 ± 3.425	0.70 ± 0.608	2.44 ± 0.985	0.30 ± 0.109
10	75.76 ± 7.134*	17.17 ± 4.940*	2.46 ± 1.395	3.76 ± 1.748	0.65 ± 0.191*
25	79.45 ± 3.690	14.90 ± 3.075	0.84 ± 1.016	4.56 ± 2.101	0.25 ± 0.058
50	75.91 ± 1.743*	17.00 ± 1.306*	2.17 ± 1.429	4.24 ± 0.304	0.68 ± 0.176*
Regimen E (single dose on postnatal day 15)					
0	86.71 ± 9.187	9.96 ± 5.892	0.88 ± 1.203	2.13 ± 2.020	0.31 ± 0.353
10	95.93 ± 2.849	2.97 ± 2.058	0.05 ± 0.070	0.97 ± 0.777	0.07 ± 0.064
25	94.00 ± 7.297	3.67 ± 3.442	0.11 ± 0.189	1.83 ± 3.126	0.38 ± 0.566
50	94.73 ± 4.169	3.32 ± 2.288	0.16 ± 0.185	1.49 ± 1.433	0.31 ± 0.327

* Significantly different ($P \leq 0.05$) from the vehicle control by Student's *t*-test for regimen B and Dunnett's test for regimen D.
[a] Approximately 2,000 cells were counted per liver. Data are expressed as percent distribution (mean ± standard deviation). Eight mice were analyzed from each group in regimen B, and four mice were analyzed per group from regimens C, D, and E; mice were killed the day after dosing.

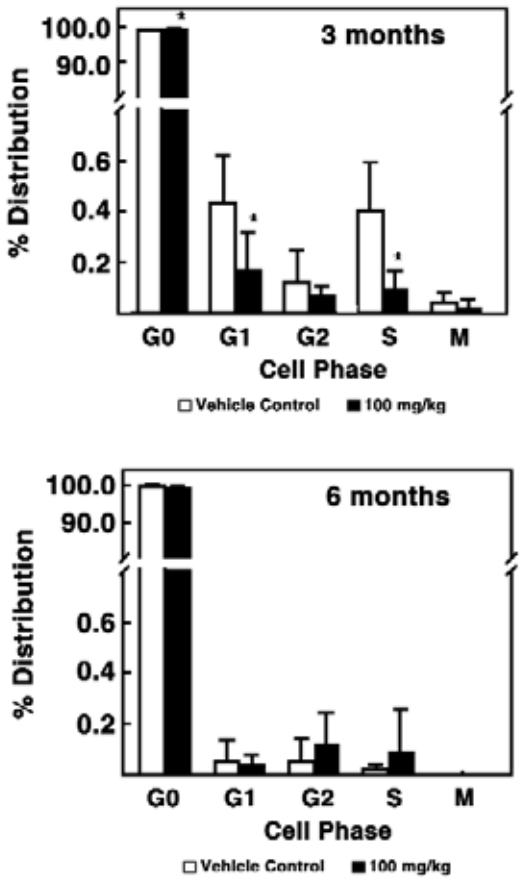


FIGURE 8
Cell Cycle Distribution in the Liver of Regimen B Female Mice Administered 100 mg/kg Chloral Hydrate for 3 or 6 Months
(Data are expressed as mean ± standard deviation. * = Significantly different (P≤0.05) from the vehicle control group)

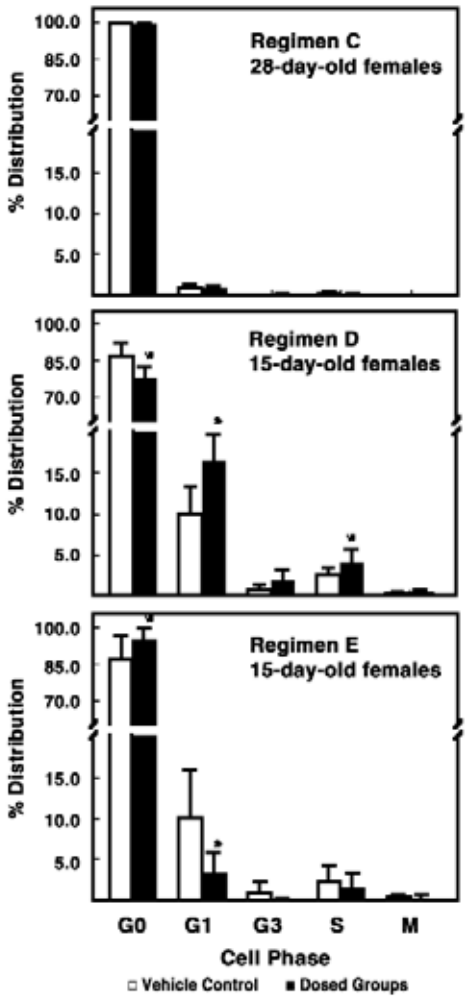


FIGURE 9
Cell Cycle Distribution in the Liver of Regimens C and D Female Mice and Regimen E Male Mice Administered a Single Dose of 10, 25, or 50 mg/kg Chloral Hydrate
(Data from dosed groups were combined and are expressed as mean ± standard deviation.
* = Significantly different ($P \leq 0.05$) from the vehicle control group)

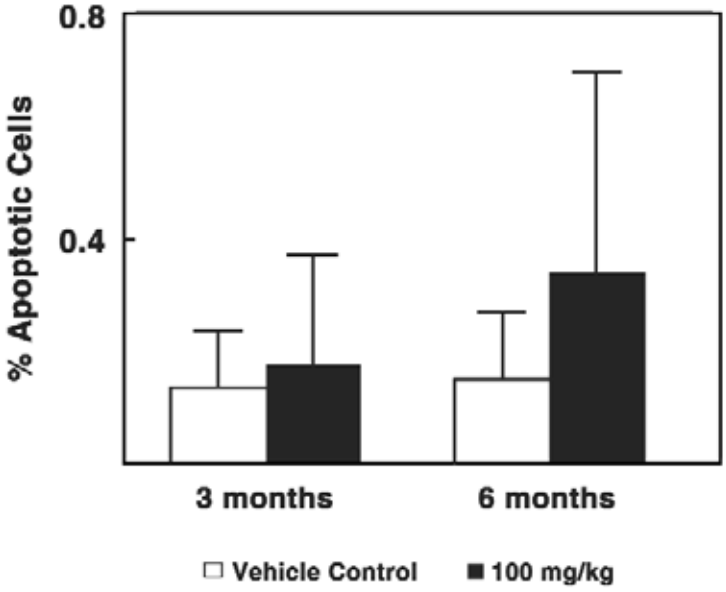


FIGURE 10
Apoptotic Cell Index in the Liver of Regimen B Female Mice Administered 100 mg/kg Chloral Hydrate for 3 or 6 Months
(Data are expressed as mean ± standard deviation.)

Liver Weights, Pathology, and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in liver weights, in the incidences of neoplasms and nonneoplastic lesions of the pituitary gland, lung, and liver, and in the incidences of malignant lymphoma. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes A, B, C, and D for female mice and Appendix E for male mice.

Pituitary gland: A dose-related and significant increase in the incidence of pars distalis adenoma occurred in regimen A 100 mg/kg females, and the incidence of this neoplasm in this group exceeded the historical control range (Tables 13, A2, and A3a). There was also a time-related increase in the incidence of adenoma in female mice administered 100 mg/kg for up to 24 months in regimen B, and the increase in the incidence of this neoplasm at 24 months was significant (Tables 14 and B2). There was a significant increase in the severity of pars distalis hyperplasia in regimen A 100 mg/kg female mice (Table 13).

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Pituitary Gland Pars Distalis in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Number Examined Microscopically	45	44	47	41
Hyperplasia				
Minimal[a]	3 (6.7%)[b]	6 (13.6%)	4 (8.5%)	0
Mild	0	0	0	7 (17.1%)
Moderate	1 (2.2%)	0	0	2 (4.9%)
Monotonic trend test[c]	0.0504	NS	NS	<0.05
Adenoma[d]				
Overall rate[e]	0/45 (0%)	2/44 (5%)	0/47 (0%)	5/41 (12%)
Adjusted rate[f]	0.0%	4.7%	0.0%	13.3%
Terminal rate[g]	0/36 (0%)	1/38 (3%)	0/42 (0%)	5/32 (16%)
First incidence (days)	—[i]	700	—	757 (T)
Poly-3 test[h]	P=0.0073	P=0.2473	—[j]	P=0.0237

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Percentage of animals with lesion of given severity
[c] Overall monotonic trend in severity with dose was tested using the Jonckheere-Terpstra test statistic. Pairwise monotonic tests of severity with dose were tested using the Williams' modification of Shirley's nonparametric test for a monotonic dose response; NS=not significant.
[d] Historical incidence for control groups in NCTR studies: 15/308 (4.9%), range 0%-6%
[e] Number of animals with neoplasm per number of animals with pituitary gland examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Pituitary Gland Pars Distalis at 2 Years in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control (Regimen A)	100 mg/kg 3 Months (Stop-Exposure)	100 mg/kg 6 Months (Stop-Exposure)	100 mg/kg 12 Months (Stop-Exposure)	100 mg/kg 24 Months (Regimen A)
Number Examined Microscopically	45	36	36	33	41
Hyperplasia					
Minimal[a]	3 (6.7%)[b]	0	0	0	0
Mild	0	1 (2.8%)	1 (2.8%)	1 (3.0%)	7 (17.1%)
Moderate	1 (2.2%)	1 (2.8%)	1 (2.8%)	1 (3.0%)	2 (4.9%)
Marked	0	1 (2.8%)	0	0	0
Monotonic trend test[c]	0.0621	NS	NS	NS	<0.05
Adenoma[d]					
Overall rate[e]	0/45 (0%)	3/36 (8%)	1/36 (3%)	1/33 (3%)	5/41 (12%)
Adjusted rate[f]	0.0%	8.9%	3.0%	3.2%	13.3%
Terminal rate[g]	0/36 (0%)	3/32 (9%)	0/29 (0%)	1/28 (4%)	5/32 (16%)
First incidence (days)	[i]	757 (T)	695	757 (T)	757 (T)
Poly-3 test[h]	P=0.0278	P=0.0849	P=0.4620	P=0.4434	P=0.0237

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Percentage of animals with lesion of given severity
[c] Overall monotonic trend in severity with dose was tested using the Jonckheere-Terpstra test statistic. Pairwise monotonic tests of severity with dose were tested using the Williams' modification of Shirley's nonparametric test for a monotonic dose response; NS=not significant.
[d] Historical incidence for control groups in NCTR studies: 15/308 (4.9%), range 0%-6%
[e] Number of animals with neoplasm per number of animals with pituitary gland examined microscopically
[f] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.
[i] Not applicable; no neoplasms in animal group

Malignant lymphoma: There was a dose-related increase in the incidence of malignant lymphoma in regimen A 100 mg/kg females, and a marginally positive time-related trend in the incidences of malignant lymphoma in regimen B (Tables 15, 16, A2, and B2). There were significant increases in the incidences of malignant lymphoma in the regimen C 10 and 50 mg/kg groups compared to that in the vehicle control group (Tables 17 and C2). The incidences in regimens A, B, and C did not exceed the historical control range (Tables 15, 16, 17, and A3b).

Lung: The incidence of alveolar/bronchiolar adenoma was increased in regimen A 100 mg/kg mice and was significantly increased in regimen B mice dosed for 12 months (Tables 18, A2, and B2); the incidences of this neoplasm increased with increasing length of exposure time in regimen B females dosed for up to 12 months. The incidences in regimen A 100 mg/kg females and in regimen B females dosed for 6, 12, or 24 months (regimen A) exceeded the historical control range (Tables 18 and A3c).

TABLE 15
Incidences of Malignant Lymphoma in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Malignant Lymphoma[a]				
Overall rate[b]	9/48 (19%)	7/48 (15%)	8/48 (17%)	15/48 (31%)
Adjusted rate[c]	20.5%	15.3%	17.1%	34.1%
Terminal rate[d]	4/37 (11%)	4/39 (10%)	7/43 (16%)	11/36 (31%)
First incidence (days)	605	622	722 (T)	555
Poly-3 test[e]	P=0.0455	P=0.3571N	P=0.4432N	P=0.1210

(T)Terminal sacrifice
[a] Historical incidence for control groups in NCTR studies: 92/374 (24.6%) range 21%-43%
[b] Number of animals with malignant lymphoma per number of animals examined microscopically (vehicle control and 100 mg/kg) or necropsied (25 and 50 mg/kg)
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.

TABLE 16
Incidences of Malignant Lymphoma at 2 Years in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	100 mg/kg			
	(Regimen A)	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)	24 Months (Regimen A)
Malignant Lymphoma[a]					
Overall rate[b]	9/48 (19%)	8/40 (20%)	13/40 (33%)	14/40 (35%)	15/48 (31%)
Adjusted rate[c]	20.5%	21.6%	34.8%	37.0%	34.1%
Terminal rate[d]	4/37 (11%)	7/34 (21%)	10/31 (32%)	13/33 (39%)	11/36 (31%)
First incidence (days)	605	747	471	694	555
Poly-3 test[e]	P=0.0834	P=0.5614	P=0.1156	P=0.0780	P=0.1210

[a] Historical incidence for control groups in NCTR studies: 92/374 (24.6%), range 21%-43%
[b] Number of animals with malignant lymphoma per number of animals examined microscopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

TABLE 17
Incidences of Malignant Lymphoma at 2 Years in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Malignant Lymphoma[a]				
Overall rate[b]	8/48 (17%)	16/48 (33%)	6/48 (13%)	16/48 (33%)
Adjusted rate[c]	17.2%	34.1%	12.6%	35.1%
Terminal rate[d]	6/42 (14%)	12/40 (30%)	5/45 (11%)	13/40 (33%)
First incidence (days)	562	604	718	543
Poly-3 test[e]	P=0.1250	P=0.0488	P=0.3714N	P=0.0404

[a] Historical incidence for untreated control groups in NCTR studies: 92/374 (24.6%), range 21%-43%
[b] Number of animals with malignant lymphoma per number of animals examined microscopically (vehicle control and 50 mg/kg) or necropsied (10 and 25 mg/kg)
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.

TABLE 18
Incidences of Alveolar/bronchiolar Adenoma of the Lung at 2 Years in Regimens A and B Female Mice in the 2-Year Gavage Study of Chloral Hydrate

Regimen A	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Alveolar/bronchiolar Adenoma[a]				
Overall rate[b]	1/48 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate[c]	2.3%	2.2%	4.3%	9.4%
Terminal rate[d]	1/37 (3%)	1/39 (3%)	2/43 (5%)	4/36 (11%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test[e]	P=0.0711	P=0.7508N	P=0.5308	P=0.1805

		100 mg/kg			
Regimen B	Vehicle Control	3 Months	6 Months	12 Months	24 Months
Alveolar/bronchiolar Adenoma[a]					
Overall rate	1/48 (2%)	2/40 (5%)	4/40 (10%)	7/40 (18%)	4/48 (8%)
Adjusted rate	2.3%	5.4%	11.0%	18.6%	9.4%
Terminal rate	1/37 (3%)	2/34 (6%)	4/31 (13%)	7/33 (21%)	4/36 (11%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.1887	P=0.4497	P=0.1309	P=0.0173	P=0.1805

(T)Terminal sacrifice
[a] Historical incidence for control groups in NCTR studies: 14/372 (3.8%), range 2%-6%
[b] Number of animals with neoplasm per number of animals with lung examined microsopically
[c] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[d] Observed incidence at terminal kill
[e] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.

Liver: A dose-related and significant increase in liver weights occurred in regimen A 100 mg/kg females (Table G1). A time-related increase in absolute liver weights occurred in regimen B; the increases were significant at 3 and 6 months and were accompanied by the significant increases in body weights (Table G2). Relative liver weights in regimen B and liver weights in regimens C, D, and E were unchanged compared to the vehicle controls (Tables G2 and G3). In regimen A, the combined incidence of hepatocellular adenoma or carcinoma varied between 4% and 6% (Tables 19 and A2). Female mice in regimen B dosed with 100 mg/kg chloral hydrate for 3, 6, or 12 months had a combined incidence of 3% to 10% (Tables 19 and B2). Female mice given a single dose of 0, 10, 25, or 50 mg/kg at 28 (regimen C) or 15 (regimen D) days of age had a combined incidence of hepatocellular adenoma or carcinoma of 2% to 13% (Tables 19, C2, and D2). The combined incidence in regimen E male mice given a single dose of 0, 10, 25, or 50 mg/kg at 15 days of age varied from 35% to 50% (Tables 19 and E2); the incidence of hepatocellular adenoma in 10 mg/kg males was significantly less than that in the vehicle controls. The combined incidences of hepatocellular adenoma or carcinoma in female mice did not exceed the historical control range (Tables 19 and A3d). The combined incidences of hepatocellular adenoma or carcinoma in male mice exceeded the historical control range [89/374 (23.8%), range 19%-28%]; however, there were no significant dose-related trends in the incidences of hepatocellular neoplasms or any increased incidences of nonneoplastic lesions related to chloral hydrate administration in male or female mice.

Tumor Mutation Analyses

DNA was isolated from all 17 liver tumors from female mice in regimen A and from regimen B mice dosed for 3, 6, or 12 months. Four mutations were detected: two in H-*ras* exon 1 (one in an adenoma from the 50 mg/kg group in regimen A, the other in a carcinoma from the vehicle control group) and two in β-catenin exon 2 (one in a carcinoma from the 100 mg/kg group sacrificed at 6 months, the other in a carcinoma from the vehicle control group).

GENETIC TOXICOLOGY

Chloral hydrate gave positive responses in both *in vitro* and *in vivo* mutagenicity assays. It induced mutations in *Salmonella typhimurium* strain TA100 at concentrations greater than or equal to 1,000 µg/plate, with and without liver S9 activation enzymes; an equivocal response was obtained in *S. typhimurium* strain TA98 in the absence of S9; and no mutagenicity was detected with strain TA1535 or TA1537, with or without S9 (Table F1; Haworth *et al.*, 1983). In addition to gene mutations in bacterial cells, chloral hydrate was shown to produce chromosomal damage in mammalian cells. It induced significant increases in sister chromatid exchanges (Table F2) and chromosomal aberrations (Table F3) in cultured Chinese hamster ovary cells, with and without S9. Concentrations of 1,000 µg/mL or higher were required to induce a significant increase in chromosomal aberrations; SCEs were induced at somewhat lower concentrations, particularly in the absence of S9. Results of sex-linked recessive lethal (SLRL) tests in *Drosophila melanogaster* were inconclusive (Table F4; Yoon *et al.*, 1985). Chloral hydrate, administered by feeding in 5% sucrose, produced only a small increase in SLRL mutations in the germ cells of male flies; this result was considered inconclusive. A second SLRL assay that used injection as the route of administration gave negative results.

An *in vivo* mammalian mutagenicity study, a mouse bone marrow micronucleus test, was performed with chloral hydrate (Table F5). In this test, male $B6C3F_1$ mice injected with 125 to 500 mg/kg showed a significant dose-related trend in the frequency of micronucleated erythrocytes in bone marrow sampled 24 hours after treatment. Thus, chloral hydrate gave positive responses in both *in vivo* and *in vitro* assays for chromosomal damage.

TABLE 19
Incidences of Hepatocellular Neoplasms at 2 Years in Regimens A, B, C, and D Female Mice and in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

Regimen A (2 years)	**Vehicle Control**	**25 mg/kg**	**50 mg/kg**	**100 mg/kg**
Number Examined Microscopically	48	48	48	48
Hepatocellular Adenoma[a]	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Hepatocellular Carcinoma	1 (2%)	0 (0%)	0 (0%)	1 (2%)
Hepatocellular Adenoma or Carcinoma[b]	2 (4%)	2 (4%)	3 (6%)	3 (6%)

Regimen B (2 years)[c]	**100 mg/kg**			
	3 Months	**6 Months**	**12 Months**	**24 Months**
Number Examined Microscopically	40	40	40	48
Hepatocellular Adenoma	1 (3%)	1 (3%)	2 (5%)	2 (4%)
Hepatocellular Carcinoma	0 (0%)	1 (3%)	2 (5%)	1 (2%)
Hepatocellular Adenoma or Carcinoma[b]	1 (3%)	2 (5%)	4 (10%)	3 (6%)

	Vehicle Control	**10 mg/kg**	**25 mg/kg**	**50 mg/kg**
Regimen C (single dose on postnatal day 28)				
Number Examined Microscopically	48	48	48	48
Hepatocellular Adenoma	3 (6%)	3 (6%)	2 (4%)	2 (4%)
Hepatocellular Carcinoma	3 (6%)	0 (0%)	0 (0%)	0 (0%)
Hepatocellular Adenoma or Carcinoma[b]	6 (13%)	3 (6%)	2 (4%)	2 (4%)
Regimen D (single dose on postnatal day 15)				
Number Examined Microscopically	48	48	48	48
Hepatocellular Adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hepatocellular Carcinoma	0 (0%)	2 (4%)	1 (2%)	1 (2%)
Hepatocellular Adenoma or Carcinoma[b]	1 (2%)	3 (6%)	2 (4%)	2 (4%)
Regimen E (single dose on postnatal day 15)				
Number Examined Microscopically	48	48	48	48
Hepatocellular Adenoma	18 (38%)	8* (17%)	12 (25%)	11 (23%)
Hepatocellular Carcinoma	10 (21%)	10 (21%)	6 (13%)	12 (25%)
Hepatocellular Adenoma or Carcinoma[d]	24 (50%)	17 (35%)	18 (38%)	21 (44%)

* Significantly different (P 0.05) from the vehicle control group by the Poly-3 test
[a] Number of animals with neoplasm; overall rate percent is given in parentheses.
[b] Historical incidence for control groups in NCTR studies: 20/371 (5.4%), range 0%-11%
[c] For statistical comparisons, regimen B groups were compared to regimen A vehicle controls.
[d] Historical incidence for control groups in NCTR studies: 89/374 (23.8%), range 19%-28%

DISCUSSION AND CONCLUSIONS

Concern about potential chloral hydrate toxicity arose following a report of the induction of liver neoplasms in male B6C3F$_1$ mice exposed to chloral hydrate for 2 years in drinking water (Daniel *et al*., 1992a). In addition, a limited study reported liver tumorigenicity of chloral hydrate in 15-day-old male B6C3F$_1$ mice given a single dose of chloral hydrate by gavage (Rijhsinghani *et al*., 1986). Due to the use of chloral hydrate in pediatric medicine, the Center for Drug Evaluation and Research of the Food and Drug Administration requested additional studies to ascertain the risk associated with the drug.

In the current study, five dose regimens were used to assess the effect of the animal's age and the duration of dosing on the tumorigenicity of chloral hydrate. This study was restricted to B6C3F$_1$ mice because ongoing U.S Environmental Protection Agency (EPA) tumorigenicity bioassays in rats suggested that rats were not susceptible (DeAngelo, personal communication, 1994). This suggested species difference has subsequently been supported (DeAngelo and George, 1995; Leuschner and Beuscher, 1998, George *et al.*, 2000). Female B6C3F$_1$ mice were chosen for regimens A, B, C, and D because female B6C3F$_1$ mice appear to be more sensitive than male B6C3F$_1$ mice to the chemical induction of neoplasms during chronic dosing (Haseman *et al*., 1984). In addition, female B6C3F$_1$ mice have a lower spontaneous liver neoplasm incidence than male B6C3F$_1$ mice (Ward *et al*., 1979; Haseman *et al*., 1985; Tamano *et al*., 1988; Chandra and Frith, 1992; Haseman *et al*., 1998), which allows hepato-carcinogenicity of low doses to be detected more readily. Furthermore, the data obtained would complement results for male B6C3F$_1$ mice reported by the EPA (Daniel *et al*., 1992a; George *et al.*, 2000) and a companion NTP study being conducted at the NCTR (NTP, 2002). When mice are dosed with carcinogens only during infancy, males are more sensitive than females to neoplasm induction (Wislocki *et al*., 1986; Fujii, 1991; Flammang *et al*., 1997; Fu *et al*., 1998, 2000). Because the data of Rijhsinghani *et al*. (1986) suggested that preweanling male B6C3F$_1$ mice may be unusually sensitive to the neoplastic effects of chloral hydrate, regimen E in the current study was modeled after their investigation.

In the present study, significant dose-related increases in the incidence of liver neoplasms did not occur in any of the dose regimens. Likewise, none of the doses caused significant increases in the incidence of liver neoplasms when compared to their respective vehicle controls. In female mice treated with chloral hydrate, the combined incidences of hepatocellular adenoma or carcinoma varied from 3% to 10%. This range is lower than the mean spontaneous incidence (23.6%, range 6%-56%) reported for control (feed studies) female B6C3F$_1$ mice given NIH-07 diet (Haseman *et al.*, 1998); however, in studies conducted at the NCTR in female B6C3F$_1$ mice, the incidence of hepatocellular adenoma or carcinoma (combined) in control groups has averaged 5.4% (range 0%-11%).

Spontaneous liver neoplasms in male and female B6C3F$_1$ mice are associated with H-*ras* mutations at codon 61, with the majority being CAA to AAA, CAA to CGA, and CAA to CTA (Richardson *et al*., 1992). The microsomal metabolism of chloral hydrate has been shown to cause lipid peroxidation (Ni *et al*., 1994, 1996) and the formation of a DNA adduct from the reaction of malondialdehyde with deoxyguanosine (Ni *et al*., 1995). Should this occur *in vivo*, the hepatic neoplasms induced by chloral hydrate could have a different pattern of H-*ras* mutations than that observed in neoplasms from control mice. This possibility was examined by screening the hepatocellular adenomas and carcinomas for H-*ras* mutations with denaturing gradient gel electrophoresis. Of the 17 neoplasms assayed from regimens A and B in the present study, only two mutations were detected; this number was insufficient for statistical comparison. The spontaneous mutation frequency for H-*ras* in B6C3F$_1$ mice has ranged from 40% to 80% (Richardson *et al.*, 1992); however, in the

B6C3F$_1$ mice from the NCTR colony, the frequency appears to be much lower (approximately 15%; Von Tungeln *et al.*, 1999), as was observed in the present study.

Mutations in the β-catenin gene are increased in hepatocellular neoplasms induced by certain chemicals (Devereaux *et al.*, 1999; Ogawa *et al.*, 1999). Accordingly, liver neoplasms in mice from regimens A and B were also assessed for β-catenin mutations. As with H-*ras*, only two mutations were detected; this limited number did not permit statistical comparison. Liver neoplasms were also screened for K-*ras* and *p53* mutations, but none were detected.

The incidences of liver neoplasms in B6C3F$_1$ mice correlated with body weight when the mice were examined at 1 year of age (Seilkop, 1995; Haseman *et al.*, 1997). Had the body weights of dosed mice been suppressed in the present study, a hepatocarcinogenic effect of chloral hydrate could have been masked. Comparisons of body weights at 1 year indicated no significant differences; thus, the failure to induce liver neoplasms was not a consequence of decreased body weight gains.

The hepatocellular neoplasm incidences in regimen E males were considerably greater than the incidences in females in regimens A, B, C, and D, which is consistent with data from previous studies (Ward *et al.*, 1979; Haseman *et al.*, 1985; Tamano *et al.*, 1988; Chandra and Frith, 1992; Haseman *et al.*, 1998). The combined incidences of hepatocellular adenoma or carcinoma in regimen E varied from 35% to 50%; these incidences are similar to the mean spontaneous incidence (42.2%, range 10%-68%) reported for control (feed studies) male B6C3F$_1$ mice given NIH-07 feed (Haseman *et al.*, 1998); however, they exceed the mean spontaneous incidence (23.8%, range 19%-28%) of hepatocellular adenoma or carcinoma (combined) in control groups in other NCTR studies using male B6C3F$_1$ mice. Compared to those observed in previous NCTR studies, the increased incidences of hepatocellular neoplasms in regimen E males in the present study may be due to stress resulting from mice being dosed by gavage at an early age. In rats, stress was reported to increase hepatocellular carcinogenesis (Laconi *et al.*, 2000). In the companion study in which male B6C3F$_1$ mice were administered chloral hydrate by gavage for 2 years, the spontaneous liver neoplasm incidence was 33%, while the incidence in treated mice ranged from 46% to 52% (NTP, 2002).

The incidence of hepatocellular neoplasms in neonatally dosed mice is typically assessed 1 year after treatment to avoid complications from the increases in the incidences of spontaneous neoplasms that occur during the second year (Flammang *et al.*, 1997; Fu *et al.*, 1998, 2000). Because regimen E males were held for 2 years after they were dosed, neoplasm induction by chloral hydrate could have been masked by an increase in the incidence of spontaneous neoplasms during the second year. In other studies conducted at the NCTR, male B6C3F$_1$ mice were treated intraperitoneally with total doses of 2,000 (30 mg/kg) or 5,000 (75 mg/kg) nmol chloral hydrate, with one-third of the dose given on day 8 and two-thirds on day 15 (Von Tungeln *et al.*, 1997; Von Tungeln, personal communication, 2000). One year after dosing, no significant increases in the incidences of liver neoplasms occurred compared to the vehicle controls. Therefore, under the dosing conditions used in the present study, chloral hydrate did not induce hepatocellular neoplasms in male or female B6C3F$_1$ mice.

The results from this study of the hepatocarcinogenicity of chloral hydrate in B6C3F$_1$ mice differ from previous reports. Daniel *et al.* (1992a) reported a 71% incidence of hepatocellular adenoma or carcinoma (combined) in male B6C3F$_1$ mice that were exposed daily to 166 mg/kg chloral hydrate in the drinking water for 2 years compared to a 15% incidence in the controls. This difference might be attributed to the greater dose (166 mg/kg per day) compared to a maximum dose of 100 mg/kg per day in the present study. In addition, the mice in Daniel *et al.* (1992a) were exposed to chloral hydrate daily in the drinking water, but in the present study, the mice were never dosed more than 5 days per week by gavage. One unusual feature of the Daniel *et al.* (1992a) bioassay was the low incidence of hepatocellular tumors in the control group (15%), a value considerably lower than the mean spontaneous incidence reported for control (feed studies) male B6C3F$_1$ mice given NIH-07 diet (42.2%, range 10%-68%) (Haseman *et al.*, 1998). As noted earlier, the incidences of liver neoplasms in B6C3F$_1$ mice are related to body weights of mice at 1 year of age (Seilkop, 1995; Haseman *et al.*, 1997). Based on the body weights of 1-year-old mice (approximately 40 g) reported by Daniel *et al.* (1992a), the spontaneous hepatocellular tumor incidence should have been approximately 25%, still significantly

different from the value for mice administered chloral hydrate in the present study.

George *et al.* (2000) exposed male B6C3F$_1$ mice to 0, 13.5, 65, or 146.6 mg/kg chloral hydrate per day in the drinking water for 2 years. Compared to the control group, the incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in mice exposed to 65 or 146.6 mg/kg; hepatocellular neoplasms were induced at a dose lower than that used in the present study. One potential shortcoming of the results reported by George *et al.* (2000) is the spontaneous hepatocellular tumor incidence, which was 64.3% for adenoma or carcinoma (combined). This percentage is considerably higher than the mean spontaneous incidence in control (feed studies) male B6C3F$_1$ mice given NIH-07 diet (42.2%, range 10%-68%) (Haseman *et al*., 1998). Even more troubling was that in the control group, the incidence of hepatocellular carcinoma occurred to a greater extent (54.8%) than the incidence of hepatocellular adenoma (21.4%). Typically, hepatocellular adenomas occur at a higher incidence than hepatocellular carcinomas; furthermore, the spontaneous incidence of hepatocellular carcinoma in the George *et al.* (2000) bioassay substantially exceeded the historical control range (6%-29%) in male B6C3F$_1$ mice given NIH-07 diet (Haseman *et al.*, 1998).

In a companion study (NTP, 2002), the incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased (46%-52%) in male B6C3F$_1$ mice given doses up to 100 mg/kg chloral hydrate per day by gavage, 5 days per week, for 2 years compared to that in the vehicle controls (33%). Although treatment with chloral hydrate increased the incidence of hepatocellular neoplasms, the magnitude of the increase was not as great as the increase in mice exposed to chloral hydrate in the drinking water (Daniel *et al.*, 1992a; George *et al.*, 2000). The weaker hepatocarcinogenic response was attributed to the differences in the dosing regimen; the 5-day-per-week gavage dosing schedule permitted a 2-day recovery period not available to mice exposed to chloral hydrate in the drinking water. It was proposed that peroxisome proliferation is involved in the tumorigenic response, and that continuous exposure to the drug is required to maintain a proliferative state (NTP, 2002). While plausible, it should be noted that George *et al.* (2000) did not detect peroxisome proliferation, measured by palmitoyl CoA oxidase activity 26 weeks after the initiation of dosing. George *et al.* (2000) did observe an increase in hepatocyte labeling indices after 26 weeks of chloral hydrate administration, but not after 52 or 78 weeks of treatment. In the present study, a decrease in the percentage of hepatocytes in S-phase was detected at 3 months; however, after 6 months of dosing, there were no differences between dosed mice and vehicle controls.

Rijhsinghani *et al.* (1986) reported that a single gavage dose of 10 mg/kg chloral hydrate resulted in a significant induction of hepatic tumors in 15-day-old male B6C3F$_1$ mice. This study was compromised by the small number of animals in the treatment groups (eight to nine mice), and in the present study, the induction of hepatic tumors was unconfirmed even at fivefold greater doses. Likewise, in another chloral hydrate bioassay with neonatal mice, an increase in the incidence of hepatic tumors did not occur (Von Tungeln *et al.*, 1997; Von Tungeln, personal communication, 2000). The spontaneous hepatic tumor incidence in the Rijhsinghani *et al.* (1986) study was 10.5%, which is considerably lower than that in other studies with B6C3F$_1$ mice (42.2%, range 10%-68%) (Haseman *et al.*, 1998). The low spontaneous hepatic tumor incidence coupled with the small number of animals used in the bioassay suggests that the increased incidence in the Rijhsinghani *et al.* (1986) study was due to normal variation in B6C3F$_1$ mice and did not result from chloral hydrate treatment.

Rijhsinghani *et al.* (1986) also reported an increase in the mitotic index of hepatocytes when measured 24 hours after dosing. In the present study, this finding was unconfirmed even at fivefold greater doses. Furthermore, a reanalysis of the Rijhsinghani *et al.* (1986) data suggests that their interpretation may not have been correct (Beland, unpublished). In addition, the purported increase in mitotic index did not occur in a dose-related manner. The results from the previously mentioned bioassays suggest that continuous daily lifetime exposure to chloral hydrate is necessary to obtain a substantial hepatocarcinogenic response in B6C3F$_1$ mice, and that under comparable dosing regimens, males are more sensitive to chloral hydrate exposure than females. This sex-related difference is consistent with data reported by Pereira and Phelps (1996) in which female B6C3F$_1$ mice were initiated with a single dose of *N*-methyl-*N*-nitrosourea and then exposed to 0, 54, 180, or 540 mg/kg trichloroacetic acid in the drinking water daily for 1 year. In the

absence of initiation with *N*-methyl-*N*-nitrosourea, there were no significant increases in the incidences of hepatic adenoma in any dosed group. However, there was an increase in the incidence of carcinoma in 540 mg/kg females. These data suggest that in female B6C3F$_1$ mice, trichloroacetic acid, and by extrapolation chloral hydrate, are hepatic tumor promoters rather than initiators.

Although chloral hydrate treatment did not result in the induction of hepatic neoplasms in the present study, a 12% incidence of pituitary gland pars distalis adenoma occurred in 100 mg/kg females in regimen A; this incidence fell well outside the control range (4.9%, range 0%-6%) observed in previous studies conducted with female B6C3F$_1$ mice at the NCTR. Higher control rates (14%, range 0%-36%) have been reported in untreated control (feed studies) and chamber control (inhalation studies) female B6C3F$_1$ mice given NIH-07 feed in other studies (Haseman *et al*., 1998).

The chronic administration of estrogenic compounds can lead to increased incidences of pituitary gland hyperplasia and adenoma (Neumann, 1991; Liebelt, 1994). The mechanism appears to be a result of estrogen inhibiting the secretion of dopamine from the hypothalamus, which results in an increase in the number of prolactin-producing cells in the anterior pituitary gland. Carcinomas are rarely observed. A similar scenario may be occurring with chloral hydrate. Plasma concentrations of prolactin were significantly decreased in ovariectomized Sprague-Dawley rats administered 17β-estradiol and chloral hydrate when compared to ovariectomized rats dosed with 17β-estradiol alone (Lawson and Gala, 1975). In contrast, when ovariectomized Sprague-Dawley rats were treated with chloral hydrate alone, there was a transient increase in plasma concentrations of prolactin (Lawson and Gala, 1974). These results indicate that chloral hydrate can interfere with the estrogen-induced secretion of prolactin. If a similar response occurs in mice, this presumably would result in decreased concentrations of serum prolactin, which would lead to a feedback stimulus directed at the anterior pituitary gland to increase the production of prolactin to normal levels. Hyperplasia and adenoma could result. A central feature of this mechanism is that it would require the chronic administration of chloral hydrate, which is consistent with the observations from this study. Increased incidences of pituitary gland neoplasms did not occur in B6C3F$_1$ mice from other chloral hydrate bioassays (Rijhsinghani *et al*., 1986; Daniel *et al*., 1992a; George *et al*., 2000; NTP, 2002); however, only males were used in these studies.

The incidence of alveolar/bronchiolar adenoma was increased significantly in regimen B 100 mg/kg female mice dosed for 12 months. Lung neoplasms have been reported in male and female B6C3F$_1$ mice exposed to up to 600 ppm trichloroethylene by inhalation for 78 weeks (reviewed by Davidson and Beliles, 1991). Although chloral hydrate is an intermediate in the metabolism of trichloroethylene (Prout *et al*., 1985; Abbas and Fisher, 1997; Greenberg *et al*., 1999), the fact that no increased incidence of alveolar/bronchiolar adenoma was observed after 2 years of dosing suggests that the increased incidence observed after 12 months of dosing may not have been due to chloral hydrate treatment. Although the observed incidence (18%) was within the historical range (5.9%, range 0%-24%) for control (feed studies) female B6C3F$_1$ mice given NIH-07 feed in other NTP studies (Haseman *et al*., 1998), it is higher than that observed in other studies conducted at the NCTR in female B6C3F$_1$ mice (3.8%, range 2%-6%). Induction of lung neoplasms has not been reported in other studies conducted with chloral hydrate (Rijhsinghani *et al*., 1986; Daniel *et al*., 1992a; DeAngelo and George, 1995; Leuschner and Beuscher, 1998).

There was a dose-related increase in the incidence of malignant lymphoma in regimen A 100 mg/kg female mice. Likewise, the incidences of malignant lymphoma in 10 and 50 mg/kg female mice in regimen C were significantly greater than that in the vehicle controls. Increased incidences of malignant lymphoma have not been reported in other chloral hydrate tumorigenicity studies (Rijhsinghani *et al*., 1986; Daniel *et al*., 1992a; DeAngelo and George, 1995; Leuschner and Beuscher, 1998; George *et al*., 2000; NTP, 2002); however, increased incidences have been reported in NMRI mice exposed to trichloroethylene by inhalation for 78 weeks (Henschler *et al*., 1980). The mean spontaneous incidence of malignant lymphoma in control (feed studies) female B6C3F$_1$ mice given NIH-07 diet in other NTP studies is 20.9% (range, 6%-42%; Haseman *et al*., 1998). In studies conducted at the NCTR using female B6C3F$_1$ mice, the mean spontaneous incidence of malignant lymphoma in control groups has been 24.6% (range, 21%-43%). The incidences of malignant lymphoma were not

affected by the duration of treatment (e.g., regimen A compared to regimen C) and were within the range of incidences in the historical controls; this suggests that the incidences observed in regimens A and C reflect the normal variation found in female $B6C3F_1$ mice.

CONCLUSIONS

Under the conditions of this 2-year gavage study, there was *equivocal evidence of carcinogenic activity* of chloral hydrate in female $B6C3F_1$ mice treated continuously for two years based on increased incidences of pituitary gland pars distalis adenomas. No increased incidences of neoplasms were seen in female $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 or 28 days of age or in male $B6C3F_1$ mice that received a single dose of chloral hydrate at 15 days of age. No hepatocarcinogenicity was seen under any dosing condition.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

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APPENDIX A
SUMMARY OF LESIONS IN REGIMEN A FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF CHLORAL HYDRATE

TABLE A1 Summary of the Incidence of Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate 84

TABLE A2 Statistical Analysis of Primary Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate 88

TABLE A3a Historical Incidence of Pituitary Gland Pars Distalis Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice 90

TABLE A3b Historical Incidence of Malignant Lymphoma in Control Female B6C3F$_1$/Nctr BR Mice 90

TABLE A3c Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice 91

TABLE A3d Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice 91

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate 92

TABLE A1
Summary of the Incidence of Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental deaths			1	1
Moribund	2	4	3	2
Natural deaths	9	5	1	9
Survivors				
Died last week of study		1		
Terminal sacrifice	37	38	43	36
Animals examined microscopically	48	48	48	48
Alimentary System				
Gallbladder	(46)	(8)	(4)	(47)
Lymphoma malignant				1 (2%)
Intestine large, cecum	(42)	(6)	(3)	(41)
Lymphoma malignant	1 (2%)			
Intestine large, colon	(46)	(9)	(3)	(43)
Lymphoma malignant	2 (4%)			
Intestine large, rectum	(44)	(8)	(3)	(43)
Lymphoma malignant				1 (2%)
Intestine small	(41)	(7)	(6)	(42)
Lymphoma malignant			1 (17%)	
Intestine small, duodenum	(40)	(7)	(4)	(42)
Polyp adenomatous				1 (2%)
Intestine small, ileum	(40)	(6)	(3)	(39)
Lymphoma malignant				1 (3%)
Intestine small, jejunum	(41)	(6)	(5)	(41)
Hemangioma			1 (20%)	
Lymphoma malignant	2 (5%)			1 (2%)
Liver	(48)	(48)	(48)	(48)
Hepatocellular adenoma	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Hepatocellular carcinoma	1 (2%)			1 (2%)
Histiocytic sarcoma	1 (2%)	1 (2%)		3 (6%)
Lymphoma malignant	6 (13%)	5 (10%)	1 (2%)	6 (13%)
Mesentery	(1)	(1)	(1)	(1)
Lymphoma malignant	1 (100%)			
Pancreas	(48)	(8)	(5)	(46)
Fibrosarcoma	1 (2%)			
Lymphoma malignant	2 (4%)	1 (13%)		3 (7%)
Salivary glands	(48)	(10)	(5)	(48)
Lymphoma malignant	3 (6%)		1 (20%)	3 (6%)
Stomach, forestomach	(47)	(10)	(4)	(45)
Papilloma squamous				1 (2%)
Stomach, glandular	(47)	(10)	(4)	(46)
Lymphoma malignant				1 (2%)
Tongue	(48)	(9)	(5)	(48)
Lymphoma malignant				1 (2%)
Papilloma squamous	1 (2%)			
Cardiovascular System				
Heart	(48)	(10)	(5)	(48)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymphoma malignant	1 (2%)			1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Endocrine System				
Adrenal gland, cortex	(46)	(9)	(5)	(47)
Histiocytic sarcoma		1 (11%)		
Lymphoma malignant	1 (2%)			2 (4%)
Adrenal gland, medulla	(46)	(9)	(3)	(46)
Lymphoma malignant	1 (2%)			1 (2%)
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(48)	(8)	(5)	(46)
Adenoma				2 (4%)
Pituitary gland	(45)	(44)	(47)	(41)
Adenoma, pars distalis		2 (5%)		5 (12%)
Adenoma, pars intermedia	2 (4%)			
Thyroid gland	(47)	(9)	(5)	(48)
Lymphoma malignant				1 (2%)
General Body System				
Tissue NOS	(1)			
Lymphoma malignant, fat	1 (100%)			
Genital System				
Clitoral gland	(43)	(8)	(4)	(43)
Lymphoma malignant				2 (5%)
Ovary	(48)	(29)	(21)	(46)
Cystadenoma	1 (2%)		1 (5%)	2 (4%)
Histiocytic sarcoma	1 (2%)			2 (4%)
Luteoma	1 (2%)			
Lymphoma malignant	2 (4%)	1 (3%)		2 (4%)
Lymphoma malignant, periovarian tissue	1 (2%)			3 (7%)
Uterus	(48)	(26)	(29)	(47)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma	2 (4%)	1 (4%)		3 (6%)
Leiomyoma			1 (3%)	
Lymphoma malignant				2 (4%)
Polyp	1 (2%)			
Vagina	(48)	(9)	(5)	(45)
Histiocytic sarcoma	2 (4%)			4 (9%)
Lymphoma malignant	2 (4%)			1 (2%)
Polyp				2 (4%)
Hematopoietic System				
Bone marrow	(47)	(10)	(5)	(47)
Hemangiosarcoma	1 (2%)			1 (2%)
Lymphoma malignant	3 (6%)			2 (4%)
Lymph node	(48)	(13)	(8)	(47)
Fibrosarcoma, inguinal		1 (8%)		
Histiocytic sarcoma, lumbar	1 (2%)			
Lymphoma malignant				1 (2%)
Lymphoma malignant, axillary		1 (8%)		
Lymphoma malignant, deep cervical			1 (13%)	
Lymphoma malignant, inguinal				1 (2%)
Lymphoma malignant, lumbar	2 (4%)	1 (8%)	1 (13%)	2 (4%)
Lymphoma malignant, mediastinal	1 (2%)	2 (15%)		
Lymphoma malignant, renal	1 (2%)	1 (8%)	2 (25%)	1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System (continued)				
Lymph node, mandibular	(47)	(11)	(5)	(46)
Lymphoma malignant	4 (9%)	3 (27%)	2 (40%)	6 (13%)
Lymph node, mesenteric	(46)	(9)	(7)	(44)
Histiocytic sarcoma	1 (2%)	1 (11%)		1 (2%)
Lymphoma malignant	6 (13%)	4 (44%)	2 (29%)	9 (20%)
Spleen	(47)	(14)	(19)	(47)
Hemangiosarcoma	1 (2%)		1 (5%)	2 (4%)
Lymphoma malignant	8 (17%)	6 (43%)	7 (37%)	12 (26%)
Thymus	(41)	(5)	(5)	(44)
Lymphoma malignant	3 (7%)	1 (20%)	2 (40%)	6 (14%)
Integumentary System				
Mammary gland	(44)	(6)	(5)	(44)
Adenocarcinoma			1 (20%)	1 (2%)
Adenoma		1 (17%)		
Fibrosarcoma	1 (2%)			
Lymphoma malignant	1 (2%)			
Skin	(45)	(10)	(5)	(46)
Fibrosarcoma				2 (4%)
Hemangiosarcoma	1 (2%)			2 (4%)
Histiocytic sarcoma		1 (10%)		
Lymphoma malignant	1 (2%)			
Osteosarcoma, metastatic, bone		1 (10%)		
Musculoskeletal System				
Bone	(47)	(10)	(5)	(48)
Osteosarcoma, lumbar, vertebra		1 (10%)		
Skeletal muscle	(48)	(11)	(5)	(48)
Fibrosarcoma		2 (18%)		
Lymphoma malignant				1 (2%)
Nervous System				
None				
Respiratory System				
Lung	(48)	(48)	(48)	(48)
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	2 (4%)	4 (8%)
Histiocytic sarcoma	1 (2%)	1 (2%)		2 (4%)
Lymphoma malignant	4 (8%)	4 (8%)	1 (2%)	9 (19%)
Osteosarcoma, metastatic, bone		1 (2%)		
Osteosarcoma, metastatic, uncertain primary site				1 (2%)
Trachea	(47)	(9)	(4)	(46)
Lymphoma malignant				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Special Senses System				
Harderian gland	(48)	(10)	(6)	(47)
Adenoma	2 (4%)	1 (10%)		2 (4%)
Carcinoma	1 (2%)			
Lymphoma malignant	2 (4%)		1 (17%)	2 (4%)
Lacrimal gland	(41)	(6)	(4)	(40)
Lymphoma malignant	1 (2%)			1 (3%)
Urinary System				
Kidney	(48)	(10)	(5)	(48)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	6 (13%)	2 (20%)		8 (17%)
Urinary bladder	(47)	(10)	(5)	(43)
Lymphoma malignant	3 (6%)			3 (7%)
Neoplasm Summary				
Total animals with primary neoplasms[b]	21	18	17	33
Total primary neoplasms	102	49	32	144
Total animals with benign neoplasms	8	6	8	16
Total benign neoplasms	10	7	8	21
Total animals with malignant neoplasms	16	12	10	23
Total malignant neoplasms	92	42	24	123
Total animals with metastatic neoplasms		1		1
Total metastatic neoplasms		2		1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Liver: Hepatocellular Adenoma				
Overall rate[a]	1/48 (2%)	2/48 (4%)	3/48 (6%)	2/48 (4%)
Adjusted rate[b]	2.3%	4.5%	6.4%	4.7%
Terminal rate[c]	1/37 (3%)	1/39 (3%)	3/43 (7%)	2/36 (6%)
First incidence (days)	757 (T)	752	757 (T)	757 (T)
Poly-3 test[d]	P=0.4037	P=0.5155	P=0.3379	P=0.5027
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/48 (4%)	2/48 (4%)	3/48 (6%)	3/48 (6%)
Adjusted rate	4.7%	4.5%	6.4%	7.0%
Terminal rate	2/37 (5%)	1/39 (3%)	3/43 (7%)	3/36 (8%)
First incidence (days)	757 (T)	752	757 (T)	757 (T)
Poly-3 test	P=0.3708	P=0.6779N	P=0.5403	P=0.5035
Lung: Alveolar/Bronchiolar Adenoma				
Overall rate	1/48 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	2.3%	2.2%	4.3%	9.4%
Terminal rate	1/37 (3%)	1/39 (3%)	2/43 (5%)	4/36 (11%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.0711	P=0.7508N	P=0.5308	P=0.1805
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	0/45 (0%)	2/44 (5%)	0/47 (0%)	5/41 (12%)
Adjusted rate	0.0%	4.7%	0.0%	13.3%
Terminal rate	0/36 (0%)	1/38 (3%)	0/42 (0%)	5/32 (16%)
First incidence (days)	—[e]	700	—[f]	757 (T)
Poly-3 test	P=0.0073	P=0.2473	—	P=0.0237
Skin: Fibrosarcoma, Hemangiosarcoma, or Histiocytic Sarcoma				
Overall rate	1/45 (2%)	1/10 (10%)	0/5 (0%)	4/46 (9%)
Adjusted rate	2.4%	14.9%	0.0%	9.6%
Terminal rate	1/37 (3%)	0/1 (0%)	0/0	3/36 (8%)
First incidence (days)	757 (T)	737	—	692
Poly-3 test	(NA)	—[g]	—[g]	P=0.1867
All Organs: Histiocytic Sarcoma				
Overall rate	3/48 (6%)	2/48 (4%)	0/48 (0%)	5/48 (10%)
Adjusted rate	7.0%	4.4%	0.0%	11.4%
Terminal rate	2/37 (5%)	0/39 (0%)	0/43 (0%)	2/36 (6%)
First incidence (days)	681	638	—	567
Poly-3 test	P=0.2238	P=0.4781N	P=0.1046N	P=0.3698
All Organs: Malignant Lymphoma				
Overall rate	9/48 (19%)	7/48 (15%)	8/48 (17%)	15/48 (31%)
Adjusted rate	20.5%	15.3%	17.1%	34.1%
Terminal rate	4/37 (11%)	4/39 (10%)	7/43 (16%)	11/36 (31%)
First incidence (days)	605	622	722 (T)	555
Poly-3 test	P=0.0455	P=0.3571N	P=0.4432N	P=0.1210

TABLE A2
Statistical Analysis of Primary Neoplasms in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
All Organs: Benign Neoplasms				
Overall rate	8/48 (17%)	6/48 (13%)	8/48 (17%)	16/48 (33%)
Adjusted rate	18.3%	13.4%	17.2%	37.5%
Terminal rate	6/37 (16%)	4/39 (10%)	8/43 (19%)	15/36 (42%)
First incidence (days)	551	700	757 (T)	747
Poly-3 test	P=0.0092	P=0.3652N	P=0.5505N	P=0.0404
All Organs: Malignant Neoplasms				
Overall rate	16/48 (33%)	12/48 (25%)	10/48 (21%)	23/48 (48%)
Adjusted rate	36.0%	25.7%	21%	51%
Terminal rate	9/37 (24%)	6/39 (15%)	9/43 (21%)	15/36 (42%)
First incidence (days)	605	564	722 (T)	555
Poly-3 test	P=0.0417	P=0.2006N	P=0.0948N	P=0.1194
All Organs: Benign or Malignant Neoplasms				
Overall rate	21/48 (44%)	18/48 (38%)	17/48 (35%)	33/48 (69%)
Adjusted rate	46.6%	38.3%	36.4%	73.0%
Terminal rate	13/37 (35%)	10/39 (26%)	16/43 (37%)	25/36 (69%)
First incidence (days)	551	564	722 (T)	555
Poly-3 test	P=0.0024	P=0.2774N	P=0.2192N	P=0.0093

(T)Terminal sacrifice
(NA)Not applicable
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.
[g] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the vehicle controls are not appropriate.

TABLE A3a
Historical Incidence of Pituitary Gland Pars Distalis Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice[a]

Study	Adenoma or Carcinoma
Doxylamine	2/38
Fumonisin B$_1$	0/29
Pyrilamine	2/45
Sulfamethazine	10/158
Triprolidine	1/38
Total (%)	15/308 (4.9%)
Mean ± standard deviation	3.7% ± 2.5%
Range	0%-6%

[a] Data as of September 1999. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE A3b
Historical Incidence of Malignant Lymphoma in Control Female B6C3F$_1$/Nctr BR Mice[a]

Study	Incidence in Controls
Doxylamine	13/48
Fumonisin B$_1$	20/47
Pyrilamine	10/48
Sulfamethazine	39/184
Triprolidine	10/47
Total (%)	92/374 (24.6%)
Mean ± standard deviation	26.6% ± 9.3%
Range	21%-43%

[a] Data as of September 1999. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed. Includes data for histiocytic, lymphocytic, mixed, unspecified, or undifferentiated cell type lymphomas

TABLE A3c
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Doxylamine	3/48	0/48	3/48
Fumonisin B$_1$	2/47	0/47	2/47
Pyrilamine	1/48	0/48	1/48
Sulfamethazine	5/182	1/182	6/182
Triprolidine	3/47	2/47	5/47
Total (%)	14/372 (3.8%)	3/372 (0.8%)	17/372 (4.6%)
Mean ± standard deviation	4.4% ± 2.0%	1.0% ± 1.9%	5.3% ± 3.3%
Range	2%-6%	0%-4%	2%-11%

[a] Data as of September 1999. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE A3d
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F$_1$/Nctr BR Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Doxylamine	0/46	0/46	0/46
Fumonisin B$_1$	5/47	0/47	5/47
Pyrilamine	1/47	0/47	1/47
Sulfamethazine	8/184	2/184	10/184
Triprolidine	2/47	2/47	4/47
Total (%)	16/371 (4.3%)	4/371 (1.1%)	20/371 (5.4%)
Mean ± standard deviation	4.3% ± 4.0%	1.1% ± 1.9%	5.3% ± 4.4%
Range	0%-11%	0%-4%	0%-11%

[a] Data as of September 1999. Studies were conducted at the National Center for Toxicological Research in animals given NIH-31 feed.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental deaths			1	1
Moribund	2	4	3	2
Natural deaths	9	5	1	9
Survivors				
Died last week of study		1		
Terminal sacrifice	37	38	43	36
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(47)	(9)	(4)	(44)
Hyperkeratosis	1 (2%)			1 (2%)
Ulcer	1 (2%)			
Gallbladder	(46)	(8)	(4)	(47)
Infiltration cellular, lymphocytic	4 (9%)			4 (9%)
Intestine large, cecum	(42)	(6)	(3)	(41)
Hyperplasia, lymphoid	5 (12%)			4 (10%)
Intestine large, rectum	(44)	(8)	(3)	(43)
Erosion	2 (5%)			
Hyperplasia, lymphoid				1 (2%)
Intestine small, duodenum	(40)	(7)	(4)	(42)
Inflammation				1 (2%)
Intestine small, ileum	(40)	(6)	(3)	(39)
Hyperplasia, lymphoid	2 (5%)			1 (3%)
Inflammation				1 (3%)
Intestine small, jejunum	(41)	(6)	(5)	(41)
Inflammation				1 (2%)
Liver	(48)	(48)	(48)	(48)
Angiectasis		2 (4%)		
Basophilic focus	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Clear cell focus		1 (2%)		
Congestion				1 (2%)
Cyst, bile duct			1 (2%)	
Degeneration			1 (2%)	
Eosinophilic focus		1 (2%)	4 (8%)	1 (2%)
Hematopoietic cell proliferation	3 (6%)	13 (27%)	16 (33%)	4 (8%)
Infiltration cellular, lymphocytic	33 (69%)	35 (73%)	40 (83%)	36 (75%)
Inflammation	2 (4%)			1 (2%)
Mineralization				1 (2%)
Necrosis	32 (67%)	35 (73%)	31 (65%)	32 (67%)
Necrosis, coagulative	1 (2%)		2 (4%)	
Regeneration			1 (2%)	
Tension lipoidosis	17 (35%)	13 (27%)	16 (33%)	16 (33%)
Vacuolization cytoplasmic	26 (54%)	36 (75%)	30 (63%)	23 (48%)
Mesentery	(1)	(1)	(1)	(1)
Necrosis, fat		1 (100%)	1 (100%)	1 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Alimentary System (continued)				
Pancreas	(48)	(8)	(5)	(46)
Atrophy	1 (2%)	1 (13%)		
Ectasia, duct				1 (2%)
Focal cellular change	2 (4%)		2 (40%)	2 (4%)
Infiltration cellular, lymphocytic	26 (54%)	3 (38%)	2 (40%)	19 (41%)
Polyarteritis			1 (20%)	
Salivary glands	(48)	(10)	(5)	(48)
Atrophy				2 (4%)
Hyperplasia, duct	1 (2%)			
Infiltration cellular, lymphocytic	38 (79%)	6 (60%)	4 (80%)	41 (85%)
Polyarteritis			1 (20%)	
Stomach, forestomach	(47)	(10)	(4)	(45)
Hyperkeratosis		3 (30%)		2 (4%)
Ulcer		1 (10%)		
Stomach, glandular	(47)	(10)	(4)	(46)
Crystals	1 (2%)			
Cyst	2 (4%)		1 (25%)	3 (7%)
Degeneration, hyaline	1 (2%)			
Mineralization		1 (10%)		2 (4%)
Tongue	(48)	(9)	(5)	(48)
Infiltration cellular, mast cell	2 (4%)			
Inflammation		1 (11%)		
Polyarteritis	1 (2%)		1 (20%)	
Cardiovascular System				
Blood vessel, aorta	(42)	(8)	(5)	(45)
Mineralization		1 (13%)		
Heart	(48)	(10)	(5)	(48)
Degeneration	1 (2%)	1 (10%)		
Dilatation		1 (10%)		
Infiltration cellular, lymphocytic				2 (4%)
Inflammation	1 (2%)			1 (2%)
Polyarteritis	1 (2%)			1 (2%)
Thrombus	1 (2%)			
Endocrine System				
Adrenal gland	(46)	(9)	(5)	(47)
Accessory adrenal cortical nodule	1 (2%)			1 (2%)
Adrenal gland, cortex	(46)	(9)	(5)	(47)
Ectopic tissue	1 (2%)			2 (4%)
Hyperplasia				1 (2%)
Hyperplasia, spindle cell	43 (93%)	6 (67%)	2 (40%)	44 (94%)
Thrombus	1 (2%)			
Vacuolization cytoplasmic	2 (4%)			
Adrenal gland, medulla	(46)	(9)	(3)	(46)
Hyperplasia				2 (4%)
Vacuolization cytoplasmic	1 (2%)			1 (2%)
Islets, pancreatic	(48)	(8)	(5)	(46)
Hyperplasia			1 (20%)	1 (2%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Endocrine System (continued)				
Parathyroid gland	(38)	(9)	(3)	(40)
Cyst				1 (3%)
Ectopic thymus	1 (3%)			2 (5%)
Infiltration cellular, lymphocytic	1 (3%)			1 (3%)
Vacuolization cytoplasmic	1 (3%)			1 (3%)
Pituitary gland	(45)	(44)	(47)	(41)
Angiectasis		1 (2%)	2 (4%)	1 (2%)
Degeneration, cystic, pars distalis		2 (5%)	1 (2%)	
Hemorrhage				1 (2%)
Hyperplasia, pars distalis	4 (9%)	6 (14%)	4 (9%)	9 (22%)
Thyroid gland	(47)	(9)	(5)	(48)
Crystals				1 (2%)
Cyst, follicle		1 (11%)		
Degeneration	1 (2%)	1 (11%)		1 (2%)
Ectopic thymus				1 (2%)
Hyperplasia, follicular cell		1 (11%)		2 (4%)
Infiltration cellular, lymphocytic	4 (9%)			
Polyarteritis			1 (20%)	
Ultimobranchial cyst	11 (23%)	2 (22%)	1 (20%)	10 (21%)
General Body System				
None				
Genital System				
Clitoral gland	(43)	(8)	(4)	(43)
Atrophy	40 (93%)	6 (75%)	4 (100%)	38 (88%)
Ovary	(48)	(29)	(21)	(46)
Atrophy	39 (81%)	7 (24%)	1 (5%)	35 (76%)
Congestion	1 (2%)			
Cyst	10 (21%)	15 (52%)	13 (62%)	14 (30%)
Cyst, periovarian tissue	16 (33%)	5 (17%)	4 (19%)	15 (33%)
Hematocyst	7 (15%)	3 (10%)	2 (10%)	3 (7%)
Hyperplasia, adenomatous	2 (4%)		1 (5%)	1 (2%)
Hyperplasia, tubular				2 (4%)
Infiltration cellular, lymphocytic	5 (10%)			2 (4%)
Mineralization		1 (3%)		1 (2%)
Polyarteritis				1 (2%)
Uterus	(48)	(26)	(29)	(47)
Angiectasis	1 (2%)		1 (3%)	1 (2%)
Atrophy	2 (4%)	5 (19%)	3 (10%)	2 (4%)
Dilatation	2 (4%)	2 (8%)	2 (7%)	
Fibrosis	1 (2%)	1 (4%)	1 (3%)	1 (2%)
Hyperplasia, cystic, endometrium	37 (77%)	16 (62%)	23 (79%)	37 (79%)
Hypertrophy, myometrium				2 (4%)
Inflammation		1 (4%)		
Metaplasia, squamous				1 (2%)
Prolapse	1 (2%)			1 (2%)
Vagina	(48)	(9)	(5)	(45)
Atrophy	2 (4%)	4 (44%)	2 (40%)	3 (7%)
Dysplasia	1 (2%)	1 (11%)		2 (4%)
Infiltration cellular, lymphocytic	3 (6%)			2 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Bone marrow	(47)	(10)	(5)	(47)
Hyperplasia	3 (6%)	5 (50%)		6 (13%)
Lymph node	(48)	(13)	(8)	(47)
Hematopoietic cell proliferation				1 (2%)
Hematopoietic cell proliferation, inguinal				1 (2%)
Hemorrhage, inguinal				1 (2%)
Hyperplasia, lymphoid, inguinal			1 (13%)	
Hyperplasia, lymphoid, thoracic			1 (13%)	
Infiltration cellular, histiocytic, inguinal				1 (2%)
Lymph node, mandibular	(47)	(11)	(5)	(46)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	3 (6%)	3 (27%)		
Hyperplasia, lymphoid	7 (15%)	1 (9%)	1 (20%)	9 (20%)
Hyperplasia, plasma cell				1 (2%)
Infiltration cellular, histiocytic		1 (9%)		
Lymph node, mesenteric	(46)	(9)	(7)	(44)
Atrophy	2 (4%)	2 (22%)		1 (2%)
Hematopoietic cell proliferation				1 (2%)
Hemorrhage	4 (9%)		1 (14%)	2 (5%)
Hyperplasia, lymphoid	2 (4%)	1 (11%)	2 (29%)	2 (5%)
Infiltration cellular, histiocytic				1 (2%)
Polyarteritis, artery			1 (14%)	
Spleen	(47)	(14)	(19)	(47)
Atrophy	2 (4%)	2 (14%)		1 (2%)
Congestion		1 (7%)		3 (6%)
Hematopoietic cell proliferation	4 (9%)	3 (21%)	5 (26%)	9 (19%)
Hyperplasia, lymphoid	13 (28%)	4 (29%)	5 (26%)	11 (23%)
Infiltration cellular, lymphocytic				1 (2%)
Infiltration cellular, plasma cell				1 (2%)
Inflammation				1 (2%)
Thymus	(41)	(5)	(5)	(44)
Atrophy, cortex	30 (73%)	3 (60%)	3 (60%)	34 (77%)
Congestion	1 (2%)			
Cyst				1 (2%)
Ectopic parathyroid gland	1 (2%)			1 (2%)
Hyperplasia, lymphoid, medulla	14 (34%)	1 (20%)		17 (39%)
Inflammation		1 (20%)		
Integumentary System				
Mammary gland	(44)	(6)	(5)	(44)
Hyperplasia	1 (2%)	1 (17%)		7 (16%)
Inflammation	1 (2%)			
Lactation	4 (9%)	1 (17%)		1 (2%)
Skin	(45)	(10)	(5)	(46)
Edema		1 (10%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Musculoskeletal System				
Bone, femur	(47)	(10)	(5)	(47)
Degeneration, cartilage				1 (2%)
Fibrous osteodystrophy	18 (38%)			10 (21%)
Polyarteritis			1 (20%)	
Bone, sternum	(47)	(10)	(5)	(48)
Fibrous osteodystrophy, multifocal	36 (77%)	3 (30%)		33 (69%)
Skeletal muscle	(48)	(11)	(5)	(48)
Infiltration cellular, lymphocytic	2 (4%)	1 (9%)		1 (2%)
Polyarteritis	1 (2%)			
Nervous System				
Brain, cerebellum	(48)	(10)	(5)	(48)
Degeneration	1 (2%)			
Thrombus	1 (2%)			
Brain, cerebrum	(48)	(10)	(5)	(48)
Degeneration	1 (2%)			
Mineralization, multifocal, thalamus	28 (58%)	3 (30%)	1 (20%)	22 (46%)
Polyarteritis			1 (20%)	
Thrombus	1 (2%)			
Spinal cord, thoracic	(48)	(10)	(5)	(47)
Degeneration	1 (2%)			
Developmental malformation				1 (2%)
Infiltration cellular, lymphocytic				1 (2%)
Thrombus	1 (2%)			
Respiratory System				
Larynx	(44)	(5)	(1)	(43)
Infiltration cellular, lymphocytic	1 (2%)			
Inflammation		1 (20%)		1 (2%)
Lung	(48)	(48)	(48)	(48)
Foreign body	1 (2%)			
Hemorrhage	2 (4%)		1 (2%)	
Hyperplasia, alveolar epithelium				1 (2%)
Hyperplasia, alveolus		1 (2%)		
Infiltration cellular, histiocytic		2 (4%)	2 (4%)	1 (2%)
Infiltration cellular, lymphocytic	37 (77%)	24 (50%)	29 (60%)	29 (60%)
Inflammation	4 (8%)			2 (4%)
Leukocytosis		1 (2%)		
Mineralization		1 (2%)		
Thrombus	1 (2%)	2 (4%)	1 (2%)	
Nose	(47)	(10)	(5)	(48)
Cyst, nasolacrimal duct	1 (2%)			
Cytoplasmic alteration, respiratory epithelium				1 (2%)
Infiltration cellular, lymphocytic, nasolacrimal duct				1 (2%)
Infiltration, glands				1 (2%)
Inflammation	1 (2%)			
Mineralization, nasolacrimal duct		1 (10%)		
Trachea	(47)	(9)	(4)	(46)
Ectasia, glands			1 (25%)	1 (2%)
Inflammation	1 (2%)	1 (11%)		

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Special Senses System				
Eye	(41)	(4)	(5)	(40)
Degeneration, retina	1 (2%)			
Thrombus	1 (2%)			
Harderian gland	(48)	(10)	(6)	(47)
Hyperplasia				1 (2%)
Infiltration cellular, lymphocytic	18 (38%)			12 (26%)
Inflammation			1 (17%)	
Thrombus	1 (2%)			
Lacrimal gland	(41)	(6)	(4)	(40)
Atrophy	1 (2%)			3 (8%)
Infiltration cellular, lymphocytic	25 (61%)	3 (50%)	3 (75%)	20 (50%)
Zymbal's gland	(43)	(6)	(3)	(40)
Inflammation	1 (2%)			1 (3%)
Urinary System				
Kidney	(48)	(10)	(5)	(48)
Accumulation hyaline droplet		3 (30%)	1 (20%)	2 (4%)
Amyloid deposition, glomerulus	2 (4%)			1 (2%)
Congestion	1 (2%)			
Cyst, renal tubule	14 (29%)	2 (20%)	4 (80%)	17 (35%)
Glomerulosclerosis			1 (20%)	
Hydronephrosis			1 (20%)	
Hydronephrosis, bilateral	1 (2%)			
Infiltration cellular, lymphocytic	39 (81%)	5 (50%)	4 (80%)	38 (79%)
Inflammation	1 (2%)			
Mineralization		1 (10%)		
Necrosis, renal tubule				1 (2%)
Nephropathy	3 (6%)	1 (10%)		
Pigmentation, renal tubule			1 (20%)	1 (2%)
Polyarteritis	1 (2%)		1 (20%)	
Urinary bladder	(47)	(10)	(5)	(43)
Infiltration cellular, lymphocytic	38 (81%)	6 (60%)	5 (100%)	35 (81%)
Polyarteritis	1 (2%)		1 (20%)	

APPENDIX B
SUMMARY OF LESIONS IN REGIMEN B FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF CHLORAL HYDRATE

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TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control[b]	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
Disposition Summary				
Animals initially in study	72	48	48	48
3-Month interim evaluation	8	8		
6-Month interim evaluation	8		8	
12-Month interim evaluation	8			8
Early deaths				
Moribund	2	2	3	
Natural deaths	9	4	6	7
Survivors				
Terminal sacrifice	37	34	31	33
Animals examined microscopically	72	48	48	48

Systems Examined at 3 Months with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

6-Month Interim Evaluation

Respiratory System		
Lung	(8)	(8)
Alveolar/bronchiolar adenoma	1 (13%)	

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
12-Month Interim Evaluation				
Alimentary System				
Mesentery	(1)			
Yolk sac carcinoma, metastatic, ovary	1 (100%)			
Genital System				
Ovary	(8)			(7)
Yolk sac carcinoma	1 (13%)			
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
2-Year Study				
Alimentary System				
Esophagus	(47)	(36)	(40)	(38)
Lymphoma malignant			1 (3%)	
Gallbladder	(46)	(37)	(36)	(36)
Lymphoma malignant		2 (5%)		
Intestine large, cecum	(42)	(37)	(37)	(32)
Lymphoma malignant	1 (2%)	2 (5%)		
Intestine large, colon	(46)	(37)	(37)	(34)
Lymphoma malignant	2 (4%)		1 (3%)	
Intestine small, jejunum	(41)	(37)	(37)	(32)
Lymphoma malignant	2 (5%)	1 (3%)	1 (3%)	
Liver	(48)	(40)	(40)	(40)
Fibrosarcoma, metastatic, skin			1 (3%)	
Hepatocellular adenoma	1 (2%)	1 (3%)	1 (3%)	2 (5%)
Hepatocellular carcinoma	1 (2%)		1 (3%)	2 (5%)
Histiocytic sarcoma	1 (2%)		1 (3%)	1 (3%)
Lymphoma malignant	6 (13%)	6 (15%)	7 (18%)	8 (20%)
Mesentery	(1)	(1)	(2)	
Fibrosarcoma, metastatic, skin			1 (50%)	
Lymphoma malignant	1 (100%)			
Pancreas	(48)	(40)	(39)	(37)
Fibrosarcoma	1 (2%)			
Fibrosarcoma, metastatic, skin			2 (5%)	
Lymphoma malignant	2 (4%)	2 (5%)	5 (13%)	1 (3%)

TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Alimentary System (continued)				
Salivary glands	(48)	(40)	(40)	(39)
Lymphoma malignant	3 (6%)	3 (8%)	3 (8%)	2 (5%)
Tongue	(48)	(39)	(39)	(40)
Lymphoma malignant			1 (3%)	
Papilloma squamous	1 (2%)			
Cardiovascular System				
Heart	(48)	(40)	(40)	(39)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	1 (2%)		1 (3%)	
Endocrine System				
Adrenal gland, cortex	(46)	(40)	(37)	(37)
Adenoma, spindle cell		1 (3%)		
Lymphoma malignant	1 (2%)			1 (3%)
Adrenal gland, medulla	(46)	(40)	(37)	(37)
Lymphoma malignant	1 (2%)			1 (3%)
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(48)	(40)	(37)	(38)
Lymphoma malignant		1 (3%)	1 (3%)	
Parathyroid gland	(38)	(31)	(33)	(36)
Adenoma			1 (3%)	
Pituitary gland	(45)	(36)	(36)	(33)
Adenoma, pars distalis		3 (8%)	1 (3%)	1 (3%)
Adenoma, pars intermedia	2 (4%)			
Thyroid gland	(47)	(40)	(40)	(39)
Adenoma, follicular cell		1 (3%)	1 (3%)	
Lymphoma malignant		1 (3%)	1 (3%)	
General Body System				
Tissue NOS	(1)		(1)	
Fibrosarcoma, metastatic, skin			1 (100%)	
Lymphoma malignant, fat	1 (100%)			
Genital System				
Clitoral gland	(43)	(37)	(33)	(33)
Lymphoma malignant			1 (3%)	
Ovary	(48)	(40)	(39)	(38)
Cystadenoma	1 (2%)		2 (5%)	
Fibrosarcoma, metastatic, periovarian tissue, skin			1 (3%)	
Histiocytic sarcoma	1 (2%)			
Luteoma	1 (2%)			
Lymphoma malignant	2 (4%)	4 (10%)	2 (5%)	
Lymphoma malignant, periovarian tissue	1 (2%)		4 (10%)	3 (8%)
Teratoma benign		1 (3%)		1 (3%)

TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Genital System (continued)				
Uterus	(48)	(40)	(40)	(39)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma	2 (4%)			1 (3%)
Lymphoma malignant			1 (3%)	1 (3%)
Polyp	1 (2%)	1 (3%)	1 (3%)	
Sarcoma stromal				1 (3%)
Vagina	(48)	(38)	(40)	(37)
Histiocytic sarcoma	2 (4%)	1 (3%)		2 (5%)
Lymphoma malignant	2 (4%)	1 (3%)	1 (3%)	
Hematopoietic System				
Bone marrow	(47)	(39)	(40)	(38)
Hemangiosarcoma	1 (2%)			
Lymphoma malignant	3 (6%)	3 (8%)	2 (5%)	
Lymph node	(48)	(40)	(40)	(40)
Histiocytic sarcoma, lumbar	1 (2%)			
Lymphoma malignant				1 (3%)
Lymphoma malignant, axillary		1 (3%)	1 (3%)	
Lymphoma malignant, lumbar	2 (4%)	1 (3%)	1 (3%)	
Lymphoma malignant, mediastinal	1 (2%)		1 (3%)	
Lymphoma malignant, renal	1 (2%)		3 (8%)	
Lymphoma malignant, thoracic			1 (3%)	1 (3%)
Squamous cell carcinoma, metastatic, lumbar, skin			1 (3%)	
Lymph node, mandibular	(47)	(40)	(39)	(39)
Lymphoma malignant	4 (9%)	4 (10%)	6 (15%)	7 (18%)
Lymph node, mesenteric	(46)	(40)	(38)	(39)
Fibrosarcoma, metastatic, skin			1 (3%)	
Histiocytic sarcoma	1 (2%)			1 (3%)
Lymphoma malignant	6 (13%)	6 (15%)	6 (16%)	8 (21%)
Spleen	(47)	(40)	(39)	(39)
Fibrosarcoma, metastatic, skin			1 (3%)	
Hemangiosarcoma	1 (2%)			1 (3%)
Histiocytic sarcoma				1 (3%)
Lymphoma malignant	8 (17%)	7 (18%)	11 (28%)	8 (21%)
Thymus	(41)	(33)	(31)	(30)
Fibrosarcoma, metastatic, skin			1 (3%)	
Lymphoma malignant	3 (7%)	4 (12%)	5 (16%)	5 (17%)
Integumentary System				
Mammary gland	(44)	(36)	(38)	(36)
Adenoacanthoma				1 (3%)
Adenocarcinoma		1 (3%)	1 (3%)	
Fibrosarcoma	1 (2%)			
Fibrosarcoma, metastatic, skin		1 (3%)		
Lymphoma malignant	1 (2%)		2 (5%)	

TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Integumentary System (continued)				
Skin	(45)	(39)	(39)	(38)
Fibrosarcoma		2 (5%)	2 (5%)	
Hemangiosarcoma	1 (2%)			
Lymphoma malignant	1 (2%)		2 (5%)	1 (3%)
Squamous cell carcinoma			1 (3%)	
Musculoskeletal System				
Skeletal muscle	(48)	(39)	(40)	(39)
Fibrosarcoma, metastatic, skin			1 (3%)	
Lymphoma malignant		1 (3%)	2 (5%)	
Nervous System				
Brain, cerebellum	(48)	(40)	(40)	(40)
Lymphoma malignant			2 (5%)	
Brain, cerebrum	(48)	(40)	(40)	(40)
Lymphoma malignant			1 (3%)	
Peripheral nerve	(48)	(38)	(39)	(38)
Lymphoma malignant		1 (3%)		
Respiratory System				
Lung	(48)	(40)	(40)	(40)
Adenoacanthoma, metastatic, mammary gland				1 (3%)
Adenocarcinoma, metastatic, mammary gland			1 (3%)	
Alveolar/bronchiolar adenoma	1 (2%)	2 (5%)	4 (10%)	7 (18%)
Alveolar/bronchiolar carcinoma		1 (3%)		
Fibrosarcoma, metastatic, skin			1 (3%)	
Histiocytic sarcoma	1 (2%)			1 (3%)
Lymphoma malignant	4 (8%)	3 (8%)	4 (10%)	8 (20%)
Squamous cell carcinoma, metastatic, skin			1 (3%)	
Nose	(47)	(40)	(40)	(40)
Fibrosarcoma, metastatic, skin		1 (3%)		
Special Senses System				
Eye	(41)	(38)	(34)	(35)
Histiocytic sarcoma, retrobulbar				1 (3%)
Harderian gland	(48)	(38)	(38)	(38)
Adenoma	2 (4%)	2 (5%)	2 (5%)	3 (8%)
Carcinoma	1 (2%)			
Lymphoma malignant	2 (4%)	2 (5%)	1 (3%)	
Lacrimal gland	(41)	(33)	(33)	(32)
Lymphoma malignant	1 (2%)	2 (6%)	2 (6%)	2 (6%)
Zymbal's gland	(43)	(38)	(36)	(36)
Fibrosarcoma, metastatic, skin		1 (3%)		
Lymphoma malignant			1 (3%)	

TABLE B1
Summary of the Incidence of Neoplasms in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Urinary System				
Kidney	(48)	(40)	(39)	(38)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	6 (13%)	6 (15%)	7 (18%)	5 (13%)
Urinary bladder	(47)	(37)	(39)	(36)
Lymphoma malignant	3 (36%)	2 (5%)	2 (5%)	3 (8%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
6-Month interim evaluation	1			
2-Year study	21	19	25	28
Total primary neoplasms				
6-Month interim evaluation	1			
2-Year study	102	83	113	93
Total animals with benign neoplasms				
6-Month interim evaluation	1			
2-Year study	8	11	13	13
Total benign neoplasms				
6-Month interim evaluation	1			
2-Year study	10	12	13	14
Total animals with malignant neoplasms				
12-Month interim evaluation	1			
2-Year study	16	13	19	21
Total malignant neoplasms				
12-Month interim evaluation	1			
2-Year study	92	71	100	79
Total animals with metastatic neoplasms				
12-Month interim evaluation	1			
2-Year study		2	4	1
Total metastatic neoplasms				
12-Month interim evaluation	1			
2-Year study		3	14	1

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Forty-eight mice served as vehicle controls for regimens A and B; the remaining 24 mice were designated for regimen B interim evaluations.
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control (Regimen A)	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)	2 Years (Regimen A)
Harderian Gland: Adenoma					
Overall rate[a]	2/48 (4%)	2/38 (5%)	2/38 (5%)	3/38 (8%)	2/47 (4%)
Adjusted rate[b]	4.7%	5.6%	5.8%	8.3%	4.8%
Terminal rate[c]	2/37 (5%)	2/33 (6%)	2/30 (7%)	3/33 (9%)	2/35 (6%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test[d]	P=0.5675	P=0.6275	P=0.6142	P=0.4216	P=0.6884
Harderian Gland: Adenoma or Carcinoma					
Overall rate	3/48 (6%)	2/38 (5%)	2/38 (5%)	3/38 (8%)	2/47 (4%)
Adjusted rate	7.0%	5.6%	5.8%	8.3%	4.8%
Terminal rate	3/37 (8%)	2/33 (6%)	2/30 (7%)	3/33 (9%)	2/35 (6%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.4707N	P=0.5829N	P=0.5984N	P=0.5814	P=0.5072N
Liver: Hepatocellular Adenoma					
Overall rate	1/48 (2%)	1/40 (3%)	1/40 (3%)	2/40 (5%)	2/48 (4%)
Adjusted rate	2.3%	2.7%	2.8%	5.3%	4.7%
Terminal rate	1/37 (3%)	1/34 (3%)	1/31 (3%)	2/33 (6%)	2/36 (6%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.3231	P=0.7287	P=0.7241	P=0.4553	P=0.5027
Liver: Hepatocellular Carcinoma					
Overall rate	1/48 (2%)	0/40 (0%)	1/40 (3%)	2/40 (5%)	1/48 (2%)
Adjusted rate	2.3%	0.0%	2.8%	5.3%	2.3%
Terminal rate	1/37 (3%)	0/34 (0%)	1/31 (3%)	2/33 (6%)	1/36 (3%)
First incidence (days)	757 (T)	—[e]	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.4537	P=0.5288N	P=0.7241	P=0.4553	P=0.7589N
Liver: Hepatocellular Adenoma or Carcinoma					
Overall rate	2/48 (4%)	1/40 (3%)	2/40 (5%)	4/40 (10%)	3/48 (6%)
Adjusted rate	4.7%	2.7%	5.5%	10.6%	7.0%
Terminal rate	2/37 (5%)	1/34 (3%)	2/31 (7%)	4/33 (12%)	3/36 (8%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.2637	P=0.5502N	P=0.6343	P=0.2787	P=0.5035
Lung: Alveolar/bronchiolar Adenoma					
Overall rate	1/48 (2%)	2/40 (5%)	4/40 (10%)	7/40 (18%)	4/48 (8%)
Adjusted rate	2.3%	5.4%	11.0%	18.6%	9.4%
Terminal rate	1/37 (3%)	2/34 (6%)	4/31 (13%)	7/33 (21%)	4/36 (11%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.1385	P=0.4497	P=0.1309	P=0.0173	P=0.1805
Lung: Alveolar/bronchiolar Adenoma or Carcinoma					
Overall rate	1/48 (2%)	3/40 (8%)	4/40 (10%)	7/40 (18%)	4/48 (8%)
Adjusted rate	2.3%	8.1%	11.0%	18.6%	9.4%
Terminal rate	1/37 (3%)	3/34 (9%)	4/31 (13%)	7/33 (21%)	4/36 (11%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.1887	P=0.2542	P=0.1309	P=0.0173	P=0.1805

TABLE B2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control (Regimen A)	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)	2 Years (Regimen A)
Pituitary Gland (Pars Distalis): Adenoma					
Overall rate	0/45 (0%)	3/36 (8%)	1/36 (3%)	1/33 (3%)	5/41 (12%)
Adjusted rate	0.0%	8.9%	3.0%	3.2%	13.3%
Terminal rate	0/36 (0%)	3/32 (9%)	0/29 (0%)	1/28 (4%)	5/32 (16%)
First incidence (days)	—	757 (T)	695	757 (T)	757 (T)
Poly-3 test	P=0.0278	P=0.0849	P=0.4620	P=0.4434	P=0.0237
Skin: Fibrosarcoma					
Overall rate	0/45 (0%)	2/39 (5%)	2/39 (5%)	0/38 (0%)	2/46 (4%)
Adjusted rate	0.0%	5.5%	5.5%	0.0%	4.8%
Terminal rate	0/37 (0%)	1/34 (3%)	0/31 (0%)	0/33 (0%)	2/36 (6%)
First incidence (days)	—	699	682	—	757 (T)
Poly-3 test	P=0.4175	P=0.2089	P=0.2078	—[f]	P=0.2419
Skin: Fibrosarcoma, Hemangiosarcoma, or Squamous Cell Carcinoma					
Overall rate	1/45 (2%)	2/39 (5%)	3/39 (8%)	0/38 (0%)	4/46 (9%)
Adjusted rate	2.4%	5.5%	8.2%	0.0%	9.6%
Terminal rate	1/37 (3%)	1/34 (3%)	0/31 (0%)	0/33 (0%)	3/36 (8%)
First incidence (days)	757 (T)	699	631	—	692
Poly-3 test	P=0.2155	P=0.4561	P=0.2627	P=0.5266N	P=0.1867
All Organs: Histiocytic Sarcoma					
Overall rate	3/48 (6%)	1/40 (3%)	1/40 (3%)	3/40 (8%)	5/48 (10%)
Adjusted rate	7.0%	2.7%	2.8%	7.9%	11.4%
Terminal rate	2/37 (5%)	1/34 (3%)	1/31 (3%)	1/33 (3%)	2/36 (6%)
First incidence (days)	681	757 (T)	757 (T)	699	567
Poly-3 test	P=0.1054	P=0.3601N	P=0.3677N	P=0.6044	P=0.3698
All Organs: Malignant Lymphoma					
Overall rate	9/48 (19%)	8/40 (20%)	13/40 (33%)	14/40 (35%)	15/48 (31%)
Adjusted rate	20.5%	21.6%	34.8%	37.0%	34.1%
Terminal rate	4/37 (11%)	7/34 (21%)	10/31 (32%)	13/33 (39%)	11/36 (31%)
First incidence (days)	605	747	471	694	555
Poly-3 test	P=0.0834	P=0.5614	P=0.1156	P=0.0780	P=0.1210
All Organs: Benign Neoplasms					
Overall rate	8/48 (17%)	11/40 (28%)	13/40 (33%)	13/40 (33%)	16/48 (33%)
Adjusted rate	18.3%	29.7%	35.6%	34.4%	37.5%
Terminal rate	6/37 (16%)	11/34 (32%)	12/31 (39%)	12/33 (36%)	15/36 (42%)
First incidence (days)	551	757 (T)	695	694	747
Poly-3 test	P=0.0672	P=0.1732	P=0.0646	P=0.0789	P=0.0404
All Organs: Malignant Neoplasms					
Overall rate	16/48 (33%)	13/40 (33%)	19/40 (48%)	21/40 (53%)	23/48 (48%)
Adjusted rate	36.0%	34.9%	49.3%	54.5%	50.9%
Terminal rate	9/37 (24%)	11/34 (32%)	12/31 (39%)	17/33 (52%)	15/36 (42%)
First incidence (days)	605	699	471	670	555
Poly-3 test	P=0.0631	P=0.5518N	P=0.1587	P=0.0681	P=0.1194

TABLE B2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control (Regimen A)	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)	2 Years (Regimen A)
All Organs: Benign or Malignant Neoplasms					
Overall rate	21/48 (44%)	19/40 (48%)	25/40 (63%)	28/40 (70%)	33/48 (69%)
Adjusted rate	46.6%	51.0%	64.8%	72.7%	73.0%
Terminal rate	13/37 (35%)	17/34 (50%)	18/31 (58%)	24/33 (73%)	25/36 (69%)
First incidence (days)	551	699	471	670	555
Poly-3 test	P=0.0037	P=0.4292	P=0.0719	P=0.0119	P=0.0093

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control[b]	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
Disposition Summary				
Animals initially in study	72	48	48	48
3-Month interim evaluation	8	8		
6-Month interim evaluation	8		8	
12-Month interim evaluation	8			8
Early deaths				
Moribund	2	2	3	
Natural deaths	9	4	6	7
Survivors				
Terminal sacrifice	37	34	31	33
Animals examined microscopically	72	48	48	48
3-Month Interim Evaluation				
Alimentary System				
Esophagus	(8)	(8)		
Inflammation, mediastinum	1 (13%)			
Liver	(8)	(8)		
Infiltration cellular, lymphocytic	1 (13%)	3 (38%)		
Necrosis	6 (75%)	5 (63%)		
Tension lipoidosis		4 (50%)		
Salivary glands	(8)	(8)		
Infiltration cellular, lymphocytic	5 (63%)			
Endocrine System				
Adrenal gland, cortex	(8)	(8)		
Hyperplasia, spindle cell	4 (50%)	3 (38%)		
Islets, pancreatic	(8)	(8)		
Hyperplasia		1 (13%)		
Thyroid gland	(7)	(8)		
Degeneration		1 (13%)		
Ultimobranchial cyst		1 (13%)		
Genital System				
Ovary	(8)	(8)		
Congestion	1 (13%)			
Hematopoietic System				
Bone marrow	(8)	(8)		
Hyperplasia	1 (13%)			
Lymph node, mandibular	(8)	(8)		
Hyperplasia, lymphoid	1 (13%)			
Lymph node, mesenteric	(7)	(8)		
Hemorrhage		1 (13%)		
Hyperplasia, lymphoid	1 (14%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion
[b] Forty-eight mice served as vehicle controls for regimens A and B; the remaining 24 mice were designated for regimen B interim evaluations.

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
3-Month Interim Evaluation (continued)				
Hematopoietic System (continued)				
Thymus	(8)	(8)		
Cyst	1 (13%)	1 (13%)		
Inflammation, mediastinum	1 (13%)			
Respiratory System				
Lung	(8)	(8)		
Congestion	1 (13%)			
Hemorrhage	1 (13%)			
Infiltration cellular, lymphocytic	1 (13%)	1 (13%)		
Inflammation		1 (13%)		
Nose	(8)	(8)		
Mineralization, nasolacrimal duct		1 (13%)		
Urinary System				
Urinary bladder	(8)	(8)		
Infiltration cellular, lymphocytic	1 (13%)			
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Special Senses System				
6-Month Interim Evaluation				
Alimentary System				
Liver	(8)		(8)	
Infiltration cellular, lymphocytic	3 (38%)		4 (50%)	
Necrosis	5 (63%)		6 (75%)	
Tension lipoidosis	1 (13%)		5 (63%)	
Vacuolization cytoplasmic	5 (63%)		7 (88%)	
Salivary glands	(8)		(8)	
Infiltration cellular, lymphocytic	4 (50%)		3 (38%)	
Stomach, forestomach	(8)		(8)	
Hyperkeratosis			1 (13%)	
Stomach, glandular	(8)		(8)	
Inflammation	1 (13%)			
Tongue	(8)		(8)	
Infiltration cellular, histiocytic	1 (13%)			

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
6-Month Interim Evaluation (continued)				
Endocrine System				
Adrenal gland, cortex	(8)		(8)	
Degeneration, fatty			1 (13%)	
Hyperplasia, spindle cell	4 (50%)		7 (88%)	
Parathyroid gland	(4)		(6)	
Ectopic thymus	1 (25%)			
Genital System				
Ovary	(8)		(8)	
Cyst	1 (13%)			
Hematocyst	1 (13%)			
Uterus	(8)		(8)	
Infiltration cellular, lymphocytic			1 (13%)	
Vagina	(7)		(8)	
Infiltration cellular, lymphocytic	1 (14%)			
Hematopoietic System				
Bone marrow	(8)		(8)	
Hyperplasia	1 (13%)		1 (13%)	
Lymph node, mandibular	(8)		(8)	
Fibrosis	1 (13%)			
Hyperplasia, lymphoid	2 (25%)		2 (25%)	
Spleen	(8)		(8)	
Congestion	1 (13%)			
Hematopoietic cell proliferation	1 (13%)		1 (13%)	
Hyperplasia, lymphoid	1 (13%)			
Thymus	(7)		(7)	
Atrophy, cortex	2 (29%)			
Hemorrhage			1 (14%)	
Hyperplasia, lymphoid, medulla			1 (14%)	
Integumentary System				
Skin	(8)		(8)	
Hyperplasia	1 (13%)			
Respiratory System				
Lung	(8)		(8)	
Infiltration cellular, lymphocytic	5 (63%)		3 (38%)	
Special Senses System				
Lacrimal gland	(6)		(7)	
Infiltration cellular, lymphocytic	2 (33%)		3 (43%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
6-Month Interim Evaluation (continued)				
Urinary System				
Kidney	(8)		(8)	
Infiltration cellular, lymphocytic	2 (25%)		4 (50%)	
Urinary bladder			(8)	
Infiltration cellular, lymphocytic			1 (13%)	
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
12-Month Interim Evaluation				
Alimentary System				
Esophagus	(8)			(8)
Hyperkeratosis	1 (13%)			
Gallbladder	(8)			(6)
Infiltration cellular, lymphocytic	1 (13%)			
Intestine large, cecum	(8)			(8)
Hyperplasia, lymphoid				1 (13%)
Intestine large, rectum	(8)			(6)
Cyst	1 (13%)			
Liver	(8)			(8)
Infiltration cellular, lymphocytic	5 (63%)			6 (75%)
Necrosis				3 (38%)
Tension lipoidosis	2 (25%)			1 (13%)
Vacuolization cytoplasmic	5 (63%)			3 (38%)
Pancreas	(8)			(8)
Infiltration cellular, lymphocytic	2 (25%)			2 (25%)
Salivary glands	(8)			(8)
Atrophy				1 (13%)
Infiltration cellular, lymphocytic	7 (88%)			7 (88%)
Stomach, forestomach	(8)			(8)
Hyperkeratosis				1 (13%)
Cardiovascular System				
Heart	(8)			(8)
Cardiomyopathy	1 (13%)			
Endocrine System				
Adrenal gland, cortex	(7)			(8)
Congestion				1 (13%)
Hyperplasia, spindle cell	6 (86%)			6 (75%)
Vacuolization cytoplasmic				2 (25%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
12-Month Interim Evaluation (continued)				
Endocrine System (continued)				
Pituitary gland	(5)			(5)
Cyst				1 (20%)
Thyroid gland	(6)			(6)
Ultimobranchial cyst				1 (17%)
Genital System				
Clitoral gland	(6)			(6)
Atrophy	5 (83%)			3 (50%)
Ovary	(8)			(7)
Atrophy	2 (25%)			1 (14%)
Cyst, periovarian tissue				2 (29%)
Hematocyst				1 (14%)
Infiltration cellular, lymphocytic				1 (14%)
Uterus	(8)			(8)
Hyperplasia, cystic, endometrium	2 (25%)			3 (38%)
Infiltration cellular, lymphocytic	1 (13%)			
Vagina	(8)			(8)
Infiltration cellular, lymphocytic	3 (38%)			
Hematopoietic System				
Bone marrow	(8)			(8)
Hyperplasia	1 (13%)			
Lymph node, mandibular	(8)			(8)
Hemorrhage				2 (25%)
Hyperplasia, lymphoid	3 (38%)			1 (13%)
Lymph node, mesenteric	(8)			(8)
Atrophy				4 (50%)
Hyperplasia, lymphoid				1 (13%)
Spleen	(8)			(8)
Hyperplasia, lymphoid	1 (13%)			
Infiltration cellular, lymphocytic				1 (13%)
Thymus	(7)			(8)
Atrophy, cortex	3 (43%)			
Cyst				1 (13%)
Hemorrhage				1 (13%)
Hyperplasia, lymphoid, medulla				6 (75%)
Musculoskeletal System				
Bone, femur	(8)			(8)
Fibrous osteodystrophy				2 (25%)
Nervous System				
Brain, cerebrum	(8)			(8)
Mineralization, multifocal, thalamus	1 (13%)			2 (25%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
12-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(8)			(8)
Infiltration cellular, lymphocytic	7 (88%)			7 (88%)
Inflammation				1 (13%)
Special Senses System				
Harderian gland	(8)			(8)
Infiltration cellular, lymphocytic	1 (13%)			
Lacrimal gland	(7)			(8)
Infiltration cellular, lymphocytic	3 (43%)			5 (63%)
Urinary System				
Kidney	(8)			(8)
Cyst, renal tubule				1 (13%)
Infiltration cellular, lymphocytic	7 (88%)			6 (75%)
Nephropathy	1 (13%)			
Urinary bladder	(8)			(8)
Infiltration cellular, lymphocytic	6 (75%)			6 (75%)
Systems Examined with No Lesions Observed				
General Body System				
Integumentary System				
2-Year Study				
Alimentary System				
Esophagus	(47)	(36)	(40)	(38)
Dilatation			1 (3%)	
Hyperkeratosis	1 (2%)			
Infiltration cellular, lymphocytic			1 (3%)	
Ulcer	1 (2%)			
Gallbladder	(46)	(37)	(36)	(36)
Infiltration cellular, lymphocytic	4 (9%)	2 (5%)	4 (11%)	6 (17%)
Inflammation			1 (3%)	
Intestine large, cecum	(42)	(37)	(37)	(32)
Hyperplasia, lymphoid	5 (12%)		2 (5%)	4 (13%)
Intestine large, colon	(46)	(37)	(37)	(34)
Hyperplasia, goblet cell				1 (3%)
Hyperplasia, lymphoid				1 (3%)
Intestine large, rectum	(44)	(37)	(37)	(32)
Erosion	2 (5%)			
Inflammation		1 (3%)		
Intestine small, duodenum	(40)	(37)	(37)	(33)
Hyperplasia, lymphoid			1 (3%)	
Infiltration cellular, lymphocytic				1 (3%)
Intestine small, ileum	(40)	(35)	(37)	(31)
Hyperplasia, lymphoid	2 (5%)	2 (6%)	3 (8%)	2 (6%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Alimentary System (continued)				
Intestine small, jejunum	(41)	(37)	(37)	(32)
Hyperplasia, goblet cell			1 (3%)	
Hyperplasia, lymphoid		2 (5%)	2 (5%)	
Liver	(48)	(40)	(40)	(40)
Apoptosis		1 (3%)		
Atrophy		1 (3%)		
Basophilic focus	1 (2%)		3 (8%)	2 (5%)
Clear cell focus				2 (5%)
Degeneration, centrilobular			1 (3%)	
Degeneration, cystic				1 (3%)
Degeneration, fatty				1 (3%)
Eosinophilic focus		1 (3%)	2 (5%)	3 (8%)
Hematopoietic cell proliferation	3 (6%)	3 (8%)	5 (13%)	1 (3%)
Hyperplasia, Kupffer cell				1 (3%)
Infiltration cellular, lymphocytic	33 (69%)	27 (68%)	29 (73%)	27 (68%)
Infiltration cellular, plasma cell		1 (3%)		
Inflammation	2 (4%)			
Leukocytosis		1 (3%)		
Mixed cell focus				1 (3%)
Necrosis	32 (67%)	24 (60%)	21 (53%)	20 (50%)
Necrosis, coagulative	1 (2%)		3 (8%)	1 (3%)
Tension lipoidosis	17 (35%)	10 (25%)	10 (25%)	8 (20%)
Vacuolization cytoplasmic	26 (54%)	29 (73%)	28 (70%)	24 (60%)
Mesentery	(1)	(1)	(2)	
Infiltration cellular, lymphocytic		1 (100%)		
Necrosis, fat			1 (50%)	
Pancreas	(48)	(40)	(39)	(37)
Atrophy	1 (2%)	1 (3%)	1 (3%)	
Ectasia, duct		1 (3%)		
Focal cellular change	2 (4%)	2 (5%)	2 (5%)	2 (5%)
Infiltration cellular, lymphocytic	26 (54%)	21 (53%)	16 (41%)	16 (43%)
Inflammation		1 (3%)		
Salivary glands	(48)	(40)	(40)	(39)
Atrophy			3 (8%)	3 (8%)
Hyperplasia, duct	1 (2%)			
Infiltration cellular, lymphocytic	38 (79%)	33 (83%)	35 (88%)	31 (79%)
Inflammation		1 (3%)		
Mineralization		1 (3%)		1 (3%)
Stomach, forestomach	(47)	(37)	(38)	(35)
Hyperkeratosis			1 (3%)	2 (6%)
Hyperplasia				1 (3%)
Ulcer				1 (3%)
Stomach, glandular	(47)	(37)	(38)	(36)
Crystals	1 (2%)			
Cyst	2 (4%)	1 (3%)	3 (8%)	2 (6%)
Degeneration, hyaline	1 (2%)			
Hyperplasia				1 (3%)
Inflammation			1 (3%)	
Tongue	(48)	(39)	(39)	(40)
Infiltration cellular, mast cell	2 (4%)	2 (5%)		
Polyarteritis	1 (2%)			

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Cardiovascular System				
Heart	(48)	(40)	(40)	(39)
Cardiomyopathy			1 (3%)	
Congestion				1 (3%)
Degeneration	1 (2%)			
Dilatation				1 (3%)
Hemorrhage, valve				1 (3%)
Infiltration cellular, lymphocytic		1 (3%)		
Inflammation	1 (2%)	1 (3%)	1 (3%)	
Polyarteritis	1 (2%)			
Thrombus	1 (2%)			
Endocrine System				
Adrenal gland	(46)	(40)	(37)	(37)
Accessory adrenal cortical nodule	1 (2%)			
Inflammation, extra adrenal tissue		1 (3%)		
Adrenal gland, cortex	(46)	(40)	(37)	(37)
Congestion				1 (3%)
Degeneration, fatty			1 (3%)	1 (3%)
Ectopic tissue	1 (2%)		1 (3%)	
Focal cellular change			2 (5%)	
Hyperplasia, spindle cell	43 (93%)	37 (93%)	34 (92%)	32 (86%)
Thrombus	1 (2%)			
Vacuolization cytoplasmic	2 (4%)	1 (3%)		1 (3%)
Adrenal gland, medulla	(46)	(40)	(37)	(37)
Congestion		1 (3%)		1 (3%)
Cytoplasmic alteration			1 (3%)	
Vacuolization cytoplasmic	1 (2%)	1 (3%)		
Islets, pancreatic	(48)	(40)	(37)	(38)
Hyperplasia				1 (3%)
Infiltration cellular, lymphocytic				1 (3%)
Parathyroid gland	(38)	(31)	(33)	(36)
Ectopic thymus	1 (3%)		1 (3%)	1 (3%)
Infiltration cellular, lymphocytic	1 (3%)			
Vacuolization cytoplasmic	1 (3%)			
Pituitary gland	(45)	(36)	(36)	(33)
Angiectasis		1 (3%)		
Cyst		1 (3%)		1 (3%)
Hyperplasia, pars distalis	4 (9%)	3 (8%)	1 (3%)	1 (3%)
Thyroid gland	(47)	(40)	(40)	(39)
Cyst, follicle		1 (3%)		3 (8%)
Degeneration	1 (2%)		1 (3%)	1 (3%)
Ectopic thymus				1 (3%)
Goiter adenomatous			1 (3%)	
Hyperplasia, follicular cell		1 (3%)	1 (3%)	1 (3%)
Hypertrophy, follicular cell				1 (3%)
Infiltration cellular, lymphocytic	4 (9%)	2 (5%)	2 (5%)	2 (5%)
Inflammation		1 (3%)		
Ultimobranchial cyst	11 (23%)	9 (23%)	8 (20%)	7 (18%)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
General Body System				
None				
Genital System				
Clitoral gland	(43)	(37)	(33)	(33)
Atrophy	40 (93%)	35 (95%)	29 (88%)	31 (94%)
Infiltration cellular, lymphocytic				1 (3%)
Inflammation		1 (3%)	2 (6%)	
Ovary	(48)	(40)	(39)	(38)
Angiectasis				1 (3%)
Atrophy	39 (81%)	33 (83%)	34 (87%)	32 (84%)
Congestion	1 (2%)			
Cyst	10 (21%)	6 (15%)	6 (15%)	9 (24%)
Cyst, periovarian tissue	16 (33%)	12 (30%)	6 (15%)	8 (21%)
Hematocyst	7 (15%)	2 (5%)	5 (13%)	4 (11%)
Hyperplasia, adenomatous	2 (4%)	3 (8%)		
Infiltration cellular, lymphocytic	5 (10%)	3 (8%)	1 (3%)	1 (3%)
Mineralization				1 (3%)
Ovotestis			1 (3%)	
Uterus	(48)	(40)	(40)	(39)
Angiectasis	1 (2%)			
Atrophy	2 (4%)	2 (5%)	2 (5%)	1 (3%)
Dilatation	2 (4%)	1 (3%)	3 (8%)	1 (3%)
Ectasia, vein				1 (3%)
Fibrosis	1 (2%)	1 (3%)	1 (3%)	
Hyperplasia, cystic, endometrium	37 (77%)	32 (80%)	26 (65%)	29 (74%)
Infiltration cellular, lymphocytic				1 (3%)
Inflammation				1 (3%)
Prolapse	1 (2%)			
Thrombus		1 (3%)		
Vagina	(48)	(38)	(40)	(37)
Atrophy	2 (4%)	2 (5%)	2 (5%)	
Dysplasia	1 (2%)	1 (3%)		
Infiltration cellular, lymphocytic	3 (6%)	2 (5%)		
Inflammation		2 (5%)		
Metaplasia				1 (3%)
Hematopoietic System				
Bone marrow	(47)	(39)	(40)	(38)
Depletion			1 (3%)	
Hyperplasia	3 (6%)	6 (15%)	4 (10%)	3 (8%)
Hypoplasia		1 (3%)		
Pigmentation		1 (3%)	1 (3%)	
Lymph node	(48)	(40)	(40)	(40)
Hyperplasia, plasma cell			1 (3%)	
Hyperplasia, plasma cell, mediastinal		1 (3%)		
Inflammation			1 (3%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node, mandibular	(47)	(40)	(39)	(39)
Atrophy		1 (3%)	1 (3%)	
Hematopoietic cell proliferation		1 (3%)		
Hemorrhage	3 (6%)	1 (3%)	1 (3%)	
Hyperplasia, lymphoid	7 (15%)	5 (13%)	10 (26%)	4 (10%)
Hyperplasia, plasma cell		1 (3%)		
Inflammation		1 (3%)		
Lymph node, mesenteric	(46)	(40)	(38)	(39)
Angiectasis			1 (3%)	
Atrophy	2 (4%)	2 (5%)		1 (3%)
Congestion, sinus				1 (3%)
Fibrosis		1 (3%)		
Hemorrhage	4 (9%)	1 (3%)	1 (3%)	1 (3%)
Hyperplasia, lymphoid	2 (4%)	2 (5%)	4 (11%)	4 (10%)
Hyperplasia, plasma cell		1 (3%)	1 (3%)	
Hyperplasia, reticulum cell			1 (3%)	
Inflammation			1 (3%)	
Spleen	(47)	(40)	(39)	(39)
Amyloid deposition			1 (3%)	
Atrophy	2 (4%)	2 (5%)		1 (3%)
Congestion				3 (8%)
Erythrophagocytosis				1 (3%)
Hematopoietic cell proliferation	4 (9%)	4 (10%)	7 (18%)	4 (10%)
Hyperplasia, lymphoid	13 (28%)	10 (25%)	11 (28%)	12 (31%)
Hyperplasia, plasma cell, red pulp		1 (3%)		
Thymus	(41)	(33)	(31)	(30)
Atrophy, cortex	30 (73%)	24 (73%)	20 (65%)	25 (83%)
Congestion	1 (2%)			1 (3%)
Cyst			1 (3%)	
Ectopic parathyroid gland	1 (2%)		1 (3%)	1 (3%)
Hyperplasia, lymphoid, medulla	14 (34%)	13 (39%)	12 (39%)	6 (20%)
Integumentary System				
Mammary gland	(44)	(36)	(38)	(36)
Galactocele		1 (3%)		
Hyperplasia	1 (2%)		1 (3%)	3 (8%)
Infiltration cellular, lymphocytic			1 (3%)	
Inflammation	1 (2%)			
Lactation	4 (9%)	1 (3%)	1 (3%)	
Metaplasia, squamous			1 (3%)	
Skin	(45)	(39)	(39)	(38)
Alopecia				1 (3%)
Infiltration cellular, lymphocytic		1 (3%)	1 (3%)	

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Musculoskeletal System				
Bone, femur	(47)	(40)	(40)	(40)
Degeneration, cartilage				1 (3%)
Fibrous osteodystrophy	18 (38%)	9 (23%)	5 (13%)	9 (23%)
Bone, sternum	(47)	(40)	(40)	(40)
Fibrous osteodystrophy, multifocal	36 (77%)	28 (70%)	25 (63%)	29 (73%)
Skeletal muscle	(48)	(39)	(40)	(39)
Infiltration cellular, lymphocytic	2 (4%)	2 (5%)		1 (3%)
Polyarteritis	1 (2%)			
Nervous System				
Brain, cerebellum	(48)	(40)	(40)	(40)
Degeneration	1 (2%)			
Thrombus	1 (2%)			
Brain, cerebrum	(48)	(40)	(40)	(40)
Degeneration	1 (2%)			
Infiltration cellular, lymphocytic		2 (5%)		
Mineralization, multifocal, thalamus	28 (58%)	21 (53%)	23 (58%)	24 (60%)
Thrombus	1 (2%)			
Peripheral nerve	(48)	(38)	(39)	(38)
Infiltration cellular, lymphocytic		1 (3%)		
Inflammation			1 (3%)	
Spinal cord, thoracic	(48)	(40)	(40)	(39)
Degeneration	1 (2%)			
Infiltration cellular, lymphocytic		1 (3%)		
Thrombus	1 (2%)			
Respiratory System				
Larynx	(44)	(40)	(34)	(34)
Concretion				1 (3%)
Crystals				1 (3%)
Infiltration cellular, lymphocytic	1 (2%)			
Lung	(48)	(40)	(40)	(40)
Congestion		1 (3%)		1 (3%)
Foreign body	1 (2%)			
Giant cell				1 (3%)
Hemorrhage	2 (4%)	1 (3%)	1 (3%)	2 (5%)
Hyperplasia, alveolar epithelium		2 (5%)	1 (3%)	2 (5%)
Infiltration cellular, histiocytic		1 (3%)	2 (5%)	2 (5%)
Infiltration cellular, lymphocytic	37 (77%)	30 (75%)	29 (73%)	27 (68%)
Inflammation	4 (8%)	4 (10%)	2 (5%)	3 (8%)
Thrombus	1 (2%)			
Thrombus, capillary				1 (3%)
Nose	(47)	(40)	(40)	(40)
Cyst, nasolacrimal duct	1 (2%)			
Dilatation, glands			1 (3%)	
Inflammation	1 (2%)			

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
2-Year Study (continued)				
Respiratory System (continued)				
Trachea	(47)	(40)	(39)	(39)
Cyst				1 (3%)
Foreign body				1 (3%)
Infiltration cellular, lymphocytic			1 (3%)	
Inflammation	1 (2%)			
Special Senses System				
Eye	(41)	(38)	(34)	(35)
Degeneration, retina	1 (2%)			
Inflammation		1 (3%)		
Thrombus	1 (2%)			
Harderian gland	(48)	(38)	(38)	(38)
Atrophy		1 (3%)		
Hyperplasia		2 (5%)		
Infiltration cellular, lymphocytic	18 (38%)	13 (34%)	16 (42%)	8 (21%)
Inflammation				1 (3%)
Thrombus	1 (2%)			
Lacrimal gland	(41)	(33)	(33)	(32)
Apoptosis		1 (3%)		
Atrophy	1 (2%)	1 (3%)		2 (6%)
Cytomegaly				1 (3%)
Ectasia, duct				1 (3%)
Infiltration cellular, lymphocytic	25 (61%)	21 (64%)	17 (52%)	13 (41%)
Necrosis		1 (3%)		
Zymbal's gland	(43)	(38)	(36)	(36)
Infiltration cellular, lymphocytic			1 (3%)	
Inflammation	1 (2%)			
Urinary System				
Kidney	(48)	(40)	(39)	(38)
Amyloid deposition, glomerulus	2 (4%)	1 (3%)	1 (3%)	2 (5%)
Congestion	1 (2%)			
Cyst, renal tubule	14 (29%)	9 (23%)	9 (23%)	8 (21%)
Glomerulosclerosis				1 (3%)
Hydronephrosis				1 (3%)
Hydronephrosis, bilateral	1 (2%)			
Infarct		1 (3%)		
Infiltration cellular, lymphocytic	39 (81%)	31 (78%)	31 (79%)	32 (84%)
Infiltration cellular, plasma cell		1 (3%)		
Inflammation	1 (2%)			
Inflammation, adventitia		1 (3%)		
Necrosis, coagulative			1 (3%)	
Nephropathy	3 (6%)		2 (5%)	4 (11%)
Pigmentation, renal tubule				1 (3%)
Polyarteritis	1 (2%)			
Urinary bladder	(47)	(37)	(39)	(36)
Infiltration cellular, lymphocytic	38 (81%)	31 (84%)	33 (85%)	28 (78%)
Polyarteritis	1 (2%)			

APPENDIX C
SUMMARY OF LESIONS IN REGIMEN C FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF CHLORAL HYDRATE
(Single Dose on Postnatal Day 28)

TABLE C1 Summary of the Incidence of Neoplasms in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate 122

TABLE C2 Statistical Analysis of Primary Neoplasms at 2 Years in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate 126

TABLE C3 Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate 128

TABLE C1
Summary of the Incidence of Neoplasms in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	3	4		2
Natural deaths	3	4	3	6
Survivors				
Terminal sacrifice	42	40	45	40
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(45)	(7)	(2)	(46)
Lymphoma malignant		1 (14%)		
Gallbladder	(43)	(8)	(3)	(44)
Lymphoma malignant		2 (25%)	1 (33%)	
Intestine large, cecum	(46)	(6)	(1)	(43)
Lymphoma malignant	2 (4%)			1 (2%)
Intestine small, duodenum	(45)	(7)	(1)	(41)
Lymphoma malignant	1 (2%)	1 (14%)		1 (2%)
Intestine small, ileum	(42)	(6)	(1)	(43)
Lymphoma malignant	1 (2%)			2 (5%)
Intestine small, jejunum	(44)	(7)	(2)	(44)
Lymphoma malignant		1 (14%)	1 (50%)	
Liver	(48)	(48)	(48)	(48)
Hemangiosarcoma			1 (2%)	
Hepatocellular adenoma	3 (6%)	3 (6%)	1 (2%)	2 (4%)
Hepatocellular adenoma, multiple			1 (2%)	
Hepatocellular carcinoma	3 (6%)			
Histiocytic sarcoma	1 (2%)	1 (2%)		
Lymphoma malignant	5 (10%)	10 (21%)	4 (8%)	9 (19%)
Mesentery	(2)	(4)	(1)	
Hemangiosarcoma			1 (100%)	
Sarcoma		1 (25%)		
Pancreas	(46)	(8)	(2)	(46)
Lymphoma malignant	2 (4%)	2 (25%)	1 (50%)	2 (4%)
Salivary glands	(48)	(9)	(3)	(48)
Lymphoma malignant	1 (2%)	3 (33%)		2 (4%)
Stomach, forestomach	(47)	(7)	(2)	(44)
Lymphoma malignant		2 (29%)		
Squamous cell carcinoma	1 (2%)			
Stomach, glandular	(47)	(7)	(2)	(44)
Lymphoma malignant		2 (29%)	1 (50%)	
Cardiovascular System				
Heart	(48)	(8)	(3)	(48)
Lymphoma malignant		1 (13%)		
Endocrine System				
Adrenal gland	(46)	(9)	(2)	(46)
Fibrosarcoma, metastatic, skin		1 (11%)		
Adrenal gland, cortex	(46)	(8)	(2)	(46)
Histiocytic sarcoma		1 (13%)		
Lymphoma malignant	1 (2%)	3 (38%)		2 (4%)

TABLE C1
Summary of the Incidence of Neoplasms in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(46)	(7)	(3)	(45)
Adenoma			1 (33%)	
Lymphoma malignant		1 (14%)	1 (33%)	
Pituitary gland	(40)	(4)		(42)
Adenoma, pars distalis	1 (3%)			1 (2%)
Thyroid gland	(48)	(8)	(2)	(48)
Lymphoma malignant		1 (13%)		
General Body System				
None				
Genital System				
Clitoral gland	(38)	(8)	(1)	(37)
Lymphoma malignant		1 (13%)		
Ovary	(47)	(28)	(28)	(45)
Cystadenoma		1 (4%)		1 (2%)
Granulosa cell tumor benign				1 (2%)
Hemangiosarcoma, periovarian tissue				1 (2%)
Histiocytic sarcoma	1 (2%)			
Luteoma				1 (2%)
Lymphoma malignant		2 (7%)	1 (4%)	1 (2%)
Lymphoma malignant, periovarian tissue	1 (2%)			
Sarcoma		1 (4%)		
Yolk sac carcinoma				1 (2%)
Uterus	(48)	(29)	(28)	(47)
Fibrosarcoma			1 (4%)	
Histiocytic sarcoma	1 (2%)			
Leiomyoma		1 (3%)		
Lymphoma malignant		1 (3%)		
Polyp	1 (2%)			
Vagina	(47)	(8)	(1)	(46)
Fibrosarcoma				1 (2%)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant		3 (38%)		
Hematopoietic System				
Bone marrow	(48)	(8)	(1)	(46)
Lymphoma malignant	3 (6%)	3 (38%)	1 (100%)	2 (4%)
Lymph node	(48)	(15)	(6)	(48)
Fibrosarcoma, metastatic, renal, uterus			1 (17%)	
Lymphoma malignant	1 (2%)			1 (2%)
Lymphoma malignant, axillary		1 (7%)		
Lymphoma malignant, inguinal		2 (13%)	1 (17%)	
Lymphoma malignant, lumbar	1 (2%)		1 (17%)	
Lymphoma malignant, mediastinal		1 (7%)		
Lymphoma malignant, renal		2 (13%)	1 (17%)	2 (4%)
Lymphoma malignant, thoracic				1 (2%)
Lymph node, mandibular	(48)	(9)	(4)	(47)
Lymphoma malignant	2 (4%)	4 (44%)	2 (50%)	7 (15%)
Lymph node, mesenteric	(47)	(11)	(4)	(43)
Lymphoma malignant	4 (9%)	6 (55%)	3 (75%)	6 (14%)
Sarcoma		1 (9%)		

TABLE C1
Summary of the Incidence of Neoplasms in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Spleen	(47)	(20)	(15)	(46)
Hemangiosarcoma		1 (5%)	2 (13%)	1 (2%)
Lymphoma malignant	7 (15%)	13 (65%)	5 (33%)	14 (30%)
Thymus	(38)	(6)	(4)	(37)
Lymphoma malignant	2 (5%)	2 (33%)	3 (75%)	2 (5%)
Sarcoma, metastatic, skin		1 (17%)		
Integumentary System				
Mammary gland	(46)	(6)	(2)	(47)
Adenocarcinoma		1 (17%)		1 (2%)
Adenoma				1 (2%)
Fibrous histiocytoma		1 (17%)		
Lymphoma malignant		1 (17%)		
Skin	(48)	(8)	(6)	(48)
Fibrosarcoma	1 (2%)	1 (13%)	1 (17%)	1 (2%)
Hemangiosarcoma			2 (33%)	
Hemangiosarcoma, metastatic, uncertain primary site		1 (13%)		
Lymphoma malignant		1 (13%)		
Sarcoma		1 (13%)		
Musculoskeletal System				
Bone, femur	(48)	(8)	(2)	(47)
Lymphoma malignant		1 (13%)		
Bone, sternum	(48)	(8)	(3)	(47)
Lymphoma malignant		1 (13%)		
Skeletal muscle	(48)	(8)	(3)	(48)
Fibrosarcoma				1 (2%)
Lymphoma malignant	1 (2%)	2 (25%)		
Nervous System				
Brain, cerebellum	(48)	(8)	(3)	(48)
Lymphoma malignant		1 (13%)		
Brain, cerebrum	(48)	(8)	(3)	(48)
Lymphoma malignant		2 (25%)		
Peripheral nerve	(48)	(8)	(3)	(48)
Lymphoma malignant		1 (13%)		
Spinal cord, thoracic	(48)	(8)	(3)	(48)
Lymphoma malignant		1 (13%)		
Respiratory System				
Lung	(48)	(8)	(4)	(48)
Alveolar/bronchiolar adenoma	3 (6%)			5 (10%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma			1 (25%)	
Carcinoma, metastatic, harderian gland	1 (2%)			
Fibrous histiocytoma		1 (13%)		
Hemangiosarcoma				1 (2%)
Lymphoma malignant	3 (6%)	4 (50%)	1 (25%)	4 (8%)
Sarcoma, metastatic, skin		1 (13%)		
Yolk sac carcinoma, metastatic, ovary				1 (2%)

TABLE C1
Summary of the Incidence of Neoplasms in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Respiratory System (continued)				
Nose	(48)	(8)	(3)	(47)
Carcinoma, metastatic, harderian gland	1 (2%)			
Lymphoma malignant		1 (13%)		
Trachea	(48)	(8)	(2)	(48)
Lymphoma malignant		1 (13%)		
Special Senses System				
Harderian gland	(48)	(10)	(5)	(47)
Adenoma	1 (2%)	2 (20%)	1 (20%)	1 (2%)
Carcinoma	1 (2%)			
Lymphoma malignant	1 (2%)	2 (20%)		
Lacrimal gland	(41)	(5)	(2)	(43)
Lymphoma malignant	1 (2%)	1 (20%)		1 (2%)
Zymbal's gland	(48)	(6)	(1)	(45)
Lymphoma malignant		1 (17%)		
Urinary System				
Kidney	(48)	(9)	(3)	(48)
Lymphoma malignant	2 (4%)	5 (56%)	1 (33%)	2 (4%)
Ureter		(1)		
Lymphoma malignant		1 (100%)		
Urinary bladder	(47)	(8)	(2)	(45)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	1 (2%)	3 (38%)		1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[b]	23	25	14	28
Total primary neoplasms	64	119	42	83
Total animals with benign neoplasms	9	7	4	12
Total benign neoplasms	10	7	4	13
Total animals with malignant neoplasms	15	22	12	21
Total malignant neoplasms	54	112	38	70
Total animals with metastatic neoplasms	1	3	1	1
Total metastatic neoplasms	2	4	1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Liver: Hepatocellular Adenoma				
Overall rate[a]	3/48 (6%)	3/48 (6%)	2/48 (4%)	2/48 (4%)
Adjusted rate[b]	6.5%	6.5%	4.2%	4.5%
Terminal rate[c]	3/42 (7%)	3/40 (8%)	2/45 (4%)	2/40 (5%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test[d]	P=0.3727N	P=0.6619N	P=0.4849N	P=0.5115N
Liver: Hepatocellular Carcinoma				
Overall rate	3/48 (6%)	0/48 (0%)	0/48 (0%)	0/48 (0%)
Adjusted rate	6.5%	0.0%	0.0%	0.0%
Terminal rate	2/42 (5%)	0/40 (0%)	0/45 (0%)	0/40 (0%)
First incidence (days)	682	—[e]	—	—
Poly-3 test	P=0.0528N	P=0.1197N	P=0.1140N	P=0.1244N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	6/48 (13%)	3/48 (6%)	2/48 (4%)	2/48 (4%)
Adjusted rate	13.0%	6.5%	4.2%	4.5%
Terminal rate	5/42 (12%)	3/40 (8%)	2/45 (4%)	2/40 (5%)
First incidence (days)	682	757 (T)	757 (T)	757 (T)
Poly-3 test	P=0.1037N	P=0.2447N	P=0.1246N	P=0.1432N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/48 (8%)	0/8 (0%)	0/4 (0%)	5/48 (10%)
Adjusted rate	8.7%	0.0%	0.0%	11.1%
Terminal rate	4/42 (10%)	0/0	0/1 (0%)	4/40 (10%)
First incidence (days)	757 (T)	—	—	707
Poly-3 test	(NA)	—[f]	—	P=0.4867
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/48 (8%)	0/8 (0%)	1/4 (25%)	5/48 (10%)
Adjusted rate	8.7%	0.0%	29.6%	11.1%
Terminal rate	4/42 (10%)	0/0	1/1 (100%)	4/40 (10%)
First incidence (days)	757 (T)	—	757 (T)	707
Poly-3 test	(NA)	—	—	P=0.4867
All Organs: Hemangiosarcoma				
Overall rate	0/48 (0%)	1/48 (2%)	3/48 (6%)	2/48 (4%)
Adjusted rate	0.0%	2.2%	6.3%	4.5%
Terminal rate	0/42 (0%)	0/40 (0%)	2/45 (4%)	1/40 (3%)
First incidence (days)	—	738	653	741
Poly-3 test	P=0.1518	P=0.5004	P=0.1264	P=0.2319
All Organs: Malignant Lymphoma				
Overall rate	8/48 (17%)	16/48 (33%)	6/48 (13%)	16/48 (33%)
Adjusted rate	17.2%	34.1%	12.6%	35.1%
Terminal rate	6/42 (14%)	12/40 (30%)	5/45 (11%)	13/40 (33%)
First incidence (days)	562	604	718	543
Poly-3 test	P=0.1250	P=0.0488	P=0.3714N	P=0.0404

TABLE C2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
All Organs: Benign Neoplasms				
Overall rate	9/48 (19%)	7/48 (15%)	4/48 (8%)	**13**/48 (27%)
Adjusted rate	19.6%	15.3%	8.4%	28.4%
Terminal rate	9/42 (21%)	7/40 (18%)	4/45 (9%)	11/40 (28%)
First incidence (days)	757 (T)	757 (T)	757 (T)	349
Poly-3 test	P=0.1399	P=0.3917N	P=0.1029N	P=0.2308
All Organs: Malignant Neoplasms				
Overall rate	15/48 (31%)	22/48 (46%)	12/48 (25%)	21/48 (44%)
Adjusted rate	31.5%	45.8%	25.1%	45.6%
Terminal rate	10/42 (24%)	14/40 (35%)	10/45 (22%)	16/40 (40%)
First incidence (days)	562	583	653	543
Poly-3 test	P=0.2432	P=0.1087	P=0.3209N	P=0.1151
All Organs: Benign or Malignant Neoplasms				
Overall rate	23/48 (48%)	25/48 (52%)	14/48 (29%)	28/48 (58%)
Adjusted rate	48.3%	52.1%	29.2%	59.7%
Terminal rate	18/42 (43%)	17/40 (43%)	12/45 (27%)	22/40 (55%)
First incidence (days)	562	583	653	349
Poly-3 test	P=0.2537	P=0.4331	P=0.0432N	P=0.1817

(T)Terminal sacrifice
(NA)Not applicable
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the vehicle controls are not appropriate.

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	3	4		2
Natural deaths	3	4	3	6
Survivors				
Terminal sacrifice	42	40	45	40
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(45)	(7)	(2)	(46)
Hyperkeratosis		1 (14%)		1 (2%)
Gallbladder	(43)	(8)	(3)	(44)
Accumulation hyaline droplet				1 (2%)
Infiltration cellular, lymphocytic	3 (7%)			2 (5%)
Intestine large, cecum	(46)	(6)	(1)	(43)
Hyperplasia, lymphoid	6 (13%)	1 (17%)		1 (2%)
Intestine large, colon	(46)	(7)	(2)	(43)
Hyperplasia, goblet cell	1 (2%)			1 (2%)
Intestine large, rectum	(46)	(7)	(2)	(43)
Fibrosis	1 (2%)			
Intestine small, ileum	(42)	(6)	(1)	(43)
Atrophy	1 (2%)			
Hyperplasia, lymphoid		1 (17%)		4 (9%)
Intestine small, jejunum	(44)	(7)	(2)	(44)
Hyperplasia, lymphoid	1 (2%)	1 (14%)		
Liver	(48)	(48)	(48)	(48)
Angiectasis			1 (2%)	
Basophilic focus	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Cyst, bile duct		1 (2%)		
Degeneration		1 (2%)	1 (2%)	1 (2%)
Degeneration, fatty		1 (2%)		
Eosinophilic focus		2 (4%)	1 (2%)	
Hematopoietic cell proliferation	5 (10%)	13 (27%)	13 (27%)	11 (23%)
Hyperplasia, Kupffer cell		2 (4%)		1 (2%)
Hyperplasia, oval cell			1 (2%)	
Infiltration cellular, lymphocytic	42 (88%)	35 (73%)	36 (75%)	33 (69%)
Infiltration cellular, plasma cell		1 (2%)	1 (2%)	
Inflammation	1 (2%)			1 (2%)
Necrosis	27 (56%)	33 (69%)	40 (83%)	34 (71%)
Necrosis, coagulative	2 (4%)	1 (2%)	4 (8%)	3 (6%)
Regeneration		1 (2%)	1 (2%)	
Tension lipoidosis	19 (40%)	14 (29%)	20 (42%)	16 (33%)
Thrombus			1 (2%)	1 (2%)
Vacuolization cytoplasmic	31 (65%)	36 (75%)	42 (88%)	34 (71%)
Mesentery	(2)	(4)	(1)	
Necrosis, fat	2 (100%)	3 (75%)		

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Alimentary System (continued)				
Pancreas	(46)	(8)	(2)	(46)
Accumulation hyaline droplet, duct				1 (2%)
Atrophy	2 (4%)			
Cyst, duct		1 (13%)		
Degeneration	1 (2%)			1 (2%)
Focal cellular change				2 (4%)
Infiltration cellular, lymphocytic	24 (52%)	5 (63%)	1 (50%)	21 (46%)
Inflammation	1 (2%)			
Necrosis				1 (2%)
Salivary glands	(48)	(9)	(3)	(48)
Atrophy				2 (4%)
Infiltration cellular, lymphocytic	41 (85%)	5 (56%)	3 (100%)	41 (85%)
Stomach, forestomach	(47)	(7)	(2)	(44)
Hyperkeratosis	1 (2%)		1 (50%)	1 (2%)
Infiltration cellular, lymphocytic				1 (2%)
Inflammation				2 (5%)
Stomach, glandular	(47)	(7)	(2)	(44)
Cyst	5 (11%)	1 (14%)		
Hyperplasia	2 (4%)			
Infiltration cellular, lymphocytic				1 (2%)
Inflammation				1 (2%)
Mineralization	1 (2%)			2 (5%)
Tongue	(48)	(8)	(3)	(48)
Infiltration cellular, mast cell	1 (2%)			
Mineralization		1 (13%)		
Polyarteritis				1 (2%)
Cardiovascular System				
Heart	(48)	(8)	(3)	(48)
Cardiomyopathy	1 (2%)			
Degeneration, valve				4 (8%)
Hyperplasia, epicardium		1 (13%)		
Infiltration cellular, lymphocytic	1 (2%)			1 (2%)
Necrosis			1 (33%)	1 (2%)
Polyarteritis				1 (2%)
Thrombus	1 (2%)			
Endocrine System				
Adrenal gland	(46)	(9)	(2)	(46)
Accessory adrenal cortical nodule	1 (2%)			
Adrenal gland, cortex	(46)	(8)	(2)	(46)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia				1 (2%)
Hyperplasia, spindle cell	44 (96%)	4 (50%)	1 (50%)	38 (83%)
Infiltration cellular, lymphocytic	1 (2%)			
Vacuolization cytoplasmic	1 (2%)			2 (4%)
Adrenal gland, medulla	(46)	(8)	(2)	(44)
Inflammation	1 (2%)			
Vacuolization cytoplasmic	1 (2%)			

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(46)	(7)	(3)	(45)
Hyperplasia				2 (4%)
Parathyroid gland	(38)	(7)		(44)
Cyst	1 (3%)			
Ectopic thymus	2 (5%)			1 (2%)
Infiltration cellular, lymphocytic	1 (3%)			1 (2%)
Pituitary gland	(40)	(4)		(42)
Angiectasis				1 (2%)
Cytomegaly	1 (3%)			
Degeneration, cystic, pars distalis	1 (3%)			
Hyperplasia, pars distalis	3 (8%)			8 (19%)
Hypertrophy, pars distalis	2 (5%)			
Polyarteritis				1 (2%)
Thyroid gland	(48)	(8)	(2)	(48)
Cyst, follicle	1 (2%)			
Degeneration				1 (2%)
Ectopic thymus	1 (2%)			
Hyperplasia, follicular cell	1 (2%)			
Infiltration cellular, lymphocytic	2 (4%)			2 (4%)
Inflammation	1 (2%)			
Polyarteritis				1 (2%)
Ultimobranchial cyst	19 (40%)	2 (13%)		20 (42%)
General Body System				
None				
Genital System				
Clitoral gland	(38)	(8)	(1)	(37)
Atrophy	37 (97%)	8 (100%)	1 (100%)	29 (78%)
Infiltration cellular, lymphocytic	1 (3%)			
Inflammation	1 (3%)			
Ovary	(47)	(28)	(28)	(45)
Angiectasis	1 (2%)			1 (2%)
Atrophy	37 (79%)	6 (21%)	1 (4%)	33 (73%)
Congestion	1 (2%)			
Cyst	9 (19%)	11 (39%)	17 (61%)	13 (29%)
Cyst, periovarian tissue	10 (21%)	9 (32%)	7 (25%)	3 (7%)
Hematocyst	4 (9%)		1 (4%)	3 (7%)
Infiltration cellular, lymphocytic	2 (4%)			
Mineralization				1 (2%)
Uterus	(48)	(29)	(28)	(47)
Angiectasis	1 (2%)			
Atrophy	2 (4%)	5 (17%)	2 (7%)	4 (9%)
Dilatation	4 (8%)	2 (7%)	8 (29%)	3 (6%)
Fibrosis				2 (4%)
Hemorrhage				1 (2%)
Hyperplasia, cystic, endometrium	40 (83%)	19 (66%)	17 (61%)	38 (81%)
Inflammation	1 (2%)		1 (4%)	1 (2%)
Vagina	(47)	(8)	(1)	(46)
Atrophy	2 (4%)	5 (63%)	1 (100%)	2 (4%)
Infiltration cellular, lymphocytic	1 (2%)			2 (4%)
Polyarteritis				1 (2%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System				
Bone marrow	(48)	(8)	(1)	(46)
Depletion		1 (13%)		
Hyperplasia	3 (6%)	1 (13%)		2 (4%)
Infarct				1 (2%)
Myelofibrosis	1 (2%)	1 (13%)		
Lymph node	(48)	(15)	(6)	(48)
Hyperplasia, lymphoid, bronchial	1 (2%)			
Hyperplasia, lymphoid, inguinal		1 (7%)		
Lymph node, mandibular	(48)	(9)	(4)	(47)
Hematopoietic cell proliferation		1 (11%)		
Hemorrhage	1 (2%)			
Hyperplasia				1 (2%)
Hyperplasia, lymphoid	8 (17%)			3 (6%)
Lymph node, mesenteric	(47)	(11)	(4)	(43)
Atrophy	1 (2%)	2 (18%)		1 (2%)
Hematopoietic cell proliferation	1 (2%)	1 (9%)		
Hemorrhage	2 (4%)	1 (9%)		1 (2%)
Hyperplasia, lymphoid	2 (4%)			1 (2%)
Hyperplasia, reticulum cell	1 (2%)			
Infiltration cellular, lymphocytic	1 (2%)			
Spleen	(47)	(20)	(15)	(46)
Atrophy	1 (2%)	2 (10%)	1 (7%)	1 (2%)
Congestion	3 (6%)	2 (10%)	1 (7%)	
Hematopoietic cell proliferation	4 (9%)	4 (20%)	2 (13%)	9 (20%)
Hyperplasia, lymphoid	21 (45%)	2 (10%)	4 (27%)	12 (26%)
Infarct				1 (2%)
Infiltration cellular, lymphocytic	1 (2%)			
Thymus	(38)	(6)	(4)	(37)
Atrophy, cortex	27 (71%)	1 (17%)	1 (25%)	15 (41%)
Congestion				1 (3%)
Cyst	1 (3%)			
Ectopic parathyroid gland	1 (3%)			
Hyperplasia, lymphoid, medulla	16 (42%)			22 (59%)
Integumentary System				
Mammary gland	(46)	(6)	(2)	(47)
Hyperplasia	1 (2%)	1 (17%)		1 (2%)
Infiltration cellular, lymphocytic	1 (2%)			
Lactation	1 (2%)			5 (11%)
Skin	(48)	(8)	(6)	(48)
Infiltration cellular, lymphocytic	1 (2%)			
Musculoskeletal System				
Bone	(48)	(8)	(3)	(47)
Fibrous osteodystrophy, turbinate				10 (21%)
Bone, femur	(48)	(8)	(2)	(47)
Fibrous osteodystrophy	13 (27%)			9 (19%)
Bone, sternum	(48)	(8)	(3)	(47)
Fibrous osteodystrophy, multifocal	36 (75%)	2 (25%)	1 (33%)	33 (70%)
Skeletal muscle	(48)	(8)	(3)	(48)
Infiltration cellular, lymphocytic	2 (4%)			2 (4%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Nervous System				
Brain, cerebellum	(48)	(8)	(3)	(48)
Mineralization, thalamus	1 (2%)			
Brain, cerebrum	(48)	(8)	(3)	(48)
Developmental malformation	1 (2%)			
Gliosis	1 (2%)			
Infiltration cellular, lymphocytic				2 (4%)
Mineralization, multifocal, thalamus	31 (65%)	4 (50%)		24 (50%)
Necrosis	1 (2%)			
Spinal cord, thoracic	(48)	(8)	(3)	(48)
Infiltration cellular, lymphocytic	1 (2%)			1 (2%)
Respiratory System				
Larynx	(44)	(4)	(2)	(42)
Infiltration cellular, lymphocytic	1 (2%)			
Lung	(48)	(8)	(4)	(48)
Congestion	1 (2%)			1 (2%)
Hyperplasia, alveolar epithelium	2 (4%)			1 (2%)
Infiltration cellular, histiocytic	2 (4%)			2 (4%)
Infiltration cellular, lymphocytic	40 (83%)	2 (25%)	2 (50%)	38 (79%)
Inflammation				1 (2%)
Nose	(48)	(8)	(3)	(47)
Focal cellular change				1 (2%)
Special Senses System				
Eye	(45)	(5)		(46)
Inflammation, cornea				1 (2%)
Harderian gland	(48)	(10)	(5)	(47)
Hyperplasia		1 (10%)	1 (20%)	1 (2%)
Infiltration cellular, lymphocytic	14 (29%)	2 (20%)	2 (40%)	16 (34%)
Lacrimal gland	(41)	(5)	(2)	(43)
Atrophy	1 (2%)			
Infiltration cellular, lymphocytic	25 (61%)	3 (60%)		26 (60%)
Zymbal's gland	(48)	(6)	(1)	(45)
Atrophy	1 (2%)			
Hyperplasia, squamous	1 (2%)			
Inflammation				1 (2%)
Urinary System				
Kidney	(48)	(9)	(3)	(48)
Accumulation hyaline droplet		1 (11%)		1 (2%)
Amyloid deposition, glomerulus	2 (4%)			
Cyst, renal tubule	10 (21%)	1 (11%)		2 (4%)
Glomerulosclerosis	2 (4%)	2 (22%)		1 (2%)
Hydronephrosis	2 (4%)			1 (2%)
Hyperplasia, renal tubule	1 (2%)			

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Regimen C Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Urinary System (continued)				
Kidney (continued)	(48)	(9)	(3)	(48)
Infiltration cellular, lymphocytic	42 (88%)	4 (23%)	2 (67%)	39 (81%)
Mineralization	1 (2%)			1 (2%)
Necrosis, renal tubule	1 (2%)			
Nephropathy	7 (15%)			2 (4%)
Pigmentation, renal tubule				1 (2%)
Vacuolization cytoplasmic, renal tubule	1 (2%)			
Urinary bladder	(47)	(8)	(2)	(45)
Fibrosis, adventitia	1 (2%)			
Infiltration cellular, lymphocytic	41 (87%)		1 (50%)	37 (82%)

APPENDIX D
SUMMARY OF LESIONS IN REGIMEN D FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF CHLORAL HYDRATE
(Single Dose on Postnatal Day 15)

TABLE D1 Summary of the Incidence of Neoplasms in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate 136

TABLE D2 Statistical Analysis of Primary Neoplasms at 2 Years in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate 140

TABLE D3 Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate 142

TABLE D1
Summary of the Incidence of Neoplasms in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental deaths	2			
Moribund	4	1	6	4
Natural deaths	6	6	3	4
Survivors				
Died last week of study		1	1	
Terminal sacrifice	36	40	38	40
Animals examined microscopically	48	48	48	48
Alimentary System				
Gallbladder	(41)	(6)	(9)	(46)
Hepatocholangiocarcinoma, metastatic, liver		1 (17%)		
Lymphoma malignant	3 (7%)			
Intestine small, duodenum	(43)	(5)	(9)	(44)
Lymphoma malignant		1 (20%)		
Intestine small, jejunum	(41)	(7)	(8)	(45)
Lymphoma malignant		2 (29%)		1 (2%)
Liver	(48)	(48)	(48)	(48)
Hemangiosarcoma			1 (2%)	
Hepatocellular adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hepatocellular carcinoma		2 (4%)	1 (2%)	1 (2%)
Hepatocholangiocarcinoma		1 (2%)		
Histiocytic sarcoma	1 (2%)	2 (4%)	2 (4%)	
Ito cell tumor NOS			1 (2%)	
Lymphoma malignant	9 (19%)	4 (8%)	3 (6%)	6 (13%)
Pancreas	(46)	(5)	(10)	(47)
Carcinoma, metastatic, uncertain primary site			1 (10%)	
Histiocytic sarcoma		1 (20%)		
Lymphoma malignant	5 (11%)			1 (2%)
Salivary glands	(47)	(7)	(10)	(48)
Lymphoma malignant	5 (11%)			
Cardiovascular System				
Heart	(48)	(8)	(10)	(48)
Hepatocholangiocarcinoma, metastatic, liver		1 (13%)		
Endocrine System				
Adrenal gland, medulla	(47)	(6)	(10)	(47)
Lymphoma malignant				1 (2%)
Pheochromocytoma benign		1 (17%)		
Islets, pancreatic	(46)	(4)	(11)	(47)
Adenoma			1 (9%)	
Histiocytic sarcoma		1 (25%)		
Parathyroid gland	(44)	(3)	(5)	(43)
Lymphoma malignant	2 (5%)			
Pituitary gland	(45)	(4)	(9)	(43)
Adenoma, pars distalis	1 (2%)		1 (11%)	1 (2%)
Adenoma, pars intermedia	1 (2%)			
Carcinoma, pars distalis				1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Thyroid gland	(46)	(5)	(9)	(47)
Adenoma, follicular cell	1 (2%)			
Lymphoma malignant	1 (2%)			
General Body System				
None				
Genital System				
Ovary	(46)	(22)	(30)	(46)
Granulosa cell tumor benign	1 (2%)			
Hepatocholangiocarcinoma, metastatic, liver		1 (5%)		
Histiocytic sarcoma		2 (9%)	2 (7%)	
Luteoma	1 (2%)			
Lymphoma malignant	2 (4%)		1 (3%)	
Lymphoma malignant, periovarian tissue	2 (4%)			
Uterus	(47)	(30)	(35)	(48)
Fibroma			1 (3%)	
Hemangiosarcoma		1 (3%)	1 (3%)	2 (4%)
Histiocytic sarcoma	1 (2%)	2 (7%)	3 (9%)	
Leiomyoma	1 (2%)			
Lymphoma malignant	1 (2%)			
Polyp				1 (2%)
Vagina	(44)	(7)	(10)	(46)
Fibrosarcoma	1 (2%)			1 (2%)
Histiocytic sarcoma		2 (29%)	3 (30%)	
Lymphoma malignant			1 (10%)	
Polyp				1 (2%)
Hematopoietic System				
Bone marrow	(47)	(8)	(10)	(48)
Histiocytic sarcoma		1 (13%)		
Lymphoma malignant	4 (9%)			1 (2%)
Lymph node	(47)	(13)	(11)	(48)
Alveolar/bronchiolar carcinoma, metastatic, mediastinal, lung				1 (2%)
Lymphoma malignant		1 (8%)		
Lymphoma malignant, inguinal	1 (2%)			
Lymphoma malignant, lumbar			1 (9%)	1 (2%)
Lymphoma malignant, mediastinal		2 (15%)		1 (2%)
Lymphoma malignant, pancreatic	1 (2%)			
Lymphoma malignant, renal		1 (8%)	1 (9%)	
Lymphoma malignant, thoracic		1 (8%)		
Lymph node, mandibular	(47)	(8)	(9)	(48)
Histiocytic sarcoma		1 (13%)		
Lymphoma malignant	7 (15%)	1 (13%)		
Squamous cell carcinoma, metastatic, nose			1 (11%)	
Lymph node, mesenteric	(46)	(10)	(8)	(45)
Carcinoma, metastatic, uncertain primary site			1 (13%)	
Histiocytic sarcoma		1 (10%)		
Lymphoma malignant	8 (17%)	5 (50%)	1 (13%)	1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Spleen	(47)	(18)	(17)	(47)
Hemangiosarcoma			1 (6%)	2 (4%)
Histiocytic sarcoma		1 (6%)		
Lymphoma malignant	11 (23%)	8 (44%)	2 (12%)	5 (11%)
Thymus	(36)	(4)	(7)	(40)
Hepatocholangiocarcinoma, metastatic, liver		1 (25%)		
Lymphoma malignant	8 (22%)		3 (43%)	
Integumentary System				
Mammary gland	(43)	(6)	(11)	(46)
Adenocarcinoma	1 (2%)	1 (17%)	2 (18%)	5 (11%)
Histiocytic sarcoma			1 (9%)	
Skin	(46)	(7)	(10)	(48)
Hemangioma	1 (2%)			
Hemangiosarcoma, metastatic, spleen				1 (2%)
Lymphoma malignant	1 (2%)			
Musculoskeletal System				
Bone	(47)	(8)	(10)	(48)
Hepatocholangiocarcinoma, metastatic, mandible, liver		1 (13%)		
Osteosarcoma, pelvis				1 (2%)
Bone, sternum	(45)	(8)	(10)	(48)
Alveolar/bronchiolar carcinoma, metastatic, adventitia, lung				1 (2%)
Nervous System				
Brain, cerebrum	(48)	(8)	(10)	(48)
Carcinoma, metastatic, pituitary gland				1 (2%)
Respiratory System				
Lung	(47)	(8)	(11)	(48)
Alveolar/bronchiolar adenoma	2 (4%)	1 (13%)	1 (9%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma		1 (13%)		2 (4%)
Alveolar/bronchiolar carcinoma, multiple			1 (9%)	
Carcinoma, metastatic, harderian gland				1 (2%)
Carcinoma, metastatic, uncertain primary site			1 (9%)	
Hepatocellular carcinoma, metastatic, liver		1 (13%)	1 (9%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (13%)		
Histiocytic sarcoma		1 (13%)	2 (18%)	
Lymphoma malignant	6 (13%)			1 (2%)
Osteosarcoma, metastatic, bone				1 (2%)
Nose	(48)	(8)	(10)	(48)
Histiocytic sarcoma		1 (13%)		
Squamous cell carcinoma			1 (10%)	

TABLE D1
Summary of the Incidence of Neoplasms in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Special Senses System				
Harderian gland	(44)	(7)	(10)	(48)
Adenoma	1 (2%)			3 (6%)
Carcinoma				1 (2%)
Histiocytic sarcoma			1 (10%)	
Lymphoma malignant	2 (5%)			
Lacrimal gland	(37)	(6)	(7)	(47)
Lymphoma malignant	2 (5%)			
Zymbal's gland	(41)	(6)	(8)	(46)
Squamous cell carcinoma			1 (13%)	
Urinary System				
Kidney	(47)	(8)	(10)	(48)
Carcinoma, metastatic, uncertain primary site			1 (10%)	
Hepatocholangiocarcinoma, metastatic, liver		1 (13%)		
Histiocytic sarcoma		1 (13%)		
Lymphoma malignant	6 (13%)		1 (10%)	2 (4%)
Urinary bladder	(46)	(5)	(10)	(48)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	3 (7%)		1 (10%)	
Neoplasm Summary				
Total animals with primary neoplasms[b]	24	20	19	25
Total primary neoplasms	107	52	45	45
Total animals with benign neoplasms	11	3	6	7
Total benign neoplasms	12	3	6	8
Total animals with malignant neoplasms	16	18	14	20
Total malignant neoplasms	95	49	38	37
Total animals with metastatic neoplasms		1	2	5
Total metastatic neoplasms		8	6	6
Total animals with uncertain neoplasms-benign or malignant			1	
Total uncertain neoplasms			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	1/44 (2%)	0/7 (0%)	0/10 (0%)	3/48 (6%)
Adjusted rate[b]	2.5%	0.0%	0.0%	6.7%
Terminal rate[c]	0/35 (0%)	0/1 (0%)	0/1 (0%)	2/40 (5%)
First incidence (days)	741	—[e]	—	738
Poly-3 test[d]	(NA)	—[f]	—	P=0.3373
Harderian Gland: Adenoma or Carcinoma				
Overall rate	1/44 (2%)	0/7 (0%)	0/10 (0%)	4/48 (8%)
Adjusted rate	2.5%	0.0%	0.0%	8.9%
Terminal rate	0/35 (0%)	0/1 (0%)	0/1 (0%)	3/40 (8%)
First incidence (days)	741	—	—	738
Poly-3 test	(NA)	—	—	P=0.2061
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	1/48 (2%)	3/48 (6%)	2/48 (4%)	2/48 (4%)
Adjusted rate	2.3%	6.7%	4.4%	4.5%
Terminal rate	1/36 (3%)	2/41 (5%)	1/39 (3%)	2/40 (5%)
First incidence (days)	757 (T)	667	678	757 (T)
Poly-3 test	P=0.5367	P=0.3200	P=0.5141	P=0.5129
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/47 (6%)	1/8 (13%)	1/11 (9%)	1/48 (2%)
Adjusted rate	7.1%	19.0%	12.8%	2.2%
Terminal rate	3/36 (8%)	1/2 (50%)	1/2 (50%)	1/40 (3%)
First incidence (days)	757 (T)	757 (T)	757 (T)	757 (T)
Poly-3 test	(NA)	—	—	P=0.2899N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/47 (6%)	2/8 (25%)	2/11 (18%)	3/48 (6%)
Adjusted rate	7.1%	38.0%	25.6%	6.6%
Terminal rate	3/36 (8%)	2/2 (100%)	2/2 (100%)	1/40 (2%)
First incidence (days)	757 (T)	757 (T)	757 (T)	618
Poly-3 test	(NA)	—	—	P=0.6361N
All Organs: Hemangiosarcoma				
Overall rate	0/48 (0%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	0.0%	2.2%	4.4%	8.7%
Terminal rate	0/36 (0%)	1/41 (2%)	0/39 (0%)	2/40 (5%)
First incidence (days)	—	757 (T)	661	485
Poly-3 test	P=0.0246	P=0.5067N	P=0.2480	P=0.0681
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	1/48 (2%)	1/48 (2%)	2/48 (4%)	4/48 (8%)
Adjusted rate	2.3%	2.2%	4.4%	8.7%
Terminal rate	1/36 (3%)	1/41 (2%)	0/39 (0%)	2/40 (5%)
First incidence (days)	757 (T)	757 (T)	661	485
Poly-3 test	P=0.0765	P=0.7530N	P=0.5167	P=0.1973

TABLE D2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
All Organs: Histiocytic Sarcoma				
Overall rate	1/48 (2%)	3/48 (6%)	4/48 (8%)	0/48 (0%)
Adjusted rate	2.3%	6.6%	8.9%	0.0%
Terminal rate	0/36 (0%)	1/41 (2%)	2/39 (5%)	0/40 (0%)
First incidence (days)	479	593	686	—
Poly-3 test	P=0.2903N	P=0.3193	P=0.1864	P=0.4960N
All Organs: Malignant Lymphoma				
Overall rate	14/48 (29%)	10/48 (21%)	5/48 (10%)	7/48 (15%)
Adjusted rate	32.4%	22.1%	11.0%	15.5%
Terminal rate	12/36 (33%)	9/41 (22%)	3/39 (8%)	6/40 (15%)
First incidence (days)	634	605	664	716
Poly-3 test	P=0.0352N	P=0.2114N	P=0.0140N	P=0.0567N
All Organs: Benign Neoplasms				
Overall rate	11/48 (23%)	3/48 (6%)	7/48 (15%)[g]	7/48 (15%)
Adjusted rate	25.6%	6.7%	15.5%	15.6%
Terminal rate	9/36 (25%)	3/41 (7%)	6/39 (15%)	6/40 (15%)
First incidence (days)	674	757 (T)	678	738
Poly-3 test	P=0.3694N	P=0.0162N	P=0.1923N	P=0.1937N
All Organs: Malignant Neoplasms				
Overall rate	16/48 (33%)	18/48 (38%)	14/48 (29%)	20/48 (42%)
Adjusted rate	36.0%	38.1%	30.0%	42.5%
Terminal rate	12/36 (33%)	13/41 (32%)	7/39 (18%)	14/40 (35%)
First incidence (days)	479	508	655	485
Poly-3 test	P=0.3166	P=0.4825	P=0.3700N	P=0.3169
All Organs: Benign or Malignant Neoplasms				
Overall rate	24/48 (50%)	20/48 (42%)	19/48 (40%)	25/48 (52%)
Adjusted rate	53.6%	42.3%	40.7%	53.0%
Terminal rate	18/36 (50%)	15/41 (37%)	12/39 (31%)	18/40 (45%)
First incidence (days)	479	508	655	485
Poly-3 test	P=0.4079	P=0.2116N	P=0.1708N	P=0.5720

(T)Terminal sacrifice
(NA)Not applicable
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the vehicle controls are not appropriate.
[g] Includes one animal with a neoplasm of uncertain malignancy

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Accidental deaths	2			
Moribund	4	1	6	4
Natural deaths	6	6	3	4
Survivors				
Died last week of study		1	1	
Terminal sacrifice	36	40	38	40
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(45)	(5)	(10)	(45)
Hyperkeratosis	2 (4%)	1 (20%)		
Gallbladder	(41)	(6)	(9)	(46)
Infiltration cellular, lymphocytic	4 (10%)			3 (7%)
Inflammation				2 (4%)
Intestine large, cecum	(43)	(4)	(8)	(45)
Hyperplasia, lymphoid	4 (9%)			
Intestine large, colon	(44)	(5)	(9)	(45)
Hyperplasia, lymphoid	1 (2%)			
Inflammation				1 (2%)
Intestine small, duodenum	(43)	(5)	(9)	(44)
Infiltration cellular, lymphocytic				1 (2%)
Inflammation			1 (11%)	
Intestine small, ileum	(40)	(4)	(7)	(45)
Hyperplasia, lymphoid				3 (7%)
Liver	(48)	(48)	(48)	(48)
Angiectasis		1 (2%)		3 (6%)
Basophilic focus	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Clear cell focus			1 (2%)	
Ectasia, vein				1 (2%)
Eosinophilic focus	1 (2%)	1 (2%)	1 (2%)	
Fibrosis		1 (2%)		
Hematopoietic cell proliferation	4 (8%)	3 (6%)	9 (19%)	7 (15%)
Hyperplasia, bile duct		1 (2%)		
Hyperplasia, Kupffer cell		1 (2%)		
Infiltration cellular, lymphocytic	32 (67%)	36 (75%)	38 (79%)	37 (77%)
Inflammation	1 (2%)			
Mineralization				1 (2%)
Necrosis	34 (71%)	36 (75%)	31 (65%)	33 (69%)
Necrosis, coagulative			1 (2%)	
Regeneration				1 (2%)
Tension lipoidosis	17 (35%)	20 (42%)	10 (21%)	11 (23%)
Thrombus				1 (2%)
Vacuolization cytoplasmic	27 (56%)	36 (75%)	36 (75%)	23 (48%)
Mesentery	(1)			(1)
Necrosis, fat	1 (100%)			1 (100%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Alimentary System (continued)				
Pancreas	(46)	(5)	(10)	(47)
Atrophy			1 (10%)	2 (4%)
Ectasia, duct		1 (20%)		
Focal cellular change	2 (4%)			2 (4%)
Infiltration cellular, lymphocytic	31 (67%)		6 (60%)	30 (64%)
Inflammation	1 (2%)			
Salivary glands	(47)	(7)	(10)	(48)
Atrophy	4 (9%)		1 (10%)	
Infiltration cellular, lymphocytic	38 (81%)	7 (100%)	6 (60%)	45 (94%)
Stomach, forestomach	(45)	(5)	(9)	(46)
Hyperkeratosis	1 (2%)	1 (20%)	1 (11%)	
Hyperplasia		1 (20%)		
Infiltration cellular, lymphocytic				1 (2%)
Stomach, glandular	(45)	(5)	(9)	(46)
Cyst	2 (4%)		1 (11%)	1 (2%)
Infiltration cellular, lymphocytic				1 (2%)
Mineralization		1 (20%)		
Tongue	(46)	(8)	(10)	(48)
Inflammation	1 (2%)			
Cardiovascular System				
Heart	(48)	(8)	(10)	(48)
Degeneration, artery	1 (2%)			
Fibrosis				1 (2%)
Infiltration cellular, lymphocytic				1 (2%)
Endocrine System				
Adrenal gland	(47)	(7)	(10)	(48)
Accessory adrenal cortical nodule			1 (10%)	
Adrenal gland, cortex	(47)	(6)	(10)	(48)
Cyst	1 (2%)			
Focal cellular change	1 (2%)			
Hyperplasia				1 (2%)
Hyperplasia, spindle cell	44 (94%)	5 (83%)	9 (90%)	44 (92%)
Vacuolization cytoplasmic	1 (2%)			
Adrenal gland, medulla	(47)	(6)	(10)	(47)
Focal cellular change	1 (2%)			
Vacuolization cytoplasmic	1 (2%)			
Islets, pancreatic	(46)	(4)	(11)	(47)
Hyperplasia				1 (2%)
Infiltration cellular, lymphocytic	2 (4%)			
Parathyroid gland	(44)	(3)	(5)	(43)
Cyst	1 (2%)			
Ectopic thymus	1 (2%)			
Pituitary gland	(45)	(4)	(9)	(43)
Angiectasis		1 (25%)		1 (2%)
Cyst			1 (11%)	
Ectasia	1 (2%)			
Hyperplasia, pars distalis	4 (9%)	1 (25%)		3 (7%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Thyroid gland	(46)	(5)	(9)	(47)
Cyst, follicle	2 (4%)			2 (4%)
Depletion secretory			1 (11%)	
Goiter adenomatous				1 (2%)
Hyperplasia, follicular cell			1 (11%)	
Infiltration cellular, lymphocytic	4 (9%)			
Inflammation			1 (11%)	
Ultimobranchial cyst	11 (24%)	2 (40%)		14 (30%)
General Body System				
None				
Genital System				
Clitoral gland	(38)	(7)	(8)	(35)
Atrophy	38 (100%)	5 (71%)	7 (88%)	32 (91%)
Inflammation	1 (3%)			
Ovary	(46)	(22)	(30)	(46)
Amyloid deposition			1 (3%)	
Angiectasis				2 (4%)
Atrophy	32 (70%)	3 (14%)	4 (13%)	29 (63%)
Congestion				1 (2%)
Cyst	15 (33%)	9 (41%)	16 (53%)	11 (24%)
Cyst, periovarian tissue	10 (22%)	5 (23%)	9 (30%)	7 (15%)
Hematocyst	4 (9%)	1 (5%)		7 (15%)
Hemorrhage			1 (3%)	
Hyperplasia, adenomatous	1 (2%)			
Hyperplasia, tubular	1 (2%)			
Infiltration cellular, lymphocytic	2 (4%)			1 (2%)
Inflammation		1 (5%)		
Mineralization				1 (2%)
Ovotestis	1 (2%)			1 (2%)
Uterus	(47)	(30)	(35)	(48)
Adenomyosis				1 (2%)
Angiectasis			1 (3%)	
Atrophy	6 (13%)	2 (7%)	4 (11%)	3 (6%)
Dilatation	2 (4%)	2 (7%)	2 (6%)	3 (6%)
Ectasia, vein	1 (2%)			
Fibrosis		2 (7%)	1 (3%)	
Hemorrhage			1 (3%)	
Hyperplasia, atypical				1 (2%)
Hyperplasia, cystic, endometrium	35 (74%)	21 (70%)	24 (69%)	38 (79%)
Hypertrophy, myometrium				1 (2%)
Inflammation		1 (3%)		
Metaplasia, squamous	1 (2%)			
Thrombus	1 (2%)			
Vagina	(44)	(7)	(10)	(46)
Amyloid deposition			1 (10%)	
Atrophy	2 (5%)	2 (29%)	2 (20%)	2 (4%)
Dysplasia			1 (10%)	
Infiltration cellular, lymphocytic	2 (5%)			1 (2%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System				
Bone marrow	(47)	(8)	(10)	(48)
Congestion			1 (10%)	
Hyperplasia	2 (4%)	2 (25%)	4 (40%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)			
Myelofibrosis			1 (10%)	1 (2%)
Lymph node	(47)	(13)	(11)	(48)
Hyperplasia, lymphoid				1 (2%)
Hyperplasia, lymphoid, axillary	1 (2%)			
Hyperplasia, lymphoid, renal				1 (2%)
Lymph node, mandibular	(47)	(8)	(9)	(48)
Atrophy	4 (9%)		1 (11%)	
Hemorrhage	1 (2%)	1 (13%)	1 (11%)	1 (2%)
Hyperplasia, lymphoid	7 (15%)	3 (38%)	2 (22%)	10 (21%)
Infiltration cellular, histiocytic				1 (2%)
Polyarteritis			1 (11%)	
Lymph node, mesenteric	(46)	(10)	(8)	(45)
Atrophy	6 (13%)		2 (25%)	
Hematopoietic cell proliferation			1 (13%)	
Hemorrhage			1 (13%)	
Hyperplasia, lymphoid	3 (7%)			3 (7%)
Infiltration cellular, histiocytic				1 (2%)
Inflammation		1 (10%)		
Spleen	(47)	(18)	(17)	(47)
Amyloid deposition			1 (6%)	
Angiectasis		1 (6%)		
Atrophy		1 (6%)	5 (29%)	
Congestion	5 (11%)			
Hematocyst				1 (2%)
Hematopoietic cell proliferation	6 (13%)	2 (11%)	8 (47%)	7 (15%)
Hyperplasia, lymphoid	16 (34%)	4 (22%)	5 (29%)	18 (38%)
Infarct	1 (2%)			
Infiltration cellular, histiocytic		1 (6%)		
Necrosis	1 (2%)	1 (6%)	1 (6%)	1 (2%)
Polyarteritis			1 (6%)	
Thymus	(36)	(4)	(7)	(40)
Atrophy, cortex	25 (69%)	3 (75%)	4 (57%)	35 (88%)
Ectopic parathyroid gland		1 (25%)		
Hyperplasia, lymphoid, medulla	10 (28%)	1 (25%)	1 (14%)	14 (35%)
Integumentary System				
Mammary gland	(43)	(6)	(11)	(46)
Hyperplasia	2 (5%)	1 (17%)		1 (2%)
Infiltration cellular, lymphocytic	1 (2%)			1 (2%)
Lactation			1 (9%)	12 (26%)
Metaplasia, squamous		1 (17%)		
Musculoskeletal System				
Bone	(47)	(8)	(10)	(48)
Fibrous osteodystrophy, turbinate				10 (21%)
Bone, femur	(47)	(8)	(10)	(48)
Degeneration, cartilage	1 (2%)			
Fibrous osteodystrophy	22 (47%)			5 (10%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Musculoskeletal System (continued)				
Bone, sternum	(45)	(8)	(10)	(48)
Fibrous osteodystrophy, multifocal	35 (78%)	1 (13%)	3 (30%)	38 (79%)
Skeletal muscle	(45)	(8)	(10)	(47)
Infiltration cellular, lymphocytic	1 (2%)			
Nervous System				
Brain, cerebellum	(48)	(8)	(10)	(48)
Mineralization, thalamus	1 (2%)			
Brain, cerebrum	(48)	(8)	(10)	(48)
Hydrocephalus				1 (2%)
Mineralization, multifocal, thalamus	30 (63%)	2 (25%)	3 (30%)	26 (54%)
Peripheral nerve	(44)	(8)	(10)	(46)
Demyelination	1 (2%)			
Spinal cord, thoracic	(48)	(8)	(9)	(48)
Degeneration, axon	1 (2%)			
Infiltration cellular, lymphocytic	1 (2%)			
Respiratory System				
Lung	(47)	(8)	(11)	(48)
Congestion	1 (2%)			
Crystals				1 (2%)
Edema	1 (2%)			
Hemorrhage	1 (2%)			
Hyperplasia, alveolar epithelium				2 (4%)
Infiltration cellular, histiocytic	4 (9%)			6 (13%)
Infiltration cellular, lymphocytic	28 (60%)	4 (50%)	4 (36%)	41 (85%)
Inflammation	4 (9%)			1 (2%)
Metaplasia, osseous			1 (9%)	
Pigmentation, hemosiderin	1 (2%)			
Thrombus				1 (2%)
Nose	(48)	(8)	(10)	(48)
Cytoplasmic alteration, respiratory epithelium	1 (2%)			
Inflammation		1 (13%)		
Special Senses System				
Eye	(42)	(3)	(5)	(45)
Cataract			1 (20%)	
Harderian gland	(44)	(7)	(10)	(48)
Atrophy			1 (10%)	
Ectasia	1 (2%)			
Hyperplasia		1 (14%)		2 (4%)
Infiltration cellular, lymphocytic	21 (48%)	1 (14%)	2 (20%)	19 (40%)
Lacrimal gland	(37)	(6)	(7)	(47)
Atrophy	5 (14%)			
Cytoplasmic alteration			1 (14%)	
Focal cellular change				1 (2%)
Infiltration cellular, lymphocytic	21 (57%)	4 (67%)	6 (86%)	34 (72%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Regimen D Female Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Urinary System				
Kidney	(47)	(8)	(10)	(48)
Accumulation hyaline droplet		1 (13%)	2 (20%)	
Amyloid deposition, glomerulus	1 (2%)		1 (10%)	2 (4%)
Cyst, renal tubule	9 (19%)	3 (38%)	2 (20%)	4 (8%)
Edema				1 (2%)
Glomerulosclerosis	1 (2%)			3 (6%)
Hematopoietic cell proliferation			1 (10%)	
Hydronephrosis			1 (10%)	
Infarct				1 (2%)
Infiltration cellular, lymphocytic	37 (79%)	6 (75%)	9 (90%)	41 (85%)
Nephropathy	2 (4%)			
Pigmentation, renal tubule	2 (4%)			1 (2%)
Vacuolization cytoplasmic, renal tubule		1 (13%)	1 (10%)	
Urinary bladder	(46)	(5)	(10)	(48)
Infiltration cellular, lymphocytic	38 (83%)	4 (80%)	5 (50%)	39 (81%)

APPENDIX E
SUMMARY OF LESIONS IN REGIMEN E MALE MICE IN THE 2-YEAR GAVAGE STUDY OF CHLORAL HYDRATE
(Single Dose on Postnatal Day 15)

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TABLE E1
Summary of the Incidence of Neoplasms in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	1	1		3
Natural deaths	2	6	2	5
Survivors				
Died last week of study				1
Terminal sacrifice	45	41	46	39
Animals examined microscopically	48	48	48	48
Alimentary System				
Intestine large, cecum	(47)	(5)	(1)	(46)
Lymphoma malignant				2 (4%)
Intestine small, duodenum	(47)	(5)	(1)	(47)
Lymphoma malignant			1 (100%)	
Intestine small, ileum	(47)	(5)		(45)
Lymphoma malignant	1 (2%)			
Intestine small, jejunum	(47)	(6)		(45)
Adenocarcinoma		1 (17%)		
Lymphoma malignant				1 (2%)
Liver	(48)	(48)	(48)	(48)
Cholangiocarcinoma		1 (2%)		
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	
Hepatoblastoma	1 (2%)			
Hepatocellular adenoma	16 (33%)	6 (13%)	12 (25%)	8 (17%)
Hepatocellular adenoma, multiple	2 (4%)	2 (4%)		3 (6%)
Hepatocellular carcinoma	9 (19%)	10 (21%)	6 (13%)	11 (23%)
Hepatocellular carcinoma, multiple	1 (2%)			1 (2%)
Hepatocholangiocarcinoma	1 (2%)			
Histiocytic sarcoma	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Lymphoma malignant	4 (8%)	3 (6%)	1 (2%)	2 (4%)
Pancreas	(47)	(6)	(2)	(47)
Lymphoma malignant				1 (2%)
Salivary glands	(47)	(7)	(3)	(48)
Histiocytic sarcoma			1 (33%)	
Lymphoma malignant		1 (14%)		1 (2%)
Stomach, forestomach	(48)	(6)	(1)	(47)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant				1 (2%)
Cardiovascular System				
None				
Endocrine System				
Adrenal gland	(48)	(6)	(2)	(47)
Carcinoma, metastatic, lung				1 (2%)
Adrenal gland, cortex	(48)	(6)	(2)	(47)
Adenoma				1 (2%)
Lymphoma malignant		1 (17%)		1 (2%)
Islets, pancreatic	(47)	(6)	(3)	(47)
Adenoma			1 (33%)	

TABLE E1
Summary of the Incidence of Neoplasms in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Endocrine System (continued)				
Thyroid gland	(48)	(6)	(2)	(47)
Adenoma	2 (4%)			
General Body System				
Tissue NOS				(1)
Lymphoma malignant, thoracic				1 (100%)
Genital System				
Coagulating gland	(48)	(7)	(1)	(46)
Lymphoma malignant				1 (2%)
Prostate	(48)	(6)	(2)	(47)
Lymphoma malignant	1 (2%)	1 (17%)		1 (2%)
Seminal vesicle	(48)	(8)	(1)	(46)
Lymphoma malignant				1 (2%)
Hematopoietic System				
Bone marrow	(48)	(7)	(2)	(48)
Hemangiosarcoma	1 (2%)			
Histiocytic sarcoma				1 (2%)
Lymphoma malignant		1 (14%)		
Lymph node	(48)	(11)	(4)	(47)
Fibrosarcoma, metastatic, axillary, skeletal muscle				1 (2%)
Fibrosarcoma, metastatic, thoracic, skeletal muscle				1 (2%)
Histiocytic sarcoma, lumbar	1 (2%)			
Histiocytic sarcoma, renal	1 (2%)	1 (9%)		
Lymphoma malignant		1 (9%)		
Lymphoma malignant, renal	1 (2%)			1 (2%)
Lymph node, mandibular	(48)	(7)	(2)	(46)
Fibrosarcoma, metastatic, skeletal muscle				1 (2%)
Histiocytic sarcoma	1 (2%)			1 (2%)
Lymphoma malignant	2 (4%)	1 (14%)		1 (2%)
Lymph node, mesenteric	(47)	(7)	(4)	(46)
Histiocytic sarcoma		1 (14%)	1 (25%)	1 (2%)
Lymphoma malignant	2 (4%)	1 (14%)		2 (4%)
Spleen	(46)	(11)	(4)	(47)
Hemangiosarcoma	1 (2%)	1 (9%)		2 (4%)
Histiocytic sarcoma		1 (9%)	1 (25%)	1 (2%)
Lymphoma malignant	4 (9%)	3 (27%)	1 (25%)	2 (4%)
Thymus	(36)	(5)	(1)	(35)
Lymphoma malignant		1 (20%)		2 (6%)
Integumentary System				
Mammary gland	(3)			(4)
Histiocytic sarcoma				1 (25%)

TABLE E1
Summary of the Incidence of Neoplasms in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Integumentary System (continued)				
Skin	(47)	(9)	(3)	(47)
Fibrosarcoma	1 (2%)		1 (33%)	
Hemangioma		1 (11%)		
Hemangiosarcoma	1 (2%)			
Lymphoma malignant	1 (2%)			1 (2%)
Papilloma		1 (11%)		
Sarcoma				1 (2%)
Schwannoma malignant		1 (11%)		
Musculoskeletal System				
Skeletal muscle	(48)	(8)	(2)	(48)
Fibrosarcoma, back				1 (2%)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant		1 (13%)		1 (2%)
Schwannoma malignant		2 (25%)		
Nervous System				
Brain, cerebrum	(47)	(7)	(2)	(48)
Lymphoma malignant		1 (14%)		
Spinal cord, thoracic	(47)	(7)	(2)	(48)
Lymphoma malignant		1 (14%)		
Respiratory System				
Lung	(48)	(8)	(4)	(47)
Alveolar/bronchiolar adenoma	4 (8%)			7 (15%)
Alveolar/bronchiolar carcinoma	4 (8%)		2 (50%)	3 (6%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Cholangiocarcinoma, metastatic, liver		1 (13%)		
Fibrosarcoma, metastatic, skeletal muscle				1 (2%)
Hepatocellular carcinoma, metastatic, liver	3 (6%)	3 (38%)	1 (25%)	2 (4%)
Histiocytic sarcoma	1 (2%)		1 (25%)	1 (2%)
Lymphoma malignant		1 (13%)		1 (2%)
Special Senses System				
Eye	(48)	(7)	(2)	(48)
Lymphoma malignant				1 (2%)
Harderian gland	(48)	(10)	(4)	(48)
Adenoma	4 (8%)	3 (30%)	2 (50%)	
Lymphoma malignant		1 (10%)		
Lacrimal gland	(48)	(7)	(1)	(47)
Lymphoma malignant		1 (14%)		1 (2%)
Urinary System				
Kidney	(48)	(8)	(2)	(47)
Carcinoma, metastatic, lung				1 (2%)
Histiocytic sarcoma	1 (2%)			
Lymphoma malignant	1 (2%)	1 (13%)		2 (4%)
Urinary bladder	(48)	(7)	(2)	(47)
Lymphoma malignant		1 (14%)		

TABLE E1
Summary of the Incidence of Neoplasms in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[b]	36	26	23	32
Total primary neoplasms	75	56	35	75
Total animals with benign neoplasms	26	10	14	17
Total benign neoplasms	28	13	15	19
Total animals with malignant neoplasms	19	18	12	21
Total malignant neoplasms	47	43	20	56
Total animals with metastatic neoplasms	3	4	1	4
Total metastatic neoplasms	3	4	1	8

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE E2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	4/48 (8%)	3/10 (30%)	2/4 (50%)	0/48 (0%)
Adjusted rate[b]	8.5%	46.2%	58.2%	0.0%
Terminal rate[c]	4/45 (9%)	3/3 (100%)	2/2 (100%)	0/40 (0%)
First incidence (days)	757	757 (T)	757 (T)	—[e]
Poly-3 test[d]	(NA)	—[f]	—	P=0.0623N
Liver: Hepatocellular Adenoma				
Overall rate	18/48 (38%)	8/48 (17%)	12/48 (25%)	11/48 (23%)
Adjusted rate	38.0%	18.0%	25.3%	23.5%
Terminal rate	17/45 (38%)	8/41 (20%)	12/46 (26%)	9/40 (23%)
First incidence (days)	709	757 (T)	757 (T)	710
Poly-3 test	P=0.1715N	P=0.0270N	P=0.1338N	P=0.0957N
Liver: Hepatocellular Carcinoma				
Overall rate	10/48 (21%)	10/48 (21%)	6/48 (13%)	12/48 (25%)
Adjusted rate	21.1%	22.2%	12.6%	25.3%
Terminal rate	9/45 (20%)	8/41 (20%)	5/46 (11%)	8/40 (20%)
First incidence (days)	721	634	718	622
Poly-3 test	P=0.4060	P=0.5496	P=0.2030N	P=0.4062
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	24/48 (50%)	17/48 (35%)	18/48 (38%)	21/48 (44%)
Adjusted rate	50.5%	37.8%	37.8%	44.2%
Terminal rate	22/45 (49%)	15/41 (37%)	17/46 (37%)	16/40 (40%)
First incidence (days)	709	634	718	622
Poly-3 test	P=0.4031N	P=0.1527N	P=0.1496N	P=0.3405N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	10/48 (21%)	10/48 (21%)	6/48 (13%)	12/48 (25%)
Adjusted rate	21.1%	22.2%	12.6%	25.3%
Terminal rate	9/45 (20%)	8/41 (20%)	5/46 (11%)	8/40 (20%)
First incidence (days)	721	634	718	622
Poly-3 test	P=0.4060	P=0.5496	P=0.2030N	P=0.4062
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	24/48 (50%)	17/48 (35%)	18/48 (38%)	21/48 (44%)
Adjusted rate	50.5%	37.8%	37.8%	44.2%
Terminal rate	22/45 (49%)	15/41 (37%)	17/46 (37%)	16/40 (40%)
First incidence (days)	709	634	718	622
Poly-3 test	P=0.4031N	P=0.1527N	P=0.1496N	P=0.3405N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/48 (8%)	0/8 (0%)	0/4 (0%)	7/47 (15%)
Adjusted rate	8.5%	0.0%	0.0%	15.2%
Terminal rate	4/45 (9%)	0/1 (0%)	0/2 (0%)	5/39 (13%)
First incidence (days)	757 (T)	—	—	622
Poly-3 test	(NA)	—	—	P=0.2469

TABLE E2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/48 (8%)	0/8 (0%)	2/4 (50%)	4/47 (9%)
Adjusted rate	8.5%	0.0%	58.2%	8.8%
Terminal rate	4/45 (9%)	0/1 (0%)	2/2 (100%)	4/39 (10%)
First incidence (days)	757 (T)	—	757 (T)	757 (T)
Poly-3 test	(NA)	—	—	P=0.6228
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	8/48 (17%)	0/8 (0%)	2/4 (50%)	11/47 (23%)
Adjusted rate	16.9%	0.0%	58.2%	23.9%
Terminal rate	8/45 (18%)	0/1 (0%)	2/2 (100%)	9/39 (23%)
First incidence (days)	757 (T)	—	757 (T)	622
Poly-3 test	(NA)	—	—	P=0.2832
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/48 (4%)	3/48 (6%)	2/48 (4%)	2/48 (4%)
Adjusted rate	4.2%	6.7%	4.2%	4.2%
Terminal rate	2/45 (4%)	3/41 (7%)	2/46 (4%)	0/40 (0%)
First incidence (days)	757 (T)	757 (T)	757 (T)	622
Poly-3 test	P=0.5031N	P=0.4732	P=0.6917N	P=0.6926
All Organs: Malignant Lymphoma				
Overall rate	5/48 (10%)	3/48 (6%)	2/48 (4%)	2/48 (4%)
Adjusted rate	10.5%	6.7%	4.2%	4.3%
Terminal rate	4/45 (9%)	2/41 (5%)	2/46 (4%)	1/40 (2%)
First incidence (days)	607	741	757 (T)	736
Poly-3 test	P=0.1585N	P=0.3946N	P=0.2189N	P=0.2262N
All Organs: Benign Neoplasms				
Overall rate	26/48 (54%)	10/48 (21%)	14/48 (29%)	17/48 (35%)
Adjusted rate	54.9%	22.5%	29.5%	35.9%
Terminal rate	25/45 (56%)	10/41 (24%)	14/46 (30%)	13/40 (33%)
First incidence (days)	709	757 (T)	757 (T)	622
Poly-3 test	P=0.1451N	P=0.0009N	P=0.0093N	P=0.0479N
All Organs: Malignant Neoplasms				
Overall rate	19/48 (40%)	18/48 (38%)	12/48 (25%)	21/48 (44%)
Adjusted rate	39.6%	39.1%	25.0%	44.0%
Terminal rate	16/45 (36%)	13/41 (32%)	10/46 (22%)	14/40 (35%)
First incidence (days)	607	587	632	622
Poly-3 test	P=0.4207	P=0.5636N	P=0.0948N	P=0.4093

TABLE E2
Statistical Analysis of Primary Neoplasms at 2 Years in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	36/48 (75%)	26/48 (54%)	23/48 (48%)	32/48 (67%)
Adjusted rate	75.0%	56.5%	47.9%	67.1%
Terminal rate	33/45 (73%)	21/41 (51%)	21/46 (46%)	25/40 (63%)
First incidence (days)	607	587	632	622
Poly-3 test	P=0.3460N	P=0.0452N	P=0.0048N	P=0.2647N

(T)Terminal sacrifice
(NA)Not applicable
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dose group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus, statistical comparisons with the vehicle controls are not appropriate.

TABLE E3
Summary of the Incidence of Nonneoplastic Lesions in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	48	48	48	48
Early deaths				
Moribund	1	1		3
Natural deaths	2	6	2	5
Survivors				
Died last week of study				1
Terminal sacrifice	45	41	46	39
Animals examined microscopically	48	48	48	48
Alimentary System				
Esophagus	(48)	(7)	(2)	(47)
Inflammation				1 (2%)
Gallbladder	(47)	(6)	(1)	(43)
Calculus microscopic observation only	1 (2%)			
Ectasia				2 (5%)
Infiltration cellular, lymphocytic	6 (13%)			2 (5%)
Inflammation	1 (2%)			1 (2%)
Intestine large, cecum	(47)	(5)	(1)	(46)
Hyperplasia, lymphoid	2 (4%)	1 (20%)		3 (7%)
Hyperplasia, lymphoid tissue	1 (2%)			1 (2%)
Intestine large, colon	(47)	(5)	(1)	(46)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Intestine large, rectum	(45)	(5)	(1)	(45)
Hyperplasia, lymphoid				1 (2%)
Intestine small, ileum	(47)	(5)		(45)
Hyperplasia, lymphoid	3 (6%)			2 (4%)
Intestine small, jejunum	(47)	(6)		(45)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Liver	(48)	(48)	(48)	(48)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Basophilic focus	10 (21%)	2 (4%)	9 (19%)	11 (23%)
Congestion	1 (2%)	1 (2%)		
Cyst, bile duct	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Degeneration				2 (4%)
Eosinophilic focus	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)		1 (2%)
Hyperplasia, ito cell			1 (2%)	
Infarct			2 (4%)	1 (2%)
Infiltration cellular, lymphocytic	11 (23%)	17 (35%)	15 (31%)	3 (6%)
Necrosis	3 (6%)		3 (6%)	6 (13%)
Regeneration	1 (2%)	1 (2%)		1 (2%)
Tension lipoidosis	16 (33%)	13 (27%)	9 (19%)	10 (21%)
Thrombus	1 (2%)	1 (2%)		
Vacuolization cytoplasmic	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Mesentery	(1)			
Infarct	1 (100%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE E3
Summary of the Incidence of Nonneoplastic Lesions in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Alimentary System (continued)				
Pancreas	(47)	(6)	(2)	(47)
Atrophy	2 (4%)			1 (2%)
Basophilic focus				1 (2%)
Focal cellular change	2 (4%)			5 (11%)
Hyperplasia	1 (2%)			
Infiltration cellular, lymphocytic	6 (13%)			6 (13%)
Inflammation	1 (2%)		1 (50%)	1 (2%)
Salivary glands	(47)	(7)	(3)	(48)
Atrophy				1 (2%)
Focal cellular change	1 (2%)			
Infiltration cellular, lymphocytic	43 (91%)	3 (43%)		42 (88%)
Mineralization		1 (14%)		
Stomach, glandular	(47)	(6)	(1)	(47)
Hyperplasia				1 (2%)
Infiltration cellular, lymphocytic	2 (4%)			2 (4%)
Inflammation				2 (4%)
Mineralization				1 (2%)
Tongue	(48)	(7)	(2)	(48)
Infiltration cellular, lymphocytic	1 (2%)			
Cardiovascular System				
Heart	(48)	(7)	(2)	(48)
Atrophy	1 (2%)			
Degeneration	1 (2%)			
Fibrosis		1 (14%)		
Infiltration cellular, lymphocytic	2 (4%)			
Necrosis		1 (14%)		
Pigmentation, valve		1 (14%)		
Endocrine System				
Adrenal gland, cortex	(48)	(6)	(2)	(47)
Atrophy	1 (2%)			
Clear cell focus	3 (6%)			4 (9%)
Congestion	1 (2%)			
Focal cellular change	1 (2%)			2 (4%)
Hyperplasia	11 (23%)			10 (21%)
Hyperplasia, spindle cell	43 (90%)	5 (83%)		42 (89%)
Necrosis				1 (2%)
Vacuolization cytoplasmic		1 (17%)		
Adrenal gland, medulla	(47)	(6)	(2)	(46)
Fibrosis				2 (4%)
Hyperplasia	7 (15%)			13 (28%)
Necrosis				1 (2%)
Parathyroid gland	(43)	(6)	(2)	(46)
Cyst				2 (4%)
Ectopic thymus				1 (2%)
Pituitary gland	(44)	(5)	(1)	(36)
Cyst	1 (2%)			
Hyperplasia, pars distalis	3 (7%)			2 (6%)
Thyroid gland	(48)	(6)	(2)	(47)
Hypertrophy, follicle		1 (17%)		
Ultimobranchial cyst	1 (2%)	1 (17%)		

TABLE E3
Summary of the Incidence of Nonneoplastic Lesions in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
General Body System				
None				
Genital System				
Coagulating gland	(48)	(7)	(1)	(46)
Atrophy				1 (2%)
Infiltration cellular, lymphocytic	3 (6%)			
Epididymis	(48)	(6)	(2)	(48)
Granuloma sperm	1 (2%)			
Infiltration cellular, lymphocytic	2 (4%)	1 (17%)		1 (2%)
Inflammation		1 (17%)		
Mineralization	1 (2%)			
Penis		(1)		
Cyst		1 (100%)		
Preputial gland	(47)	(10)	(6)	(46)
Atrophy	2 (4%)			1 (2%)
Cyst	4 (9%)	5 (50%)	4 (67%)	5 (11%)
Cyst, multiple		1 (10%)		
Ectasia	30 (64%)	4 (40%)	2 (33%)	26 (57%)
Infiltration cellular, lymphocytic	1 (2%)		1 (17%)	4 (9%)
Inflammation	1 (2%)		1 (17%)	2 (4%)
Necrosis, fat			1 (17%)	
Prostate	(48)	(6)	(2)	(47)
Fibrosis				1 (2%)
Infiltration cellular, lymphocytic	16 (33%)			10 (21%)
Inflammation				1 (2%)
Seminal vesicle	(48)	(8)	(1)	(46)
Atrophy				1 (2%)
Ectasia		1 (13%)		
Testes	(48)	(7)	(2)	(48)
Degeneration		1 (14%)		1 (2%)
Infiltration cellular, lymphocytic				1 (2%)
Mineralization				2 (4%)
Hematopoietic System				
Bone marrow	(48)	(7)	(2)	(48)
Atrophy	1 (2%)			
Congestion				1 (2%)
Hyperplasia	4 (8%)			4 (8%)
Pigmentation	1 (2%)	2 (29%)		3 (6%)
Lymph node, mandibular	(48)	(7)	(2)	(46)
Hematopoietic cell proliferation				1 (2%)
Hyperplasia, lymphoid				2 (4%)
Infiltration cellular, lymphocytic	4 (8%)			
Lymph node, mesenteric	(47)	(7)	(4)	(46)
Angiectasis		1 (14%)		
Congestion	4 (9%)		1 (25%)	2 (4%)
Hematopoietic cell proliferation	3 (6%)			1 (2%)
Hemorrhage	10 (21%)	4 (57%)	1 (25%)	20 (43%)
Hyperplasia, lymphoid			1 (25%)	1 (2%)
Infiltration cellular, lymphocytic	2 (4%)			
Inflammation				2 (4%)

TABLE E3
Summary of the Incidence of Nonneoplastic Lesions in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Spleen	(46)	(11)	(4)	(47)
Angiectasis				1 (2%)
Atrophy	1 (2%)			2 (4%)
Congestion	2 (4%)			1 (2%)
Cyst	1 (2%)			
Hematopoietic cell proliferation	8 (17%)	5 (45%)	1 (25%)	10 (21%)
Hyperplasia, lymphoid	5 (11%)		1 (25%)	6 (13%)
Infarct				2 (4%)
Thymus	(36)	(5)	(1)	(35)
Atrophy, cortex			1 (100%)	1 (3%)
Cyst	1 (3%)			1 (3%)
Hyperplasia, lymphoid, medulla				1 (3%)
Infiltration cellular, histiocytic				1 (3%)
Necrosis		1 (20%)		
Pigmentation		1 (20%)		
Integumentary System				
Skin	(47)	(9)	(3)	(47)
Atrophy, subcutaneous tissue		1 (11%)	1 (33%)	
Musculoskeletal System				
Bone, femur	(48)	(7)	(2)	(48)
Infiltration cellular, lymphocytic	1 (2%)			
Bone, sternum	(48)	(7)	(2)	(48)
Fibrous osteodystrophy, multifocal	1 (2%)	1 (14%)		
Skeletal muscle	(48)	(8)	(2)	(48)
Infiltration cellular, lymphocytic	2 (4%)			1 (2%)
Nervous System				
Brain, cerebrum	(47)	(7)	(2)	(48)
Hemorrhage		1 (14%)		
Infiltration cellular, lymphocytic				1 (2%)
Mineralization, multifocal, thalamus	25 (53%)	3 (43%)	1 (50%)	13 (27%)
Peripheral nerve	(47)	(7)	(2)	(48)
Infiltration cellular, lymphocytic	1 (2%)			
Spinal cord, thoracic	(47)	(7)	(2)	(48)
Cyst, meninges	1 (2%)			
Respiratory System				
Lung	(48)	(8)	(4)	(47)
Congestion	3 (6%)			1 (2%)
Cyst	1 (2%)			
Hemorrhage	1 (2%)	1 (13%)		
Hyperplasia, alveolar epithelium	11 (23%)			7 (15%)
Infiltration cellular, histiocytic	1 (2%)	1 (13%)		3 (6%)
Infiltration cellular, lymphocytic	42 (88%)	3 (38%)		37 (79%)
Inflammation	3 (6%)	1 (13%)		
Mineralization	1 (2%)			

TABLE E3
Summary of the Incidence of Nonneoplastic Lesions in Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Respiratory System (continued)				
Nose	(48)	(7)	(2)	(48)
Infiltration cellular, lymphocytic				1 (2%)
Inflammation				2 (4%)
Special Senses System				
Eye	(48)	(7)	(2)	(48)
Inflammation, cornea		1 (14%)		
Harderian gland	(48)	(10)	(4)	(48)
Degeneration	1 (2%)			
Hyperplasia	1 (2%)			
Infiltration cellular, lymphocytic	25 (52%)	3 (30%)	2 (50%)	29 (60%)
Inflammation				1 (2%)
Lacrimal gland	(48)	(7)	(1)	(47)
Atrophy	6 (13%)			2 (4%)
Focal cellular change	1 (2%)			
Infiltration cellular, lymphocytic	18 (38%)	1 (14%)		8 (17%)
Vacuolization cytoplasmic				1 (2%)
Zymbal's gland	(44)	(4)	(2)	(45)
Infiltration cellular, lymphocytic	3 (7%)			
Inflammation				1 (2%)
Urinary System				
Kidney	(48)	(8)	(2)	(47)
Congestion		1 (13%)		2 (4%)
Cyst, renal tubule	1 (2%)			
Focal cellular change				1 (2%)
Infarct				1 (2%)
Infiltration cellular, lymphocytic	45 (94%)	4 (50%)	1 (50%)	42 (89%)
Infiltration cellular, plasma cell				1 (2%)
Inflammation		1 (13%)		1 (2%)
Mineralization	2 (4%)			1 (2%)
Nephropathy	6 (13%)		2 (100%)	4 (10%)
Pigmentation, renal tubule				1 (2%)
Vacuolization cytoplasmic	1 (2%)			
Urinary bladder	(48)	(7)	(2)	(47)
Ectasia		1 (14%)		
Infiltration cellular, lymphocytic	28 (58%)	1 (14%)		22 (47%)
Inflammation		1 (14%)		1 (2%)

APPENDIX F
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983). Chloral hydrate was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of chloral hydrate. The high dose was limited by experimental design to 10,000 µg/plate. All positive assays were repeated under the conditions that elicited the positive response.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Galloway *et al.* (1987). Chloral hydrate was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of chloral hydrate; the high dose was limited by toxicity. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with chloral hydrate in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing chloral hydrate was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with chloral hydrate, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no chloral hydrate, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9. All slides were scored blind and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level. Because significant chemical-induced cell cycle delay was seen at some of the higher doses tested, incubation time for these cultures was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one

dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with chloral hydrate for 18.5 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with chloral hydrate and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test: because cell cycle delay was anticipated in the absence of S9, the incubation period for these cultures was extended.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. One hundred first-division metaphase cells were scored at most dose levels; occasionally, when a high percentage of aberrant cells was present in the culture, fewer cells were scored. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Chromosomal aberration data are presented as the percentage of cells with aberrations. To arrive at a statistical decision, analyses were conducted on both the dose-response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) was considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend, in the absence of a statistically significant increase at any one dose resulted in an equivocal response (Galloway *et al.,* 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Yoon *et al.* (1985). Chloral hydrate was supplied as a coded aliquot from Radian Corporation. It was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because a clearly positive response was not obtained, chloral hydrate was retested by injection into adult males.

To administer chloral hydrate by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anaesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of chloral hydrate at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on an aqueous solution of chloral hydrate in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of chloral hydrate dissolved in saline and allowed to recover for 24 hours. A concurrent saline control group was also included.

Treated males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings was treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason *et al*., 1992) using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

MOUSE BONE MARROW MICRONUCLEUS TEST PROTOCOL

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by chloral hydrate exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male $B6C3F_1$ mice were injected intraperitoneally three times at 24-hour intervals with chloral hydrate dissolved in phosphate-buffered saline. Solvent control animals were injected with phosphate-buffered saline only. The positive control mice received injections of cyclophosphamide. The mice were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of four or five animals per dose group.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of dose groups. A final determination of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final decision is made by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitude of those effects.

EVALUATION PROTOCOL

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

Chloral hydrate gave positive responses in both *in vitro* and *in vivo* mutagenicity assays. It induced mutations in *Salmonella typhimurium* strain TA100, with and without liver S9 activation enzymes; an equivocal response was obtained in *S. typhimurium* strain TA98 in the absence of S9; and no mutagenicity was detected with strain TA1535 or TA1537, with or without S9 (Table F1; Haworth *et al.*, 1983). In addition to gene mutations in bacterial cells, chloral hydrate was shown to produce chromosomal damage in mammalian cells. It induced significant increases in SCEs (Table F2) and Abs (Table F3) in cultured CHO cells, with and without S9. Results of SLRL tests in *D. melanogaster* were inconclusive (Table F4; Yoon *et al.*, 1985). Chloral hydrate, administered by feeding in 5% sucrose, produced only a small increase in SLRL mutations in the germ cells of male flies; this result was considered inconclusive. A second SLRL assay that used injection as the route of administration gave negative results.

An *in vivo* mammalian mutagenicity study, a mouse bone marrow micronucleus test, was performed with chloral hydrate (Table F5). In this test, male $B6C3F_1$ mice injected with 125 to 500 mg/kg showed a significant dose-related trend in the frequency of micronucleated erythrocytes in bone marrow sampled 24 hours after treatment. Thus, chloral hydrate gave positive responses in both *in vivo* and *in vitro* assays for chromosomal damage.

TABLE F1
Mutagenicity of Chloral Hydrate in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b]					
		−S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100							
	0	134 ± 7.4	125 ± 8.7	139 ± 2.4	125 ± 5.8	126 ± 5.8	142 ± 5.5
	100	141 ± 3.9	163 ± 6.6	161 ± 1.3	153 ± 3.9	149 ± 4.4	159 ± 2.3
	333	197 ± 16.3		255 ± 7.6		200 ± 11.8	
	1,000	306 ± 6.0	297 ± 1.2	366 ± 17.5	351 ± 19.3	327 ± 4.2	306 ± 3.5
	3,333	423 ± 17.1	422 ± 12.7	530 ± 8.2	524 ± 20.0	494 ± 9.0	495 ± 3.4
	4,000		456 ± 5.6				
	5,000		501 ± 1.0		584 ± 22.5		565 ± 12.9
	6,667	468 ± 17.4					
	7,500		518 ± 7.2				
	10,000			689 ± 37.8	632 ± 12.8	704 ± 66.8	650 ± 8.6
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		1,894 ± 86.8	2,025 ± 20.2	1,431 ± 42.4	2,068 ± 92.8	1,485 ± 83.6	1,612 ± 23.0
TA1535							
	0	17 ± 3.2		12 ± 5.2		10 ± 3.1	
	100	19 ± 0.6		10 ± 2.0		15 ± 4.6	
	333	19 ± 1.7		14 ± 0.7		7 ± 0.9	
	1,000	18 ± 1.5		12 ± 0.7		11 ± 1.0	
	3,333	15 ± 1.2		13 ± 0.9		15 ± 1.0	
	6,667	13 ± 2.9					
	10,000			9 ± 0.6		11 ± 1.5	
Trial summary		Negative		Negative		Negative	
Positive control		1,156 ± 37.8		126 ± 9.6		109 ± 4.0	
TA1537							
	0	7 ± 1.2		7 ± 1.5		6 ± 1.5	
	100	9 ± 2.5		7 ± 1.3		8 ± 0.9	
	333	5 ± 0.6		5 ± 1.2		7 ± 1.2	
	1,000	11 ± 1.8		6 ± 0.6		7 ± 0.6	
	3,333	11 ± 1.8		9 ± 1.3		5 ± 0.7	
	6,667	12 ± 2.9					
	10,000			4 ± 0.3		5 ± 0.9	
Trial summary		Negative		Negative		Negative	
Positive control		478 ± 54.9		133 ± 4.0		124 ± 12.8	

TABLE F1
Mutagenicity of Chloral Hydrate in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA98							
	0	21 ± 2.0	22 ± 3.7	29 ± 2.0	31 ± 3.2	30 ± 0.9	37 ± 4.7
	100	20 ± 6.0	21 ± 2.3	24 ± 2.3	26 ± 1.7	29 ± 2.9	29 ± 1.5
	333	21 ± 0.7		33 ± 1.5		31 ± 3.6	
	1,000	31 ± 0.3	27 ± 2.5	28 ± 5.0	32 ± 2.3	30 ± 1.2	38 ± 4.7
	3,333	46 ± 5.6	42 ± 4.0	42 ± 5.2	43 ± 0.6	39 ± 0.6	45 ± 3.7
	4,000		43 ± 1.5				
	5,000		41 ± 3.1		45 ± 1.3		46 ± 1.5
	6,667	39 ± 4.1					
	7,500		27 ± 6.9[d]				
	10,000			27 ± 4.6	43 ± 2.8	31 ± 2.3	38 ± 1.7
Trial summary		Equivocal	Equivocal	Negative	Equivocal	Negative	Negative
Positive control		1,526 ± 14.2	1,738 ± 33.8	1,345 ± 157.3	1,775 ± 14.9	1,245 ± 89.2	1,476 ± 27.3

[a] Study was performed at EG&G Mason. The detailed protocol and these data are presented by Haworth *et al.* (1983). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[d] Slight toxicity

TABLE F2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Chloral Hydrate[a]

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
−S9								
Trial 1								
Summary: Weakly positive								
Dimethylsulfoxide[c]		50	1,039	488	0.46	9.8	26.0	
Chloral hydrate	16.7	50	1,017	463	0.45	9.3	26.0	−3.07
	50.0	50	1,040	520	0.50	10.4	26.0	6.46
	167.0	50	1,042	747	0.71	14.9	26.0	52.63*
	500.0	0					26.0	
					P<0.001[d]			
Mitomycin-C[e]	0.001	50	1,040	635	0.61	12.7	26.0	30.00
	0.010	5	105	267	2.54	53.4	26.0	441.40
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,041	415	0.39	8.3	26.0	
Chloral hydrate	100	50	1,023	593	0.57	11.9	26.0	45.41*
	150	50	1,032	625	0.60	12.5	26.0	51.92*
	200	50	1,033	831	0.80	16.6	34.8[f]	101.79*
	350	0					34.8[f]	
					P<0.001			
Mitomycin-C	0.001	50	1,035	515	0.49	10.3	26.0	24.82
	0.010	5	103	196	1.90	39.2	26.0	377.34

TABLE F2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Chloral Hydrate

Compound	Dose (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Weakly positive								
Dimethylsulfoxide		50	1,036	464	0.44	9.3	26.0	
Chloral hydrate	167	50	1,037	391	0.37	7.8	26.0	−15.82
	500	50	1,017	386	0.37	7.7	26.0	−15.26
	1,700	50	1,040	567	0.54	11.3	26.0	21.73*
	5,000	0					26.0	
					P<0.001			
Cyclophosphamide[e]	0.4	50	1,042	651	0.62	13.0	26.0	39.50
	2.0	5	105	194	1.84	38.8	26.0	312.53
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,040	365	0.35	7.3	26.0	
Chloral hydrate	3,000	50	1,030	504	0.48	10.1	26.0	39.42*
	4,000	50	1,033	587	0.56	11.7	26.0	61.91*
	5,000	50	1,036	712	0.68	14.2	34.8[f]	95.82*
					P<0.001			
Cyclophosphamide	0.4	50	1,020	629	0.61	12.6	26.0	75.71
	2.0	5	105	162	1.54	32.4	26.0	339.61

* Positive response (≥20% increase over solvent control)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Solvent control
[d] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[e] Positive control
[f] Because chloral hydrate induced a delay in the cell division cycle, harvest time was extended to maximize the number of second-division metaphase cells available for analysis.

TABLE F3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Chloral Hydrate[a]

−S9					+S9				
Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (µg/mL)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 - Harvest time: 20.5 hours[b] Summary: Negative					**Trial 1** - Harvest time: 12.5 Summary: Positive				
Dimethylsulfoxide[c]					Dimethylsulfoxide				
	100	3	0.03	2.0		100	1	0.01	1.0
Chloral hydrate					Chloral hydrate				
400	100	2	0.02	2.0	3,000	100	30	0.30	24.0*
600	100	0	0.00	0.0	3,500	50	10	0.20	20.0*
800	100	3	0.03	3.0	4,000	25	8	0.32	24.0*
						4,500	0		
				P=0.473 [d]					P<0.001
Mitomycin-C[e]					Cyclophosphamide[e]				
0.0400	100	23	0.23	13.0	7.5	100	14	0.14	11.0
0.0625	25	28	1.12	36.0	37.5	25	14	0.56	28.0
Trial 2 - Harvest time: 20.5 hours[b] Summary: Positive					**Trial 2** - Harvest time: 12.5 hours Summary: Weakly positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	3	0.03	3.0
Chloral hydrate					Chloral hydrate				
750	100	6	0.06	6.0	2,500	100	9	0.09	7.0
1,000	100	23	0.23	18.0*	3,000	100	46	0.46	33.0*
1,250	100	57	0.57	36.0*	3,500	0			
1,500	0								
				P<0.001					P<0.001
Mitomycin-C					Cyclophosphamide				
0.0400	100	7	0.07	7.0	7.5	100	13	0.13	10.0
0.0625	25	15	0.60	48.0	37.5	25	13	0.52	32.0

* Positive ($P \leq 0.05$)
[a] Study was performed at Litton Bionetics, Inc. The detailed protocol is presented by Galloway *et al*. (1987). Abs=aberrations
[b] Because chloral hydrate induced a delay in the cell division cycle, incubation time prior to addition of Colcemid was lengthened to provide sufficient first-division metaphase cells at harvest.
[c] Solvent control
[d] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[e] Positive control

TABLE F4
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Chloral Hydrate[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			Total[b]
				Mating 1	Mating 2	Mating 3	
Feeding	5,500	6	0	4/2,140	2/2,315	3/2,287	9/6,742 (0.13%)
	0			1/2,167	1/2,362	1/2,260	3/6,789 (0.04%)
Injection	10,000	10	0	0/1,910	2/2,394	1/2,183	3/6,487 (0.05%)
	0			2/1,370	0/3,003	1/2,120	3/6,493 (0.05%)

[a] Study was performed at Brown University. The detailed protocol and these data are presented by Yoon *et al*. (1985). Significance of total number of lethal mutations/total number of X chromosomes was tested by a normal approximation to the binomial test (Margolin *et al.*, 1983). Results of the feeding experiment were inconclusive. Results of the injection experiment were negative at the 5% level.
[b] Total number of lethal mutations/total number of X chromosomes tested for three mating trials

TABLE F5
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Chloral Hydrate by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs[b]
Trial 1			
Phosphate-buffered saline[c]		4	2.9 ± 0.5
Chloral hydrate	125	5	2.1 ± 0.5
	250	5	2.7 ± 0.6
	500	5	4.4 ± 0.8
			P=0.006[d]
Cyclophosphamide[e]	15	4	19.1 ± 2.2
Trial 2			
Phosphate-buffered saline		5	1.7 ± 0.3
Chloral hydrate	125	5	2.2 ± 0.5
	250	5	2.1 ± 0.3
	500	5	3.5 ± 0.5
			P=0.004
Cyclophosphamide	15	5	17.4 ± 1.7

[a] Study was performed at Oak Ridge Associated Universities. The detailed protocol is presented by Shelby *et al*. (1993). PCE=polychromatic erythrocyte. 2,000 PCEs were scored for each animal in each group.
[b] Mean ± standard error
[c] Solvent control
[d] Significance of micronucleated PCEs/1,000 cells PCEs by the one-tailed trend test (ILS, 1990); significant at $P \leq 0.025$
[e] Positive control

APPENDIX G
LIVER WEIGHTS
AND LIVER-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE G1 **Liver Weights and Liver-Weight-to-Body-Weight Ratios for Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate** **176**
TABLE G2 **Liver Weights and Liver-Weight-to-Body-Weight Ratios at 2 Years for Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate** **176**
TABLE G3 **Liver Weights and Liver-Weight-to-Body-Weight Ratios at 2 Years for Regimen C Female Mice, Regimen D Female Mice, and Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate** **177**

TABLE G1
Liver Weights and Liver-Weight-to-Body-Weight Ratios for Regimen A Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
n	37	38	43	36
Necropsy body wt	32.4 ± 0.8	32.8 ± 0.6	32.0 ± 0.7	31.8 ± 0.6
Liver				
Absolute	1.408 ± 0.037	1.458 ± 0.037	1.395 ± 0.028	1.536 ± 0.056*
Relative	0.044 ± 0.001	0.045 ± 0.001	0.044 ± 0.001	0.048 ± 0.001*

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Liver weights (absolute weights) and body weights are given in grams; liver-weight-to-body-weight ratios (relative weights) are given as g liver weight/g body weight (mean ± standard error).

TABLE G2
Liver Weights and Liver-Weight-to-Body-Weight Ratios at 2 Years for Regimen B 100 mg/kg Female Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control (Regimen A)	3 Months (Stop-Exposure)	6 Months (Stop-Exposure)	12 Months (Stop-Exposure)
n	37	34	31	33
Necropsy body wt	32.4 ± 0.8	34.9 ± 0.7**	35.6 ± 1.0**	34.6 ± 0.7**
Liver				
Absolute	1.408 ± 0.037	1.584 ± 0.071**	1.555 ± 0.073*	1.502 ± 0.051
Relative	0.044 ± 0.001	0.046 ± 0.003	0.044 ± 0.002	0.044 ± 0.001

* Significantly different (P≤0.05) from the control group by Dunnett's test
** P≤0.01
[a] Liver weights (absolute weights) and body weights are given in grams; liver-weight-to-body-weight ratios (relative weights) are given as g liver weight/g body weight (mean ± standard error).

TABLE G3
Liver Weights and Liver-Weight-to-Body-Weight Ratios at 2 Years for Regimen C Female Mice, Regimen D Female Mice, and Regimen E Male Mice in the 2-Year Gavage Study of Chloral Hydrate[a]

	Vehicle Control	10 mg/kg	25 mg/kg	50 mg/kg
Regimen C (single dose on postnatal day 28)				
n	42	40	45	40
Necropsy body wt	38.5 ± 1.1	36.1 ± 0.9	37.0 ± 0.9	39.3 ± 0.9
Liver				
Absolute	1.654 ± 0.062	1.579 ± 0.053	1.527 ± 0.039	1.645 ± 0.043
Relative	0.044 ± 0.002	0.044 ± 0.002	0.042 ± 0.001	0.043 ± 0.002
Regimen D (single dose on postnatal day 15)				
n	36	40	38	40
Necropsy body wt	34.6 ± 0.7	34.5 ± 0.7	35.1 ± 0.9	35.9 ± 0.8
Liver				
Absolute	1.565 ± 0.083	1.551 ± 0.072	1.470 ± 0.034	1.563 ± 0.057
Relative	0.045 ± 0.002	0.046 ± 0.003	0.043 ± 0.001	0.044 ± 0.002
Regimen E (single dose on postnatal day 15)				
n	45	41	46	39
Necropsy body wt	38.2 ± 0.6	36.2 ± 0.5*	35.9 ± 0.6*	35.7 ± 0.6*
Liver				
Absolute	2.174 ± 0.130	2.037 ± 0.122	1.895 ± 0.086	2.194 ± 0.172
Relative	0.059 ± 0.005	0.058 ± 0.004	0.054 ± 0.003	0.064 ± 0.006

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Liver weights (absolute weights) and body weights are given in grams; liver-weight-to-body-weight ratios (relative weights) are given as g liver weight/g body weight (mean ± standard error).

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF CHLORAL HYDRATE 180
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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF CHLORAL HYDRATE

Chloral hydrate was obtained from Sigma Chemical Company (St. Louis, MO) in one lot (12H0289), which was used during the 2-year study. Identity, purity, and stability analyses were conducted by the study laboratory.

Lot 12H0289, a white, crystalline solid, was identified as chloral hydrate by gas chromatography/mass spectrometry by system A (Table H1) and proton nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of chloral hydrate; the mass spectra were also consistent with the literature spectra (*NIST Spectra*) of chloral hydrate. The spectra are presented in Figures H1 and H2.

The purity of the lot 12H0289 was determined by gas chromatography (system B). Samples from each of the two bottles of lot 12H0289 received from the manufacturer were analyzed. One impurity peak was detected for each sample. For one sample, the impurity peak had an area of approximately 0.5% relative to the major peak area; the relative area of the impurity peak for the second sample was too small to be quantified. The results indicated a purity of approximately 99.5% or greater.

The bulk chemical was stored in the original amber glass bottles at room temperature, protected from light, in a container of dry indicating silica gel. Stability was monitored during the 2-year study with gas chromatography/mass spectrometry (system A) and with gas chromatography (system B). No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing chloral hydrate with distilled deionized water to give the required concentrations (Table H2). The dose formulations were prepared monthly and stored for up to 4 weeks at room temperature in amber glass bottles with Teflon®-lined caps, protected from light.

Stability studies of the 0.85, 1.4, and 2.5 mg/mL dose formulations were performed by the study laboratory using gas chromatography by system C. Stability was confirmed for 24 days for the 0.85 mg/mL formulation, 78 days for the 2.5 mg/mL formulation, and 7 months for the 1.4 mg/mL formulation when stored in amber glass vials at room temperature.

Periodic analyses of the dose formulations were conducted by the study laboratory with gas chromatography (system C). During the 2-year study, the dose formulations were analyzed approximately every 3 months, and animal room samples were analyzed every 6 months (Table H3). Of the 42 dose formulations analyzed, all were within 10% of the target concentrations, with no value greater than 109% of the target concentration. Of the animal room samples analyzed, 69 of 78 were within 10% of the target concentrations. Samples outside the 10% range were those obtained from the first sample collection. By the third collection, all samples were within the required specification. Animals were dosed after a minimum of three samples had been drawn through the dosing machine.

TABLE H1
Gas Chromatography Systems Used in the Gavage Study of Chloral Hydrate[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometer with electron impact ionization (70 eV)	DB-5, 30 m × 0.25 mm, 0.25 µm film (J&W Scientific, Folsom, CA)	Helium at 1 mL/minute	30° C for 1 minute, then 50° C/minute to 225° C, held for 6 minutes
System B			
Flame ionization	DB-1701, 30 m × 0.25 mm, 0.25 µm film (J&W Scientific)	Helium at approximately 0.8 mL/minute	40° C for 5 minutes, then 20° C/minute to 150° C, held for 5 minutes
System C			
Flame ionization	GP 60/80 Carbopak-B, 5% Carbowax 20 M, 6 feet × 2 mm (Supelco, Bellefonte, PA)	Helium or nitrogen at 20 mL/minute	110° C isothermal

[a] The gas chromatographs were manufactured by Varian, Inc. (Palo Alto, CA) (system A) or Hewlett-Packard (Palo Alto, CA) (systems B and C).

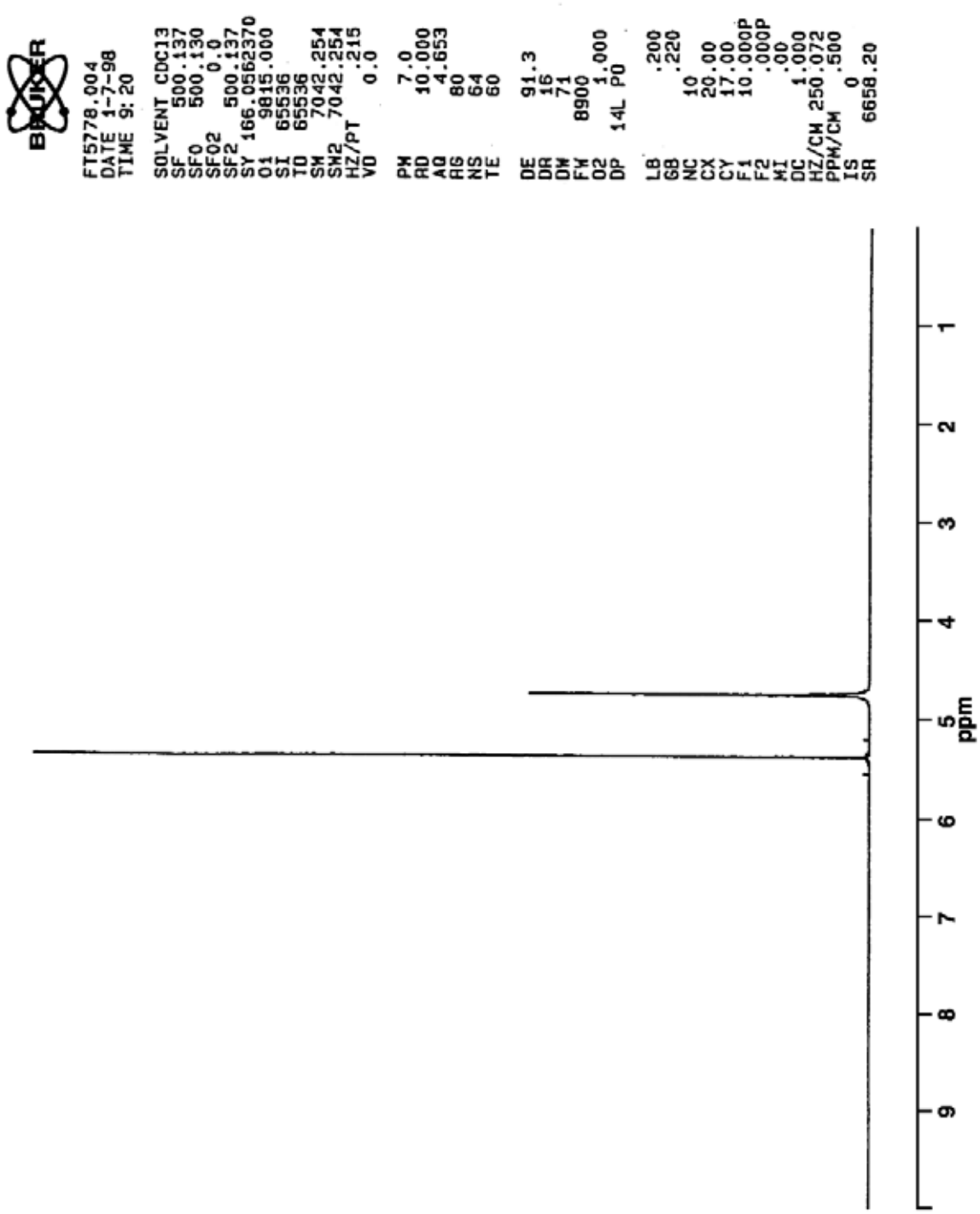

FIGURE H1
Mass Spectrum of Chloral Hydrate

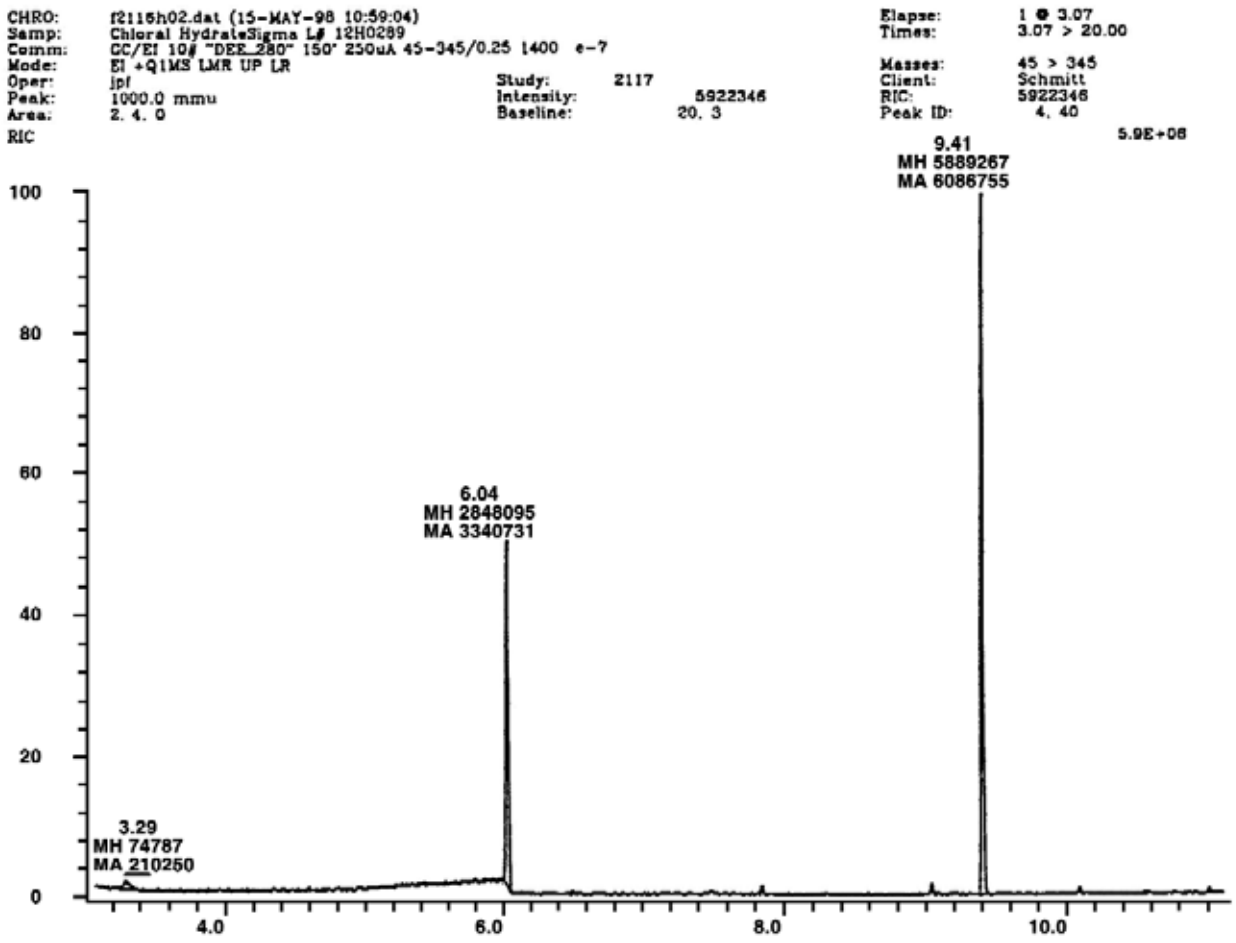

FIGURE H2
Proton Nuclear Magnetic Resonance Spectrum of Chloral Hydrate

TABLE H2
Preparation and Storage of Dose Formulations in the Gavage Study of Chloral Hydrate

Preparation
The dosing vehicle was prepared by mixing chloral hydrate with distilled water. The mixing cylinders were inverted several times by hand, and the dose formulations were then diluted to the proper volume with additional distilled water. The dose formulations were prepared every 4 weeks for regimens A and B and once for regimens C, D, and E.

Chemical Lot Number
12H0289

Maximum Storage Time
4 weeks

Storage Conditions
Stored in amber glass bottles at room temperature, protected from light

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

TABLE H3
Results of Analyses of Dose Formulations Administered to Mice in the Gavage Study of Chloral Hydrate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration[a] (mg/mL)	Difference from Target (%)
Regimens A and B				
6 November 1995	8 November 1995	2.5	2.711	+8
		2.5	2.716	+8
		5.0	5.119	+2
		5.0	5.065	+1
		10.0	9.962	0
		10.0	9.937	−1
20 December 1995	28 December 1995[b]	1.225	0.880	−28
		1.225	1.064	−13
		1.225	1.135	−7
		1.41	1.393	−1
		1.41	1.326	−6
		1.41	1.405	0
		4.76	5.006	+5
		4.76	4.784	+1
		4.76	4.869	+2
		5.46	5.675	+4
		5.46	5.386	−1
		5.46	5.677	+4
12 February 1996	20 February 1996	2.5	2.496	0
		2.5	2.463	−1
		5.0	5.051	+1
		5.0	5.029	+1
		10.0	10.16	+2
		10.0	9.902	−1
7 May 1996	16 May 1996	2.5	2.518	+1
		2.5	2.450	−2
		5.0	4.876	−2
		5.0	5.009	0
		10.0	10.09	+1
		10.0	9.839	−2
7 May 1996	16 May 1996[b]	1.27	0.707	−44
		1.27	1.105	−13
		1.27	1.142	−10
		1.73	1.942	+12
		1.73	1.685	−3
		1.73	1.679	−3
		5.42	6.792	+25
		5.42	5.389	−1
		5.42	5.382	−1
		5.48	6.672	+22
		5.48	5.678	+4
		5.48	5.581	+2

TABLE H3
Results of Analyses of Dose Formulations Administered to Mice in the Gavage Study of Chloral Hydrate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
30 July 1996	8 August 1996	2.5	2.506	0
		2.5	2.481	−1
		5.0	5.100	+2
		5.0	4.995	0
		10.0	9.928	−1
		10.0	9.968	0
2 July 1996	19 July 1996[b]	1.415	1.417	0
		1.415	1.409	0
		1.415	1.392	−2
		1.76	1.874	+6
		1.76	1.749	−1
		1.76	1.731	−2
		5.50	6.459	+17
		5.50	5.489	0
		5.50	5.544	+1
		5.96	5.886	−1
		5.96	5.950	0
		5.96	5.869	−2
23 October 1996	15 November 1996[b]	1.30	1.265	−3
		1.30	1.219	−6
		1.30	1.239	−5
		1.67	1.604	−4
		1.67	1.585	−5
		1.67	1.598	−4
		4.59	4.601	0
		4.59	4.526	−1
		4.59	4.464	−3
		5.48	5.337	−3
		5.48	5.306	−3
		5.48	5.291	−3
18 November 1996	21 November 1996	2.5	2.360	−6
		2.5	2.328	−7
		5.0	5.207	+4
		5.0	5.217	+4
		10.0	10.25	+3
		10.0	9.811	−2
11 February 1997	18 February 1997	2.5	2.508	0
		2.5	2.518	+1
		5.0	5.223	+5
		5.0	5.058	+1
		10.0	9.900	−1
		10.0	10.03	0
7 May 1997	10 June 1997	2.5	2.501	0
		2.5	2.565	+3
		5.0	5.027	+1
		5.0	4.903	−2
		10.0	10.12	+1
		10.0	10.04	0

TABLE H3
Results of Analyses of Dose Formulations Administered to Mice in the Gavage Study of Chloral Hydrate

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
7 May 1997	19 May 1997[b]	1.542	1.630	+6
		1.542	1.522	−1
		1.542	1.559	+1
		1.754	1.790	+2
		1.754	1.735	−1
		1.754	1.691	−4
		5.283	5.774	+9
		5.283	5.297	0
		5.283	5.209	−1
		5.833	6.059	+4
		5.833	5.747	−1
		5.833	5.617	−4
Regimens D and E				
1 May 1995	2 May 1995[b]	0.85	0.91	+7
		0.85	0.84	−1
		0.85	0.89	+5
		4.25	3.87	−9
		4.25	3.78	−11
		4.25	4.01	−6
5 May 1995	8 May 1995[b]	0.85	0.84	−1
		0.85	0.89	+5
		0.85	0.85	0
		4.25	4.06	−4
		4.25	4.31	+1
		4.25	4.33	+2
10 May 1995	15 May 1995[b]	0.85	0.89	+5
		0.85	0.89	+5
		0.85	0.91	+7
		4.25	4.34	+2
		4.25	4.26	0
		4.25	4.23	0

[a] Results of single analyses
[b] Animal room samples

APPENDIX I
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-31 RAT AND MOUSE RATION

TABLE I1 Ingredients of NIH-31 Rat and Mouse Ration 190
TABLE I2 Vitamins and Minerals in NIH-31 Rat and Mouse Ration 190
TABLE I3 Nutrient Composition of NIH-31 Rat and Mouse Ration 191
TABLE I4 Contaminant Levels in NIH-31 Rat and Mouse Ration 191

TABLE I1
Ingredients of NIH-31 Rat and Mouse Ration

Ingredients[a]	Percent by Weight
Ground #2 yellow shelled corn	21.0
Ground whole hard wheat	35.5
Ground whole oats	10.0
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	9.0
Wheat middlings	10.0
Alfalfa meal (17% protein)	2.0
Corn gluten meal (60% protein)	2.0
Soy oil	1.5
Dried brewer's yeast	1.0
Dicalcium phosphate (food grade)	1.5
Ground limestone	0.5
Salt	0.5
Premixes (vitamin and mineral)	0.5

[a] Ingredients were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE I2
Vitamins and Minerals in NIH-31 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	22,000,000 IU	Vitamin A palmitate or acetate
D_3	3,800,000 IU	D-activated animal sterol
K_3	20 g	Menadione activity
d-α-Tocopheryl acetate	15 g	
Choline	700 g	Choline chloride
Folic acid	1 g	
Niacin	20 g	
d-Pantothenic acid	25 g	*d*-Calcium pantothenate
Riboflavin	5 g	
Thiamine	65 g	Thiamine mononitrate
B_{12}	14 g	
Pyridoxine	2 g	Pyridoxine hydrochloride
Biotin	0.120 g	*d*-Biotin
Minerals		
Iron	60 g	Iron sulfate
Magnesium	400 g	Magnesium oxide
Manganese	100 g	Manganous oxide
Zinc	10 g	Zinc oxide
Copper	4 g	Copper sulfate
Iodine	1.5 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

[a] Per ton (2,000 lb) of finished product

TABLE I3
Nutrient Composition of NIH-31 Rat and Mouse Ration[a]

Nutrient	Mean ± Standard Deviation[b]
Crude protein (% by weight)	19.2 ± 1.3
Crude fat (% by weight)	5.14 ± 0.91
Vitamins	
Vitamin A (ppm)	11,200 ± 1,900
Vitamin B_1 (mg/g)	0.095 ± 0.028
Vitamin E (ppm)	61.3 ± 7.0
Minerals	
Selenium (ppm)	0.33 ± 0.11

[a] Prior to autoclaving
[b] Average of 17 diet production lots

TABLE I4
Contaminant Levels in NIH-31 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]
Contaminants	
Arsenic (ppb)	83 ± 38
Cadmium (ppb)	81 ± 35
Lead (ppm)	0.37 ± 0.26
Aflatoxin B_1 (ppb)	<0.25
Aflatoxin B_2 (ppb)	<0.25
Aflatoxin G_1 (ppb)	<0.25
Aflatoxin G_2 (ppb)	<0.12
Fumonisin B_1 (ppb)	44.9 ± 21.9
Total fumonisin (ppb)	76.1 ± 23.4
Volatile nitrosamines (ppb)	6.39 ± 1.30
Pesticides (ppb)	
Heptachlor	<10
DDT, total[c]	<5
Dieldrin	<5
PCB	19 ± 21
Malathion	101 ± 99
Lindane	<1.0

[a] Prior to autoclaving
[b] Average of 4, 9, 12, or 17 diet production lots; for values less than the limit of detection, the detection limit is given as the mean.
[c] DDE+DDT+DDD

APPENDIX J
SENTINEL ANIMAL PROGRAM

METHODS 194
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SENTINEL ANIMAL PROGRAM

Methods

Rodents used in the Carcinogenesis Program of the National Center for Toxicological Research (NCTR) are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from randomly selected mice during the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to the Surveillance/Diagnostic Program, Division of Microbiology, at the NCTR for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
MICE	
2-Year Study	
ELISA	
Ectromelia virus	17, 21, and 24 months, study termination
GDVII (mouse encephalomyelitis virus)	17, 21, and 24 months, study termination
LCM (lymphocytic choriomeningitis virus)	17, 21, and 24 months, study termination
MVM (minute virus of mice)	17, 21, and 24 months, study termination
MHV (mouse hepatitis virus)	17, 21, and 24 months, study termination
Mycoplasma arthritidis	17, 21, and 24 months, study termination
Mycoplasma pulmonis	17, 21, and 24 months, study termination
PVM (pneumonia virus of mice)	17, 21, and 24 months, study termination
Polyoma virus	17, 21, and 24 months, study termination
Reovirus 3	17, 21, and 24 months, study termination
Sendai	17, 21, and 24 months, study termination

RESULTS

All test results were negative.

National Toxicology Program Technical Reports

Printed as of February 2002

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Chemical	TR No.
Acetaminophen	394
Acetonitrile	447
Acrylonitrile	506
Agar	230
Allyl Glycidyl Ether	376
Allyl Isothiocyanate	234
Allyl Isovalerate	253
1-Amino-2,4-Dibromoanthraquinone	383
2-Amino-4-Nitrophenol	339
2-Amino-5-Nitrophenol	334
11-Aminoundecanoic Acid	216
dl-Amphetamine Sulfate	387
Ampicillin Trihydrate	318
Anthraquinone	494
Asbestos, Amosite (Hamsters)	249
Asbestos, Amosite (Rats)	279
Asbestos, Chrysotile (Hamsters)	246
Asbestos, Chrysotile (Rats)	295
Asbestos, Crocidolite	280
Asbestos, Tremolite	277
L-Ascorbic Acid	247
AZT and AZT/α-Interferon A/D	469
Barium Chloride Dihydrate	432
Benzaldehyde	378
Benzene	289
Benzethonium Chloride	438
Benzofuran	370
Benzyl Acetate (Gavage)	250
Benzyl Acetate (Feed)	431
Benzyl Alcohol	343
o-Benzyl-*p*-Chlorophenol (Gavage)	424
o-Benzyl-*p*-Chlorophenol (Mouse Skin)	444
2-Biphenylamine Hydrochloride	233
2,2-Bis(Bromomethyl)-1,3-Propanediol	452
Bis(2-Chloro-1-Methylethyl) Ether	239
Bisphenol A	215
Boric Acid	324
Bromodichloromethane	321
Bromoethane	363
1,3-Butadiene	288
1,3-Butadiene	434
t-Butyl Alcohol	436
Butyl Benzyl Phthalate	213
Butyl Benzyl Phthalate	458
N-Butyl Chloride	312
t-Butylhydroquinone	459
γ-Butyrolactone	406
Caprolactam	214
d-Carvone	381
Chloral Hydrate	502
Chlorinated and Chloraminated Water	392
Chlorendic Acid	304
Chlorinated Paraffins: C_{23}, 43% Chlorine	305
Chlorinated Paraffins: C_{12}, 60% Chlorine	308
Chlorinated Trisodium Phosphate	294
2-Chloroacetophenone	379
p-Chloroaniline Hydrochloride	351
CS2	377
Chlorobenzene	261
Chlorodibromomethane	282
Chloroethane	346
2-Chloroethanol	275
3-Chloro-2-Methylpropene	300
Chloroprene	467

Chemical	TR No.
1-Chloro-2-Propanol	477
Chlorpheniramine Maleate	317
C.I. Acid Orange 3	335
C.I. Acid Orange 10	211
C.I. Acid Red 14	220
C.I. Acid Red 114	405
C.I. Basic Red 9 Monohydrochloride	285
C.I. Direct Blue 15	397
C.I. Direct Blue 218	430
C.I. Disperse Blue 1	299
C.I. Disperse Yellow 3	222
C.I. Pigment Red 3	407
C.I. Pigment Red 23	411
C.I. Solvent Yellow 14	226
Cobalt Sulfate Heptahydrate	471
Coconut Oil Acid Diethanolamine Condensate	479
Codeine	455
Comparative Initiation/Promotion Studies (Mouse Skin)	441
Corn Oil, Safflower Oil, and Tricaprylin	426
Coumarin	422
Cytembena	207
D&C Red No. 9	225
D&C Yellow No. 11	463
Decabromodiphenyl Oxide	309
Diallyl Phthalate (Mice)	242
Diallyl Phthalate (Rats)	284
4,4′-Diamino-2,2′-Stilbenedisulfonic Acid, Disodium Salt	412
2,4-Diaminophenol Dihydrochloride	401
1,2-Dibromo-3-Chloropropane	206
1,2-Dibromoethane	210
2,3-Dibromo-1-Propanol	400
1,2-Dichlorobenzene (*o*-Dichlorobenzene)	255
1,4-Dichlorobenzene (*p*-Dichlorobenzene)	319
p,p′-Dichlorodiphenyl sulfone	501
2,4-Dichlorophenol	353
2,6-Dichloro-*p*-Phenylenediamine	219
1,2-Dichloropropane	263
1,3-Dichloropropene (Telone II)	269
Dichlorvos	342
Dietary Restriction	460
Diethanolamine	478
Di(2-Ethylhexyl) Adipate	212
Di(2-Ethylhexyl) Phthalate	217
Diethyl Phthalate	429
Diglycidyl Resorcinol Ether	257
3,4-Dihydrocoumarin	423
1,2-Dihydro-2,2,4-Trimethylquinoline (Monomer)	456
Dimethoxane	354
3,3′-Dimethoxybenzidine Dihydrochloride	372
N,N-Dimethylaniline	360
3,3′-Dimethylbenzidine Dihydrochloride	390
Dimethyl Hydrogen Phosphite	287
Dimethyl Methylphosphonate	323
Dimethyl Morpholinophosphoramidate	298
Dimethylvinyl Chloride	316
Diphenhydramine Hydrochloride	355
5,5-Diphenylhydantoin	404
Emodin	493
Ephedrine Sulfate	307
Epinephrine Hydrochloride	380
1,2-Epoxybutane	329
Erythromycin Stearate	338
Ethyl Acrylate	259
Ethylbenzene	466

Chemical	TR No.
Ethylene Glycol	413
Ethylene Glycol Monobutyl Ether	484
Ethylene Oxide	326
Ethylene Thiourea	388
Eugenol	223
FD&C Yellow No. 6	208
Fumonisin B_1	496
Furan	402
Furfural	382
Furfuryl Alcohol	482
Furosemide	356
Gallium Arsenide	492
Geranyl Acetate	252
Glutaraldehyde	490
Glycidol	374
Guar Gum	229
Gum Arabic	227
HC Blue 1	271
HC Blue 2	293
HC Red 3	281
HC Yellow 4	419
Hexachlorocyclopentadiene	437
Hexachloroethane	361
4-Hexylresorcinol	330
Hydrochlorothiazide	357
Hydroquinone	366
8-Hydroxyquinoline	276
Indium Phosphide	499
Iodinated Glycerol	340
Isobutene	487
Isobutyl Nitrite	448
Isobutyraldehyde	472
Isophorone	291
Isoprene	486
Lauric Acid Diethanolamine Condensate	480
d-Limonene	347
Locust Bean Gum	221
60-Hz Magnetic Fields	488
Magnetic Field Promotion	489
Malonaldehyde, Sodium Salt	331
Manganese Sulfate Monohydrate	428
D-Mannitol	236
Marine Diesel Fuel and JP-5 Navy Fuel	310
Melamine	245
2-Mercaptobenzothiazole	332
Mercuric Chloride	408
Methacrylonitrile	497
8-Methoxypsoralen	359
α-Methylbenzyl Alcohol	369
Methyl Bromide	385
Methyl Carbamate	328
Methyldopa Sesquihydrate	348
Methylene Chloride	306
4,4′-Methylenedianiline Dihydrochloride	248
Methyleugenol	491
Methyl Methacrylate	314
N-Methylolacrylamide	352
Methylphenidate Hydrochloride	439
Mirex	313
Molybdenum Trioxide	462
Monochloroacetic Acid	396
Monuron	266
Nalidixic Acid	368
Naphthalene (Mice)	410
Naphthalene (Rats)	500
Nickel (II) Oxide	451
Nickel Sulfate Hexahydrate	454
Nickel Subsulfide	453
p-Nitroaniline	418
o-Nitroanisole	416
p-Nitrobenzoic Acid	442

Chemical	TR No.
Nitrofurantoin	341
Nitrofurazone	337
Nitromethane	461
p-Nitrophenol	417
o-Nitrotoluene	504
Ochratoxin A	358
Oleic Acid Diethanolamine Condensate	481
Oxazepam (Mice)	443
Oxazepam (Rats)	468
Oxymetholone	485
Oxytetracycline Hydrochloride	315
Ozone and Ozone/NNK	440
Penicillin VK	336
Pentachloroanisole	414
Pentachloroethane	232
Pentachloronitrobenzene	325
Pentachlorophenol, Purified	483
Pentachlorophenol, Technical Grade	349
Pentaerythritol Tetranitrate	365
Phenolphthalein	465
Phenylbutazone	367
Phenylephrine Hydrochloride	322
N-Phenyl-2-Naphthylamine	333
o-Phenylphenol	301
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Gavage)	244
Polybrominated Biphenyl Mixture (Firemaster FF-1) (Feed)	398
Polysorbate 80 (Glycol)	415
Polyvinyl Alcohol	474
Primidone	476
Probenecid	395
Promethazine Hydrochloride	425
Propylene	272
1,2-Propylene Oxide	267
Propyl Gallate	240
Pyridine	470
Quercetin	409
Resorcinol	403
Rhodamine 6G	364
Rotenone	320
Roxarsone	345
Salicylazosulfapyridine	457
Scopolamine Hydrobromide Trihydrate	445
Sodium Azide	389
Sodium Fluoride	393
Sodium Nitrite	495
Sodium Xylenesulfonate	464
Stannous Chloride	231
Succinic Anhydride	373
Talc	421
Tara Gum	224
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Dermal)	201
2,3,7,8-Tetrachlorodibenzo-*p*-Dioxin (Gavage)	209
1,1,1,2-Tetrachloroethane	237
Tetrachloroethylene	311
Tetracycline Hydrochloride	344
Tetrafluoroethylene	450
1-Trans-Delta^9-Tetrahydrocannabinol	446
Tetrahydrofuran	475
Tetrakis(Hydroxymethyl)Phosphonium Sulfate	296
Tetrakis(Hydroxymethyl)Phosphonium Chloride	296
Tetranitromethane	386
Theophylline	473
4,4-Thiobis(6-*t*-Butyl-*m*-Cresol)	435
Titanocene Dichloride	399
Toluene	371
2,4- & 2,6-Toluene Diisocyanate	251
o-Toluidine Hydrochloride	153
Triamterene	420
Tribromomethane	350
Trichloroethylene	243
Trichloroethylene	273

Chemical	TR No.
1,2,3-Trichloropropane	384
Tricresyl Phosphate	433
Triethanolamine	449
Tris(2-Chloroethyl) Phosphate	391
Tris(2-Ethylhexyl) Phosphate	274
Turmeric Oleoresin (Curcumin)	427
4-Vinylcyclohexene	303
4-Vinyl-1-Cyclohexene Diepoxide	362
Vinylidene Chloride	228
Vinyl Toluene	375
Xylenes (Mixed)	327
2,6-Xylidine	278
Zearalenone	235
Ziram	238