



NTP

National Toxicology Program

U.S. Department of Health and Human Services

NTP TECHNICAL REPORT ON
THE TOXICOLOGY AND
CARCINOGENESIS STUDIES OF

BIS(2-CHLOROETHOXY)METHANE
(CAS No. 111-91-1)
IN F344/N RATS AND
B6C3F1 MICE
(DERMAL STUDIES)

NTP TR 536

AUGUST 2011

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

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

FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

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SUMMARY

Background

Bis(2-chloroethoxy)methane is used as a solvent and to make fungicides and polymers. We studied the effects of bis(2-chloroethoxy)methane on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We painted solutions containing bis(2-chloroethoxy)methane in ethanol on the backs of male and female rats and mice. Groups of 50 male and female rats received 75, 150, or 300 milligrams of bis(2-chloroethoxy)methane per kilogram of body weight 5 days per week for 2 years; similar groups of male mice received 150, 300, or 600 mg/kg and female mice received 100, 200, or 400 mg/kg. Groups of animals receiving ethanol alone served as the control groups. At the end of the study, tissues from more than 40 sites were examined for every animal.

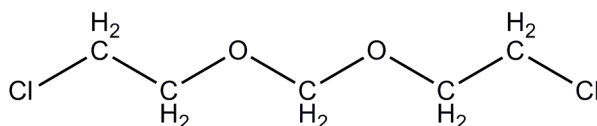
Results

The only effects observed in male and female rats were degeneration of the olfactory epithelium of the nose, and some instances of stomach inflammation or ulcer were observed in males. Male and female mice receiving bis(2-chloroethoxy)methane had increased rates of some alterations of heart tissue that were termed cardiomyopathy. Male mice also had inflammation of the skin and stomach ulcers.

Conclusions

We conclude that exposure to bis(2-chloroethoxy)methane did not cause cancer in male or female rats or mice. Exposure to bis(2-chloroethoxy)methane caused some non-cancerous lesions of the nose in rats and the heart in mice.

ABSTRACT



BIS(2-CHLOROETHOXY)METHANE

CAS No. 111-91-1

Chemical Formula: $C_5H_{10}Cl_2O_2$ Molecular Weight: 173.04

Synonyms: Bis(2-chloroethyl)formal; bis(β -chloroethyl)formal; dichlorodiethyl formal; dichlorodiethyl methylal; di(2-chloroethyl) acetal; dichloroethyl formal; 2,2-dichloroethyl formal; dichloromethoxy ethane; ethane, 1,1-[methylenebis(oxy)]bis(2-chloro-); formaldehyde bis(2-chloroethyl) acetal; formaldehyde bis(β -chloroethyl) acetal; methane, bis(2-chloroethoxy)-; 1,1-[methylenebis(oxy)]bis(2-chloroethane)

Bis(2-chloroethoxy)methane is used as a solvent and the starting agent in the production of fungicides and polysulfide polymers. Bis(2-chloroethoxy)methane was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as a starting material to produce polysulfide elastomers, and because there were no 2-year carcinogenicity studies reported in the literature. Male and female F344/N rats and B6C3F1 mice received dermal applications of bis(2-chloroethoxy)methane in ethanol (greater than 98% pure) for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and *Escherichia coli*, rat bone marrow cells, and mouse peripheral blood erythrocytes.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were dermally administered 0, 12.5, 25, 50, 100, or 200 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 16 days. All rats survived to the end of the study. Mean body weights of dosed rats were similar to those of the vehicle control groups. There were no histopathologic lesions related to bis(2-chloroethoxy)methane administration.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were dermally administered 0, 12.5, 25, 50, 100, or 200 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 17 days. All mice survived to the end of the study. Mean body weights of dosed mice were similar to those of the vehicle control groups. There were no histopathologic lesions related to bis(2-chloroethoxy)methane administration.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were dermally administered 0, 50, 100, 200, 400, or 600 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 14 weeks. Additional clinical pathology groups of 10 male and 10 female rats were administered the same doses for 23 days. All core study 600 mg/kg males and females and two 400 mg/kg females died before the end of the study. The cause of death was considered to be related to the cardiotoxic effect of bis(2-chloroethoxy)methane. There were no significant differences between final mean body weights of dosed rats and those of the vehicle control groups; the mean body weight gain of 400 mg/kg males was significantly less than that of the vehicle controls.

Clinical findings included prostration and ataxia in 600 mg/kg rats during the first week of the study and nasal/eye discharge, lethargy, ataxia, and abnormal breathing in 400 and 600 mg/kg females beginning week 5. An enlarged heart was noted in one 100 mg/kg female rat. Relative kidney weights of 100, 200, and 400 mg/kg males were significantly greater than that of the vehicle control group. Increased incidences and severities of myofiber cytoplasmic vacuolization and interstitial mononuclear cell infiltration in the heart occurred in 400 and 600 mg/kg male and female rats and in 200 mg/kg females. Increased incidences and severities of myofiber necrosis occurred in 600 mg/kg males and females; one female each in the 200 and 400 mg/kg groups also had this lesion. Three 600 mg/kg males had atrial thrombosis.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were dermally administered 0, 50, 100, 200, 400, or 600 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 14 weeks. Except for three 600 mg/kg females, all mice survived to the end of the study. Mean body weights of dosed and vehicle control mice were similar. One 600 mg/kg female that died early exhibited lethargy, abnormal breathing, and tremors, and one animal had clonic seizures. One 600 mg/kg female that died early had focal erosion of the glandular stomach and a focus in the duodenum found to consist of acute suppurative inflammation and thrombosis. Absolute and relative kidney weights of 400 and 600 mg/kg males and 600 mg/kg females were significantly greater than those of the vehicle control groups. Absolute liver weights of 400 and 600 mg/kg females were also significantly increased. Significantly increased incidences of myofiber cytoplasmic vacuolization occurred in 400 and 600 mg/kg females.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were dermally administered 0, 75, 150, or 300 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 105 weeks. Survival of all dosed groups of rats was generally similar to that of the vehicle controls. Mean body weights of dosed rats were similar to those of the vehicle controls throughout the study. Clinical findings in 300 mg/kg females that died during the first year of the study included abnormal breathing, lethargy, thinness, nasal discharge, and ataxia.

Significantly increased incidences of degeneration of the olfactory epithelium in the nose occurred in all

dosed groups of males and in 150 and 300 mg/kg females. The incidences of inflammation of the forestomach were significantly increased in 150 and 300 mg/kg males, and the incidence of ulcers was significantly increased in 300 mg/kg males. Increased incidences of cystic degeneration of the liver occurred in 150 and 300 mg/kg male rats; the incidence was significantly increased in the 300 mg/kg group.

2-YEAR STUDY IN MICE

Groups of 50 male mice were dermally administered 0, 150, 300, or 600 mg bis(2-chloroethoxy)methane/kg body weight in ethanol, 5 days per week for 105 weeks. Groups of 50 female mice were dermally administered 0, 100, 200, or 400 mg/kg in ethanol, 5 days per week for 104 weeks. Survival of 600 mg/kg male mice was significantly less than that of the vehicle control group. Mean body weights of dosed mice were generally similar to those of the vehicle controls throughout the study. Clinical findings observed in 600 mg/kg male mice that died during the first year of the study included lethargy and thinness.

Myocardial heart changes were recorded according to the characteristic lesions of cardiomyopathy syndrome (necrosis, mononuclear cell infiltration, myocardial cell vacuolization, and interstitial fibrosis) separately, and in addition, where appropriate, they were also categorized as cardiomyopathy. Increased incidences of cardiomyopathy and mononuclear cell infiltration occurred in 600 mg/kg males and 400 mg/kg females; the incidences were significantly increased in 600 mg/kg males compared to the vehicle controls. Significantly increased incidences of cardiomyocyte vacuolization and interstitial fibrosis occurred in 600 mg/kg males. A few early deaths in the 600 mg/kg males were considered to be due, at least in part and probably exclusively, to bis(2-chloroethoxy)methane-induced cardiotoxicity.

The incidence of ulceration of the forestomach was significantly increased in 600 mg/kg males.

Significantly increased incidences of dermal inflammation and fibrosis and epidermal hyperplasia at the site of application occurred in 600 mg/kg male mice.

GENETIC TOXICOLOGY

Bis(2-chloroethoxy)methane was mutagenic in *S. typhimurium* strains TA100 and TA1535 in the presence of exogenous metabolic activation enzymes (S9) in one study; results from a second bacterial mutagenicity test were judged to be equivocal based on responses

observed in TA100 and in *E. coli* strain WP2*uvrA*/pKM101 in the presence of S9. No mutagenicity was observed in other tester strains or in the absence of S9. Bis(2-chloroethoxy)methane did not increase the frequency of micronucleated reticulocytes in bone marrow of male F344/N rats following three daily treatments by gavage or micronucleated erythrocytes in peripheral blood of male or female mice after 3 months of dermal exposure.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of

bis(2-chloroethoxy)methane in male or female F344/N rats administered 75, 150, or 300 mg/kg. There was *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male B6C3F1 mice administered 150, 300, or 600 mg/kg or in female B6C3F1 mice administered 100, 200, or 400 mg/kg.

The administration of bis(2-chloroethoxy)methane for 2 years resulted in increased incidences of non-neoplastic lesions in the nose of male and female rats, the forestomach of male rats, the heart of male and female mice, and the forestomach and skin of male mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Bis(2-chloroethoxy)methane

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in ethanol by dermal application	0, 75, 150, or 300 mg/kg	0, 75, 150, or 300 mg/kg	0, 150, 300, or 600 mg/kg	0, 100, 200, or 400 mg/kg
Body weights	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group	Dosed groups similar to the vehicle control group
Survival rates	21/50, 29/50, 14/50, 24/50	19/50, 30/50, 28/50, 18/50	37/50, 40/50, 42/50, 28/50	31/50, 38/50, 37/50, 37/50
Nonneoplastic effects	<u>Nose:</u> olfactory epithelium, degeneration (5/50, 17/49, 30/50, 48/49) <u>Forestomach:</u> inflammation (0/50, 2/50, 6/50, 10/50); ulcer (0/50, 2/50, 2/50, 7/50)	<u>Nose:</u> olfactory epithelium, degeneration (5/49, 4/49, 18/50, 49/49)	<u>Heart:</u> myocardium, cardiomyopathy (10/50, 12/50, 7/50, 28/50); myocardium, fibrosis (0/50, 3/50, 3/50, 13/50); myocardium, infiltration cellular, mononuclear cell (11/50, 12/50, 8/50, 28/50); myocardium, vacuolization cytoplasmic (10/50, 15/50, 11/50, 29/50) <u>Forestomach:</u> ulcer (1/50, 1/50, 1/50, 7/50) <u>Skin (site of application):</u> dermis, fibrosis (6/50, 1/50, 2/50, 25/50); dermis, inflammation (3/50, 1/50, 3/50, 13/50); epidermis, hyperplasia (8/50, 1/50, 4/50, 28/50)	<u>Heart:</u> myocardium, cardiomyopathy (10/50, 7/50, 10/50, 17/50); myocardium, infiltration cellular, mononuclear cell (9/50, 7/50, 10/50, 17,50); myocardium, vacuolization cytoplasmic (14/50, 4/50, 6/50, 13/50)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Bacterial gene mutations:		Positive in one study in <i>S. typhimurium</i> strains TA100 and TA1535 with S9; equivocal in a second study in TA100 and in <i>E. coli</i> with S9; negative in TA100, TA1535, and <i>E. coli</i> without S9 and TA98 with or without S9		
Micronucleated erythrocytes				
Rat bone marrow <i>in vivo</i> :		Negative in male rats		
Mouse peripheral blood <i>in vivo</i> :		Negative in male and female mice		

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 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 on 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 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 bis(2-chloroethoxy)methane on November 19, 2009, 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 November 19, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of bis(2-chloroethoxy)methane 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. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of bis(2-chloroethoxy)methane by describing the uses of the solvent, the results of mutagenicity tests, background information on the absorption and metabolism of the chemical, and the design and results of the short- and long-term dermal exposure studies. The proposed conclusions for the 2-year studies were *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male or female F344/N rats and *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male or female B6C3F1 mice.

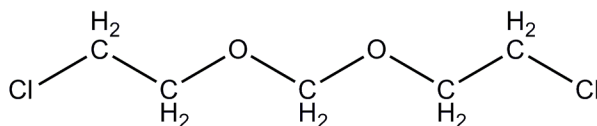
Dr. Riviere, the first principal reviewer, felt the studies were well performed and reported. He asked that more details of the dermal exposure protocols be included.

Dr. Nagarkatti, the second principal reviewer, inquired if the thymic atrophy and bone marrow depletion warranted other studies on immunosuppression. She inquired also if the dermal application could result in hypersensitivity. She thought the report explained well the different forms of cardiotoxicity and cardiomyopathy observed. Dr. Dunnick said the discussion would be expanded where appropriate.

Dr. Bunton, the third principal reviewer, inquired about the criteria for distinguishing the cardiotoxicity from cardiomyopathy, with the former sometimes masking the latter. Dr. A. Nyska, contractor to the NTP, explained that the damage associated with cardiotoxicity is more widespread, consisting of vacuolization, necrosis, and mononuclear cell infiltration, which mask the focal lesions characteristic of cardiomyopathy.

Dr. Riviere moved, and Dr. Nagarkatti seconded, that the conclusions be accepted as written. The motion was approved with seven yes votes. Drs. Teeguarden and Novak were recused from participation in the peer review and vote because of potential conflicts of interest; therefore, Dr. Portier served as Chairperson of the committee for this report. Dr. Eastmond was absent for the vote.

INTRODUCTION



BIS(2-CHLOROETHOXY)METHANE

CAS No. 111-91-1

Chemical Formula: C₅H₁₀Cl₂O₂ Molecular Weight: 173.04

Synonyms: Bis(2-chloroethyl)formal; bis(β -chloroethyl)formal; dichlorodiethyl formal; dichlorodiethyl methylal; di(2-chloroethyl) acetal; dichloroethyl formal; 2,2-dichloroethyl formal; dichloromethoxy ethane; ethane, 1,1-[methylenebis(oxy)]bis(2-chloro-); formaldehyde bis(2-chloroethyl) acetal; formaldehyde bis(β -chloroethyl) acetal; methane, bis(2-chloroethoxy)-; 1,1-[methylenebis(oxy)]bis(2-chloroethane)

CHEMICAL AND PHYSICAL PROPERTIES

Bis(2-chloroethoxy)methane is a colorless liquid with a flash point (open cup) of 230° C, a freezing point of -32.8° C, a boiling point of 218.1° C, a specific gravity (20° C/20° C) of 1.2339, and a vapor pressure of 0.1 mm Hg at 25° C (*Hawley's*, 1997; HSDB, 2009). The solubility is less than 1.000 ppm in water (Battelle, 1999).

In water, bis(2-chloroethoxy)methane can be decomposed by mineral acids (*Hawley's*, 1997). Toxic chloride fumes are formed on contact with acid or acid fumes (Sax, 1984). Hydrolysis is relatively slow, with a minimum calculated half-life in pure water (pH = 7 and 25° C) of 6 months to several years (SRI, 1989; HSDB, 2009). Hydrolysis can occur at two sites on the molecule: the carbon-oxygen bonds of the acetal linkages and the carbon-chlorine bonds (HSDB, 2009). The calculated hydrolytic half-life is approximately 6 months for the carbon-chlorine bonds. Bis(2-chloroethoxy)methane is slightly flammable when exposed to heat or flame (Sax, 1984). Upon thermal decomposition, hydrogen chloride fumes are released (HSDB, 2009).

PRODUCTION, USE, AND HUMAN EXPOSURE

Approximately 10 to 50 million pounds of bis(2-chloroethoxy)methane were produced in the United States in 1977 (HSDB, 2009). No current data on import volumes were found. Bis(2-chloroethoxy)methane is produced by the reaction of ethylene chlorohydrin and formaldehyde under acid catalysis (*Kirk-Othmer*, 1984).

Bis(2-chloroethoxy)methane is used as a solvent and a starting agent in the production of fungicides and polysulfide polymers (Mutuc *et al.*, 2008). Bis(2-chloroethoxy)methane polysulfide elastomers are used extensively in a variety of sealant applications because of their resistance to degradation by many solvents and resistance to high temperatures (Vietti and Scherrer, 1992). Over 95% of polysulfide polymers are made from bis(2-chloroethoxy)methane and sodium polysulfide (HSDB, 2009).

Bis(2-chloroethoxy)methane does not occur naturally (HSDB, 2009). Release of the chemical can occur by volatilization during its manufacture, formulation in

polysulfides, or use as a solvent. The chemical has been detected as a subsurface contaminant at a number of industrial sites (Thomann, 1995; Mutuc *et al.*, 2008). Bis(2-chloroethoxy)methane was found (levels not reported) in the industrial wastes of metal finishing, plastics and chemical manufacturing, and steam electric power industries that discharge effluents to the combined sewerage system along the lower Passaic River in New Jersey (Shear *et al.*, 1996). The volatility of bis(2-chloroethoxy)methane does not contribute significantly to its environmental fate in water (HSDB, 1998). It is estimated that in a model pond, the half-life of bis(2-chloroethoxy)methane would be 11 years. This is compared to a half-life of 0.5 to 2.0 years for structurally similar haloethers. Bis(2-chloroethoxy)methane has been detected in water from inland waterways (van Steenderen *et al.*, 1987). Samples of treated effluent taken from a synthetic rubber manufacturing plant revealed bis(2-chloroethoxy)methane levels of 140 mg/L (USEPA, 1973). Clams sampled from the Chef Menteur River that flows from Lake Pontchartrain, Louisiana, to the Gulf of Mexico had wet weight tissue concentrations of 12 ng bis(2-chloroethoxy)methane/g, even though the chemical was not detected in the lake's sediment samples (McFall *et al.*, 1985). Bis(2-chloroethoxy)methane was not detected in fish taken from 14 river tributaries of Lake Michigan (Camanzo *et al.*, 1987).

Bis(2-chloroethoxy)methane is reported to have a low soil organic carbon content normalized sorption coefficient value of 32 (Tao and Lu, 1999) and is only slightly soluble in water (HSDB, 2009). Given the vapor pressure and water solubility of bis(2-chloroethoxy)methane, along with an estimated Henry's Law constant of 3.9×10^{-6} atm·m³/mole at 25° C, volatilization from moist soil is expected to be an important fate process for bis(2-chloroethoxy)methane; it is not expected to volatilize from dry soil surfaces based on its vapor pressure (HSDB, 2009). Gas chromatography with mass spectrometry analysis of plants grown in sludge-treated coal refuse showed that they contained less than the minimum detection limit of 4 mg bis(2-chloroethoxy)methane/kg dry weight (Webber *et al.*, 1994). A study by Patterson and Kodukala (1981) showed that when the influent of a sludge treatment facility was spiked with 0.24 g bis(2-chloroethoxy)methane/L, 60% of the pollutant was removed. In the atmosphere, bis(2-chloroethoxy)methane has an estimated half-life of 2.1 days when reacted with photochemically produced hydroxyl radicals (HSDB, 2009). Exposure to bis(2-chloroethoxy)methane may occur by dermal contact and/or inhalation from its manufacture and use as a solvent and intermediate for polysulfide rubbers (HSDB, 2009).

No human exposure data are available (HSDB, 2009).

REGULATORY STATUS

Bis(2-chloroethoxy)methane is listed as a hazardous waste in 40 CFR § 261.32 because it is an industrial wastewater sludge generated by chlorinated aliphatic chemical manufacturing facilities. The Code of Federal Regulations establishes limits of pollutants in wastewater and nonwastewater; wastewaters are wastes that contain 1% by weight total organic carbon and less than 1% by weight total suspended solids; nonwastewaters are wastes that do not meet the criteria for wastewaters (40 CFR § 268.48). Bis(2-chloroethoxy)methane has a wastewater standard of 0.036 mg/L and a non-wastewater standard of 7.2 mg/kg.

ABSORPTION, DISTRIBUTION, METABOLISM, EXCRETION, AND TOXICOKINETICS

Experimental Animals

In a study conducted for the NTP, [¹⁴C]-bis(2-chloroethoxy)methane was found to be readily absorbed and rapidly excreted following gavage administration of 0.1 or 10 mg/kg to male F344 rats (Black *et al.*, 2007). The majority of the ¹⁴C was excreted within 24 hours of administration, primarily in the urine. Cumulative 72-hour excretion of the dose amounted to 90% to 94% in urine, a trace in feces, and up to 7% exhaled as ¹⁴CO₂. Results were similar following intravenous injection of 1 mg/kg. Male and female B6C3F1 mice also excreted the majority of either a gavage or intravenous dose of [¹⁴C]-bis(2-chloroethoxy)methane in the urine. Compared to rats, mice appeared to excrete bis(2-chloroethoxy)methane-derived radioactivity at a slower rate over time, and a notable amount, approximately 25% of a 10 mg/kg gavage dose, was detected in the feces. The distribution of ¹⁴C to tissues was rapid following intravenous administration to male rats. Approximately 80% of the total dose was present in tissues at 15 minutes, with the blood containing approximately 2% of the dose; respective concentrations in the kidney, liver, adipose tissue, and lung were 30-, 4-, 3-, and 1.5-fold higher than in the blood. Elimination of ¹⁴C from tissues was rapid over time, with the peak concentration observed at or before the 15-minute time point in most tissues. A notable exception was the thymus, where the ¹⁴C concentration increased threefold between 15 minutes and 4 hours and remained constant through 8 hours postdosing. Over 90% of the ¹⁴C in the thymus at 8 hours appeared to be bound to macromolecules. The tissue-to-blood ratio of ¹⁴C in the heart was less than 1 at all time points.

Individual tissues were not collected and analyzed from animals in the gavage studies; however, only a small amount (less than or equal to 1.5%) of the total dose remained in the carcasses of rats and mice 72 hours after dosing.

Black *et al.* (2007) investigated absorption of [¹⁴C]-bis(2-chloroethoxy)methane following dermal administration of 0.1 or 10 mg/kg to male F344 rats and B6C3F1 mice. Total recovery of the radiolabel for 0.1 mg/kg mice was approximately 41% of the total dose. No species-related effects were observed at 10 mg/kg, with mean recovery from 73% to 87% of the total dose. In 10 mg/kg rats and mice, bis(2-chloroethoxy)methane was described as being moderately absorbed from the dosing site (protected from grooming) with total dose absorption ranging from 12% to 18% for rats and 14% to 22% for mice. Most of the absorbed dose was excreted in the urine. Up to 50% of the total dose volatilized from the skin of some animals during these 24-hour experiments. Total dose absorption over 24 hours ranged from 33% to 55% for rats and 17% to 25% for mice following dermal application of 10 mg/kg to an unprotected site (RTI, 2002). These results indicated that rats, but not mice, ingested a significant amount of bis(2-chloroethoxy)methane from grooming at the administration site.

[¹⁴C]-bis(2-chloroethoxy)methane was rapidly metabolized following intravenous administration to rats (Black *et al.*, 2007). Most of the ¹⁴C in blood at 15 minutes after dosing consisted of bis(2-chloroethoxy)methane-derived metabolites or was bound to blood constituents. Parent chemical was near the limit of detection in blood within 4 hours of dosing and was not detected in the urine of rats. Bis(2-chloroethoxy)methane underwent rapid metabolism in mice as well, although species-dependent differences in metabolism were observed in these studies. Five major bis(2-chloroethoxy)methane-derived metabolites were detected in rat urine, whereas only three were detected in mouse urine. As in rats, no parent chemical was detected in the urine of dosed mice. None of the bis(2-chloroethoxy)methane-derived metabolites in rat or mouse urine appeared to be glucuronide or sulfate conjugates. The major metabolite in rat urine, approximately 40% of the total dose, was isolated and identified as thiodiglycolic acid. The metabolites of bis(2-chloroethoxy)methane in mouse urine were not identified. No bis(2-chloroethoxy)methane-derived metabolite in mouse urine coeluted with thiodiglycolic acid in rat urine in high-performance liquid chromatography analysis. However, thiodiglycolic acid was present in tissues of mice administered bis(2-chloroethoxy)methane in toxicokinetic studies conducted by the NTP (Appendix L). Therefore, the inability to

detect thiodiglycolic acid in mouse urine in the study conducted by Black *et al.* (2007) may indicate complete metabolism of the chemical in mice. A proposed metabolic scheme for bis(2-chloroethoxy)methane metabolism in rats is shown in Figure 1. Bis(2-chloroethoxy)methane has been described as being environmentally persistent (HSDB, 2009); however, the results of the studies conducted by Black *et al.* (2007) indicated that the ether bonds of bis(2-chloroethoxy)methane are readily cleaved *in vivo*, resulting in likely formation of 2-chloroethanol and chloroacetaldehyde. Chloroacetaldehyde has been shown to be neurotoxic to mice (Sood and O'Brien, 1996), a cardiotoxicant in rabbits (Joqueviel *et al.*, 1997), and mutagenic in *Escherichia coli* transfected with single-strand DNA adducts of the chemical (Jacobsen *et al.*, 1989). The metabolism of 2-chloroethanol, chloroacetaldehyde, and chemicals, such as ifosfamide and vinylidene chloride, that form chloroacetaldehyde has been studied (Jones and Hathway, 1978; Grunow and Altmann, 1982; Joqueviel *et al.*, 1997). Chloroacetaldehyde and its product chloroacetic acid are conjugated with glutathione and further metabolized to form thiodiglycolic acid.

Thiodiglycolic acid, shown to inhibit mitochondrial function in rat liver (Visarius *et al.*, 1998), can be further metabolized to thiodiglycolic acid sulfoxide, thioglycolic acid, and dithiodiglycolic acid (Jones and Hathway, 1978; Grunow and Altmann, 1982; Joqueviel *et al.*, 1997). Thioglycolic acid has been reported to elicit potentially toxic effects in rats and mice (Gan *et al.*, 2003).

The NTP conducted single-dose intravenous toxicokinetic studies in F344/N rats and B6C3F1 mice of bis(2-chloroethoxy)methane (up to 40 mg/kg in rats and 100 mg/kg in mice) and its metabolite thiodiglycolic acid (20 mg/kg in rats and 50 mg/kg in mice) (Appendix L). Single-dose dermal toxicokinetic studies of bis(2-chloroethoxy)methane were also conducted by applying the chemical (up to 400 mg/kg in rats and 600 mg/kg in mice) to a site unprotected from oral grooming. Bioavailability of bis(2-chloroethoxy)methane estimated by the measurement of parent chemical in plasma was low in both species but was higher in rats (11.2% to 17.9% in males and 20.0% to 28.7% in females) than in mice (5.09% to 6.86% in males and 11.9% in females). In a previous study conducted by the NTP in which 0.1 or 10 mg/kg [¹⁴C]bis(2-chloroethoxy)methane was applied under similar conditions to male F344 rats and male B6C3F1 mice, dermal bioavailability 24 hours after the application was estimated to be 33% to 55% in rats and 17% to 25% in mice (RTI, 2002). Factors such as hepatic first-pass metabolism of the parent chemical,

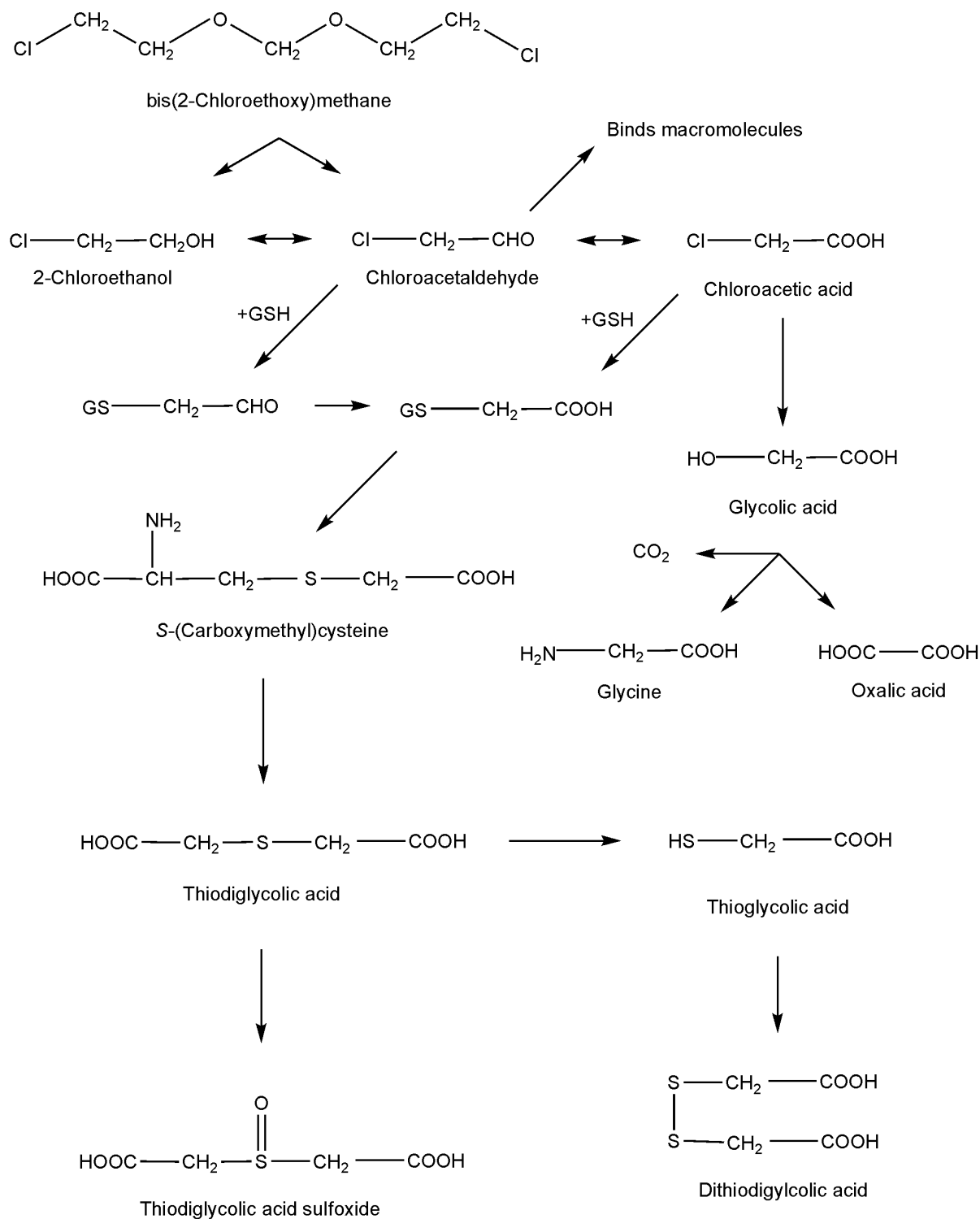


FIGURE 1
Proposed Scheme for Metabolism of Bis(2-chloroethoxy)methane in Rats
 (Adapted from Jones and Hathway, 1978; Grunow and Altman, 1982; Black *et al.*, 2007)

differences in the applied dose, and differences in the methodology used in the estimation of bioavailability (parent chemical plasma levels versus total radioactivity in excreta and tissues) may have played a role in the observed differences in bioavailability between the two studies.

Humans

No studies reporting the fate of bis(2-chloroethoxy)methane in humans were found in the literature; therefore, no direct comparisons of bis(2-chloroethoxy)methane metabolism can be made between rodents and humans. However, it could be speculated that bis(2-chloroethoxy)methane would be metabolized to thiodiglycolic acid in humans. *S*-(carboxymethyl)-cysteine, a putative bis(2-chloroethoxy)methane-derived metabolite in rodents, was metabolized to thiodiglycolic acid and thiodiglycolic acid sulfoxide following oral administration to human volunteers (Hofmann *et al.*, 1991). Further, chloroacetaldehyde was detected in human microsomes incubated with vinylidene chloride and in patients following ifosfamide administration (Dowsley *et al.*, 1999; Brüggemann *et al.*, 2007). Visarius *et al.* (1998) detected increasing concentrations of thiodiglycolic acid in the urine of patients infused with ifosfamide for 5 days. These results indicated formation of 2-chloroethanol, chloroacetaldehyde, and chloroacetic acid from ifosfamide and their subsequent conjugation with glutathione (or cysteine) to form *S*-(carboxymethyl)cysteine and thiodiglycolic acid.

TOXICITY

Experimental Animals

Smyth and Carpenter (1948) reported an oral LD₅₀ for Sherman rats (sex not specified) of 65 mg bis(2-chloroethoxy)methane/kg (0.38 mmol/kg) and a dermal LD₅₀ in guinea pigs (sex and strain not specified) of 170 mg/kg (0.98 mmol/kg). Gersich and Mayes (1986) reported a 48-hour LC₅₀ (lowest concentration to kill 50% of test animals) of 201 mg/L (1.16 mmol/L) for water fleas (*Daphnia magna* Straus) and a 96-hour LC₅₀ of 184 mg/L (1.06 mmol/L) for fathead minnows (*Pimephales promelas* Rafinesque).

In a 4-hour inhalation study of groups of six rats, no animals died when exposed to 60 ppm bis(2-chloroethoxy)methane and all of the animals died when exposed to 120 ppm (Smyth and Carpenter, 1948). In a subsequent 4-hour inhalation study conducted by these investigators, four of six rats exposed to 62 ppm died; no other signs or symptoms of exposure were observed (Carpenter *et al.*, 1949). A single oral dose of 60 mg bis(2-chloroethoxy)methane/kg (0.35 mmol/kg)

killed two of five female rats; a single 100 mg/kg oral dose of bis(2-chloroethoxy)methane was lethal when administered at a concentration of 100 mg/mL (0.58 mmol/mL) but was essentially nontoxic at a concentration of 10 mg/mL (0.06 mmol/mL) (Bio/dynamics, 1990a). Bis(2-chloroethoxy)methane (0.5 mL, undiluted) was not irritating to the cornea of albino rabbits (sex not specified) in an ocular toxicity study (Carpenter and Smyth, 1946).

In a subchronic 3-month gavage toxicity study, Sprague-Dawley rats were given daily doses of 10, 20, 40, 80, or 120 mg bis(2-chloroethoxy)methane/kg (0.06, 0.12, 0.23, 0.46, or 0.69 mmol/kg) for at least 90 days (Bio/dynamics, 1990b). Of the 20 rats that received 120 mg/kg, all 10 males and seven of 10 females died or were killed in a moribund condition during the study. One death occurred after a single dose, and seven more occurred during the first week of the study. One 80 mg/kg female died on study day 78. No deaths were observed in the 10, 20, or 40 mg/kg dose groups. Rats that died exhibited emaciation, poor food consumption, hypothermia, lethargy/prostration, dyspnea/gasping, moist rales, ataxia, abnormal posture, slight tremors, salivation, and brown-yellow stains on the snout, paws, ventral surface, and/or anogenital area. Microscopic examination revealed degeneration of the myocardium in all animals that died after day 14; this was considered to be a possible cause of death.

Humans

No studies on the toxicity of bis(2-chloroethoxy)methane in humans were found in a review of the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

No information on the reproductive or developmental toxicity of bis(2-chloroethoxy)methane in experimental animals or humans was found in a review of the literature.

CARCINOGENICITY

No studies of bis(2-chloroethoxy)methane in experimental animals or epidemiology studies in humans were found in a review of the literature. The NTP found no evidence of carcinogenic activity for a number of bis(2-chloroethoxy)methane metabolites (Figure 1). Oral administration of monochloroacetic acid for up to 2 years showed no evidence of carcinogenic activity in F344/N rats or B6C3F1 mice

(NTP, 1992). Dermal application of 2-chloroethanol for up to 2 years showed no evidence of carcinogenic activity in F344/N rats or Swiss CD-1[®] mice (NTP, 1985). Dermal application of glycolic acid did not alter the photocarcinogenesis of simulated solar light (generated using a filtered 6.5 KW xenon arc light source) in a 1-year photocarcinogenicity study in SKH-1 hairless mice (NTP, 2007).

GENETIC TOXICITY

No reports of mutagenicity studies with bis(2-chloroethoxy)methane or a major metabolite, thiodiglycolic acid, were found in a search of the literature. However, thioglycolic acid, a possible metabolite of thiodiglycolic acid (Jones and Hathway, 1978), was reported to be negative in a bacterial mutagenicity assay, and the sodium salt of thioglycolic acid was also found to be negative for mutation induction in bacteria (Zeiger *et al.*, 1987). Both of these studies were conducted in multiple strains of *Salmonella typhimurium*, with and without exogenous metabolic activation. Two other metabolites of bis(2-chloroethoxy)methane, monochloroacetic acid and 2-chloroethanol, showed some evidence of mutagenic activity *in vitro* (Haworth *et al.*, 1983; Galloway *et al.*, 1987; McGregor *et al.*,

1987,1988; Ivett *et al.*, 1989); there is no clear evidence of *in vivo* genotoxicity.

In addition to the bacterial mutagenicity assays, the NTP conducted an assessment of micronucleus induction in erythrocytes of male and female B6C3F1 mice following 3 months of dermal exposure to 22.5 to 360 mg sodium thioglycolate/kg per day (NTP, unpublished data). Although frequencies of micronucleated erythrocytes in all treated groups of male mice were elevated over the control value, none reached statistical significance, and sodium thioglycolate was judged to be negative in the male mouse micronucleus assay. In females, results were positive, based on a greater than twofold increase in micronucleated erythrocytes observed at the high dose of 360 mg/kg.

STUDY RATIONALE

Bis(2-chloroethoxy)methane was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as a starting material to produce polysulfide elastomers and because there were no 2-year carcinogenicity studies reported in the literature.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION Bis(2-chloroethoxy)methane

Bis(2-chloroethoxy)methane was obtained from Karl Industries, Inc. (Aurora, OH), in two lots (B007269977 and B004160277). Lot B007269977 was used in the 2-week and 3-month studies, and lot B004160277 was used in the 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Chemistry Support Services, Columbus, OH) (Appendix I). Identity analyses were conducted by the study laboratory at Battelle Columbus Operations (Columbus, OH). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the bis(2-chloroethoxy)methane studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless liquid, was identified as bis(2-chloroethoxy)methane using infrared (IR) and proton and carbon-13 nuclear magnetic resonance spectroscopy and boiling point determinations. The purity of each lot was determined using gas chromatography (GC), and the purity of lot B004160277 was determined using differential scanning calorimetry (DSC).

For lot B007269977, Karl Fischer titration indicated 0.06% water, and elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for bis(2-chloroethoxy)methane. GC indicated one major peak and two impurities with areas of at least 0.1% of the total peak area. The overall purity of lot B007269977 was determined to be approximately 98.5%.

For lot B004160277, Karl Fischer titration indicated 0.12% water. Elemental analyses for carbon and hydrogen were consistent with the theoretical values for bis(2-chloroethoxy)methane, but the elemental analysis value for chlorine was approximately 6% less than the theoretical value; this low value appeared to be anomalous. DSC indicated a purity of 98.62%. GC indicated a major peak and three impurities; the

impurities together represented 1.7% of the total peak area. The overall purity of lot B004160277 was determined to be approximately 98.2%.

To ensure stability, the bulk chemical was stored at less than or equal to -20° C, protected from light in glass bottles. Periodic reanalyses of the bulk chemical were performed during the 2-week, 3-month, and 2-year studies using GC; no degradation of the bulk chemical was observed.

Ethanol

USP-grade 95% ethanol was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and used as the vehicle during the 2-week, 3-month, and 2-year studies. Identity was confirmed by the study laboratory using IR spectroscopy, and purity was determined by the study laboratory using GC. No impurities with areas of 0.1% or greater relative to the major peak area were found in any of the lots, and benzene was not found in any of the lots.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing bis(2-chloroethoxy)methane with 95% ethanol to give the required concentrations (Table I2). Stability studies of a 5 mg/mL formulation were performed by the analytical chemistry laboratory using GC. Stability was confirmed for at least 42 days for dose formulations stored at room temperature in amber glass bottles sealed with Teflon[®]-lined lids and for at least 3 hours under simulated animal room conditions provided the bottle was kept sealed between the brief periods of formulation removal.

Periodic analyses of the dose formulations of bis(2-chloroethoxy)methane were conducted by the study laboratory using GC. During the 2-week studies, the dose formulations were analyzed once; all five formulations for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all

10 animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I4). Of the dose formulations analyzed, all 15 for rats and mice were within 10% of the target concentrations; all 30 animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 36 for rats and all 72 for mice were within 10% of the target concentrations; all 36 animal room samples were within 10% of the target concentrations.

2-WEEK STUDIES

Male and female F344/N and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats were 4 to 5 weeks old, and the mice were 5 to 6 weeks old. Animals were quarantined for 11 days and were approximately 5 to 6 (rats) or 6 to 7 (mice) weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease.

The dermal route was selected as the route of exposure to mimic potential human exposure to the chemical from its manufacture and use as a solvent or sealant. The doses selected for the 2-week dermal studies (0, 12.5, 25, 50, 100, and 200 mg/kg) were based on the expectation that approximately 50% of the chemical would be absorbed after dermal application (RTI, 2002). It was estimated that a dermal dose of 200 mg/kg would result in an actual dose of approximately 100 mg/kg (RTI, 2002), a dose somewhat above the reported oral rat LD₅₀ of 65 mg/kg (Smyth and Carpenter, 1948).

Groups of five male and five female rats and mice received dermal applications of 0, 12.5, 25, 50, 100, or 200 mg bis(2-chloroethoxy)methane/kg body weight per day in ethanol, 5 days per week for 16 (rats) or 17 (mice) days; dosing volumes were 0.5 mL/kg body weight for rats and 2.0 mL/kg for mice. The doses were applied to the dorsal region from the mid-back to the interscapular area, with the longitudinal axis oriented along that of the rodent. Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, on study day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on vehicle control and 200 mg/kg rats and mice. Table 1 lists the tissues and organs examined.

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to bis(2-chloroethoxy)methane and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 11 (male rats), 12 (female rats), 13 (female mice), or 14 (male mice) days and were 5 to 6 (rats) or 6 to 7 (mice) weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At 1 month and at the end of the studies, serologic analyses were performed on five male and five female sentinel rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix K).

Groups of 10 male and 10 female rats and mice received dermal applications of 0, 50, 100, 200, 400, or 600 mg bis(2-chloroethoxy)methane/kg body weight per day in ethanol, 5 days per week for 14 weeks; dosing volumes were 0.5 (rats) or 2 (mice) mL/kg. Additional clinical pathology groups of 10 male and 10 female rats received the same doses for 23 days. The doses were applied to the dorsal region from the mid-back to the interscapular area, with the longitudinal axis oriented along that of the rodent. Feed and water were available *ad libitum*. Rats and mice were housed individually. Clinical findings were recorded weekly and at study termination for core study rats and mice. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 23 and from core study rats and mice at the end of the study for hematology and clinical chemistry (rats). Blood samples for hematology analyses were placed in tubes containing potassium EDTA. Erythrocyte, leukocyte, and platelet counts; hemoglobin concentrations; packed cell volume; mean cell volume; mean cell hemoglobin;

and mean cell hemoglobin concentration were determined using a Cell Dyn 3500 (Abbott Diagnostics, Abbott Park, IL). Manual hematocrit values were compared to Cobas values for packed cell volume. Blood smears for rats and mice were stained with Wright-Giemsa stain. Leukocyte differential counts for rats and mice were based on classifying a minimum of 100 white cells. Reticulocytes were stained with new methylene blue and enumerated as a reticulocyte: erythrocyte ratio using the Miller disc method (Brecher and Schneiderman, 1950). Blood samples for clinical chemistry analyses were placed in tubes containing separator gel and allowed to clot. After clot retraction occurred, the samples were centrifuged, and the serum was aliquoted for assay of serum chemistry analytes using a Hitachi 911 (Roche Diagnostics Corporation, Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice administered 0, 50 (female rats), 100 (rats and female mice), 200, 400 (male rats and male and female mice), or 600 (male mice) mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (eyes were fixed in Davidson's solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of approximately 5 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control rats and mice, core study 400 and 600 mg/kg rats, and 600 mg/kg mice. The following tissues were examined to a no-effect level: the Harderian gland, heart, kidney, mesenteric lymph node, nose, spleen, and glandular stomach, and thymus of rats; the uterus of female rats; the liver of mice; and the heart, kidney, mandibular and mesenteric lymph nodes, and thymus of female mice.

After a review of the laboratory reports and selected histopathology slides by a quality assessment pathologist, the findings and reviewed slides were submitted to a NTP Pathology Working Group (PWG) coordinator for a second independent review. Any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists were resolved by the NTP pathology peer review process. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, NTP pathologist, reviewing pathologist(s), if any, and the PWG coordinator. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats received dermal applications of 0, 75, 150, or 300 mg bis(2-chloroethoxy)methane/kg body weight per day in ethanol (dosing volume, 0.5 mL/kg), 5 days per week for 105 weeks. Groups of 50 male mice received dermal applications of 0, 150, 300, or 600 mg/kg in ethanol, 5 days per week for 105 weeks. Groups of 50 female mice received dermal applications of 0, 100, 200, or 400 mg/kg in ethanol, 5 days per week for 104 weeks. The dosing volume for mice was 2 mL/kg. For rats and mice, the doses were applied to the dorsal region from the mid-back to the interscapular area, with the longitudinal axis oriented along that of the rodent.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Animals were quarantined

for 11 (male rats), 12 (female rats), 13 (female mice), or 14 (male mice) days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats were 5 to 6 weeks old and mice were 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were housed individually. Feed and water were available *ad libitum*. Racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded on study day 29, every 4 weeks thereafter, and at the end of the studies. All animals were weighed initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 μm , and stained with hematoxylin and eosin for microscopic examination; heart sections from early death female rats and male mice were stained with Gomori's Methenamine Silver, Masson's Trichrome, and Movat's Pentachrome. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. 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 Toxicology Data Management System. The report slides, paraffin blocks, residual wet tissues, and pathology data were sent to the NTP Archives for inventory, slide/block match, wet tissue audit, and storage. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histo-technique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the heart and skin of rats and mice and the stomach (forestomach and glandular) of rats.

The quality assessment report and the reviewed slides were submitted to the NTP PWG coordinator, who reviewed the selected tissues and addressed any inconsistencies in 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 coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups. 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(s), 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 Dermal Studies of Bis(2-chloroethoxy)methane

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies 11 days	Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days	Rats: 11 (males) or 12 (females) days Mice: 13 (females) or 14 (males) days
Average Age When Studies Began Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 5 to 6 weeks Mice: 6 to 7 weeks	Rats: 5 to 6 weeks Mice: 6 to 7 weeks
Date of First Dose September 10, 2001	Rats: February 4 (males) or 5 (females), 2002 Mice: February 6 (females) or 7 (males), 2002	Rats: September 16 (males) or 17 (females), 2002 Mice: October 2 (females) or 3 (males), 2002
Duration of Dosing 5 doses per week for 16 (rats) or 17 (mice) days	5 doses per week for 14 weeks	Rats: 5 doses per week for 105 weeks Mice: 5 doses per week for 104 (females) or 105 (males) weeks
Date of Last Dose Rats: September 25, 2001 Mice: September 26, 2001	Rats: May 6 (males) or 7 (females), 2002 Mice: May 8 (females) or 9 (males), 2002	Rats: September 13 (males) or 15 (females), 2004 Mice: September 28 (females) or 30 (males), 2004
Necropsy Dates Rats: September 26, 2001 Mice: September 27, 2001	Rats: May 7 (males) or 8 (females), 2002 Mice: May 9 (females) or 10 (males), 2002	Rats: September 13-14 (males) or 15-16 (females), 2004 Mice: September 27-29 (females) or September 29-October 1 (males), 2004
Average Age at Necropsy 8 weeks (rats) 9 to 10 weeks (mice)	19 to 20 weeks	109 to 111 weeks
Size of Study Groups 5 males and 5 females	10 males and 10 females	50 males and 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage 1	1	1

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Bis(2-chloroethoxy)methane

2-Week Studies	3-Month Studies	2-Year Studies
Method of Animal Identification		
Tail tattoo	Tail tattoo	Tail tattoo
Diet		
Irradiated NTP-2000 pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available <i>ad libitum</i> , changed weekly	Same as 2-week studies	Same as 2-week studies
Water		
Tap water (City of Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available <i>ad libitum</i>	Same as 2-week studies	Same as 2-week studies
Cages		
Polycarbonate (Lab Products, Inc., Seaford, DE), changed weekly	Same as 2-week studies	Same as 2-week studies, except rat cages changed twice weekly beginning week 14
Bedding		
Irradiated Sani-Chips (P.J. Murphy Forest Products Corporation, Montville, NJ), changed weekly	Same as 2-week studies	Same as 2-week studies, except rat bedding changed twice weekly beginning week 14
Rack Filters		
Spun-bonded polyester (Snow Filtration Company, Cincinnati, OH), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks		
Stainless steel (Lab Products, Inc., Seaford, DE), changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses		
0, 12.5, 25, 50, 100, or 200 mg/kg in ethanol (dosing volumes, 0.5 mL/kg for rats and 2 mL/kg for mice)	0, 50, 100, 200, 400, or 600 mg/kg in ethanol (dosing volumes, 0.5 mL/kg for rats and 2 mL/kg for mice)	Rats: 0, 75, 150, or 300 mg/kg in ethanol (dosing volume, 0.5 mL/kg) Mice: 0, 150, 300, or 600 (males) or 0, 100, 200, or 400 (females) mg/kg in ethanol (dosing volume, 2 mL/kg)
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on study day 8, and at the end of the studies; clinical findings were recorded daily.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly and at the end of the studies.	All animals were observed twice daily. Clinical findings were recorded on study day 29, every 4 weeks thereafter, and at the end of the studies. All animals were weighed initially, weekly for the first 13 weeks, every 4 weeks thereafter, and at the end of the studies.
Method of Sacrifice		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Bis(2-chloroethoxy)methane

2-Week Studies	3-Month Studies	2-Year Studies
<p>Necropsy Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.</p>	<p>Necropsies were performed on all core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.</p>	<p>Necropsies were performed on all animals.</p>
<p>Clinical Pathology None</p>	<p>Blood was collected from the retroorbital sinus of clinical pathology rats on days 4 and 23 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats). Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids</p>	<p>None</p>
<p>Histopathology Histopathology was performed on vehicle control and 200 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: colon, small intestine, kidney, liver, skin (site of application), and stomach (forestomach and glandular).</p>	<p>Complete histopathology was performed on all vehicle control rats and mice, core study 400 and 600 mg/kg rats, and 600 mg/kg mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus. The following tissues were examined to a no-effect level: Harderian gland, heart, kidney, mesenteric lymph node, nose, spleen, glandular stomach, and thymus of rats; the uterus of female rats; the liver of mice; and the heart, kidney, mandibular and mesenteric lymph nodes, and thymus of female mice.</p>	<p>Complete histopathology was performed on all rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eyes, gallbladder (mice), Harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (with mainstem bronchus), lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin (site of application), spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.</p>

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of Bis(2-chloroethoxy)methane

2-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology None	At the end of the studies, sperm samples were collected from male animals in the 0, 100 (rats), 200, 400, and 600 (mice) mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility, count, and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females administered 0, 50 (rats), 100, 200, or 400 (mice) mg/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

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; 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 two sided. Clusters of early death times were determined by cluster analysis, using the centroid method (Gower, 1967).

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A3, B1, B3, C1, C3, D1, and D3 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, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., mesentery, pleura, peripheral nerve, skeletal muscle, tongue, tooth, and Zymbal's gland) before microscopic evaluation, the denominators consist of the number of animals that had a gross abnormality. When neoplasms had multiple

potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 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, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion 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 lesion 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 lesion 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 B6C3F1 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 lesion 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 lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as $1-P$ with the letter N added (e.g., $P=0.99$ is presented as $P=0.01N$).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were

investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regular cycling females in each exposed group were compared to the control group using the Fisher exact test (Gart *et al.*, 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. 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 NIEHS. The audit findings were reviewed and assessed

by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of bis(2-chloroethoxy)methane was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, micronucleated polychromatic erythrocytes (reticulocytes) in rat bone marrow, and increases in the frequency of micronucleated normochromatic erythrocytes in mouse peripheral blood. Micronuclei (literally “small nuclei” or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical's carcinogenicity in experimental animals based on numerous considerations, 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). The short-term tests were originally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests 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 have high predictivity for rodent carcinogenicity; a weak response in one sex only or negative results in both sexes in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies (Witt *et al.*, 2000). 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

RATS 2-WEEK STUDY

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of dosed rats were similar to those of the vehicle control groups. There were no biologically significant differences in organ weights between the dosed and vehicle control

groups (Table G1). No clinical findings or histopathologic lesions related to bis(2-chloroethoxy)methane administration were observed.

Dose Selection Rationale: Because of the lack of toxicity in the 2-week study, doses of 0, 50, 100, 200, 400, and 600 mg/kg were selected for the 3-month study in rats.

TABLE 2
Survival and Body Weights of Rats in the 2-Week Dermal Study of Bis(2-chloroethoxy)methane

Dose (mg/kg)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	89 ± 4	154 ± 4	65 ± 4	
12.5	5/5	87 ± 4	153 ± 6	65 ± 3	99
25	5/5	87 ± 4	149 ± 4	62 ± 2	97
50	5/5	89 ± 4	155 ± 7	67 ± 3	101
100	5/5	88 ± 4	152 ± 5	64 ± 3	99
200	5/5	87 ± 4	152 ± 5	66 ± 2	99
Female					
0	5/5	79 ± 3	121 ± 4	43 ± 2	
12.5	5/5	78 ± 3	119 ± 4	41 ± 2	98
25	5/5	78 ± 2	117 ± 3	39 ± 3	97
50	5/5	79 ± 3	119 ± 3	40 ± 2	98
100	5/5	79 ± 3	118 ± 3	39 ± 1	98
200	5/5	78 ± 2	119 ± 1	40 ± 2	98

^a Number of animals surviving at 2 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

3-MONTH STUDY

All 600 mg/kg males and females and two 400 mg/kg females died before the end of the study (Table 3). The cause of death was considered to be related to the cardiotoxic effect of bis(2-chloroethoxy)methane. Of the surviving groups, final mean body weights were similar to those of the vehicle controls; the body weight gain of 400 mg/kg males was significantly less than that of the vehicle controls (Table 3; Figure 2). Clinical

findings included prostration and ataxia in 600 mg/kg rats during the first week of the study and nasal/eye discharge, lethargy, ataxia, and abnormal breathing in 400 and 600 mg/kg females beginning week 5. An enlarged heart was noted in one 100 mg/kg female rat. Otherwise, gross findings were interpreted to be spontaneous and unrelated to bis(2-chloroethoxy)-methane administration.

TABLE 3
Survival and Body Weights of Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

Dose (mg/kg)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	93 ± 3	332 ± 8	239 ± 6	
50	10/10	93 ± 3	322 ± 7	229 ± 6	97
100	10/10	93 ± 3	320 ± 8	227 ± 7	96
200	10/10	93 ± 3	329 ± 7	236 ± 7	99
400	10/10	92 ± 3	307 ± 7	215 ± 5*	92
600	0/10 ^c	93 ± 3	—	—	—
Female					
0	10/10	89 ± 2	194 ± 4	106 ± 4	
50	10/10	89 ± 3	193 ± 3	104 ± 3	99
100	10/10	89 ± 3	189 ± 3	100 ± 3	97
200	10/10	89 ± 3	190 ± 3	101 ± 3	98
400	8/10 ^d	88 ± 3	195 ± 4	106 ± 3	100
600	0/10 ^e	89 ± 3	—	—	—

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test

^a Number of animals surviving at 3 months/number initially in group

^b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.

^c Weeks of death: 1, 1, 1, 1, 6, 7, 7, 8, 8, 10

^d Week of deaths: 11

^e Weeks of death: 4, 4, 5, 5, 5, 5, 5, 6, 6, 6

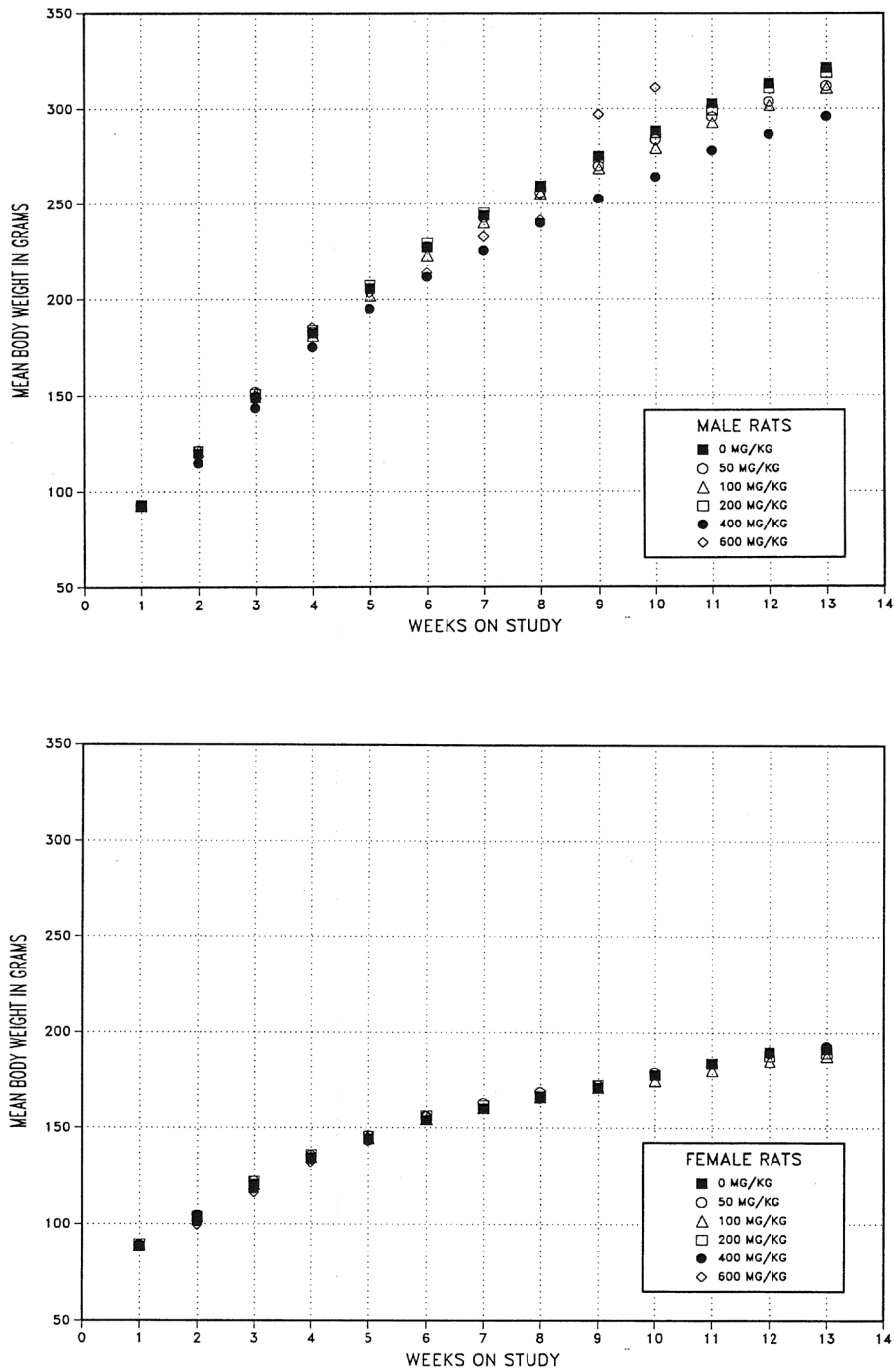


FIGURE 2
Growth Curves for Rats Administered Bis(2-chloroethoxy)methane Dermally for 3 Months

There were no changes in the hematology or clinical chemistry endpoints attributable to the administration of bis(2-chloroethoxy)methane (Table F1).

Relative kidney weights of 100, 200, and 400 mg/kg males were significantly greater than that of the vehicle control group (Table G2).

There were no significant differences in sperm parameters of male rats administered 100, 200, or 400 mg/kg and no significant differences in the estrous cyclicity of female rats administered 50, 100, or 200 mg/kg when compared to the vehicle controls (Tables H1 and H2).

Treatment-related target organs included the heart, lymphoid organs, and Harderian gland. While the lesions in the heart are considered to be a direct toxic effect of bis(2-chloroethoxy)methane, the changes noted in the other organs are considered to reflect indirect stress effects. Generally, dose-related increased incidences and severities, ranging from minimal to moderate, of myofiber cytoplasmic vacuolization and interstitial mononuclear cell infiltration in the heart occurred in 400 and 600 mg/kg male and female rats and in 200 mg/kg females (Table 4). Increased incidences and severities of myofiber necrosis occurred in 600 mg/kg males and females; one female each in the 200 and 400 mg/kg groups also had this lesion. Three 600 mg/kg males had atrial thrombosis. Significantly decreased incidences of spontaneous cardiomyopathy occurred in 400 and 600 mg/kg males and females and in 200 mg/kg females (male: vehicle control, 10/10; 50 mg/kg, 8/10; 100 mg/kg, 10/10; 200 mg/kg, 10/10; 400 mg/kg, 2/10; 600 mg/kg, 1/10; female: 8/10, 6/10, 8/10, 4/10, 3/10, 0/10). This was likely due to the overwhelming chemical-related cardiotoxicity which masked the spontaneous cardiomyopathy. Cardiomyopathy was recorded separately from the presumptive cardiotoxic lesions. The decreases in incidences of cardiomyopathy in the 400 and 600 mg/kg groups were due to increases in cardiotoxic lesions. A semiquantitative grading scheme was used to evaluate the extent of the lesions in the heart sections as follows: minimal (grade 1), lesions involved less than 10% of the heart section; mild (grade 2), lesions involved 11% to 40% of the heart section; moderate (grade 3), lesions involved 41% to 80% of the heart section; marked (grade 4), lesions involved 81% to 100% of the heart section. The no-observed-adverse-effect level for the heart lesions was 200 mg/kg for male rats and 100 mg/kg for female rats.

Mononuclear cell infiltration consisted of a widespread increase in nuclear density attributable to an increase in cells in the interstitium. These cells were not immediately identifiable, having indistinct borders, pale eosinophilic, sometimes fibrillar cytoplasm and elongated nuclei. Sometimes these cells' nuclei had a central condensed core (Anitschkow cells), which suggests a histiocytic origin. Cardiomyopathy is a common, spontaneously occurring degenerative lesion of the myocardium of rats and mice that increases in frequency with age. Its cause is uncertain, although it has been suggested to be due to focal ischemia resulting from myocardial vascular disease (Ayers and Jones, 1978). The age of onset and the severity are affected by diet, environment, and stress (MacKenzie and Alison, 1990). It is relatively common in rats, especially in males where it can be observed by 3 or 4 months of age (Ruben *et al.*, 2000). Although cardiomyopathy is a spontaneous pathology, increased incidences may be chemical related (Jokinen *et al.*, 2005). Cardiomyopathy consists of a spectrum of degenerating/necrotic myofibers with mononuclear cells, mostly macrophages, that are present for cleanup and repair. The lesions are generally small (less than 30 cells) and focal to multifocal. The cardiomyocytes in these areas are often missing or appear to be hypereosinophilic with hyalinized cytoplasm. Infrequent cardiomyocyte vacuolization is present, but it is a minor component of the overall lesion (Plate 1). Fibrosis is often observed in older lesions and therefore is more obvious in 2-year studies than in 3-month studies. Atrial thrombosis consisted of a mature blood clot present within the lumen of the atrium, consisting of alternating areas of fibrin and layered cellular elements (white blood cells and platelets) with irregular pockets of erythrocytes (Plate 2). Diffuse myocardial vacuolization is generally considered to be related to treatment and was described as the main characteristic of chemically induced myofiber degeneration (Jokinen *et al.*, 2005). Cardiomyocyte vacuolization consisted of a widespread accumulation of multiple, well demarcated, round, variably sized (primarily small), clear vacuoles within the cardiomyocyte sarcoplasm that occasionally coalesced into larger vacuoles (Plates 3 and 4). Vacuoles, often similar in appearance, in the interstitium were interpreted as a spontaneous background change consisting of a minimal amount of vacuolar change within a focus of spontaneous cardiomyopathy and were not included in this diagnosis. Cardiomyocyte necrosis was characterized by small areas containing fragmented, angular, brightly eosinophilic myofibers with dark, shrunken nuclei. The same cardiomyocytes may also have contained variable numbers of clear

TABLE 4
Incidences of Nonneoplastic Lesions of the Heart in Rats in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male						
Number Examined Microscopically	10	10	10	10	10	10
Infiltration Cellular, Mononuclear Cell ^a	0	0	0	0	7** (1.0) ^b	10** (1.4)
Atrial Thrombosis Myocardium, Vacuolization Cytoplasmic	0	0	0	0	0	3 (3.7)
Myocardium, Necrosis	0	0	0	0	6** (1.3)	10** (1.5)
	0	0	0	0	0	7** (1.7)
Female						
Number Examined Microscopically	10	10	10	10	10	10
Infiltration, Cellular, Mononuclear Cell	0	0	0	7** (1.0)	6** (1.2)	10** (1.9)
Myocardium, Vacuolization Cytoplasmic	0	0	0	2 (1.0)	5* (1.4)	9** (1.8)
Myocardium, Necrosis	0	0	0	1 (1.0)	1 (1.0)	5* (1.4)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test

** $P \leq 0.01$

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

vacuoles or vacuoles containing pale-staining fibrillar material.

Increased incidences, mostly significant, of lymphoid necrosis and atrophy in the thymus, mesenteric lymph node, and spleen, as well as porphyrin pigmentation in the Harderian gland, occurred in 600 mg/kg male and female rats (data not shown). Increased porphyrin secretion and lymphoid tissue changes are suggested to be related to stress (Greaves, 2000).

Dose Selection Rationale: In the 3-month rat study, cardiac toxicity was seen at 400 and 600 mg/kg in male and female rats. Most of the male rats at 600 mg/kg died early due to the cardiac toxicity and associated side effects in other organ systems. Treatment-related early deaths were seen in female rats at 400 and 600 mg/kg. The top dose for the 2-year rat study was set at 300 mg/kg, a dose below the level where cardiac toxicity was seen in the 3-month study. The doses selected for the 2-year rat study were 0, 75, 150, and 300 mg/kg.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 3). One male rat in the 150 mg/kg group died on study day 282; all other male rats survived at least 316 days. Among female rats during the first year of the study, there were two clusters of early deaths that occurred only in the 300 mg/kg group. The earlier cluster ranged from study days 66 to 121 and involved nine animals; the later cluster ranged

from study days 189 to 262 and involved five animals. There were no significant differences in the incidences or severities of histopathologic heart lesions in rats that died during the first year of the study compared to those that died during the second year (data not shown). All animals in the 75 and 150 mg/kg groups survived at least 332 days, and except for one vehicle control animal that died on study day 39, female vehicle controls survived at least 469 days.

TABLE 5
Survival of Rats in the 2-Year Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	21	17	31	20
Natural deaths	8	4	5	6
Animals surviving to study termination	21	29 ^a	14	24
Percent probability of survival at end of study ^b	42	58	28	48
Mean survival (days) ^c	676	669	638	688
Survival analysis ^d	P=0.815N	P=0.331N	P=0.088	P=0.409N
Female				
Animals initially in study	50	50	50	50
Moribund	19	18	15	19
Natural deaths	12	2	7	13
Animals surviving to study termination	19	30	28	18
Percent probability of survival at end of study	38	60	56	36
Mean survival (days)	646	680	686	518
Survival analysis	P=0.169	P=0.041N	P=0.061N	P=0.437

^a Includes one animal that died during the last week of the study

^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.

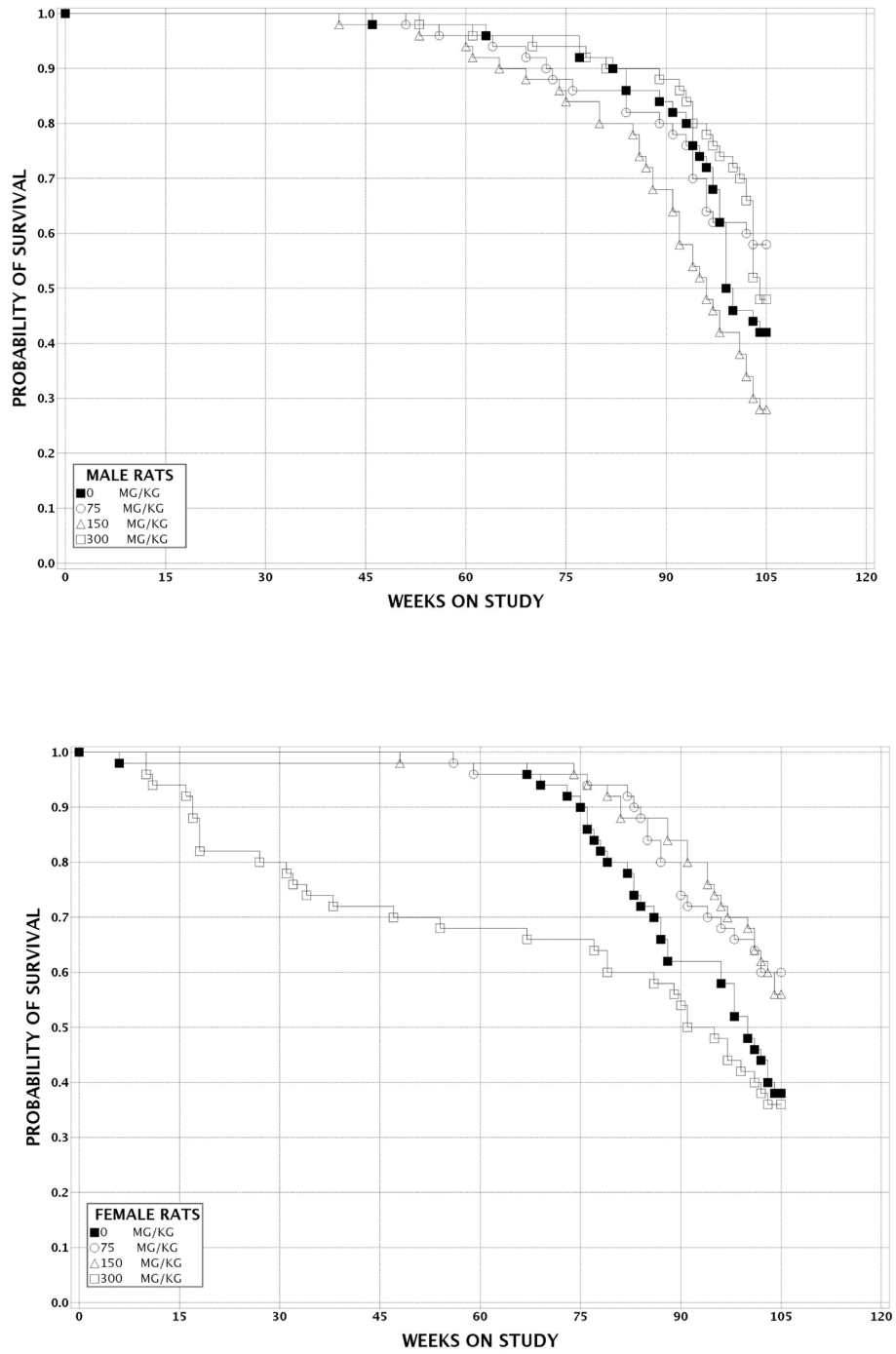


FIGURE 3
Kaplan-Meier Survival Curves for Rats
Administered Bis(2-chloroethoxy)methane Dermally for 2 Years

Body Weights and Clinical Findings

Mean body weights of dosed rats were similar to those of the vehicle controls throughout the study (Figure 4; Tables 6 and 7). Clinical findings in 300 mg/kg females that died during the first year of the study

included abnormal breathing, lethargy, thinness, nasal discharge, and ataxia. No chemical-related clinical findings were observed in the other dosed groups or in 300 mg/kg females during the second year of the study.

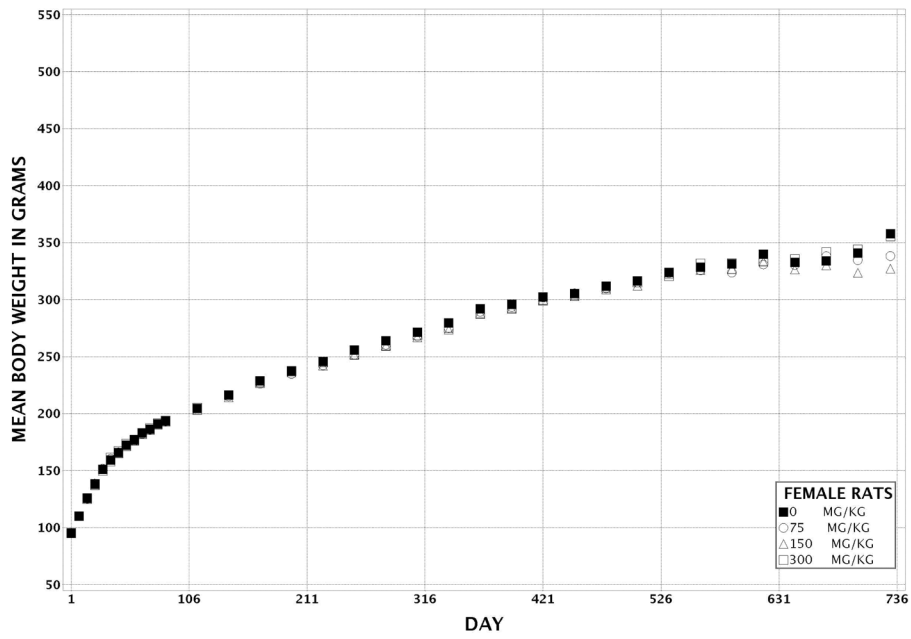
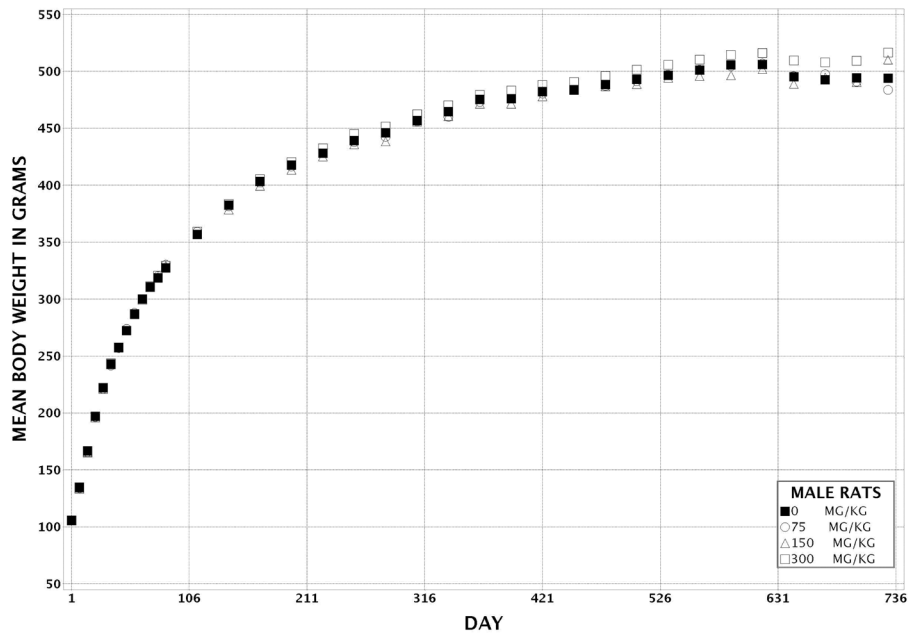


FIGURE 4
Growth Curves for Rats Administered Bis(2-chloroethoxy)methane Dermally for 2 Years

TABLE 6
Mean Body Weights and Survival of Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

Days on Study	Vehicle Control		75 mg/kg			150 mg/kg			300 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	106	50	106	100	50	106	100	50	106	100	50
8	135	50	134	99	50	135	100	50	134	99	50
15	167	50	166	99	50	166	99	50	165	99	50
22	197	50	197	100	50	196	100	50	197	100	50
29	222	50	221	100	50	221	100	50	222	100	50
36	243	50	242	99	50	243	100	50	244	100	50
43	258	50	257	100	50	258	100	50	258	100	50
50	273	50	274	101	50	273	100	50	272	100	50
57	287	50	288	100	50	287	100	50	287	100	50
64	300	50	300	100	50	300	100	50	300	100	50
71	311	50	311	100	50	311	100	50	312	100	50
78	319	50	320	101	50	320	100	50	321	101	50
85	328	50	330	101	50	329	101	50	330	101	50
113	357	50	359	101	50	357	100	50	360	101	50
141	383	50	382	100	50	379	99	50	384	100	50
169	403	50	402	100	50	400	99	50	405	101	50
197	418	50	417	100	50	414	99	50	421	101	50
225	428	50	428	100	50	425	99	50	432	101	50
253	439	50	438	100	50	436	99	50	445	101	50
281	446	50	443	99	50	439	98	50	452	101	50
309	457	50	456	100	50	456	100	49	462	101	50
337	465	49	460	99	50	461	99	49	470	101	50
365	475	49	473	100	49	472	99	49	479	101	50
393	476	49	476	100	48	472	99	48	483	102	49
421	482	49	481	100	48	478	99	47	488	101	49
449	484	48	484	100	47	484	100	46	491	101	48
477	488	48	487	100	47	487	100	45	496	102	48
505	493	48	491	100	44	489	99	44	502	102	47
533	497	47	497	100	43	495	100	42	506	102	47
561	501	46	502	100	43	496	99	40	510	102	46
589	506	43	505	100	41	497	98	40	515	102	45
617	506	43	507	100	40	502	99	34	516	102	45
645	495	40	496	100	38	489	99	29	510	103	43
673	493	36	498	101	31	495	101	24	508	103	39
701	494	23	491	99	31	491	99	21	509	103	36
Mean for weeks											
1-13	242		242	100		242	100		242	100	
14-52	422		420	100		418	99		426	101	
53-101	492		491	100		488	99		501	102	

TABLE 7
Mean Body Weights and Survival of Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

Days on Study	Vehicle Control		75 mg/kg			150 mg/kg			300 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	95	50	95	100	50	95	100	50	95	100	50
8	110	50	110	100	50	110	100	50	110	100	50
15	126	50	126	100	50	125	100	50	126	100	50
22	139	50	139	100	50	137	99	50	138	100	50
29	151	50	152	100	50	150	99	50	151	100	50
36	159	50	161	101	50	158	99	50	162	101	50
43	166	49	166	100	50	165	100	50	167	101	50
50	172	49	172	100	50	172	100	50	174	101	50
57	177	49	176	100	50	176	100	50	177	100	50
64	183	49	182	100	50	182	100	50	183	100	50
71	187	49	186	100	50	186	100	50	187	101	47
78	191	49	191	100	50	190	100	50	192	100	47
85	194	49	193	100	50	193	100	50	194	100	47
113	205	49	205	100	50	203	99	50	205	100	46
141	217	49	215	99	50	214	99	50	217	100	41
169	229	49	226	99	50	227	99	50	228	100	41
197	237	49	235	99	50	236	100	50	238	100	40
225	246	49	242	99	50	242	99	50	245	100	38
253	256	49	252	98	50	252	98	50	252	99	37
281	264	49	260	99	50	259	98	50	260	98	36
309	271	49	268	99	50	267	98	50	269	99	36
337	280	49	275	98	50	274	98	49	276	99	35
365	292	49	289	99	50	288	99	49	288	99	35
393	296	49	294	99	49	292	99	49	293	99	34
421	302	49	302	100	48	300	99	49	299	99	34
449	305	49	306	100	48	303	99	49	304	100	34
477	312	48	310	99	48	309	99	49	311	100	33
505	317	47	315	100	48	312	99	49	315	100	33
533	324	43	323	100	47	323	100	47	321	99	33
561	328	40	326	99	47	326	99	45	332	101	30
589	331	36	324	98	44	327	99	44	332	100	30
617	340	31	331	97	40	333	98	42	334	98	29
645	333	31	331	99	36	326	98	40	336	101	25
673	334	29	338	101	34	330	99	35	342	102	24
701	341	24	335	98	33	324	95	34	344	101	21
Mean for weeks											
1-13	158		158	100		157	100		158	100	
14-52	245		242	99		242	99		243	99	
53-101	320		317	99		315	99		319	100	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the nose, forestomach, adrenal cortex, liver, and lung. 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 Appendix A for male rats and Appendix B for female rats.

Nose: Significantly increased incidences of degeneration of the olfactory epithelium occurred in all dosed groups of males and in 150 and 300 mg/kg females (Tables 8, A3, and B3). Olfactory degeneration consisted of focal areas of disorganization and loss of the normal olfactory epithelium with accompanying loss of the underlying olfactory nerve processes and varying numbers of small vacuoles within the olfactory epithelium (Plates 5 and 6). This lesion was most commonly located in the dorsal region of Level II. The olfactory epithelial changes were graded in a semiquantitative manner as follows: 0, within normal limits; 1, 20% or less of the olfactory epithelium was affected; 2, 21% to 40% of the olfactory epithelium was affected; 3, 41% to 70% of the olfactory epithelium was affected; 4, greater than 71% of the olfactory epithelium was affected.

Forestomach: Increased incidences of inflammation and ulcers occurred in all groups of dosed males; the increases in the incidences of inflammation at 150 and 300 mg/kg and of ulcers at 300 mg/kg were significant (Tables 8 and A3). Inflammation consisted of accumulation within the lamina propria of inflammatory cells, predominantly lymphocytes, with occasional macrophages and plasma cells. Ulceration consisted of a loss of the stratified squamous epithelium with exposure of the underlying lamina propria. Usually only a single ulcer was present.

Adrenal cortex: A significantly increased incidence of necrosis occurred in 300 mg/kg female rats (Tables 8 and B3). Microscopically, necrosis consisted of a well-demarcated ovoid area of coagulation, hyper-eosinophilia, and loss of cellular detail within the

adrenal cortex. Six of the seven animals that had necrosis died during the first year of the study, and it has been suggested that such necrosis is stress related (Greaves, 2000).

Liver: Increased incidences of cystic degeneration occurred in 150 and 300 mg/kg male rats; the incidence was significantly increased in the 300 mg/kg group (Tables 8 and A3). Cystic degeneration consisted of a multilocular cystic area containing a finely granular or flocculent eosinophilic material, apparently resulting from the distention and occasional rupture of adjacent hepatocytes.

Lung: Incidences of alveolar/bronchiolar adenoma in female rats occurred with a positive trend (vehicle control, 0/50; 75 mg/kg, 0/50; 150 mg/kg, 3/50; 300 mg/kg, 2/50; Tables B1 and B2) but were within the historical control ranges for dermal studies with ethanol vehicle control groups and for all routes [dermal studies: 4/150 (3% ± 5%), range 0%-8%; all routes: 24/1,350 (2% ± 3%), range 0%-8%]. The incidences of alveolar/bronchiolar adenoma were decreased in dosed groups of male rats compared to the vehicle controls (4/50, 1/50, 3/50, 0/50; Tables A1 and A2). Significantly increased incidences of pulmonary alveolar epithelial hyperplasia occurred in dosed groups of female rats (1/50, 7/50, 7/50, 6/50); the incidences in males were increased but not significantly (7/50, 12/50, 9/50, 8/50) (Tables A3 and B3). Because the incidences of alveolar/bronchiolar adenoma in females were within the historical control ranges, the incidences of adenoma in dosed males were decreased, and incidences of pulmonary alveolar epithelial hyperplasia were not significantly increased in dosed males, the lung changes were not considered to be chemical related. Alveolar epithelial hyperplasia consisted of focal thickening of the alveolar septa caused by increased numbers of prominent, cuboidal type II pneumocytes, with maintenance of normal alveolar septal architecture. Alveolar/bronchiolar adenoma consisted of well-demarcated hypercellular masses distorting the normal septal architecture that were characterized by well-differentiated cuboidal to round cells forming papillary projections into the alveolar or bronchiolar lumens and by slight compression of the surrounding parenchyma.

TABLE 8
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Male				
Nose ^a	50	49	50	49
Olfactory Epithelium, Degeneration ^b	5 (1.2) ^c	17** (1.3)	30** (1.3)	48** (1.9)
Forestomach	50	50	50	50
Inflammation	0	2 (3.5)	6* (3.3)	10** (2.6)
Ulcer	0	2 (3.5)	2 (4.0)	7** (3.0)
Liver	50	50	50	50
Degeneration, Cystic	6 (1.5)	5 (1.4)	10 (1.4)	15* (1.5)
Female				
Nose	49	49	50	49
Olfactory Epithelium, Degeneration	5 (1.0)	4 (1.3)	18** (1.1)	49** (2.3)
Adrenal Cortex	50	50	50	50
Necrosis	0	0	0	7** (2.6)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly 3 test

** $P \leq 0.01$

^a Number of animals with tissue examined microscopically

^b Number of animals with lesion

^c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

MICE**2-WEEK STUDY**

All mice survived to the end of the study (Table 9). Final mean body weights and body weight gains of dosed mice were similar to those of the vehicle control groups. There were no biologically significant differences between organ weights of the dosed groups and those of the vehicle control groups (Table G3). No clinical findings or histopathologic lesions related

to bis(2-chloroethoxy)methane administration were observed.

Dose Selection Rationale: Because of the lack of toxicity in the 2-week study, doses of 0, 50, 100, 200, 400, and 600 mg/kg were selected for the 3-month study in mice.

TABLE 9
Survival and Body Weights of Mice in the 2-Week Dermal Study of Bis(2-chloroethoxy)methane

Dose (mg/kg)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	22.1 ± 0.6	25.0 ± 0.6	2.9 ± 0.3	
12.5	5/5	22.4 ± 0.7	25.2 ± 0.4	2.8 ± 0.6	101
25	5/5	22.5 ± 0.6	24.9 ± 0.5	2.4 ± 0.1	100
50	5/5	23.1 ± 1.0	25.3 ± 0.7	2.2 ± 0.7	102
100	5/5	22.5 ± 0.7	24.3 ± 0.7	1.8 ± 0.1	97
200	5/5	22.5 ± 0.7	25.9 ± 0.8	3.4 ± 1.1	104
Female					
0	5/5	18.6 ± 0.3	21.6 ± 0.6	3.0 ± 0.5	
12.5	5/5	18.4 ± 0.3	21.5 ± 0.7	3.1 ± 0.5	99
25	5/5	18.2 ± 0.5	21.3 ± 0.4	3.1 ± 0.7	99
50	5/5	18.5 ± 0.3	21.6 ± 0.6	3.0 ± 0.5	100
100	5/5	17.8 ± 0.7	21.2 ± 0.8	3.4 ± 0.3	98
200	5/5	18.7 ± 0.4	22.3 ± 0.7	3.7 ± 0.5	103

^a Number of animals surviving at 2 weeks/number initially in group

^b Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

3-MONTH STUDY

Except for three 600 mg/kg females, all mice survived to the end of the study (Table 10). Final mean body weights and body weight gains of dosed and vehicle control mice were similar (Table 10; Figure 5). One 600 mg/kg female that died early exhibited lethargy, abnormal breathing, and tremors, and one animal

had clonic seizures; otherwise, no clinical findings related to bis(2-chloroethoxy)methane administration were observed. One 600 mg/kg female that died early had focal erosion of the glandular stomach. The same animal had a focus in the duodenum, found to consist of acute suppurative inflammation and thrombosis.

TABLE 10
Survival and Body Weights of Mice in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

Dose (mg/kg)	Survival ^a	Mean Body Weight ^b (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	22.8 ± 0.2	37.8 ± 0.6	15.0 ± 0.6	
50	10/10	22.8 ± 0.3	37.8 ± 1.0	14.9 ± 0.9	100
100	10/10	22.9 ± 0.2	38.3 ± 0.6	15.4 ± 0.5	101
200	10/10	22.9 ± 0.2	38.1 ± 1.0	15.2 ± 0.9	101
400	10/10	23.0 ± 0.3	36.9 ± 0.8	13.9 ± 0.6	98
600	10/10	23.0 ± 0.3	37.3 ± 1.1	14.4 ± 1.0	99
Female					
0	10/10	18.6 ± 0.2	30.5 ± 1.0	11.9 ± 1.0	
50	10/10	18.7 ± 0.2	32.5 ± 0.7	13.8 ± 0.6	107
100	10/10	18.3 ± 0.3	32.2 ± 0.9	13.8 ± 0.8	106
200	10/10	18.5 ± 0.2	31.9 ± 0.7	13.3 ± 0.6	105
400	10/10	18.7 ± 0.2	32.3 ± 0.9	13.5 ± 0.9	106
600	7/10 ^c	18.5 ± 0.1	30.9 ± 1.5	12.3 ± 1.5	101

^a Number of animals surviving at 3 months/number initially in group

^b Weights and weight changes are given as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test.

^c Weeks of death: 6, 8, 11

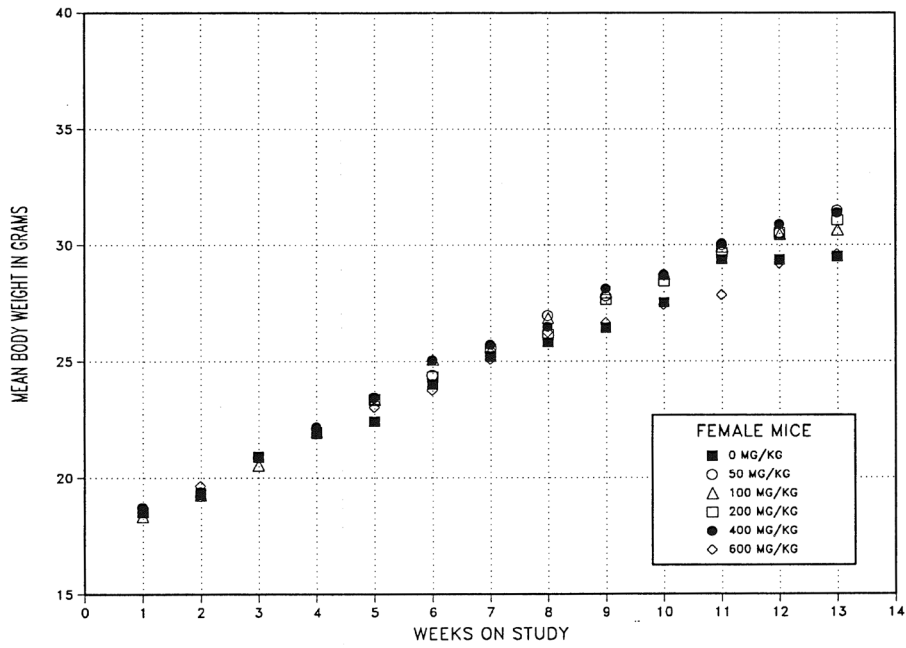
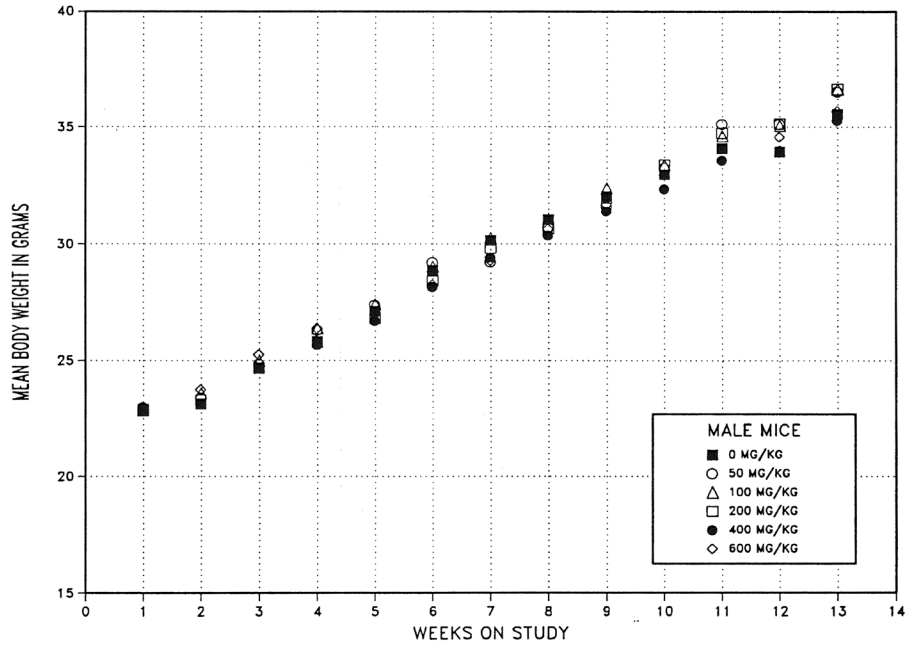


FIGURE 5
Growth Curves for Mice Administered Bis(2-chloroethoxy)methane Dermal for 3 Months

The hematology data for mice in the 3-month toxicity study of bis(2-chloroethoxy)methane are listed in Table F2. There was a decrease in the erythron, evidenced by decreases in the hematocrit values, hemoglobin concentrations, and erythrocyte counts, in 400 and 600 mg/kg males; the decreases were minimal (less than or equal to 7%). There were no changes in the reticulocyte counts of the affected males, suggesting that the decreased erythron was too small to stimulate an erythropoietic response or it was related to an erythropoietic suppression. The cause for the erythron decrease was unknown.

Absolute and relative kidney weights of 400 and 600 mg/kg males and 600 mg/kg females were significantly greater than those of the vehicle control groups (Table G4). Absolute liver weights of 400 and 600 mg/kg females were also significantly increased.

There were no significant differences in sperm parameters of male mice administered 200, 400, or 600 mg/kg and no significant differences in the estrous cyclicity of female mice administered 100, 200, or

400 mg/kg when compared to the vehicle controls (Tables H3 and H4).

Treatment-related target tissues were the heart and lymphoid organs of females. Significantly increased incidences of myofiber cytoplasmic vacuolization occurred in 400 and 600 mg/kg females (Table 11). Cardiomyocyte vacuolization consisted of a widespread accumulation of multiple, round, variably sized (primarily small), and clear vacuoles located within the cardiomyocyte sarcoplasm which were well-demarcated, clear, and occasionally coalesced into larger vacuoles (Plate 7). Vacuoles in the interstitium, often similar in appearance, were interpreted as a background change and were not included in this diagnosis. In some instances, myofiber cytoplasmic vacuolization may have been spontaneous, consisting of a minimal amount of vacuolar change within a focus of spontaneous cardiomyopathy; in which case, the lesion was not diagnosed separately. The severity of the heart lesions was graded as described for 3-month rats. The no-observed-adverse-effect level was at least 600 mg/kg for male mice and 200 mg/kg for female mice.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Female Mice in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Heart ^a	10	10	10	10	10	10
Myocardium, Vacuolization						
Cytoplasmic ^b	0	0	0	0	4* (1.0) ^c	5* (1.6)
Mandibular Lymph Node	10	10	10	10	10	10
Necrosis, Lymphoid	0	0	0	1 (1.0)	0	5* (1.4)
Mesenteric Lymph Node	10	10	10	10	10	10
Necrosis, Lymphoid	0	0	0	0	0	3 (1.7)
Spleen	10	9	9	8	7	10
Necrosis, Lymphoid	0	0	0	0	0	3 (1.7)
Thymus	10	10	10	10	10	10
Necrosis, Lymphoid	0	0	0	0	0	4* (2.8)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test

^a Number of animals with tissue examined microscopically

^b Number of animals with lesion

^c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

The incidences of lymphoid necrosis were increased in the mandibular and mesenteric lymph nodes, the spleen, and the thymus of 600 mg/kg females; one 200 mg/kg female also had lymphoid necrosis in the mandibular lymph node (Table 11). Lymphoid necrosis was characterized by diffuse necrosis (pyknosis, karyorrhexis, and lysis of lymphocyte nuclei) of lymphocytes scattered throughout the tissue and was considered to be secondary to stress.

Dose Selection Rationale: In the 3-month male mouse study, no cardiac toxicity, body weight effect, or mortality occurred at 600 mg/kg. An increase in kidney weights at 600 mg/kg was not considered to be life threatening. The doses selected for the 2-year male mouse study were 0, 150, 300, and 600 mg/kg. A dose of 600 mg/kg was considered to be too high for a 2-year female mouse study because at this dose in the 3-month study, cardiac toxicity and early death occurred in some of the female mice. The doses selected for the 2-year female mouse study were 0, 100, 200, and 400 mg/kg.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 6). Survival of 600 mg/kg male mice was significantly less than that of the vehicle control group; 10% of the 600 mg/kg males died within the first 13 weeks of the study, and 18% died within the first year. In comparison, no females and only two males in the other dose groups died during

the first year. Among the male mice, there were three clusters of deaths during the first year of the study. The earliest cluster ranged from study days 4 to 72 and involved five 600 mg/kg male mice. The second cluster ranged from study days 123 to 168 and involved three animals, one each from the 150, 300, and 600 mg/kg groups. The latest cluster ranged from study days 226 to 251 and involved three 600 mg/kg animals.

TABLE 12
Survival of Mice in the 2-Year Dermal Study of Bis(2-chloroethoxy)methane

Male	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Animals initially in study	50	50	50	50
Moribund	7	4	2	8
Natural deaths	6	6	6	14
Animals surviving to study termination	37	40 ^a	42	28 ^b
Percent probability of survival at end of study ^c	74	78	84	54
Mean survival (days) ^d	682	695	697	575
Survival analysis ^e	P=0.008	P=0.726N	P=0.307N	P=0.037
Female	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Animals initially in study	50	50	50	50
Moribund	11	5	7	11
Natural deaths	8	7	6	2
Animals surviving to study termination	31	38	37	37 ^b
Percent probability of survival at end of study	62	76	74	74
Mean survival (days)	692	697	690	713
Survival analysis	P=0.321N	P=0.222N	P=0.366N	P=0.240N

^a Includes two animals that died during the last week of the study

^b Includes one animal that died during the last week of the study

^c Kaplan-Meier determinations

^d Mean of all deaths (uncensored, censored, and terminal sacrifice)

^e 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.

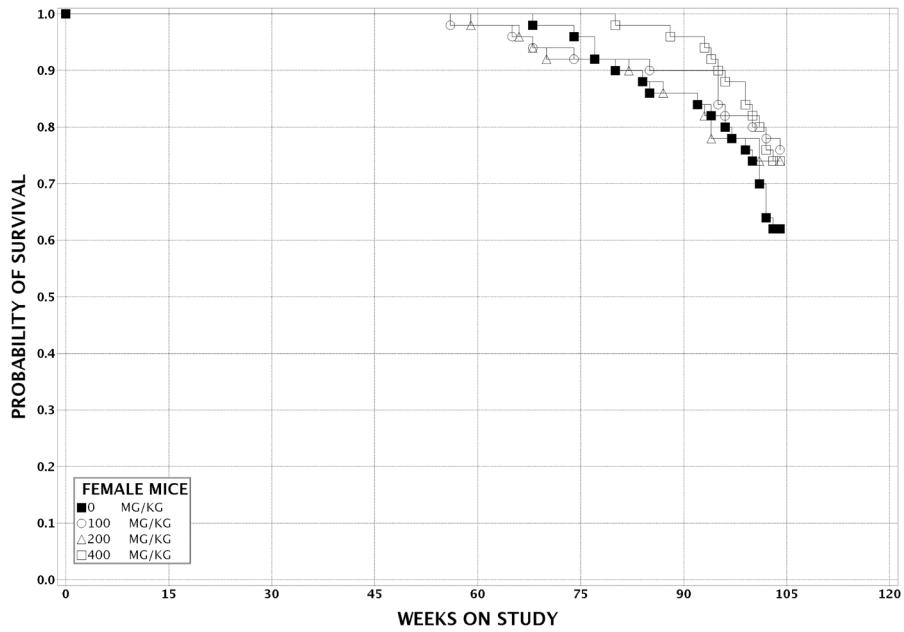
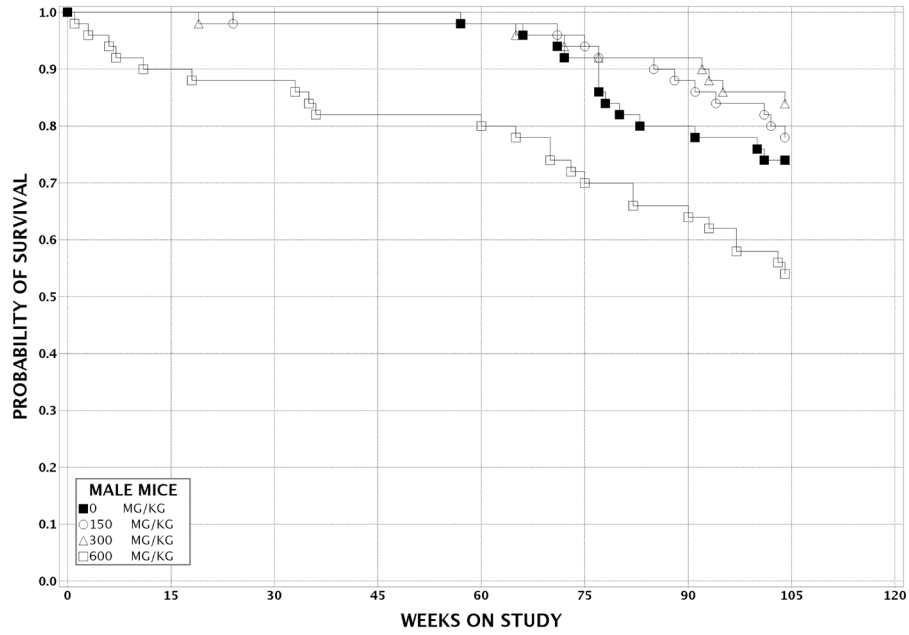


FIGURE 6
Kaplan-Meier Survival Curves for Mice
Administered Bis(2-chloroethoxy)methane Dermally for 2 Years

Body Weights and Clinical Findings

Mean body weights of dosed mice were generally similar ($\pm 10\%$) to those of the vehicle controls throughout the study (Tables 13 and 14; Figure 7). Clinical findings observed in 600 mg/kg male mice that

died during the first year of the study included lethargy and thinness. No chemical-related clinical findings were observed in other groups of male or female mice or during the second year of the study.

TABLE 13
Mean Body Weights and Survival of Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

Days on Study	Vehicle Control		150 mg/kg			300 mg/kg			600 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	22.5	50	22.5	100	50	22.5	100	50	22.6	100	50
8	23.1	50	23.3	101	50	23.4	101	50	23.4	101	49
15	24.6	50	24.5	99	50	24.9	101	50	24.5	100	49
22	26.0	50	26.0	100	50	25.7	99	50	25.9	100	48
29	27.1	50	27.0	100	50	26.9	99	50	26.9	99	48
36	28.1	50	28.2	101	50	28.4	101	50	28.0	100	48
43	29.1	50	29.2	101	50	29.2	101	50	29.1	100	47
50	29.9	50	30.2	101	50	30.1	101	50	29.9	100	46
57	31.1	50	31.0	100	50	31.3	101	50	30.8	99	46
64	32.2	50	32.3	100	50	32.6	101	50	31.6	98	46
71	33.4	50	33.2	100	50	33.5	101	50	32.7	98	46
78	33.7	50	33.9	101	50	33.9	101	50	33.0	98	45
85	35.5	50	35.5	100	50	35.7	101	50	34.5	97	45
113	39.6	50	39.9	101	50	39.7	100	50	38.3	97	45
141	43.3	50	43.3	100	50	43.2	100	49	41.6	96	44
169	45.2	50	46.3	103	49	45.7	101	49	44.0	97	44
197	48.2	50	48.7	101	49	48.7	101	49	46.8	97	44
225	48.7	50	49.6	102	49	50.1	103	49	47.6	98	44
253	50.3	50	51.1	102	49	51.2	102	49	50.0	99	41
281	50.8	50	51.7	102	49	52.0	102	49	51.4	101	41
309	51.3	50	52.1	102	49	52.5	102	49	52.6	103	41
337	51.9	50	52.9	102	49	53.5	103	49	53.6	103	41
365	52.4	50	53.4	102	49	54.5	104	49	54.2	104	41
393	52.8	50	54.2	103	49	54.8	104	49	54.4	103	41
421	52.7	49	54.4	103	49	55.0	104	49	54.4	103	40
449	52.8	49	53.5	101	49	55.6	105	49	53.6	102	40
477	51.8	48	53.7	104	49	55.4	107	48	54.5	105	39
505	52.6	46	54.3	103	48	55.9	106	47	54.8	104	37
533	53.5	44	55.4	104	46	56.8	106	46	56.0	105	35
561	52.6	41	54.2	103	46	56.1	107	46	55.5	106	35
589	52.6	40	53.8	102	45	56.3	107	46	55.7	106	33
617	52.1	40	53.1	102	44	55.6	107	46	54.9	105	33
645	52.6	39	53.7	102	43	54.7	104	45	54.5	104	32
673	51.9	39	53.5	103	42	55.7	107	43	55.4	107	31
701	51.6	38	53.1	103	41	55.6	108	43	54.1	105	29
Mean for weeks											
1-13	28.9		29.0	100		29.1	101		28.7	99	
14-52	47.7		48.4	101		48.5	102		47.3	99	
53-101	52.5		53.9	103		55.5	106		54.8	104	

TABLE 14
Mean Body Weights and Survival of Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

Days on Study	Vehicle Control		100 mg/kg			200 mg/kg			400 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	18.7	50	18.8	101	50	18.7	100	50	18.8	101	50
8	19.0	50	19.5	103	50	18.9	99	50	19.1	101	50
15	20.6	50	21.1	103	50	20.2	98	50	20.7	101	50
22	21.9	50	22.0	101	50	21.5	98	50	22.0	101	50
29	23.3	50	23.6	101	50	23.0	99	50	23.5	101	50
36	24.5	50	24.4	100	50	24.2	99	50	24.5	100	50
43	25.0	50	25.3	101	50	24.9	100	50	25.2	101	50
50	25.7	50	25.9	101	50	25.8	100	50	26.3	103	50
57	26.9	50	27.0	100	50	26.9	100	50	27.4	102	50
64	27.3	50	27.6	101	50	27.2	100	50	27.6	101	50
71	28.3	50	28.9	102	50	28.2	100	50	28.8	102	50
78	28.6	50	29.2	102	50	28.6	100	50	29.5	103	50
85	30.2	50	30.9	102	50	30.1	100	50	30.6	101	50
113	33.1	50	34.4	104	50	33.5	101	50	34.2	104	50
141	37.1	50	38.4	103	50	37.6	101	50	38.0	102	50
169	40.1	50	41.0	102	50	40.5	101	50	40.7	102	50
197	43.6	50	44.6	102	50	44.1	101	50	44.5	102	50
225	45.9	50	46.0	100	50	45.1	98	50	46.1	100	50
253	48.4	50	49.1	101	50	47.8	99	50	48.0	99	50
281	50.6	50	51.1	101	50	50.5	100	50	50.6	100	50
309	52.1	50	52.7	101	50	52.9	102	50	51.9	100	50
337	54.1	50	54.2	100	50	54.6	101	50	53.8	100	50
365	56.2	50	56.3	100	50	56.7	101	50	56.4	100	50
393	56.3	50	57.0	101	49	57.4	102	50	57.4	102	50
421	57.0	50	57.8	102	49	57.8	101	49	57.3	101	50
449	57.8	50	57.9	100	49	58.5	101	49	58.0	100	50
477	58.4	49	59.1	101	47	58.1	100	47	57.7	99	50
505	58.6	49	59.5	102	47	59.2	101	46	57.8	99	50
533	59.7	48	61.2	103	46	61.3	103	46	59.9	100	50
561	60.0	45	60.3	101	46	60.3	101	46	59.8	100	49
589	60.0	44	60.1	100	45	60.0	100	44	59.8	100	49
617	59.5	43	57.9	97	45	57.7	97	43	57.9	97	48
645	60.8	42	57.7	95	45	58.6	97	41	58.3	96	48
673	60.8	40	57.8	95	41	59.4	98	39	57.6	95	44
701	59.6	35	55.9	94	40	59.1	99	38	55.9	94	40
Mean for weeks											
1-13	24.6		24.9	101		24.5	99		24.9	101	
14-52	45.0		45.7	102		45.2	100		45.3	101	
53-101	58.8		58.3	99		58.8	100		58.0	99	

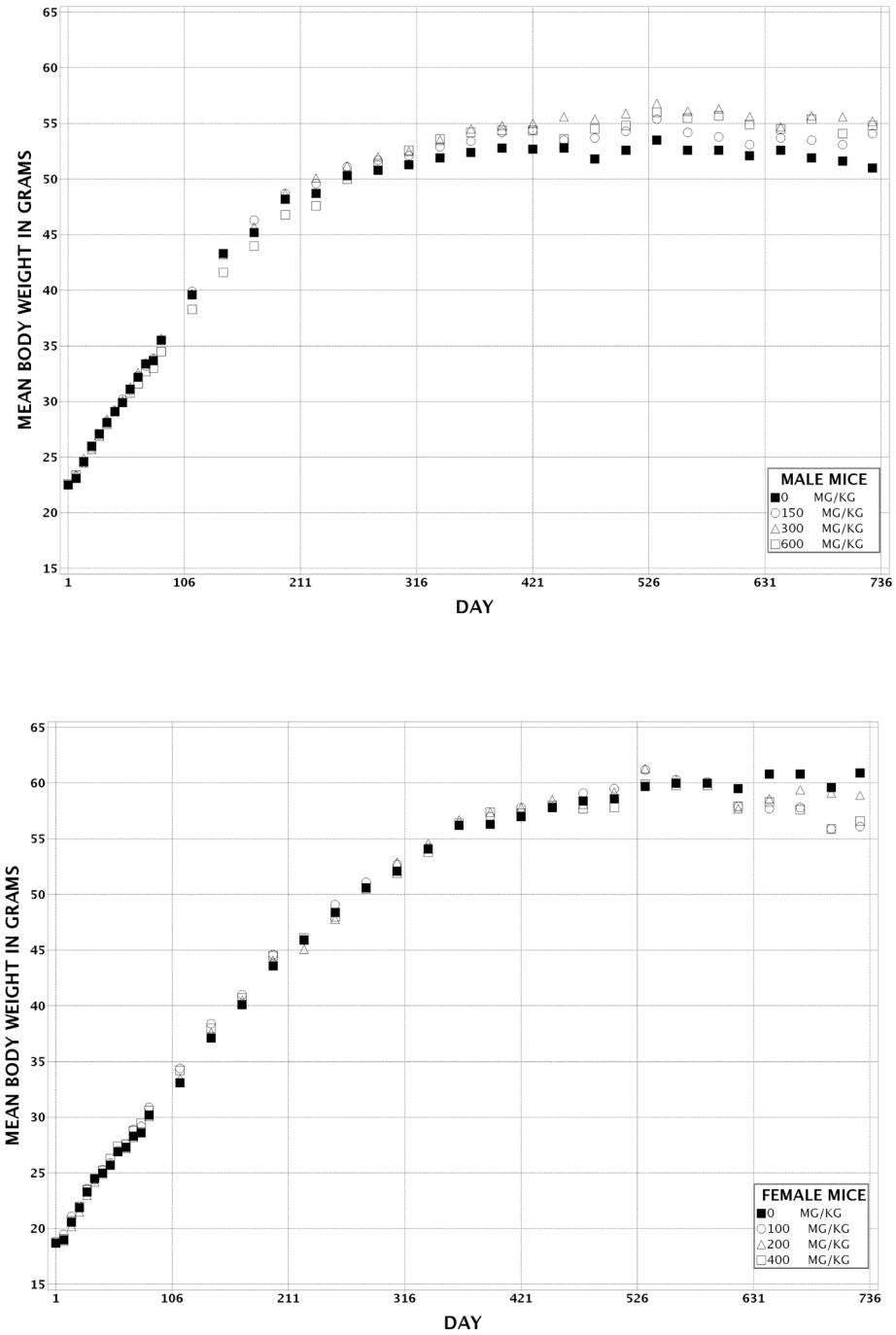


FIGURE 7
Growth Curves for Mice Administered Bis(2-chloroethoxy)methane Dermally for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the heart, forestomach, skin, uterus, and lung. 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 Appendix C for male mice and Appendix D for female mice.

Heart: Chemical-related increases in cardiac lesions were seen in male mice at 600 mg/kg (Tables 15 and C3). In these animals, the microscopic heart lesions closely resembled those reported in the 3-month mouse study. A few early deaths in 600 mg/kg males were considered to be due, at least in part and probably exclusively, to bis(2-chloroethoxy)methane-induced cardiotoxicity. Although the chemical-related histopathologic findings ranged from minimal to mild, the changes were still considered significant. The nature and severity of heart lesions in mice that died in the first year of the study and those surviving to the end of the study were similar. It is assumed that very small lesions may significantly affect the function of the heart and produce profound clinical findings and even death. It is suggested that in those male mice that died during the first year of the study, some “critically” located myofibers may have been damaged, ultimately affecting the heart function and leading to early death. However, overall, there were no significant differences between the severities or incidences of heart lesions in 600 mg/kg male mice that died during the first year of the study and those that died during the second year of the study.

As in rats, cardiomyopathy in mice is a common, spontaneously occurring degenerative lesion of the myocardium that increases in frequency and severity with age, although cardiomyopathy is usually less severe in mice than in rats. Due to the age-related cardiomyopathy and the suspected chemical-related cardiotoxicity, the myocardial heart changes were recorded according to the characteristic lesions of cardiomyopathy (necrosis, mononuclear cell infiltration, myocardial cell vacuolization, and interstitial fibrosis) separately, and in addition, where appropriate, they were also categorized as cardiomyopathy. Increased incidences of cardiomyopathy and mononuclear cell infiltration occurred in 600 mg/kg males and 400 mg/kg females; the increases were significant in 600 mg/kg males (Tables 15, C3, and D3). Significantly increased incidences of cardiomyocyte vacuolization and interstitial fibrosis occurred in 600 mg/kg males. Severity scores for cardiomyopathy

and mononuclear cell infiltration were significantly higher in 600 mg/kg males than in the vehicle controls. In females, severity scores did not differ notably between the vehicle control group and the 400 mg/kg group for any of the lesions. Microscopically, mononuclear cell infiltration consisted of irregular areas of increased nuclear density due to the addition of round to ovoid mononuclear cells, often interpreted as macrophages, within the interstitium. Myocardial cell vacuolization consisted of small clusters of clear, discrete vacuoles within myocardial cells or the interstitium (Plate 8). Fibrosis consisted of an increased amount of collagenous matrix within the interstitium, with or without an increase in fibroblasts. Necrosis consisted of scattered, small areas of pyknotic and karyorrhectic nuclear debris, often surrounded by hypereosinophilic, hyalinized cardiomyocyte cytoplasm. Cardiomyopathy was diagnosed as an overall syndrome consisting of a combination of the four lesions described above when one or more of these lesions was felt to exceed the historical threshold for the normal appearance of the aged mouse heart. Most often, more than one lesion was present, and lesions were present at more than one location throughout the myocardium. The lesions comprising cardiomyopathy were present as infrequent, widely separated small foci of myocardial vacuolization or interstitial mononuclear cell infiltration with variable collagen deposition. Most of the mononuclear cells were recognizable as macrophages, with fewer lymphocytes. Approximately one fourth of them were indeterminate cells with indistinct borders, pale eosinophilic cytoplasm, and elongated nuclei. The cardiomyocytes in these areas were often missing or appeared to be hypereosinophilic with hyalinized cytoplasm. Infrequent cardiomyocyte vacuolization was present, but it was a minor component of the overall lesion. All of these heart lesions occurred most often in the left ventricle and interventricular septum and tended to be located toward the base of the heart.

Forestomach: Incidences of squamous cell papilloma were numerically increased in dosed female mice, but the trend ($P=0.135$) and pairwise comparisons were not significant (vehicle control, 1/50; 100 mg/kg, 2/50; 200 mg/kg, 4/50; 400 mg/kg, 4/50; Tables D1 and D2). While the incidences in the dosed groups exceeded the historical control ranges for dermal studies with ethanol vehicle control groups and for all routes (except in the 100 mg/kg group) [dermal studies: 3/150 ($2\% \pm 0\%$), range 2%; all routes: 26/1,498 ($2\% \pm 2\%$), range 0%-6%], this finding was not considered to be chemical-related because the incidences were only slightly outside the historical control range for ethanol dermal studies

TABLE 15
Incidences of Nonneoplastic Lesions of the Heart in Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

Male	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Number Examined Microscopically	50	50	50	50
Myocardium, Cardiomyopathy ^a	10 (1.0) ^b	12 (1.1)	7 (1.0)	28** (1.4)▲
Myocardium, Fibrosis	0	3 (1.0)	3 (1.0)	13** (1.1)
Myocardium, Infiltration Cellular, Mononuclear Cell	11 (1.0)	12 (1.1)	8 (1.0)	28** (1.4)▲
Myocardium, Vacuolization Cytoplasmic	10 (1.0)	15 (1.1)	11 (1.0)	29** (1.3)
Female	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Number Examined Microscopically	50	50	50	50
Myocardium, Cardiomyopathy	10 (1.2)	7 (1.0)	10 (1.0)	17 (1.1)
Myocardium, Fibrosis	1 (1.0)	1 (1.0)	0	2 (1.0)
Myocardium, Infiltration Cellular, Mononuclear Cell	9 (1.2)	7 (1.0)	10 (1.0)	17 (1.1)
Myocardium, Vacuolization Cytoplasmic	14 (1.1)	4** (1.0)	6* (1.0)	13 (1.1)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test

** $P \leq 0.01$

▲ Severity differs ($P \leq 0.05$) from the vehicle control group by the Mann-Whitney U test

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

and there was no supporting evidence in males. The incidence of ulceration of the forestomach was significantly increased in 600 mg/kg males (vehicle control, 1/50; 150 mg/kg, 1/50; 300 mg/kg, 1/50; 600 mg/kg, 7/50; Table C3). Squamous cell papilloma consisted of small papillary projections of lamina propria protruding into the lumen covered by proliferating, well-differentiated epithelium. Ulceration consisted of a loss of the stratified squamous epithelium with exposure of the underlying lamina propria, and usually only a single ulcer was present.

Skin: The incidences of subcutaneous fibrous histiocytoma, fibrosarcoma, myxosarcoma, or malignant schwannoma (combined) occurred with a slightly positive trend ($P=0.030$) in females, but the increases were not significant in any dosed group (2/50, 2/50, 3/50, 7/50; Tables D1 and D2). All incidences were within the historical control range for all routes of administration (0%-16%). The occurrences of these neoplasms were considered to be incidental and not chemical related.

Significantly increased incidences of dermal inflammation and fibrosis and epidermal hyperplasia at the site of application occurred in 600 mg/kg male mice (Tables 16 and C3) Plate 9). Inflammation consisted of accumulation within the lamina propria of a few inflammatory cells, predominantly lymphocytes with occasional macrophages, plasma cells, and neutrophils. Dermal fibrosis and epidermal hyperplasia are considered to be consequences of the inflammation and the adjacent epidermis (Plate 9).

Uterus: The incidences of uterine adenoma were marginally increased by the trend test ($P=0.048$) in female mice (vehicle control, 0/50; 100 mg/kg, 0/50; 200 mg/kg, 0/50; 400 mg/kg, 2/50; Table D2). The incidences of uterine adenoma or carcinoma (combined) were increased, but not significantly, in 200 and 400 mg/kg females (0/50, 0/50, 1/50, 2/50). The incidence of adenoma or carcinoma (combined) in the 400 mg/kg group slightly exceeded the historical control range for 2-year dermal studies with ethanol vehicle control groups (0%-2%) but was within the

TABLE 16
Incidences of Nonneoplastic Lesions of the Skin (Site of Application) in Male Mice
in the 2-Year Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Number Examined Microscopically	50	50	50	50
Dermis, Fibrosis ^a	6 (1.7) ^b	1* (3.0)	2 (3.0)	25** (1.3)
Dermis, Inflammation	3 (2.3)	1 (3.0)	3 (2.0)	13** (1.4)
Epidermis, Hyperplasia	8 (1.8)	1* (2.0)	4 (1.8)	28** (1.2)

* Significantly different (P<0.05) from the vehicle control group by the Poly-3 test

** P<0.01

^a Number of animals with lesion

^b Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

historical control range for all routes (0%-6%). The occurrences of these neoplasms were considered to be incidental and not chemical related.

Lung: Incidences of alveolar/bronchiolar adenoma in female mice occurred with a positive trend (P=0.045) (1/50, 3/50, 4/50, 6/50; Tables D1 and D2) but were within the historical control range for all routes [73/1,496 (5% ± 3%), range 0%-12%]. The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) did not show a treatment-related trend in dosed male or female mice compared to the vehicle controls (male: 11/50, 18/50, 12/50, 8/50; female: 5/50, 3/50, 4/50, 8/50). The lung changes in dosed female mice were not considered to be chemical related because the incidences of alveolar/bronchiolar adenoma or carcinoma (combined) were within the historical control range for all routes [128/1,496 (9% ± 4%), range 2%-18%] and incidences of pulmonary alveolar epithelial hyperplasia (Table D3) were not increased.

GENETIC TOXICOLOGY

Bis(2-chloroethoxy)methane was tested in two independent bacterial mutagenicity tests (Table E1). In the first test, bis(2-chloroethoxy)methane induced significant increases in mutant colonies in *Salmonella typhimurium* strain TA100 in the presence of 10% and

30% induced hamster liver S9; equivocal responses were seen in TA100 when treatment was carried out with 10% or 30% induced rat liver S9. Positive results were seen in strain TA1535 in the presence of either induced hamster (10% or 30%) or rat (30%) liver S9 enzymes. No induction of gene mutations was seen in strain TA98, with or without S9. In the second bacterial mutagenicity test conducted with bis(2-chloroethoxy)methane, small increases in mutant colonies, judged to be equivocal, were seen in *S. typhimurium* strain TA100 and in *Escherichia coli* strain WP2uvrA/pKM101 in the presence of 10% induced rat liver S9. As in the first test, no induction of gene mutations was seen in *S. typhimurium* strain TA98, with or without S9.

Bis(2-chloroethoxy)methane was tested in two independent rodent micronucleus assays. The first assessed frequencies of micronucleated reticulocytes (polychromatic erythrocytes) in bone marrow of male F344/N rats administered bis(2-chloroethoxy)methane by gavage three times at 24-hour intervals at doses up to 65 mg/kg per day (Table E2). The second assay assessed micronucleated normochromatic erythrocyte frequencies in peripheral blood of male and female B6C3F1 mice exposed to bis(2-chloroethoxy)methane dermally for 3 months at doses up to 600 mg/kg per day (Table E3). Results of both tests were negative. In neither test was the percentage of reticulocytes significantly altered, indicating an absence of bis(2-chloroethoxy)methane-related bone marrow toxicity.

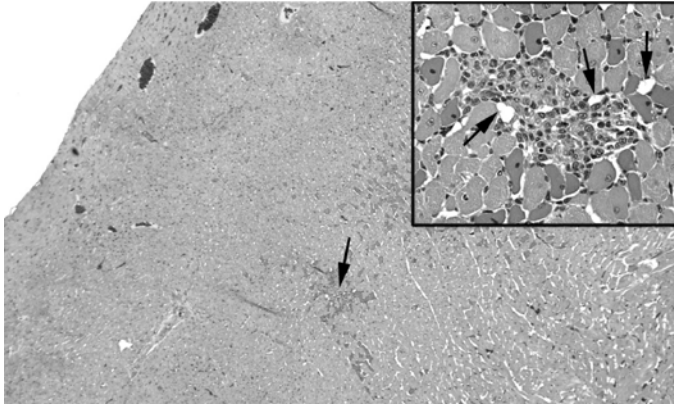


PLATE 1

Mononuclear cell infiltration and interstitial vacuolization (arrows) in the heart of a male F344/N vehicle control rat in the 3-month dermal study of bis(2-chloroethoxy)methane. These myocardial changes are consistent with early stages of “murine progressive cardiomyopathy” (Ruben *et al.*, 2000). H&E

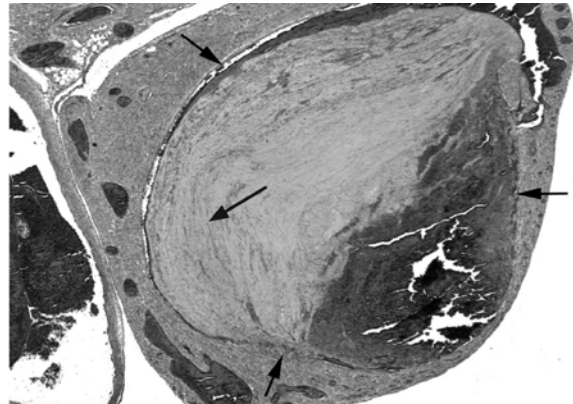


PLATE 2

Atrial thrombosis (arrows) in the heart of a male F344/N rat administered 600 mg/kg bis(2-chloroethoxy)methane for 5 days in the 3-month dermal study. Multifocal, extensive myofiber vacuolization associated with myocardial necrosis and interstitial mononuclear cell infiltration was present. H&E

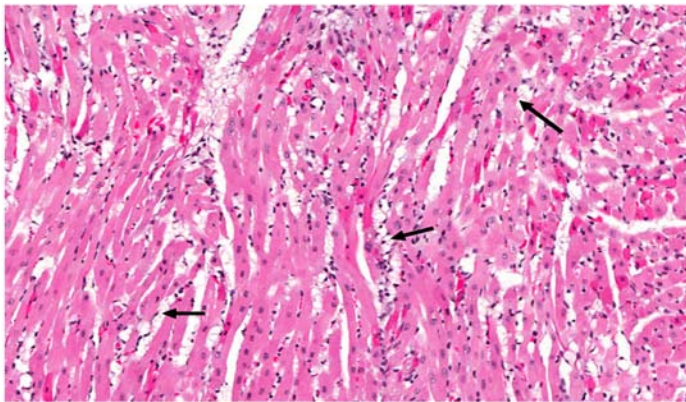


PLATE 3

Heart of a male F344/N rat administered 600 mg/kg bis(2-chloroethoxy)methane dermally for 3 months. Note the multiple foci of cytoplasmic vacuolization (arrows) associated with mononuclear cell infiltration. H&E

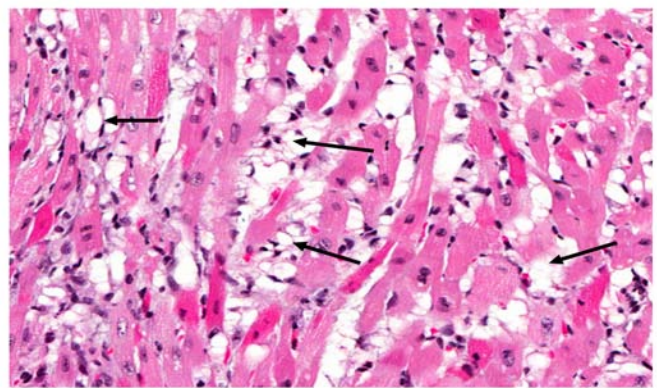


PLATE 4

Higher magnification of Plate 3. Note the multiple foci of cytoplasmic vacuolization (arrows) associated with mononuclear cell infiltration. H&E

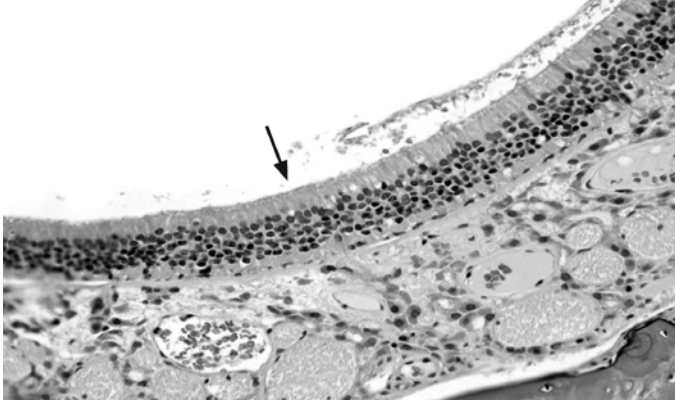


PLATE 5

Normal aspect and thickness of the olfactory epithelium (arrow) at level II in the nasal cavity of a male F344/N vehicle control rat in the 2-year dermal study of bis(2-chloroethoxy)methane. H&E

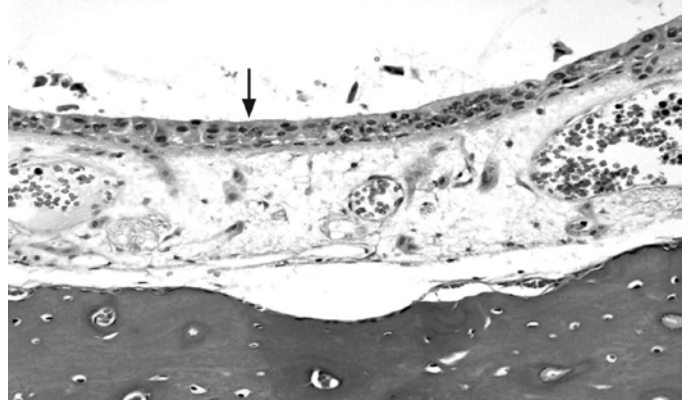


PLATE 6

Olfactory degeneration (arrow) at level II in the nasal cavity of a male F344/N rat administered 300 mg/kg bis(2-chloroethoxy)methane for 2 years. The change consists of focal areas of disorganization and loss of the normal olfactory epithelium with accompanying loss of the underlying olfactory nerve processes. Compare to Plate 5. H&E

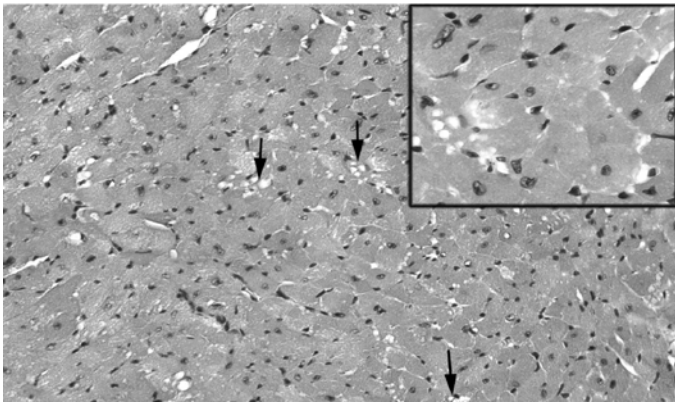


PLATE 7

Multifocal myofiber vacuolization (arrows) in the heart of a female B6C3F1 mouse administered 600 mg/kg bis(2-chloroethoxy)methane dermally for 3 months. H&E

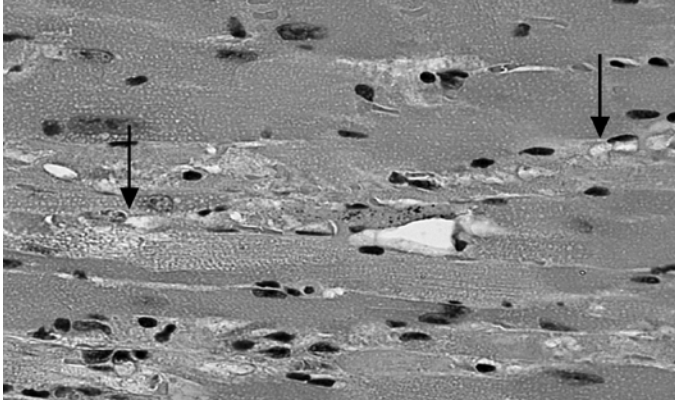


PLATE 8

Mild myocardial cytoplasmic vacuolization (arrows) in the heart of a male B6C3F1 mouse administered 600 mg/kg bis(2-chloroethoxy)methane dermally for 2 years. H&E

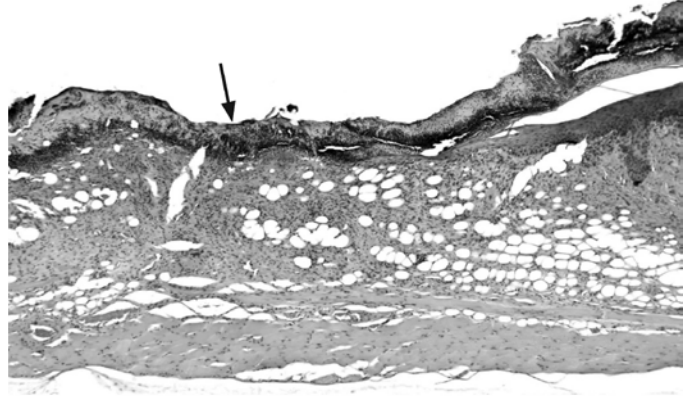


PLATE 9

Ulceration (arrow), fibrosis, inflammation, and epidermal hyperplasia in the skin at the site of application of a male B6C3F1 mouse administered 600 mg/kg bis(2-chloroethoxy)methane dermally for 2 years. H&E

DISCUSSION AND CONCLUSIONS

Bis(2-chloroethoxy)methane, a chlorinated aliphatic hydrocarbon, is resistant to degradation by solvents or high temperatures. Because of these physical properties, the chemical is used as a starting compound to produce polysulfide elastomers for a variety of sealant applications. It has been found in a variety of environmental locations including sludges and water supplies (USEPA, 2000). Bis(2-chloroethoxy)methane was nominated for study by the National Institute of Environmental Health Sciences because of its widespread use as a starting material to produce polysulfide elastomers and because there were no 2-year carcinogenicity studies reported in the literature. The dermal route of administration was selected to mimic potential human exposure to the chemical during its manufacture and from its use in solvents or sealants.

The primary toxicity in the 3-month studies was to the heart. In the 3-month rat study, no rats administered 600 mg/kg bis(2-chloroethoxy)methane survived. Chemical-related cardiac lesions occurred in both male and female rats, primarily in the 400 and 600 mg/kg groups, and consisted of cardiomyocytic cytoplasmic vacuolization and interstitial mononuclear cell infiltration of minimal to moderate severity. Atrial thrombosis occurred in three 600 mg/kg male rats and was considered likely to be secondary to direct cardiotoxicity. The main localization of the bis(2-chloroethoxy)methane-induced cardiac thromboses occurred in the left atrium. The literature survey suggested that chemical-induced atrial thrombosis might be closely related to myocardial injury, endothelial injury, circulatory stasis, and impaired atrial mechanical activity, such as atrial fibrillation, which could cause stasis of blood within the left atrial appendage, contributing to left atrial thrombosis (Yoshizawa *et al.*, 2005). Historical control data for cardiac thrombosis in both sexes of F344/N rats and B6C3F1 mice from NTP reports indicated that no cardiac thrombosis occurred spontaneously in any 3-month studies (Yoshizawa *et al.*, 2005).

Spontaneous (background) cardiomyopathy occurs in a high percentage of control F344/N rats and B6C3F1 mice and consists of multifocal myocardial fiber

eosinophilia, loss of striations, occasional cardiomyocyte necrosis, infiltration of mononuclear and rare polymorphonuclear inflammatory cells, and eventually fibrosis (Elwell and Mahler, 1999; Ruben *et al.*, 2000). The severity of this background cardiomyopathy increases as the rats and mice age (Ruben *et al.*, 2000; Jokinen *et al.*, 2005). The individual lesions of spontaneous cardiomyopathy in chronic rat and mouse studies are generally qualitatively indistinguishable from chemical-related cardiotoxic lesions. However, in acute and subchronic studies, when the fibrosis of the damaged myofibers is not yet prominent, it is still possible to distinguish between two potential mechanisms of cardiotoxicity. When a direct toxic effect on the myocardial cells does occur, the degree and extent of myocardial involvement is more diffuse, compared to spontaneous cardiomyopathy and cardiotoxic lesions induced by chemicals that primarily affect the coronary vasculature (with secondary effects on the myocardium), in which case, lesions tend to be multifocal (Jokinen *et al.*, 2005). In the case of bis(2-chloroethoxy)methane, the damage to the myocardial cells was direct (i.e., damage to the mitochondria). Paradoxically, by the end of the current 3-month study, the incidence of spontaneous cardiomyopathy appeared reduced in 600 mg/kg rats. This was likely due to the overwhelming chemical-related cardiotoxicity exhibiting diffuse myofiber cytoplasmic vacuolization, interstitial mononuclear cell infiltration, and necrosis which masked the spontaneous cardiomyopathy (characterized by focal lesions). Sporadic foci of the background cardiomyopathy in untreated control rats most often occur in the papillary muscles of the heart (Ruben *et al.*, 2000). The heart base, left and right ventricular free walls, interventricular septum, and atria also exhibit this focal, spontaneous background cardiomyopathy at times. In the case of chemically induced direct damage to the cardiomyocyte, the main morphological characteristic that distinguishes the chemical-related cardiotoxicity from the spontaneous cardiomyopathy is the widespread involvement of the myocardium in the former, versus the random foci in the latter (Jokinen *et al.*, 2005). Other lesions occurring with increased incidences in dosed rats in the 3-month study, particularly at 600 mg/kg, included atrophy

and/or necrosis of the thymus, spleen, and mesenteric lymph node. These lesions may have resulted from the general toxicity of the chemical, leading to early death.

In the 3-month mouse study, chemical-related myocardial vacuolization consisted of widespread cardiomyocytes exhibiting vacuolization of the sarcoplasm and was seen primarily in 400 and 600 mg/kg female mice. In other studies, the primary subcellular damage was identified in the mitochondria by electron microscopic analysis (Nyska *et al.*, 2009). Bis(2-chloroethoxy)methane-induced cardiotoxicity was less severe in mice than in rats, with all male and all but three female mice surviving until the end of the dosing period. The cause of death may have been related to cardiotoxicity. It is hypothesized that when bis(2-chloroethoxy)methane-induced cardiotoxicity occurs in "critical" myofibers, it may affect heart function and lead to death. In B6C3F1 mice, in contrast to F344/N rats, spontaneous cardiomyopathy occurs infrequently in 3-month studies.

No other chemical-related lesions were seen in the 3-month rat and mouse studies.

Based on the sperm parameters and estrous cyclicity results, the reproductive organ weights, and histopathology of the reproductive organs, there was no evidence of toxicity to the reproductive systems of rats or mice in the 3-month studies.

For the 2-year studies, bis(2-chloroethoxy)methane doses were selected below those expected to produce extensive cardiotoxicity and/or mortality (rats: 0, 75, 100, or 300 mg/kg per day; male mice: 0, 150, 300, or 600 mg/kg; female mice: 0, 100, 200, or 400 mg/kg). The objective of the 2-year studies was to look at long-term sequelae, including potential carcinogenicity, from chemical exposure. While this 2-year rat study did not find chemical-related increases in incidences or severities of cardiotoxic lesions, other studies suggest that evidence for chemical-induced acute cardiac effects may be detected by electro-cardiogram (ECG) endpoints (Howden *et al.*, 2005), gene transcript analysis (Dunnick *et al.*, 2006), or electron microscopic analysis (Dunnick *et al.*, 2004a) in cases where histopathologic cardiac lesions are not observed. After bis(2-chloroethoxy)methane exposure at concentrations that do not induce chemical-related cardiac lesions in rats, an "occult" toxicity may occur that leaves the animals more susceptible to subsequent cardiotoxic events (Golomb *et al.*, 2007).

In the 2-year rat study, the nature and severity of heart lesions in dosed rats that died during the first year of the study and those surviving to the end of the study were

comparable, and no consistent differences in chemical-related cardiac lesions could be identified. Regardless of cardiac lesion presence, dosed animals were not more likely to die during the first year of the study. It is possible that the commonly seen spontaneous age-related cardiomyopathy (Ayers and Jones, 1978; MacKenzie and Alison, 1990; Jokinen *et al.*, 2005) masked a chemical-related cardiotoxic change. The early death or moribund condition of 300 mg/kg females may have been related to bis(2-chloroethoxy)methane-induced cardiotoxic events that could not be detected by histopathologic analysis of heart tissue. It is possible that the chemical caused fatal physiologic cardiac alterations and/or dysrhythmias in some animals. This likelihood is supported by bis(2-chloroethoxy)methane being a known cardiotoxicant in rats and mice after acute exposure (Dunnick *et al.*, 2004a).

Chemical-related lesions in the 2-year rat study included degeneration of the olfactory epithelium in the nose of males and females and inflammation of the forestomach in males.

In the 2-year mouse study, more deaths occurred in 600 mg/kg male mice during the first year of the study than in the other groups. The myocardial changes seen in the hearts of these mice were considered to be excessive for relatively young mice, and thus the changes were interpreted to be chemical related. Cardiotoxicity may have played a role in the deaths of 600 mg/kg male mice during the first year of the study. Increased incidences of myocardial vacuolization, interstitial fibrosis, mononuclear cell infiltration, and cardiomyopathy occurred in 600 mg/kg male mice after 2 years of dosing, and these increases were significantly greater than those in the vehicle control group, which had a low level background of cardiomyopathy (Elwell and Mahler, 1999).

Chemicals metabolized to thiodiglycolic acid in humans, such as ifosfamide, cause cardiotoxicity via mitochondrial toxicity (Visarius *et al.*, 1998), and bis(2-chloroethoxy)methane similarly causes mitochondrial toxicity in rodents within 3 days of dosing (Dunnick *et al.*, 2004b). Mitochondrial toxicity has been attributed in part to inhibition of fatty acid metabolism (Visarius *et al.*, 1998, 1999; Marthaler *et al.*, 1999) and is accompanied by decreases in gene transcript levels of ATP synthase subunits (Dunnick *et al.*, 2006) and dehydrogenase levels (Golomb *et al.*, 2007), enzymes critical in fatty acid metabolism, a main source of energy for the heart (Neubauer, 2007).

Cardiac disease results when the energy supply generated in the mitochondria fails to meet the energy demand (Hochachka *et al.*, 1996; Neubauer, 2007). In

most forms of heart disease, damage to the heart eventually results in mitochondrial damage and cardiotoxicity, whether the initial event was vascular disease, atherosclerosis, or direct damage to heart tissue (Ballinger *et al.*, 2002; Ballinger, 2005; Madamanchi *et al.*, 2005a,b; Madamanchi and Runge, 2007; Neubauer, 2007). In the current studies, there was no evidence of increased accumulation of bis(2-chloroethoxy)methane in the hearts of rats or mice versus that in other tissues (Black *et al.*, 2007). However, the vulnerability of the heart to bis(2-chloroethoxy)methane-induced damage at the dose levels used in the 3-month studies could have been because the heart contains more mitochondria per gram of tissue than skeletal muscle (Miller *et al.*, 2003). In addition, recent studies indicate that mitochondrial proteins in the heart may differ from those in the skeletal muscle or liver (Forner *et al.*, 2006), perhaps suggesting different susceptibilities to chemical-induced mitochondrial damage in these different tissues.

Bis(2-chloroethoxy)methane was mutagenic in the presence of exogenous metabolic activation in strains of *Salmonella typhimurium* that revert by base substitution.

There are no data from mammalian cell mutagenicity studies with bis(2-chloroethoxy)methane. However, assessments of micronucleus induction in peripheral blood erythrocytes of male and female B6C3F1 mice and the bone marrow of male F344/N rats administered bis(2-chloroethoxy)methane were negative.

CONCLUSIONS

Under the conditions of these 2-year dermal studies, there was *no evidence of carcinogenic activity** of bis(2-chloroethoxy)methane in male or female F344/N rats administered 75, 150, or 300 mg/kg. There was *no evidence of carcinogenic activity* of bis(2-chloroethoxy)methane in male B6C3F1 mice administered 150, 300, or 600 mg/kg or in female B6C3F1 mice administered 100, 200, or 400 mg/kg.

The administration of bis(2-chloroethoxy)methane for 2 years resulted in increased incidences of non-neoplastic lesions in the nose of male and female rats, the forestomach of male rats, the heart of male and female mice, and the forestomach and skin of male mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS
IN THE 2-YEAR DERMAL STUDY
OF BIS(2-CHLOROETHOXY)METHANE

TABLE A1	Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study of Bis(2-chloroethoxy)methane	70
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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	21	17	31	20
Natural deaths	8	4	5	6
Survivors				
Died last week of study		1		
Terminal sacrifice	21	28	14	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(49)	(50)	(50)	(50)
Adenoma		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Granular cell tumor malignant	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Mesentery	(10)	(10)	(9)	(9)
Oral mucosa	(2)	(0)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Schwannoma malignant, metastatic, skin			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Ganglioneuroma			1 (2%)	1 (2%)
Pheochromocytoma benign	2 (4%)	4 (8%)	5 (10%)	6 (12%)
Pheochromocytoma complex		1 (2%)		
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)	1 (2%)	
Bilateral, pheochromocytoma benign, multiple			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	7 (14%)	2 (4%)	3 (6%)
Carcinoma			1 (2%)	1 (2%)
Parathyroid gland	(49)	(44)	(45)	(47)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Endocrine System (continued)				
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma	32 (65%)	23 (46%)	22 (44%)	29 (58%)
Pars distalis, adenoma, multiple	3 (6%)	1 (2%)	4 (8%)	
Pars intermedia, adenoma		1 (2%)		1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma			1 (2%)	
Bilateral, C-cell, adenoma, multiple	1 (2%)			
C-cell, adenoma	8 (16%)	9 (18%)	7 (14%)	7 (14%)
C-cell, adenoma, multiple	1 (2%)			1 (2%)
C-cell, carcinoma	1 (2%)			
Follicle, adenoma				1 (2%)
Follicle, carcinoma			1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Penis	(0)	(0)	(1)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Carcinoma			1 (2%)	
Prostate gland	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	18 (36%)	21 (42%)	17 (34%)	19 (38%)
Interstitial cell, adenoma	12 (24%)	8 (16%)	14 (28%)	14 (28%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(4)	(0)	(6)	(5)
Deep cervical, rhabdomyosarcoma, metastatic, skeletal muscle			1 (17%)	
Mediastinal, rhabdomyosarcoma, metastatic, skeletal muscle			1 (17%)	
Mediastinal, sarcoma			1 (17%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Spleen	(50)	(50)	(49)	(50)
Hemangioma		1 (2%)		
Hemangiosarcoma				1 (2%)
Sarcoma	1 (2%)			
Thymus	(49)	(50)	(50)	(48)
Schwannoma malignant, metastatic, skin			1 (2%)	
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma			1 (2%)	
Fibroadenoma	3 (6%)	1 (2%)		1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		2 (4%)		
Basal cell carcinoma		1 (2%)		
Squamous cell papilloma			1 (2%)	
Trichoepithelioma	1 (2%)		1 (2%)	1 (2%)
Dermis, fibroma				1 (2%)
Epidermis, squamous cell carcinoma	1 (2%)			
Site of application, epidermis, basal cell adenoma		1 (2%)		
Subcutaneous tissue, fibroma	1 (2%)		2 (4%)	
Subcutaneous tissue, fibrosarcoma	2 (4%)			
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)		1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosarcoma		1 (2%)		
Osteosarcoma			1 (2%)	
Skeletal muscle	(1)	(0)	(1)	(0)
Hemangiosarcoma	1 (100%)			
Rhabdomyosarcoma			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Glioma malignant				1 (2%)
Cranial nerve, schwannoma malignant	1 (2%)			
Spinal cord	(1)	(1)	(0)	(0)
Astrocytoma malignant	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	1 (2%)	3 (6%)	
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	1 (2%)	
Carcinoma, metastatic, mammary gland			1 (2%)	
Carcinoma, metastatic, thyroid gland			1 (2%)	
Hemangiosarcoma, metastatic, skeletal muscle	1 (2%)			
Hemangiosarcoma, metastatic, spleen				1 (2%)
Nose	(50)	(49)	(50)	(49)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Zymbal's gland	(1)	(0)	(0)	(1)
Adenoma	1 (100%)			
Carcinoma				1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Hemangiosarcoma			1 (2%)	
Lipoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia granulocytic				1 (2%)
Leukemia mononuclear	17 (34%)	14 (28%)	19 (38%)	12 (24%)
Mesothelioma malignant	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	49	47	49	49
Total primary neoplasms	127	108	120	109
Total animals with benign neoplasms	49	45	47	46
Total benign neoplasms	96	87	89	89
Total animals with malignant neoplasms	26	18	26	19
Total malignant neoplasms	31	21	31	20
Total animals with metastatic neoplasms	2	1	5	1
Total metastatic neoplasms	2	1	10	1

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Adrenal Medulla: Pheochromocytoma Benign				
Overall rate ^a	3/50 (6%)	5/50 (10%)	7/50 (14%)	6/50 (12%)
Adjusted rate ^b	7.2%	12.1%	18.6%	13.7%
Terminal rate ^c	1/21 (5%)	4/29 (14%)	3/14 (21%)	4/24 (17%)
First incidence (days)	682	631	554	677
Poly-3 test ^d	P=0.236	P=0.350	P=0.115	P=0.265
Adrenal Medulla: Pheochromocytoma Benign or Complex				
Overall rate	3/50 (6%)	6/50 (12%)	7/50 (14%)	6/50 (12%)
Adjusted rate	7.2%	14.2%	18.6%	13.7%
Terminal rate	1/21 (5%)	4/29 (14%)	3/14 (21%)	4/24 (17%)
First incidence (days)	682	386	554	677
Poly-3 test	P=0.275	P=0.245	P=0.115	P=0.265
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	9.5%	2.4%	8.0%	0.0%
Terminal rate	2/21 (10%)	1/29 (3%)	0/14 (0%)	0/24 (0%)
First incidence (days)	583	729 (T)	481	— ^e
Poly-3 test	P=0.063N	P=0.185N	P=0.560N	P=0.055N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	11.9%	4.9%	10.6%	0.0%
Terminal rate	3/21 (14%)	2/29 (7%)	1/14 (7%)	0/24 (0%)
First incidence (days)	583	729 (T)	481	—
Poly-3 test	P=0.034N	P=0.225N	P=0.569N	P=0.027N
Mammary Gland: Fibroadenoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	1/50 (2%)
Adjusted rate	7.2%	2.4%	0.0%	2.3%
Terminal rate	1/21 (5%)	1/29 (3%)	0/14 (0%)	0/24 (0%)
First incidence (days)	655	729 (T)	—	719
Poly-3 test	P=0.200N	P=0.312N	P=0.146N	P=0.290N
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	1/50 (2%)	1/50 (2%)
Adjusted rate	7.2%	2.4%	2.8%	2.3%
Terminal rate	1/21 (5%)	1/29 (3%)	0/14 (0%)	0/24 (0%)
First incidence (days)	655	729 (T)	704	719
Poly-3 test	P=0.236N	P=0.312N	P=0.357N	P=0.290N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	7.2%	2.4%	5.4%	2.3%
Terminal rate	1/21 (5%)	1/29 (3%)	0/14 (0%)	0/24 (0%)
First incidence (days)	655	729 (T)	481	719
Poly-3 test	P=0.267N	P=0.312N	P=0.556N	P=0.290N
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	7/50 (14%)	2/50 (4%)	3/50 (6%)
Adjusted rate	7.2%	17%	5.5%	6.8%
Terminal rate	2/21 (10%)	5/29 (17%)	0/14 (0%)	1/24 (4%)
First incidence (days)	682	670	643	647
Poly-3 test	P=0.318N	P=0.151	P=0.559N	P=0.637N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	7/50 (14%)	3/50 (6%)	4/50 (8%)
Adjusted rate	7.2%	17.0%	8.2%	9.1%
Terminal rate	2/21 (10%)	5/29 (17%)	0/14 (0%)	2/24 (8%)
First incidence (days)	682	670	643	647
Poly-3 test	P=0.489N	P=0.151	P=0.603	P=0.530
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	35/49 (71%)	24/50 (48%)	26/50 (52%)	29/50 (58%)
Adjusted rate	75.2%	54.3%	61.1%	61.4%
Terminal rate	15/21 (71%)	15/29 (52%)	6/14 (43%)	13/24 (54%)
First incidence (days)	316	505	453	485
Poly-3 test	P=0.188N	P=0.025N	P=0.102N	P=0.106N
Preputial Gland: Adenoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.8%	7.3%	5.4%	2.3%
Terminal rate	0/21 (0%)	3/29 (10%)	0/14 (0%)	1/24 (4%)
First incidence (days)	533	729 (T)	426	729 (T)
Poly-3 test	P=0.302N	P=0.487	P=0.649	P=0.488N
Skin: Basal Cell Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.3%	0.0%	0.0%
Terminal rate	0/21 (0%)	3/29 (10%)	0/14 (0%)	0/24 (0%)
First incidence (days)	—	729 (T)	—	—
Poly-3 test	P=0.305N	P=0.116	— ^f	—
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.4%	7.3%	2.7%	2.3%
Terminal rate	0/21 (0%)	3/29 (10%)	0/14 (0%)	0/24 (0%)
First incidence (days)	570	729 (T)	643	712
Poly-3 test	P=0.437N	P=0.296	P=0.729	P=0.752N
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.4%	9.7%	2.7%	2.3%
Terminal rate	0/21 (0%)	3/29 (10%)	0/14 (0%)	0/24 (0%)
First incidence (days)	570	654	643	712
Poly-3 test	P=0.364N	P=0.172	P=0.729	P=0.752N
Skin: Squamous Cell Papilloma, Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.7%	9.7%	5.5%	2.3%
Terminal rate	0/21 (0%)	3/29 (10%)	1/14 (7%)	0/24 (0%)
First incidence (days)	570	643	643	712
Poly-3 test	P=0.253N	P=0.326	P=0.641	P=0.488N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	1/50 (2%)
Adjusted rate	7.2%	0.0%	5.5%	2.3%
Terminal rate	1/21 (5%)	0/29 (0%)	0/14 (0%)	0/24 (0%)
First incidence (days)	645	—	654	724
Poly-3 test	P=0.324N	P=0.122N	P=0.563N	P=0.291N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Testes: Adenoma				
Overall rate	30/50 (60%)	29/50 (58%)	31/50 (62%)	33/50 (66%)
Adjusted rate	66.2%	67.5%	76.1%	72.4%
Terminal rate	15/21 (71%)	21/29 (72%)	13/14 (93%)	17/24 (71%)
First incidence (days)	533	584	554	620
Poly-3 test	P=0.244	P=0.536	P=0.201	P=0.332
Thyroid gland (C-cell): Adenoma				
Overall rate	10/50 (20%)	9/50 (18%)	8/50 (16%)	8/50 (16%)
Adjusted rate	23.5%	21.5%	21.2%	18.2%
Terminal rate	5/21 (24%)	7/29 (24%)	2/14 (14%)	5/24 (21%)
First incidence (days)	570	502	596	669
Poly-3 test	P=0.315N	P=0.514N	P=0.507N	P=0.366N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	11/50 (22%)	9/50 (18%)	8/50 (16%)	8/50 (16%)
Adjusted rate	25.9%	21.5%	21.2%	18.2%
Terminal rate	6/21 (29%)	7/29 (24%)	2/14 (14%)	5/24 (21%)
First incidence (days)	570	502	596	669
Poly-3 test	P=0.241N	P=0.412N	P=0.409N	P=0.273N
All Organs: Mononuclear Cell Leukemia				
Overall rate	17/50 (34%)	14/50 (28%)	19/50 (38%)	12/50 (24%)
Adjusted rate	38.3%	31.8%	45.7%	26.6%
Terminal rate	5/21 (24%)	6/29 (21%)	4/14 (29%)	6/24 (25%)
First incidence (days)	533	478	371	423
Poly-3 test	P=0.188N	P=0.338N	P=0.313	P=0.165N
All Organs: Malignant Mesothelioma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rate	4.8%	7.3%	5.5%	2.3%
Terminal rate	1/21 (5%)	3/29 (10%)	1/14 (7%)	0/24 (0%)
First incidence (days)	655	729 (T)	643	716
Poly-3 test	P=0.298N	P=0.492	P=0.646	P=0.484N
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	45/50 (90%)	47/50 (94%)	46/50 (92%)
Adjusted rate	99.6%	96.7%	99.2%	95.7%
Terminal rate	21/21 (100%)	29/29 (100%)	14/14 (100%)	23/24 (96%)
First incidence (days)	316	502	426	485
Poly-3 test	P=0.170N	P=0.339N	P=0.995N	P=0.251N
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	18/50 (36%)	26/50 (52%)	19/50 (38%)
Adjusted rate	56.4%	40.2%	59.1%	40.9%
Terminal rate	9/21 (43%)	9/29 (31%)	5/14 (36%)	8/24 (33%)
First incidence (days)	316	386	371	367
Poly-3 test	P=0.163N	P=0.085N	P=0.478	P=0.095N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	47/50 (94%)	49/50 (98%)	49/50 (98%)
Adjusted rate	99.6%	97.7%	99.9%	98.0%
Terminal rate	21/21 (100%)	29/29 (100%)	14/14 (100%)	23/24 (96%)
First incidence (days)	316	386	371	367
Poly-3 test	P=0.498N	P=0.539N	P=1.000	P=0.589N

(T) Terminal sacrifice

- ^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, lung, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
- ^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 A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	21	17	31	20
Natural deaths	8	4	5	6
Survivors				
Died last week of study		1		
Terminal sacrifice	21	28	14	24
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Ulcer		1 (2%)		
Intestine large, cecum	(49)	(50)	(50)	(50)
Edema	1 (2%)			
Inflammation	1 (2%)			
Parasite metazoan		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan		2 (4%)		
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	11 (22%)	5 (10%)	7 (14%)	7 (14%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Ulcer		1 (2%)		1 (2%)
Epithelium, hyperplasia		1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	7 (14%)	3 (6%)	6 (12%)
Basophilic focus	19 (38%)	19 (38%)	16 (32%)	19 (38%)
Clear cell focus	22 (44%)	24 (48%)	14 (28%)	22 (44%)
Degeneration, cystic	6 (12%)	5 (10%)	10 (20%)	15 (30%)
Eosinophilic focus	10 (20%)	15 (30%)	8 (16%)	11 (22%)
Fatty change	19 (38%)	20 (40%)	16 (32%)	20 (40%)
Fibrosis	39 (78%)	25 (50%)	23 (46%)	31 (62%)
Hematopoietic cell proliferation	2 (4%)			
Hepatodiaphragmatic nodule	4 (8%)	5 (10%)	6 (12%)	9 (18%)
Inflammation	32 (64%)	38 (76%)	33 (66%)	41 (82%)
Mixed cell focus	4 (8%)	10 (20%)	8 (16%)	3 (6%)
Necrosis	4 (8%)	2 (4%)	5 (10%)	3 (6%)
Thrombosis			2 (4%)	
Bile duct, cyst		1 (2%)		1 (2%)
Bile duct, hyperplasia	46 (92%)	46 (92%)	44 (88%)	47 (94%)
Centrilobular, necrosis	1 (2%)	4 (8%)	8 (16%)	4 (8%)
Mesentery	(10)	(10)	(9)	(9)
Accessory spleen	1 (10%)			
Fat, necrosis	8 (80%)	9 (90%)	8 (89%)	8 (89%)
Oral mucosa	(2)	(0)	(0)	(0)
Inflammation, granulomatous	1 (50%)			
Gingival, cyst	2 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy	17 (34%)	21 (42%)	17 (34%)	17 (34%)
Acinus, hyperplasia	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Duct, cyst	11 (22%)	5 (10%)	4 (8%)	5 (10%)

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

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation		2 (4%)	6 (12%)	10 (20%)
Ulcer		2 (4%)	2 (4%)	7 (14%)
Epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Ectopic tissue	1 (2%)			
Inflammation	1 (2%)	2 (4%)	1 (2%)	
Mineralization		1 (2%)		
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	48 (96%)	45 (90%)	49 (98%)
Fibrosis	50 (100%)	50 (100%)	49 (98%)	49 (98%)
Inflammation, chronic active	1 (2%)			
Thrombosis	3 (6%)	1 (2%)	4 (8%)	3 (6%)
Coronary artery, inflammation		1 (2%)		
Endothelium, hyperplasia	1 (2%)			
Myocardium, infiltration cellular, mononuclear cell	50 (100%)	50 (100%)	49 (98%)	50 (100%)
Myocardium, necrosis	50 (100%)	50 (100%)	49 (98%)	50 (100%)
Myocardium, vacuolization cytoplasmic	50 (100%)	50 (100%)	49 (98%)	50 (100%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Hyperplasia	16 (32%)	22 (44%)	13 (26%)	16 (32%)
Vacuolization cytoplasmic	33 (66%)	38 (76%)	35 (70%)	39 (78%)
Adrenal medulla	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		2 (4%)
Hemorrhage	1 (2%)			
Hyperplasia	9 (18%)	7 (14%)	8 (16%)	10 (20%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	9 (18%)	11 (22%)	5 (10%)
Parathyroid gland	(49)	(44)	(45)	(47)
Hyperplasia		1 (2%)		
Hyperplasia, focal				1 (2%)
Pituitary gland	(49)	(50)	(50)	(50)
Angiectasis	37 (76%)	30 (60%)	26 (52%)	32 (64%)
Atrophy	1 (2%)			
Cyst	6 (12%)	6 (12%)	10 (20%)	8 (16%)
Pars distalis, hyperplasia	13 (27%)	16 (32%)	12 (24%)	17 (34%)
Thyroid gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation		1 (2%)		
C-cell, hyperplasia	21 (42%)	22 (44%)	23 (46%)	14 (28%)
Follicle, cyst		1 (2%)		
Follicle, hyperplasia	1 (2%)	4 (8%)	1 (2%)	2 (4%)
General Body System				
None				

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Inflammation	1 (2%)			1 (2%)
Penis	(0)	(0)	(1)	(0)
Inflammation			1 (100%)	
Preputial gland	(50)	(50)	(50)	(50)
Cyst		3 (6%)	2 (4%)	
Hyperplasia			1 (2%)	2 (4%)
Inflammation	50 (100%)	50 (100%)	47 (94%)	49 (98%)
Duct, ectasia			1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Hyperplasia	39 (78%)	41 (82%)	39 (78%)	40 (80%)
Inflammation	44 (88%)	37 (74%)	32 (64%)	42 (84%)
Inflammation, suppurative	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation	2 (4%)			2 (4%)
Testes	(50)	(50)	(50)	(50)
Inflammation, granulomatous		1 (2%)		
Necrosis			1 (2%)	
Germinal epithelium, atrophy	2 (4%)	1 (2%)	1 (2%)	
Germinal epithelium, mineralization	1 (2%)	1 (2%)		2 (4%)
Tunic, fibrosis		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy			4 (8%)	
Hyperplasia	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Lymph node	(4)	(0)	(6)	(5)
Deep cervical, ectasia			1 (17%)	
Deep cervical, hyperplasia, lymphoid			1 (17%)	1 (20%)
Mediastinal, hyperplasia, lymphoid				1 (20%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	1 (2%)			
Hyperplasia, lymphoid				1 (2%)
Inflammation, chronic active	1 (2%)			
Spleen	(50)	(50)	(49)	(50)
Atrophy			3 (6%)	4 (8%)
Fibrosis			2 (4%)	1 (2%)
Hematopoietic cell proliferation	24 (48%)	25 (50%)	15 (31%)	24 (48%)
Infarct			1 (2%)	
Stromal hyperplasia			1 (2%)	
Thymus	(49)	(50)	(50)	(48)
Atrophy	45 (92%)	50 (100%)	46 (92%)	47 (98%)
Ectopic parathyroid gland	1 (2%)			
Ectopic thyroid				1 (2%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Hyperplasia	13 (27%)	10 (20%)	7 (14%)	12 (24%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	3 (6%)		
Edema	1 (2%)			
Hemorrhage		1 (2%)		
Artery, site of application, dermis, inflammation		1 (2%)		
Control dermis, fibrosis			1 (2%)	

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Integumentary System (continued)				
Skin (continued)	(50)	(50)	(50)	(50)
Dermis, inflammation		1 (2%)		
Hair follicle, site of application, epidermis, atrophy	1 (2%)			
Sebaceous gland, hyperplasia			1 (2%)	
Site of application, dermis, fibrosis		1 (2%)		
Site of application dermis, inflammation	2 (4%)	2 (4%)	2 (4%)	
Site of application, epidermis, hyperkeratosis		1 (2%)	1 (2%)	
Site of application , epidermis, ulcer	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteopetrosis			1 (2%)	
Joint, inflammation	2 (4%)			1 (2%)
Skeletal muscle	(1)	(0)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hydrocephalus	17 (34%)	14 (28%)	14 (28%)	14 (28%)
Hippocampus, necrosis			1 (2%)	
Hypothalamus, compression	17 (34%)	8 (16%)	12 (24%)	14 (28%)
Thalamus, necrosis			1 (2%)	
Spinal cord	(1)	(1)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion		1 (2%)		
Inflammation	17 (34%)	22 (44%)	20 (40%)	16 (32%)
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	7 (14%)	12 (24%)	9 (18%)	8 (16%)
Artery, hyperplasia		1 (2%)		
Nose	(50)	(49)	(50)	(49)
Foreign body	7 (14%)	12 (24%)	4 (8%)	5 (10%)
Inflammation	27 (54%)	32 (65%)	22 (44%)	18 (37%)
Polyp, inflammatory	1 (2%)			
Glands, cyst		1 (2%)	2 (4%)	1 (2%)
Olfactory epithelium, accumulation, hyaline droplet	1 (2%)	2 (4%)		
Olfactory epithelium, degeneration	5 (10%)	17 (35%)	30 (60%)	48 (98%)
Olfactory epithelium, metaplasia, respiratory	9 (18%)	9 (18%)	3 (6%)	4 (8%)
Olfactory epithelium, metaplasia, squamous	1 (2%)			
Trachea	(50)	(50)	(50)	(50)
Inflammation	1 (2%)		3 (6%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	2 (4%)	1 (2%)		2 (4%)
Retina, atrophy	2 (4%)	1 (2%)		2 (4%)
Sclera, metaplasia, osseous	38 (76%)	35 (70%)	28 (56%)	32 (64%)

TABLE A3
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Special Senses System (continued)				
Harderian gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	2 (4%)
Hyperplasia		1 (2%)		1 (2%)
Inflammation	3 (6%)	3 (6%)	2 (4%)	1 (2%)
Pigmentation, porphyrin	1 (2%)			
Zymbal's gland	(1)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Infarct		1 (2%)		
Inflammation				1 (2%)
Nephropathy	47 (94%)	48 (96%)	43 (86%)	48 (96%)
Renal tubule, cyst		1 (2%)		
Renal tubule, hyperplasia				1 (2%)
Renal tubule, pigmentation, lipofuscin	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation				2 (4%)
Muscularis, mineralization		1 (2%)		
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)	1 (2%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR DERMAL STUDY
OF BIS(2-CHLOROETHOXY)METHANE

TABLE B1	Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study of Bis(2-chloroethoxy)methane	84
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TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	18	15	19
Natural deaths	12	2	7	13
Survivors				
Terminal sacrifice	19	30	28	18
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Periesophageal tissue, lipoma	1 (2%)			
Intestine large, cecum	(50)	(50)	(50)	(50)
Lipoma			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular carcinoma				1 (2%)
Mesentery	(13)	(13)	(17)	(6)
Leiomyosarcoma, metastatic, uterus		1 (8%)		
Oral mucosa	(1)	(0)	(0)	(0)
Squamous cell papilloma	1 (100%)			
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(0)	(0)	(1)
Squamous cell papilloma				1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			2 (4%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	1 (2%)	1 (2%)		2 (4%)
Pheochromocytoma complex		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma	1 (2%)			
Parathyroid gland	(43)	(46)	(48)	(50)
Adenoma			1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	20 (40%)	23 (46%)	22 (44%)	17 (34%)
Pars distalis, adenoma, multiple	2 (4%)	5 (10%)	2 (4%)	1 (2%)
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, C-cell, adenoma	1 (2%)			
C-cell, adenoma	5 (10%)	9 (18%)	11 (22%)	2 (4%)
C-cell, adenoma, multiple		2 (4%)		
C-cell, carcinoma			1 (2%)	
Follicle, adenoma	1 (2%)	1 (2%)		
Follicle, carcinoma	1 (2%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150mg/kg	300 mg/kg
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	2 (4%)	4 (8%)	5 (10%)	3 (6%)
Carcinoma			2 (4%)	1 (2%)
Ovary	(50)	(50)	(50)	(50)
Granulosa cell tumor malignant	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Leiomyosarcoma		1 (2%)		
Polyp stromal	5 (10%)	5 (10%)	8 (16%)	4 (8%)
Sarcoma stromal	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Bilateral, polyp stromal				1 (2%)
Vagina	(0)	(0)	(0)	(1)
Polyp				1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Lymph node	(3)	(1)	(5)	(1)
Deep cervical, carcinoma, metastatic, thyroid gland			1 (20%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(49)	(50)	(50)	(49)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		1 (2%)
Carcinoma	1 (2%)	2 (4%)	1 (2%)	
Fibroadenoma	12 (24%)	18 (36%)	12 (24%)	14 (28%)
Fibroadenoma, multiple	4 (8%)	4 (8%)	6 (12%)	2 (4%)
Skin	(50)	(50)	(50)	(49)
Subcutaneous tissue, fibroma	2 (4%)	1 (2%)		
Subcutaneous tissue, fibrosarcoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Oligodendroglioma malignant	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			3 (6%)	2 (4%)
Nose	(49)	(49)	(50)	(49)
Trachea	(50)	(50)	(50)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150mg/kg	300 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Lacrimal gland	(0)	(1)	(0)	(0)
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Lipoma			1 (2%)	
Sarcoma	1 (2%)			
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Leukemia mononuclear	12 (24%)	9 (18%)	12 (24%)	13 (26%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	44	43	45	34
Total primary neoplasms	79	92	93	69
Total animals with benign neoplasms	37	41	41	31
Total benign neoplasms	59	77	74	51
Total animals with malignant neoplasms	19	15	18	16
Total malignant neoplasms	20	15	19	18
Total animals with metastatic neoplasms		1	1	
Total metastatic neoplasms		1	1	

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Clitoral Gland: Adenoma				
Overall rate ^a	2/50 (4%)	4/50 (8%)	5/50 (10%)	3/50 (6%)
Adjusted rate ^b	5.3%	9.5%	11.5%	10.3%
Terminal rate ^c	2/19 (11%)	4/30 (13%)	3/28 (11%)	2/18 (11%)
First incidence (days)	730 (T)	730 (T)	561	674
Poly-3 test ^d	P=0.285	P=0.389	P=0.279	P=0.386
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	7/50 (14%)	4/50 (8%)
Adjusted rate	5.3%	9.5%	16.1%	13.6%
Terminal rate	2/19 (11%)	4/30 (13%)	5/28 (18%)	2/18 (11%)
First incidence (days)	730 (T)	730 (T)	561	674
Poly-3 test	P=0.131	P=0.389	P=0.117	P=0.230
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	6.9%	6.7%
Terminal rate	0/19 (0%)	0/30 (0%)	2/28 (7%)	0/18 (0%)
First incidence (days)	— ^e	—	637	213
Poly-3 test	P=0.045	— ^f	P=0.145	P=0.190
Mammary Gland: Fibroadenoma				
Overall rate	16/50 (32%)	22/50 (44%) ^g	18/50 (36%)	16/50 (32%) ^g
Adjusted rate	39.6%	48.2%	40.8%	53.5%
Terminal rate	9/19 (47%)	12/30 (40%)	11/28 (39%)	11/18 (61%)
First incidence (days)	511	532	635	597
Poly-3 test	P=0.240	P=0.275	P=0.544	P=0.177
Mammary Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.6%	7.1%	2.3%	3.4%
Terminal rate	0/19 (0%)	2/30 (7%)	1/28 (4%)	0/18 (0%)
First incidence (days)	539	603	730 (T)	674
Poly-3 test	P=0.503N	P=0.344	P=0.734N	P=0.697
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	17/50 (34%)	23/50 (46%)	18/50 (36%)	16/50 (32%)
Adjusted rate	41.5%	49.9%	40.8%	53.5%
Terminal rate	9/19 (47%)	12/30 (40%)	11/28 (39%)	11/18 (61%)
First incidence (days)	511	532	635	597
Poly-3 test	P=0.304	P=0.279	P=0.564N	P=0.221
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/50 (44%)	28/50 (56%)	24/50 (48%)	18/50 (36%)
Adjusted rate	52.7%	61.1%	53.5%	55.2%
Terminal rate	8/19 (42%)	16/30 (53%)	15/28 (54%)	7/18 (39%)
First incidence (days)	539	388	526	464
Poly-3 test	P=0.518N	P=0.275	P=0.560	P=0.508
Thyroid Gland (C-cell): Adenoma				
Overall rate	6/50 (12%)	11/50 (22%)	11/50 (22%)	2/50 (4%)
Adjusted rate	15.8%	26.1%	25.1%	6.9%
Terminal rate	2/19 (11%)	10/30 (33%)	4/28 (14%)	2/18 (11%)
First incidence (days)	672	711	616	730 (T)
Poly-3 test	P=0.243N	P=0.194	P=0.223	P=0.235N

TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	6/50 (12%)	11/50 (22%)	11/50 (22%)	2/50 (4%)
Adjusted rate	15.8%	26.1%	25.1%	6.9%
Terminal rate	2/19 (11%)	10/30 (33%)	4/28 (14%)	2/18 (11%)
First incidence (days)	672	711	616	730 (T)
Poly-3 test	P=0.243N	P=0.194	P=0.223	P=0.235N
Uterus: Stromal Polyp				
Overall rate	5/50 (10%)	5/50 (10%)	8/50 (16%)	5/50 (10%)
Adjusted rate	13.0%	11.8%	18.1%	16.5%
Terminal rate	2/19 (11%)	4/30 (13%)	4/28 (14%)	2/18 (11%)
First incidence (days)	614	586	561	378
Poly-3 test	P=0.306	P=0.568N	P=0.369	P=0.473
All Organs: Mononuclear Cell Leukemia				
Overall rate	12/50 (24%)	9/50 (18%)	12/50 (24%)	13/50 (26%)
Adjusted rate	29.1%	20.8%	26.4%	40.9%
Terminal rate	3/19 (16%)	4/30 (13%)	2/28 (7%)	4/18 (22%)
First incidence (days)	519	595	561	378
Poly-3 test	P=0.142	P=0.263N	P=0.485N	P=0.211
All Organs: Benign Neoplasms				
Overall rate	37/50 (74%)	41/50 (82%)	41/50 (82%)	31/50 (62%)
Adjusted rate	84.1%	85.0%	87.9%	88.0%
Terminal rate	16/19 (84%)	24/30 (80%)	25/28 (89%)	16/18 (89%)
First incidence (days)	511	388	526	213
Poly-3 test	P=0.311	P=0.573	P=0.403	P=0.423
All Organs: Malignant Neoplasms				
Overall rate	19/50 (38%)	15/50 (30%)	18/50 (36%)	16/50 (32%)
Adjusted rate	44.4%	33.6%	39.1%	48.5%
Terminal rate	5/19 (26%)	7/30 (23%)	7/28 (25%)	5/18 (28%)
First incidence (days)	519	575	550	189
Poly-3 test	P=0.346	P=0.205N	P=0.387N	P=0.450
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	43/50 (86%)	45/50 (90%)	34/50 (68%)
Adjusted rate	93.5%	88.3%	92.9%	92.8%
Terminal rate	17/19 (90%)	25/30 (83%)	25/28 (89%)	16/18 (89%)
First incidence (days)	511	388	526	189
Poly-3 test	P=0.506	P=0.287N	P=0.620N	P=0.629N

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for clitoral gland, lung, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.

^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.

^g One adenoma occurred in an animal that also had a fibroadenoma

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	19	18	15	19
Natural deaths	12	2	7	13
Survivors				
Terminal sacrifice	19	30	28	18
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	3 (6%)	2 (4%)	1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan		4 (8%)	6 (12%)	5 (10%)
Ulcer	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Ulcer	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis	4 (8%)	5 (10%)	1 (2%)	2 (4%)
Basophilic focus	41 (82%)	48 (96%)	42 (84%)	30 (60%)
Clear cell focus	9 (18%)	9 (18%)	10 (20%)	5 (10%)
Degeneration, cystic		2 (4%)		
Eosinophilic focus	5 (10%)	8 (16%)	6 (12%)	6 (12%)
Fatty change	9 (18%)	9 (18%)	4 (8%)	9 (18%)
Fibrosis	5 (10%)	3 (6%)	10 (20%)	2 (4%)
Hepatodiaphragmatic nodule	7 (14%)	6 (12%)	8 (16%)	8 (16%)
Inflammation	40 (80%)	45 (90%)	39 (78%)	33 (66%)
Mixed cell focus	4 (8%)	10 (20%)	10 (20%)	3 (6%)
Necrosis	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Bile duct, cyst		1 (2%)	1 (2%)	
Bile duct, hyperplasia	17 (34%)	18 (36%)	19 (38%)	12 (24%)
Centrilobular, necrosis	4 (8%)	3 (6%)	5 (10%)	5 (10%)
Periportal, cytoplasmic alteration	1 (2%)			
Mesentery	(13)	(13)	(17)	(6)
Necrosis	1 (8%)			
Fat, necrosis	13 (100%)	12 (92%)	17 (100%)	6 (100%)
Oral mucosa	(1)	(0)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Inflammation	2 (4%)			1 (2%)
Acinus, atrophy	13 (26%)	12 (24%)	9 (18%)	6 (12%)
Acinus, hyperplasia	1 (2%)			
Duct, cyst	4 (8%)	6 (12%)	1 (2%)	2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	6 (12%)	3 (6%)	1 (2%)	2 (4%)
Ulcer	4 (8%)	3 (6%)		1 (2%)
Epithelium, hyperplasia	1 (2%)	2 (4%)		
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Inflammation	2 (4%)			1 (2%)
Mineralization				2 (4%)
Ulcer	1 (2%)	1 (2%)		2 (4%)
Tongue	(0)	(0)	(0)	(1)

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

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	40 (80%)	45 (90%)	42 (84%)	46 (92%)
Fibrosis	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Thrombosis	1 (2%)	1 (2%)		
Coronary artery, inflammation	1 (2%)			
Myocardium, infiltration cellular, mononuclear cell	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Myocardium, necrosis	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Myocardium, vacuolization cytoplasmic	50 (100%)	50 (100%)	50 (100%)	50 (100%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			1 (2%)
Hemorrhage				2 (4%)
Hyperplasia	11 (22%)	26 (52%)	18 (36%)	14 (28%)
Necrosis				7 (14%)
Vacuolization cytoplasmic	15 (30%)	17 (34%)	21 (42%)	15 (30%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	3 (6%)	3 (6%)	4 (8%)
Necrosis			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	10 (20%)	6 (12%)	5 (10%)	6 (12%)
Parathyroid gland	(43)	(46)	(48)	(50)
Pituitary gland	(50)	(50)	(50)	(50)
Angiectasis	32 (64%)	39 (78%)	32 (64%)	26 (52%)
Atrophy	1 (2%)			
Cyst	16 (32%)	21 (42%)	17 (34%)	18 (36%)
Hemorrhage				1 (2%)
Pars distalis, hyperplasia	16 (32%)	26 (52%)	19 (38%)	18 (36%)
Pars intermedia, hyperplasia	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cyst			1 (2%)	
C-cell, hyperplasia	33 (66%)	38 (76%)	34 (68%)	19 (38%)
Follicle, hyperplasia		2 (4%)	3 (6%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)	2 (4%)	
Hyperplasia	2 (4%)	6 (12%)	6 (12%)	3 (6%)
Inflammation	32 (64%)	32 (64%)	40 (80%)	23 (46%)
Ovary	(50)	(50)	(50)	(50)
Atrophy		2 (4%)	2 (4%)	
Cyst	5 (10%)	4 (8%)	7 (14%)	5 (10%)
Bilateral, Cyst			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Inflammation, suppurative	1 (2%)			
Cervix, endometrium, hyperplasia		1 (2%)		
Cervix, myometrium, hyperplasia	1 (2%)			
Endometrium, hyperplasia, cystic	6 (12%)	4 (8%)	4 (8%)	5 (10%)
Vagina	(0)	(0)	(0)	(1)

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(49)
Atrophy	2 (4%)			
Hyperplasia	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Lymph node	(3)	(1)	(5)	(1)
Deep cervical, hyperplasia, lymphoid			1 (20%)	
Mediastinal, ectasia		1 (100%)		
Mediastinal, hyperplasia, lymphoid	1 (33%)			
Pancreatic, ectasia	1 (33%)			
Pancreatic, hyperplasia, lymphoid	1 (33%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Atrophy	3 (6%)			2 (4%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	29 (58%)	37 (74%)	34 (68%)	26 (52%)
Infarct				1 (2%)
Thymus	(49)	(50)	(50)	(49)
Atrophy	43 (88%)	50 (100%)	47 (94%)	35 (71%)
Hemorrhage				1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)		3 (6%)	2 (4%)
Inflammation	1 (2%)			
Skin	(50)	(50)	(50)	(49)
Cyst epithelial inclusion			1 (2%)	
Control dermis, inflammation	1 (2%)			
Control epidermis, hyperplasia	1 (2%)			
Dermis, inflammation		1 (2%)	1 (2%)	
Epidermis, hyperplasia		1 (2%)		
Epidermis, ulcer		1 (2%)	1 (2%)	
Sebaceous gland, hyperplasia			1 (2%)	
Site of application, dermis, fibrosis	3 (6%)		1 (2%)	1 (2%)
Site of application, dermis, inflammation		2 (4%)	3 (6%)	2 (4%)
Site of application epidermis, hyperkeratosis			1 (2%)	
Site of application, epidermis, hyperplasia	1 (2%)	2 (4%)	5 (10%)	1 (2%)
Site of application, epidermis, ulcer	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Subcutaneous tissue, necrosis			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Joint, inflammation	1 (2%)			3 (6%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Hydrocephalus	11 (22%)	14 (28%)	8 (16%)	5 (10%)
Hippocampus, necrosis		1 (2%)		
Hypothalamus, compression	13 (26%)	12 (24%)	8 (16%)	5 (10%)
Vein, infiltration cellular, mononuclear cell		1 (2%)		

TABLE B3
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	75 mg/kg	150 mg/kg	300 mg/kg
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)		2 (4%)	1 (2%)
Inflammation	18 (36%)	31 (62%)	24 (48%)	19 (38%)
Metaplasia, osseous			1 (2%)	
Thrombosis	1 (2%)			
Alveolar epithelium, hyperplasia	1 (2%)	7 (14%)	7 (14%)	6 (12%)
Alveolar epithelium, metaplasia, squamous			3 (6%)	
Bronchus, hyperplasia		1 (2%)		
Nose	(49)	(49)	(50)	(49)
Foreign body	1 (2%)	2 (4%)	2 (4%)	
Inflammation	10 (20%)	14 (29%)	16 (32%)	6 (12%)
Glands, cyst				1 (2%)
Olfactory epithelium, accumulation, hyaline droplet		1 (2%)	1 (2%)	
Olfactory epithelium, degeneration	5 (10%)	4 (8%)	18 (36%)	49 (100%)
Olfactory epithelium, metaplasia, respiratory			1 (2%)	1 (2%)
Olfactory epithelium, metaplasia, squamous				1 (2%)
Respiratory epithelium, degeneration			1 (2%)	
Trachea	(50)	(50)	(50)	(50)
Glands, cyst		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	1 (2%)	2 (4%)	3 (6%)	5 (10%)
Retina, atrophy	2 (4%)	3 (6%)	4 (8%)	5 (10%)
Sclera, metaplasia, osseous	7 (14%)	2 (4%)	6 (12%)	4 (8%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Inflammation	5 (10%)	11 (22%)	9 (18%)	10 (20%)
Capillary, hyperplasia		1 (2%)		
Lacrimal gland	(0)	(1)	(0)	(0)
Hyperplasia		1 (100%)		
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hydronephrosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, suppurative		1 (2%)		
Nephropathy	30 (60%)	30 (60%)	34 (68%)	25 (50%)
Renal tubule, cyst	1 (2%)			
Urinary bladder	(50)	(50)	(49)	(50)
Inflammation	1 (2%)			
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE
IN THE 2-YEAR DERMAL STUDY
OF BIS(2-CHLOROETHOXY)METHANE

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TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	4	2	8
Natural deaths	6	6	6	14
Survivors				
Died last week of study		2		1
Terminal sacrifice	37	38	42	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(49)	(49)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma		2 (4%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Hemangiosarcoma	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Hemangiosarcoma, metastatic, skin				1 (2%)
Hemangiosarcoma, metastatic, spleen				1 (2%)
Hepatoblastoma	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Hepatoblastoma, multiple		1 (2%)		1 (2%)
Hepatocellular adenoma	13 (26%)	14 (28%)	12 (24%)	15 (30%)
Hepatocellular adenoma, multiple	20 (40%)	26 (52%)	25 (50%)	11 (22%)
Hepatocellular carcinoma	8 (16%)	14 (28%)	8 (16%)	7 (14%)
Hepatocellular carcinoma, multiple	6 (12%)	1 (2%)	2 (4%)	1 (2%)
Hepatocholangiocarcinoma		1 (2%)		
Ito cell tumor benign	1 (2%)			
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Mesentery	(5)	(8)	(11)	(1)
Hemangioma	1 (20%)			
Osteosarcoma, metastatic, uncertain primary site			1 (9%)	
Pancreas	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Hemangioma		1 (2%)		
Squamous cell papilloma		1 (2%)		1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(0)	(0)
Tooth	(5)	(5)	(4)	(1)
Odontoma	1 (20%)		2 (50%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Bilateral, adenoma	1 (2%)			
Subcapsular, adenoma	5 (10%)		3 (6%)	6 (12%)
Subcapsular, carcinoma				1 (2%)
Adrenal medulla	(49)	(50)	(50)	(50)
Islets, pancreatic	(50)	(50)	(50)	(50)
Parathyroid gland	(47)	(33)	(45)	(46)
Pituitary gland	(50)	(49)	(49)	(49)
Pars distalis, adenoma	1 (2%)			
Pars intermedia, adenoma	1 (2%)	1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Bilateral, follicular cell, adenoma				1 (2%)
Follicular cell, adenoma		1 (2%)	3 (6%)	1 (2%)
Follicular cell, carcinoma	1 (2%)			
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	2 (4%)	1 (2%)	2 (4%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Hemangiosarcoma	1 (2%)	4 (8%)	1 (2%)	1 (2%)
Lymph node	(2)	(1)	(2)	(1)
Mediastinal, carcinoma, metastatic, thyroid gland	1 (50%)			
Mediastinal, osteosarcoma, metastatic, uncertain primary site			1 (50%)	
Lymph node, mandibular	(50)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Hemangiosarcoma			1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	3 (6%)	1 (2%)	3 (6%)	2 (4%)
Thymus	(49)	(49)	(47)	(49)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Integumentary System				
Mammary gland	(1)	(0)	(0)	(0)
Carcinoma	1 (100%)			
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma				1 (2%)
Keratoacanthoma		1 (2%)		
Site of application, dermis, hemangiosarcoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, schwannoma malignant				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(0)	(1)
Hepatoblastoma, metastatic, liver				1 (100%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	10 (20%)	7 (14%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma	4 (8%)	4 (8%)	4 (8%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)			
Carcinoma, metastatic, Harderian gland	1 (2%)			
Carcinoma, metastatic, thyroid gland	1 (2%)			
Hemangiosarcoma, metastatic, skin			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	3 (6%)	6 (12%)	3 (6%)	4 (8%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Mediastinum, hemangioma		1 (2%)		
Mediastinum, hemangiosarcoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland		1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Sarcoma	1 (2%)			
Harderian gland	(50)	(50)	(50)	(49)
Adenoma	10 (20%)	10 (20%)	8 (16%)	9 (18%)
Carcinoma	2 (4%)	1 (2%)		
Sarcoma	1 (2%)			
Bilateral, adenoma				1 (2%)
Zymbal's gland	(1)	(0)	(0)	(1)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (2%)		
Carcinoma, metastatic, thyroid gland	1 (2%)			
Renal tubule, adenoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions^b				
Multiple organs	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	
Lymphoma malignant		3 (6%)	2 (4%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	48	46	45	37
Total primary neoplasms	97	110	94	76
Total animals with benign neoplasms	41	42	39	34
Total benign neoplasms	63	71	63	53
Total animals with malignant neoplasms	25	32	25	16
Total malignant neoplasms	34	39	31	23
Total animals with metastatic neoplasms	5	8	5	7
Total metastatic neoplasms	11	8	10	7
Total animals with malignant neoplasms of uncertain primary site			1	

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Adrenal Cortex: Adenoma				
Overall rate ^a	6/49 (12%)	0/50 (0%)	3/50 (6%)	6/50 (12%)
Adjusted rate ^b	14.1%	0.0%	6.5%	17.3%
Terminal rate ^c	5/36 (14%)	0/39 (0%)	3/42 (7%)	6/27 (22%)
First incidence (days)	533	— ^c	728 (T)	728 (T)
Poly-3 test ^d	P=0.242	P=0.012N	P=0.205N	P=0.471
Bone Marrow: Hemangiosarcoma				
Overall rate	1/50 (2%)	4/50 (8%)	1/50 (2%)	1/50 (2%)
Adjusted rate	2.3%	8.8%	2.2%	2.9%
Terminal rate	1/37 (3%)	4/39 (10%)	1/42 (2%)	1/27 (4%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.433N	P=0.195	P=0.745N	P=0.710
Harderian Gland: Adenoma				
Overall rate	10/50 (20%)	10/50 (20%)	8/50 (16%)	10/50 (20%)
Adjusted rate	22.6%	22%	17.1%	28.2%
Terminal rate	8/37 (22%)	8/39 (21%)	7/42 (17%)	8/27 (30%)
First incidence (days)	533	701	533	486
Poly-3 test	P=0.384	P=0.572N	P=0.349N	P=0.378
Harderian Gland: Adenoma or Carcinoma				
Overall rate	12/50 (24%)	11/50 (22%)	8/50 (16%)	10/50 (20%)
Adjusted rate	26.7%	24.2%	17.1%	28.2%
Terminal rate	9/37 (24%)	9/39 (23%)	7/42 (17%)	8/27 (30%)
First incidence (days)	461	701	533	486
Poly-3 test	P=0.510N	P=0.487N	P=0.197N	P=0.538
Liver: Hemangiosarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	2.3%	4.4%	8.7%	2.9%
Terminal rate	0/37 (0%)	2/39 (5%)	3/42 (7%)	1/27 (4%)
First incidence (days)	578	728 (T)	722	728 (T)
Poly-3 test	P=0.433	P=0.515	P=0.196	P=0.708
Liver: Hepatocellular Adenoma				
Overall rate	33/50 (66%)	40/50 (80%)	37/50 (74%)	26/50 (52%)
Adjusted rate	71.5%	86.1%	77.7%	70.0%
Terminal rate	27/37 (73%)	35/39 (90%)	34/42 (81%)	19/27 (70%)
First incidence (days)	461	589	501	506
Poly-3 test	P=0.354N	P=0.060	P=0.320	P=0.537N
Liver: Hepatocellular Carcinoma				
Overall rate	14/50 (28%)	15/50 (30%)	10/50 (20%)	8/50 (16%)
Adjusted rate	30.8%	32%	21.3%	22.3%
Terminal rate	9/37 (24%)	10/39 (26%)	8/42 (19%)	4/27 (15%)
First incidence (days)	495	521	501	522
Poly-3 test	P=0.151N	P=0.539	P=0.209N	P=0.275N
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	40/50 (80%)	43/50 (86%)	39/50 (78%)	30/50 (60%)
Adjusted rate	82.4%	90.8%	81.4%	79.4%
Terminal rate	29/37 (78%)	36/39 (92%)	35/42 (83%)	21/27 (78%)
First incidence (days)	461	521	501	506
Poly-3 test	P=0.275N	P=0.175	P=0.555N	P=0.469N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Liver: Hepatoblastoma				
Overall rate	2/50 (4%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.7%	6.6%	4.3%	8.6%
Terminal rate	2/37 (5%)	3/39 (8%)	2/42 (5%)	3/27 (11%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.361	P=0.524	P=0.669N	P=0.403
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	15/50 (30%)	17/50 (34%)	11/50 (22%)	10/50 (20%)
Adjusted rate	33.0%	36.3%	23.4%	27.9%
Terminal rate	10/37 (27%)	12/39 (31%)	9/42 (21%)	6/27 (22%)
First incidence (days)	495	521	501	522
Poly-3 test	P=0.224N	P=0.455	P=0.212N	P=0.401N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	40/50 (80%)	43/50 (86%)	39/50 (78%)	30/50 (60%)
Adjusted rate	82.4%	90.8%	81.4%	79.4%
Terminal rate	29/37 (78%)	36/39 (92%)	35/42 (83%)	21/27 (78%)
First incidence (days)	461	521	501	506
Poly-3 test	P=0.275N	P=0.175	P=0.555N	P=0.469N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	14/50 (28%)	8/50 (16%)	7/50 (14%)
Adjusted rate	13.9%	30.3%	17.4%	20.0%
Terminal rate	6/37 (16%)	12/39 (31%)	8/42 (19%)	5/27 (19%)
First incidence (days)	728 (T)	495	728 (T)	678
Poly-3 test	P=0.503	P=0.053	P=0.439	P=0.341
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	5/50 (10%)	4/50 (8%)	4/50 (8%)	1/50 (2%)
Adjusted rate	11.5%	8.7%	8.7%	2.9%
Terminal rate	4/37 (11%)	2/39 (5%)	4/42 (10%)	1/27 (4%)
First incidence (days)	539	631	728 (T)	728 (T)
Poly-3 test	P=0.138N	P=0.469N	P=0.466N	P=0.163N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	11/50 (22%)	18/50 (36%)	12/50 (24%)	8/50 (16%)
Adjusted rate	25.2%	38.6%	26.1%	22.9%
Terminal rate	10/37 (27%)	14/39 (36%)	12/42 (29%)	6/27 (22%)
First incidence (days)	539	495	728 (T)	678
Poly-3 test	P=0.294N	P=0.126	P=0.559	P=0.511N
Spleen: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.0%	2.2%	6.5%	5.8%
Terminal rate	3/37 (8%)	1/39 (3%)	3/42 (7%)	2/27 (7%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.547	P=0.286N	P=0.631N	P=0.596N
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	2.2%	6.5%	5.8%
Terminal rate	0/37 (0%)	1/39 (3%)	3/42 (7%)	2/27 (7%)
First incidence (days)	—	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.094	P=0.511	P=0.131	P=0.193

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.3%	2.2%	6.5%	5.8%
Terminal rate	1/37 (3%)	1/39 (3%)	3/42 (7%)	2/27 (7%)
First incidence (days)	728 (T)	728 (T)	728 (T)	728 (T)
Poly-3 test	P=0.222	P=0.749N	P=0.330	P=0.426
All Organs: Hemangioma				
Overall rate	2/50 (4%)	2/50 (4%)	0/50 (0%)	0/50 (0%)
Adjusted rate	4.7%	4.4%	0.0%	0.0%
Terminal rate	2/37 (5%)	2/39 (5%)	0/42 (0%)	0/27 (0%)
First incidence (days)	728 (T)	728 (T)	—	—
Poly-3 test	P=0.091N	P=0.675N	P=0.222N	P=0.288N
All Organs: Hemangiosarcoma				
Overall rate	5/50 (10%)	6/50 (12%)	9/50 (18%)	4/50 (8%)
Adjusted rate	11.5%	13.2%	19.4%	11.5%
Terminal rate	4/37 (11%)	6/39 (15%)	7/42 (17%)	4/27 (15%)
First incidence (days)	578	728 (T)	648	728 (T)
Poly-3 test	P=0.453	P=0.529	P=0.229	P=0.635
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	7/50 (14%)	8/50 (16%)	9/50 (18%)	4/50 (8%)
Adjusted rate	16.1%	17.6%	19.4%	11.5%
Terminal rate	6/37 (16%)	8/39 (21%)	7/42 (17%)	4/27 (15%)
First incidence (days)	578	728 (T)	648	728 (T)
Poly-3 test	P=0.376N	P=0.535	P=0.446	P=0.403N
All Organs: Malignant Lymphoma				
Overall rate	0/50 (0%)	3/50 (6%)	2/50 (4%)	2/50 (4%)
Adjusted rate	0.0%	6.5%	4.3%	5.6%
Terminal rate	0/37 (0%)	2/39 (5%)	1/42 (2%)	1/27 (4%)
First incidence (days)	—	495	127	451
Poly-3 test	P=0.239	P=0.131	P=0.258	P=0.197
All Organs: Benign Neoplasms				
Overall rate	41/50 (82%)	42/50 (84%)	39/50 (78%)	34/50 (68%)
Adjusted rate	86.1%	89.1%	81.9%	89.8%
Terminal rate	33/37 (89%)	36/39 (92%)	36/42 (86%)	25/27 (93%)
First incidence (days)	397	495	501	486
Poly-3 test	P=0.478	P=0.448	P=0.386N	P=0.423
All Organs: Malignant Neoplasms				
Overall rate	26/50 (52%)	32/50 (64%)	26/50 (52%)	16/50 (32%)
Adjusted rate	55.4%	66.1%	52.3%	43.7%
Terminal rate	18/37 (49%)	23/39 (59%)	19/42 (45%)	11/27 (41%)
First incidence (days)	461	495	127	451
Poly-3 test	P=0.095N	P=0.193	P=0.458N	P=0.200N

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	46/50 (92%)	46/50 (92%)	37/50 (74%)
Adjusted rate	96.0%	95.0%	92.0%	94.3%
Terminal rate	35/37 (95%)	37/39 (95%)	38/42 (91%)	26/27 (96%)
First incidence (days)	397	495	127	451
Poly-3 test	P=0.367N	P=0.603N	P=0.338N	P=0.549N

(T) Terminal sacrifice

- ^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, liver, lung, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.
- ^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

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	7	4	2	8
Natural deaths	6	6	6	14
Survivors				
Died last week of study		2		1
Terminal sacrifice	37	38	42	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	1 (2%)
Ulcer			1 (2%)	
Gallbladder	(50)	(49)	(49)	(50)
Inflammation				1 (2%)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Epithelium, necrosis	1 (2%)			
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Mineralization				1 (2%)
Epithelium, diverticulum				1 (2%)
Peyer's patch, hyperplasia	1 (2%)		1 (2%)	1 (2%)
Serosa, inflammation				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Basophilic focus	3 (6%)	1 (2%)	1 (2%)	3 (6%)
Clear cell focus	26 (52%)	29 (58%)	34 (68%)	16 (32%)
Congestion				1 (2%)
Eosinophilic focus	26 (52%)	28 (56%)	27 (54%)	27 (54%)
Fibrosis			1 (2%)	
Hemorrhage				1 (2%)
Infarct	1 (2%)	2 (4%)		2 (4%)
Inflammation	5 (10%)	3 (6%)	5 (10%)	5 (10%)
Mixed cell focus	9 (18%)	9 (18%)	9 (18%)	7 (14%)
Regeneration				1 (2%)
Tension lipidosis	9 (18%)	1 (2%)	3 (6%)	6 (12%)
Thrombosis			2 (4%)	1 (2%)
Bile duct, cyst				1 (2%)
Centrilobular, degeneration				3 (6%)
Hepatocyte, hypertrophy		1 (2%)		1 (2%)
Hepatocyte, necrosis	3 (6%)	2 (4%)	10 (20%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic, diffuse	14 (28%)	11 (22%)	12 (24%)	13 (26%)
Oval cell, hyperplasia			2 (4%)	
Serosa, inflammation		1 (2%)		
Mesentery	(5)	(8)	(11)	(1)
Inflammation			3 (27%)	1 (100%)
Necrosis	4 (80%)	7 (88%)	7 (64%)	

^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 Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Alimentary System (continued)				
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus	1 (2%)			1 (2%)
Acinus, atrophy	1 (2%)		1 (2%)	1 (2%)
Acinus, hyperplasia	8 (16%)	4 (8%)	13 (26%)	2 (4%)
Artery, inflammation		1 (2%)		
Duct, cyst		2 (4%)	2 (4%)	2 (4%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema				1 (2%)
Erosion		1 (2%)		1 (2%)
Inflammation	1 (2%)	3 (6%)		2 (4%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	7 (14%)
Epithelium, hyperplasia		3 (6%)	1 (2%)	2 (4%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion				4 (8%)
Inflammation			1 (2%)	1 (2%)
Glands, hyperplasia			1 (2%)	
Serosa, inflammation			1 (2%)	
Tongue	(1)	(0)	(0)	(0)
Mineralization	1 (100%)			
Tooth	(5)	(5)	(4)	(1)
Dysplasia	4 (80%)	4 (80%)		1 (100%)
Inflammation			1 (25%)	
Peridontal tissue, fibrosis		1 (20%)	2 (50%)	1 (100%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Adventitia, inflammation			2 (4%)	
Heart	(50)	(50)	(50)	(50)
Artery, inflammation	2 (4%)		2 (4%)	
Atrium, thrombosis				1 (2%)
Epicardium, hyperplasia			1 (2%)	
Epicardium, inflammation	1 (2%)		1 (2%)	
Myocardium, cardiomyopathy	10 (20%)	12 (24%)	7 (14%)	28 (56%)
Myocardium, fibrosis		3 (6%)	3 (6%)	13 (26%)
Myocardium, infiltration cellular, mononuclear cell	11 (22%)	12 (24%)	8 (16%)	28 (56%)
Myocardium, mineralization		1 (2%)	2 (4%)	3 (6%)
Myocardium, necrosis	3 (6%)	2 (4%)		1 (2%)
Myocardium, vacuolization cytoplasmic	10 (20%)	15 (30%)	11 (22%)	29 (58%)
Endocrine System				
Adrenal cortex	(49)	(50)	(50)	(50)
Degeneration	1 (2%)			
Hyperplasia	13 (27%)	4 (8%)	11 (22%)	2 (4%)
Hypertrophy	24 (49%)	34 (68%)	26 (52%)	22 (44%)
Subcapsular, hyperplasia	43 (88%)	44 (88%)	50 (100%)	39 (78%)
Adrenal medulla	(49)	(50)	(50)	(50)
Hyperplasia		2 (4%)	1 (2%)	
Pigmentation, melanin				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	32 (64%)	35 (70%)	39 (78%)	21 (42%)
Parathyroid gland	(47)	(33)	(45)	(46)
Cyst	1 (2%)			2 (4%)

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Endocrine System (continued)				
Pituitary gland	(50)	(49)	(49)	(49)
Pars distalis, cyst	6 (12%)	1 (2%)	3 (6%)	3 (6%)
Pars distalis, hyperplasia	20 (40%)	5 (10%)	17 (35%)	5 (10%)
Pars intermedia, cyst	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)			
Follicle, cyst			2 (4%)	
Follicular cell, hyperplasia				2 (4%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Granuloma sperm		1 (2%)	2 (4%)	
Inflammation	4 (8%)		1 (2%)	4 (8%)
Preputial gland	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	
Inflammation	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Duct, ectasia	3 (6%)	4 (8%)		1 (2%)
Prostate	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Inflammation	1 (2%)		2 (4%)	3 (6%)
Epithelium, hyperplasia	16 (32%)	4 (8%)	3 (6%)	6 (12%)
Seminal vesicle	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Dilatation			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Mineralization	1 (2%)	1 (2%)		
Germinal epithelium, degeneration	11 (22%)	7 (14%)	6 (12%)	9 (18%)
Interstitial cell, hyperplasia	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Atrophy	1 (2%)			
Hemorrhage	1 (2%)			2 (4%)
Myelofibrosis			1 (2%)	
Lymph node	(2)	(1)	(2)	(1)
Lymph node, mandibular	(50)	(50)	(50)	(50)
Hyperplasia, plasma cell	1 (2%)	1 (2%)		2 (4%)
Lymph node, mesenteric	(50)	(50)	(50)	(49)
Hemorrhage	1 (2%)			2 (4%)
Hyperplasia, lymphoid			1 (2%)	
Hyperplasia, plasma cell		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Atrophy	3 (6%)	1 (2%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation			1 (2%)	1 (2%)
Thymus	(49)	(49)	(47)	(49)
Inflammation			1 (2%)	

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Integumentary System				
Mammary gland	(1)	(0)	(0)	(0)
Duct, dilatation	1 (100%)			
Skin	(50)	(50)	(50)	(50)
Ulcer	1 (2%)	1 (2%)		1 (2%)
Site of application, dermis, fibrosis	6 (12%)	1 (2%)	2 (4%)	25 (50%)
Site of application, dermis, inflammation	3 (6%)	1 (2%)	3 (6%)	13 (26%)
Site of application, epidermis, hyperplasia	8 (16%)	1 (2%)	4 (8%)	28 (56%)
Site of application, epidermis, ulcer	3 (6%)	1 (2%)	1 (2%)	4 (8%)
Subcutaneous tissue, edema				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cartilage, femur, joint, hyperplasia	1 (2%)			
Femur, joint, inflammation	1 (2%)			
Skeletal muscle	(0)	(0)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Ventricle, inflammation	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Inflammation	3 (6%)	3 (6%)		
Thrombosis		1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Alveolus, infiltration cellular, histiocyte	3 (6%)	1 (2%)		
Mediastinum, inflammation		1 (2%)		
Perivascular, cyst		1 (2%)		
Serosa, hyperplasia		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Inflammation	8 (16%)	3 (6%)	2 (4%)	3 (6%)
Respiratory epithelium, hyperplasia	1 (2%)		1 (2%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Arteriole, inflammation	1 (2%)			
Cornea, hyperplasia				1 (2%)
Cornea, inflammation	3 (6%)			
Retrolbulbar, inflammation	1 (2%)			
Harderian gland	(50)	(50)	(50)	(49)
Cyst	1 (2%)			
Hemorrhage				1 (2%)
Epithelium, hyperplasia			2 (4%)	
Zymbal's gland	(1)	(0)	(0)	(1)
Cyst	1 (100%)			1 (100%)
Inflammation	1 (100%)			

TABLE C3
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	150 mg/kg	300 mg/kg	600 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	7 (14%)	8 (16%)	11 (22%)	11 (22%)
Hydronephrosis	5 (10%)	1 (2%)	3 (6%)	1 (2%)
Infarct		1 (2%)	1 (2%)	
Infiltration cellular, mononuclear cell		3 (6%)		
Inflammation	4 (8%)	2 (4%)	4 (8%)	1 (2%)
Mineralization	22 (44%)	26 (52%)	21 (42%)	25 (50%)
Nephropathy	45 (90%)	42 (84%)	44 (88%)	37 (74%)
Artery, inflammation	2 (4%)		2 (4%)	
Urinary bladder	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE
IN THE 2-YEAR DERMAL STUDY
OF BIS(2-CHLOROETHOXY)METHANE

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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	5	7	11
Natural deaths	8	7	6	2
Survivors				
Died last week of study				1
Terminal sacrifice	31	38	37	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Gallbladder	(50)	(50)	(50)	(50)
Intestine large, cecum	(50)	(50)	(50)	(50)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Intestine small, ileum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(50)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)			
Hepatocellular adenoma	11 (22%)	11 (22%)	9 (18%)	18 (36%)
Hepatocellular adenoma, multiple	11 (22%)	13 (26%)	6 (12%)	9 (18%)
Hepatocellular carcinoma	1 (2%)	7 (14%)	8 (16%)	5 (10%)
Hepatocellular carcinoma, multiple	2 (4%)	1 (2%)		
Hepatocholangiocarcinoma			1 (2%)	
Mesentery	(16)	(13)	(11)	(10)
Fibrous histiocytoma				2 (20%)
Hepatocholangiocarcinoma, metastatic, liver			1 (9%)	
Sarcoma	1 (6%)			
Pancreas	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Stomach, glandular	(50)	(50)	(50)	(50)
Tooth	(0)	(0)	(2)	(3)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			1 (2%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign		1 (2%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, adenoma	4 (8%)	11 (22%)	6 (12%)	4 (8%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma		2 (4%)	1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Hemangioma	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Cystadenoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Hemangioma		1 (2%)		2 (4%)
Hemangiosarcoma		1 (2%)	1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Adenoma				2 (4%)
Carcinoma			1 (2%)	
Hemangioma		1 (2%)		1 (2%)
Hemangiosarcoma		1 (2%)	2 (4%)	
Polyp stromal		2 (4%)		1 (2%)
Vagina	(0)	(0)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	2 (4%)	
Lymph node	(8)	(9)	(3)	(5)
Fibrosarcoma, metastatic, skin				1 (20%)
Pancreatic, hepatocholangiocarcinoma, metastatic, liver			1 (33%)	
Lymph node, mandibular	(50)	(50)	(50)	(49)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Fibrous histiocytoma, metastatic, Hemangiosarcoma		1 (2%)		1 (2%)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	5 (10%)	2 (4%)	2 (4%)	1 (2%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Thymus	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Fibrous histiocytoma, metastatic, uncertain primary site				1 (2%)
Thymoma malignant				1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Carcinoma	2 (4%)			
Skin	(50)	(50)	(50)	(50)
Site of application, dermis, hemangiosarcoma			1 (2%)	
Site of application, dermis, schwannoma malignant	1 (2%)	1 (2%)		
Subcutaneous tissue, fibrosarcoma				2 (4%)
Subcutaneous tissue, fibrous histiocytoma		1 (2%)	1 (2%)	2 (4%)
Subcutaneous tissue, hemangioma			1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)			
Subcutaneous tissue, myxosarcoma			1 (2%)	
Subcutaneous tissue, schwannoma malignant	1 (2%)		1 (2%)	3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteosarcoma	1 (2%)			
Vertebra, osteosarcoma	1 (2%)		1 (2%)	1 (2%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord	(2)	(0)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)	3 (6%)	4 (8%)	6 (12%)
Alveolar/bronchiolar carcinoma	4 (8%)			2 (4%)
Carcinoma, metastatic, Harderian gland		1 (2%)		
Fibrous histiocytoma, metastatic, mesentery				1 (2%)
Hepatocellular carcinoma, metastatic, liver			2 (4%)	2 (4%)
Hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)		1 (2%)	1 (2%)
Mediastinum, hepatocholangiocarcinoma, metastatic, liver			1 (2%)	
Nose	(50)	(50)	(50)	(50)
Pleura	(0)	(0)	(1)	(0)
Hepatocholangiocarcinoma, metastatic, liver			1 (100%)	
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	6 (12%)	5 (10%)	5 (10%)
Carcinoma		3 (6%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Systemic Lesions				
Multiple organs ^b	(50)	(50)	(50)	(50)
Histiocytic sarcoma	3 (6%)		4 (8%)	4 (8%)
Lymphoma malignant	9 (18%)	13 (26%)	5 (10%)	10 (20%)
Neoplasm Summary				
Total animals with primary neoplasms ^c	44	43	38	44
Total primary neoplasms	71	88	70	89
Total animals with benign neoplasms	27	35	30	37
Total benign neoplasms	34	55	39	55
Total animals with malignant neoplasms	31	24	22	26
Total malignant neoplasms	37	33	31	34
Total animals with metastatic neoplasms	1	1	5	6
Total metastatic neoplasms	1	1	14	8
Total animals with malignant neoplasms of uncertain primary site				1

^a Number of animals examined microscopically at the site and the number of animals with neoplasm

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Harderian Gland: Adenoma				
Overall rate ^a	3/50 (6%)	6/50 (12%)	5/50 (10%)	5/50 (10%)
Adjusted rate ^b	6.7%	13.2%	11.1%	10.5%
Terminal rate ^c	2/31 (7%)	6/38 (16%)	4/37 (11%)	4/37 (11%)
First incidence (days)	534	727 (T)	411	701
Poly-3 test ^d	P=0.430	P=0.248	P=0.359	P=0.388
Harderian Gland: Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	6.5%	0.0%	0.0%
Terminal rate	0/31 (0%)	1/38 (3%)	0/37 (0%)	0/37 (0%)
First incidence (days)	— ^e	589	—	—
Poly-3 test	P=0.289N	P=0.126	— ^f	—
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	5/50 (10%)	5/50 (10%)
Adjusted rate	6.7%	19.6%	11.1%	10.5%
Terminal rate	2/31 (7%)	7/38 (18%)	4/37 (11%)	4/37 (11%)
First incidence (days)	534	589	411	701
Poly-3 test	P=0.548N	P=0.064	P=0.359	P=0.388
Liver: Hepatocellular Adenoma				
Overall rate	22/50 (44%)	24/50 (48%)	15/50 (30%)	27/50 (54%)
Adjusted rate	48.8%	52.6%	33.1%	56.5%
Terminal rate	18/31 (58%)	23/38 (61%)	12/37 (32%)	24/37 (65%)
First incidence (days)	595	664	584	650
Poly-3 test	P=0.321	P=0.440	P=0.092N	P=0.294
Liver: Hepatocellular Carcinoma				
Overall rate	3/50 (6%)	8/50 (16%)	8/50 (16%)	5/50 (10%)
Adjusted rate	6.8%	17.1%	17.8%	10.5%
Terminal rate	2/31 (7%)	5/38 (13%)	6/37 (16%)	3/37 (8%)
First incidence (days)	714	386	638	689
Poly-3 test	P=0.504	P=0.116	P=0.101	P=0.395
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	22/50 (44%)	26/50 (52%)	21/50 (42%)	30/50 (60%)
Adjusted rate	48.8%	55.6%	45.8%	62.5%
Terminal rate	18/31 (58%)	23/38 (61%)	16/37 (43%)	25/37 (68%)
First incidence (days)	595	386	584	650
Poly-3 test	P=0.134	P=0.326	P=0.468N	P=0.125
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	6/50 (12%)
Adjusted rate	2.2%	6.6%	9.0%	12.6%
Terminal rate	0/31 (0%)	3/38 (8%)	4/37 (11%)	5/37 (14%)
First incidence (days)	639	727 (T)	727 (T)	689
Poly-3 test	P=0.045	P=0.312	P=0.176	P=0.067
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate	9.0%	0.0%	0.0%	4.2%
Terminal rate	3/31 (10%)	0/38 (0%)	0/37 (0%)	2/37 (5%)
First incidence (days)	652	—	—	727 (T)
Poly-3 test	P=0.338N	P=0.058N	P=0.061N	P=0.309N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	5/50 (10%)	3/50 (6%)	4/50 (8%)	8/50 (16%)
Adjusted rate	11.1%	6.6%	9.0%	16.8%
Terminal rate	3/31 (10%)	3/38 (8%)	4/37 (11%)	7/37 (19%)
First incidence (days)	639	727 (T)	727 (T)	689
Poly-3 test	P=0.149	P=0.349N	P=0.509N	P=0.314
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/49 (8%)	11/50 (22%)	6/50 (12%)	4/50 (8%)
Adjusted rate	9.2%	24.2%	13.6%	8.4%
Terminal rate	4/30 (13%)	10/38 (26%)	6/37 (16%)	3/37 (8%)
First incidence (days)	727 (T)	700	727 (T)	671
Poly-3 test	P=0.226N	P=0.053	P=0.383	P=0.590N
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Myxosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	0.0%	2.2%	4.5%	8.4%
Terminal rate	0/31 (0%)	1/38 (3%)	1/37 (3%)	1/37 (3%)
First incidence (days)	—	727 (T)	645	659
Poly-3 test	P=0.026	P=0.505	P=0.238	P=0.071
Skin (Subcutaneous Tissue): Malignant Schwannoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	4.5%	2.2%	2.3%	6.3%
Terminal rate	1/31 (3%)	1/38 (3%)	0/37 (0%)	0/37 (0%)
First incidence (days)	701	727 (T)	701	650
Poly-3 test	P=0.345	P=0.492N	P=0.500N	P=0.534
Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, Myxosarcoma, or Malignant Schwannoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	7/50 (14%)
Adjusted rate	4.5%	4.4%	6.7%	14.5%
Terminal rate	1/31 (3%)	2/38 (5%)	1/37 (3%)	1/37 (3%)
First incidence (days)	701	727 (T)	645	650
Poly-3 test	P=0.030	P=0.686N	P=0.503	P=0.101
Spleen: Hemangiosarcoma				
Overall rate	5/50 (10%)	2/50 (4%)	2/50 (4%)	1/50 (2%)
Adjusted rate	11.2%	4.4%	4.4%	2.1%
Terminal rate	2/31 (7%)	1/38 (3%)	0/37 (0%)	1/37 (3%)
First incidence (days)	588	664	411	727 (T)
Poly-3 test	P=0.070N	P=0.209N	P=0.211N	P=0.089N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	4/50 (8%)
Adjusted rate	2.3%	4.4%	8.9%	8.4%
Terminal rate	1/31 (3%)	1/38 (3%)	3/37 (8%)	2/37 (5%)
First incidence (days)	727 (T)	671	584	696
Poly-3 test	P=0.135	P=0.511	P=0.181	P=0.201
Uterus: Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	0.0%	4.2%
Terminal rate	0/31 (0%)	0/38 (0%)	0/37 (0%)	2/37 (5%)
First incidence (days)	—	—	—	727 (T)
Poly-3 test	P=0.048	—	—	P=0.253

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Uterus: Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	2.3%	4.2%
Terminal rate	0/31 (0%)	0/38 (0%)	0/37 (0%)	2/37 (5%)
First incidence (days)	—	—	701	727 (T)
Poly-3 test	P=0.077	—	P=0.500	P=0.253
All Organs: Hemangioma				
Overall rate	1/50 (2%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	2.3%	4.4%	2.3%	8.3%
Terminal rate	1/31 (3%)	2/38 (5%)	1/37 (3%)	1/37 (3%)
First incidence (days)	727 (T)	727 (T)	727 (T)	650
Poly-3 test	P=0.126	P=0.509	P=0.761	P=0.205
All Organs: Hemangiosarcoma				
Overall rate	7/50 (14%)	5/50 (10%)	4/50 (8%)	2/50 (4%)
Adjusted rate	15.6%	10.9%	8.7%	4.2%
Terminal rate	3/31 (10%)	3/38 (8%)	1/37 (3%)	1/37 (3%)
First incidence (days)	588	664	411	701
Poly-3 test	P=0.047N	P=0.364N	P=0.250N	P=0.066N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	8/50 (16%)	7/50 (14%)	5/50 (10%)	5/50 (10%)
Adjusted rate	17.8%	15.3%	10.9%	10.4%
Terminal rate	4/31 (13%)	5/38 (13%)	2/37 (5%)	2/37 (5%)
First incidence (days)	588	664	411	650
Poly-3 test	P=0.172N	P=0.482N	P=0.263N	P=0.234N
All Organs: Histiocytic Sarcoma				
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)	4/50 (8%)
Adjusted rate	6.8%	0.0%	9.0%	8.4%
Terminal rate	3/31 (10%)	0/38 (0%)	2/37 (5%)	3/37 (8%)
First incidence (days)	727 (T)	—	657	713
Poly-3 test	P=0.238	P=0.114N	P=0.504	P=0.537
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	13/50 (26%)	5/50 (10%)	10/50 (20%)
Adjusted rate	19.6%	28.1%	11.1%	20.8%
Terminal rate	4/31 (13%)	10/38 (26%)	4/37 (11%)	7/37 (19%)
First incidence (days)	472	454	472	615
Poly-3 test	P=0.429N	P=0.236	P=0.204N	P=0.541
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	35/50 (70%)	30/50 (60%)	37/50 (74%)
Adjusted rate	58.7%	75.7%	64.5%	76.1%
Terminal rate	21/31 (68%)	30/38 (79%)	24/37 (65%)	29/37 (78%)
First incidence (days)	534	659	411	650
Poly-3 test	P=0.088	P=0.057	P=0.354	P=0.049
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	24/50 (48%)	22/50 (44%)	26/50 (52%)
Adjusted rate	64.6%	49.3%	45.1%	52.5%
Terminal rate	16/31 (52%)	14/38 (37%)	12/37 (32%)	15/37 (41%)
First incidence (days)	472	386	411	554
Poly-3 test	P=0.198N	P=0.094N	P=0.040N	P=0.156N

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	43/50 (86%)	38/50 (76%)	44/50 (88%)
Adjusted rate	89.1%	88.4%	76.8%	88.0%
Terminal rate	26/31 (84%)	33/38 (87%)	26/37 (70%)	31/37 (84%)
First incidence (days)	472	386	411	554
Poly-3 test	P=0.426N	P=0.586N	P=0.084N	P=0.558N

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, pituitary gland, and spleen; for other tissues, denominator is number of animals necropsied.

^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 D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	11	5	7	11
Natural deaths	8	7	6	2
Survivors				
Died last week of study				1
Terminal sacrifice	31	38	37	36
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Inflammation			1 (2%)	
Gallbladder	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)			
Epithelium, cyst				1 (2%)
Epithelium, hyperplasia	1 (2%)			
Intestine large, cecum	(50)	(50)	(50)	(50)
Arteriole, inflammation		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Arteriole, inflammation		1 (2%)		
Epithelium, diverticulum	1 (2%)			
Intestine small, duodenum	(50)	(50)	(50)	(50)
Arteriole, inflammation		1 (2%)		
Intestine small, ileum	(50)	(50)	(50)	(50)
Inflammation		1 (2%)	1 (2%)	
Ulcer		1 (2%)		
Epithelium, diverticulum		1 (2%)		
Intestine small, jejunum	(50)	(50)	(50)	(50)
Epithelium, hyperplasia				1 (2%)
Peyer's patch, diverticulum	1 (2%)			
Peyer's patch, hyperplasia		2 (4%)		1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis			2 (4%)	2 (4%)
Basophilic focus	1 (2%)	2 (4%)	2 (4%)	
Clear cell focus	1 (2%)	3 (6%)		5 (10%)
Eosinophilic focus	12 (24%)	9 (18%)	11 (22%)	12 (24%)
Hematopoietic cell proliferation				1 (2%)
Infarct	1 (2%)			
Inflammation	8 (16%)	10 (20%)	15 (30%)	10 (20%)
Mitotic alteration			1 (2%)	
Mixed cell focus	1 (2%)	1 (2%)		1 (2%)
Pigmentation, hemosiderin			1 (2%)	1 (2%)
Regeneration		1 (2%)	1 (2%)	
Tension lipidosis	7 (14%)	6 (12%)	5 (10%)	8 (16%)
Bile duct, cyst			1 (2%)	1 (2%)
Hepatocyte, hypertrophy				1 (2%)
Hepatocyte, mineralization	1 (2%)			
Hepatocyte, necrosis	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Hepatocyte, vacuolization cytoplasmic		1 (2%)		
Hepatocyte, vacuolization cytoplasmic, diffuse	3 (6%)	1 (2%)	5 (10%)	3 (6%)
Serosa, fibrosis	1 (2%)			1 (2%)
Serosa, inflammation	2 (4%)	1 (2%)		

^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 Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Alimentary System (continued)				
Mesentery	(16)	(13)	(11)	(10)
Inflammation			2 (18%)	2 (20%)
Necrosis	14 (88%)	13 (100%)	8 (73%)	7 (70%)
Pancreas	(50)	(50)	(50)	(50)
Basophilic focus				2 (4%)
Inflammation	1 (2%)			
Acinus, atrophy		2 (4%)		
Acinus, hyperplasia	6 (12%)	3 (6%)	7 (14%)	9 (18%)
Arteriole, inflammation				1 (2%)
Duct, cyst	1 (2%)	2 (4%)		
Duct, inflammation			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Inflammation	2 (4%)			
Ulcer	1 (2%)			
Epithelium, hyperplasia	5 (10%)	5 (10%)	13 (26%)	5 (10%)
Stomach, glandular	(50)	(50)	(50)	(50)
Mineralization		2 (4%)		
Epithelium, cyst				1 (2%)
Epithelium, hyperplasia	2 (4%)	1 (2%)	1 (2%)	
Glands, cyst			1 (2%)	3 (6%)
Glands, hyperplasia		2 (4%)		
Tooth	(0)	(0)	(2)	(3)
Dysplasia				2 (67%)
Inflammation			1 (50%)	
Gingiva, hyperplasia				1 (33%)
Peridontal tissue, fibrosis				2 (67%)
Peridontal tissue, inflammation				1 (33%)
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Aorta, inflammation		1 (2%)		
Heart	(50)	(50)	(50)	(50)
Artery, inflammation	1 (2%)	3 (6%)	2 (4%)	1 (2%)
Atrium, thrombosis			1 (2%)	1 (2%)
Epicardium, inflammation				1 (2%)
Myocardium, cardiomyopathy	10 (20%)	7 (14%)	10 (20%)	17 (34%)
Myocardium, fibrosis	1 (2%)	1 (2%)		2 (4%)
Myocardium, infiltration cellular, mononuclear cell	9 (18%)	7 (14%)	10 (20%)	17 (34%)
Myocardium, mineralization	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Myocardium, necrosis	1 (2%)			
Myocardium, vacuolization cytoplasmic	14 (28%)	4 (8%)	6 (12%)	13 (26%)
Valve, thrombosis	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Atrophy	1 (2%)			
Hyperplasia	11 (22%)	11 (22%)	7 (14%)	11 (22%)
Hypertrophy	38 (76%)	46 (92%)	42 (84%)	40 (80%)
Subcapsular, hyperplasia	50 (100%)	50 (100%)	50 (100%)	48 (96%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	2 (4%)	7 (14%)	2 (4%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	23 (46%)	23 (46%)	31 (62%)	21 (42%)
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, angiectasis	1 (2%)	2 (4%)		
Pars distalis, cyst	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Pars distalis, hyperplasia	24 (49%)	27 (54%)	24 (48%)	27 (54%)
Pars intermedia, hyperplasia			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Inflammation	1 (2%)	1 (2%)		
C-cell, hyperplasia			1 (2%)	
Follicle, cyst	1 (2%)		1 (2%)	1 (2%)
Follicular cell, hyperplasia		2 (4%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(49)	(50)
Inflammation		1 (2%)	1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		
Cyst	13 (26%)	7 (14%)	7 (14%)	15 (30%)
Hemorrhage	2 (4%)	1 (2%)		3 (6%)
Mineralization		1 (2%)		
Pigmentation, hemosiderin	1 (2%)			
Thrombosis				1 (2%)
Interstitial cell, hyperplasia				1 (2%)
Periovarian tissue, inflammation	2 (4%)			
Uterus	(50)	(50)	(50)	(50)
Hemorrhage				1 (2%)
Endometrium, hyperplasia, cystic	49 (98%)	47 (94%)	48 (96%)	50 (100%)
Myometrium, angiectasis	1 (2%)			
Myometrium, cyst				1 (2%)
Vagina	(0)	(0)	(1)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Myelofibrosis	8 (16%)	3 (6%)	8 (16%)	11 (22%)
Lymph node	(8)	(9)	(3)	(5)
Iliac, degeneration, cystic	1 (13%)			
Iliac, hemorrhage	1 (13%)			
Lumbar, hyperplasia, lymphoid	1 (13%)			
Pancreatic, hyperplasia, lymphoid		1 (11%)		
Renal, hyperplasia, lymphoid	1 (13%)			
Lymph node, mandibular	(50)	(50)	(50)	(49)
Infiltration cellular, plasma cell				1 (2%)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Infiltration cellular, plasma cell			1 (2%)	
Necrosis			1 (2%)	
Arteriole, inflammation		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Hyperplasia, lymphoid	1 (2%)	4 (8%)		1 (2%)
Infiltration cellular, plasma cell			2 (4%)	
Capsule, hyperplasia	1 (2%)			

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Hematopoietic System (continued)				
Thymus	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia, lymphoid			1 (2%)	3 (6%)
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Skin	(50)	(50)	(50)	(50)
Site of application, dermis, fibrosis	4 (8%)	1 (2%)		2 (4%)
Site of application, dermis, inflammation	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Site of application, epidermis, hyperplasia	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Site of application, epidermis, ulcer	1 (2%)		1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Spinal cord	(2)	(0)	(1)	(0)
Demyelination	1 (50%)		1 (100%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	1 (2%)			
Infiltration cellular		1 (2%)		
Inflammation	1 (2%)	1 (2%)		1 (2%)
Alveolar epithelium, hyperplasia	5 (10%)	1 (2%)	4 (8%)	4 (8%)
Alveolus, infiltration cellular, histiocyte	1 (2%)	1 (2%)		2 (4%)
Arteriole, inflammation		1 (2%)		
Serosa, hyperplasia				1 (2%)
Nose	(50)	(50)	(50)	(50)
Inflammation	13 (26%)	8 (16%)	7 (14%)	6 (12%)
Polyp, inflammatory			1 (2%)	
Glands, olfactory epithelium, dilatation	1 (2%)		1 (2%)	
Glands, respiratory epithelium, dilatation		1 (2%)	1 (2%)	
Respiratory epithelium, hyperplasia				2 (4%)
Septum, cyst			1 (2%)	
Pleura	(0)	(0)	(1)	(0)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cornea, inflammation		2 (4%)		
Retina, dysplasia			1 (2%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Epithelium, hyperplasia	12 (24%)	7 (14%)	6 (12%)	4 (8%)

TABLE D3
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Dermal Study
of Bis(2-chloroethoxy)methane

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct	1 (2%)	1 (2%)	3 (6%)	3 (6%)
Infiltration cellular, mononuclear cell	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Inflammation	4 (8%)			
Mineralization	9 (18%)	8 (16%)	11 (22%)	9 (18%)
Nephropathy	37 (74%)	37 (74%)	42 (84%)	38 (76%)
Capsule, fibrosis	1 (2%)			
Renal tubule, dilatation		1 (2%)		
Renal tubule, pigmentation		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Amyloid deposition				1 (2%)
Hyperplasia, lymphoid				1 (2%)
Inflammation				1 (2%)
Inflammation, chronic	1 (2%)			
Arteriole, inflammation			1 (2%)	
Arteriole, muscularis, degeneration		1 (2%)		

APPENDIX E

GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

BACTERIAL MUTAGENICITY TEST PROTOCOL

Two independent assays for bacterial mutagenicity were conducted with bis(2-chloroethoxy)methane. The first assay was performed at SRI International (Menlo Park, CA) and followed protocols reported by Zeiger *et al.* (1992) using *Salmonella typhimurium* tester strains TA98, TA100, and TA1535, either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague Dawley rat or Syrian hamster liver). The second assay, conducted with the same lot (B007269977) of bis(2-chloroethoxy)methane used in the 2-week and 3-month dermal studies, was performed at SITEK Research Laboratories (Rockville, MD) using a slightly modified protocol (activation only with rat liver S9) and *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain, in addition to *S. typhimurium* strains TA98 and TA100. In both assays, bis(2-chloroethoxy)methane was sent to the laboratory as a coded aliquot and incubated with the bacterial tester strains 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 bis(2-chloroethoxy)methane. The high dose was limited by toxicity in some trials and by the limit dose of 10,000 µg/plate in those trials where only slight toxicity was observed. All trials were repeated, except TA98 at SRI International, and those that were conducted with S9 activation enzymes were repeated using the same or higher concentrations of S9.

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.

RAT BONE MARROW MICRONUCLEUS TEST PROTOCOL

An initial dose-setting test was conducted to select the range of doses in these acute (three exposure) studies. Bone marrow tests were performed in male F344/N rats using the standard NTP three-treatment protocol (Shelby *et al.*, 1993). Five animals per dose group were administered bis(2-chloroethoxy)methane in corn oil by gavage at 24 hour intervals. Vehicle control animals received corn oil alone. The positive control was cyclophosphamide. Twenty-four hours after the final treatment, the animals were euthanized and smears of the bone marrow cells obtained from the femurs were prepared. Air-dried smears were fixed and stained. Two thousand polychromatic erythrocytes (PCEs; reticulocytes) were scored per animal for frequency of micronucleated cells. In addition, the percentage of PCEs among 500 erythrocytes was scored per animal as a measure of bone marrow toxicity.

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 using a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group. 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 dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects.

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs; mature erythrocytes) in each of five animals per dose group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

Results were tabulated and analyzed as described for PCEs in the rat bone marrow micronucleus test. Results of the 3-month study were accepted without repeat tests because additional data could not be obtained.

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 judgment of the overall evidence for activity of the chemical in an assay.

RESULTS

Bis(2-chloroethoxy)methane was tested in two independent bacterial mutagenicity tests (Table E1). In the first test, bis(2-chloroethoxy)methane induced significant increases in mutant colonies in *S. typhimurium* strain TA100 in the presence of 10% and 30% induced hamster liver S9; equivocal responses were seen in TA100 when treatment was carried out with 10% or 30% induced rat liver S9. Positive results were seen in strain TA1535 in the presence of either induced hamster (10% or 30%) or rat (30%) liver S9 enzymes. No induction of gene mutations was seen in strain TA98, with or without S9. In the second bacterial mutagenicity test conducted with bis(2-chloroethoxy)methane, small increases in mutant colonies, judged to be equivocal, were seen in *S. typhimurium* strain TA100 and in *E. coli* strain WP2 *uvrA*/pKM101 in the presence of 10% induced rat liver S9. As in the first test, no induction of gene mutations was seen in *S. typhimurium* strain TA98, with or without S9.

Bis(2-chloroethoxy)methane was tested in two independent rodent micronucleus assays. The first assessed frequencies of micronucleated reticulocytes (PCEs) in bone marrow of male F344 rats administered bis(2-chloroethoxy)methane by gavage three times at 24 hour intervals at doses up to 65 mg/kg per day (Table E2). The second assay assessed micronucleated normochromatic erythrocyte frequencies in peripheral blood of male and female B6C3F1 mice exposed to bis(2-chloroethoxy)methane dermally for 3 months at doses up to 600 mg/kg per day (Table E3). Results of both tests were negative. In neither test was the percentage of reticulocytes significantly altered, indicating an absence of bis(2-chloroethoxy)methane-related bone marrow toxicity.

TABLE E1
Mutagenicity of Bis(2-chloroethoxy)methane in Bacterial Tester Strains

Strain	Dose ($\mu\text{g}/\text{plate}$)	Revertants/Plate ^a			
		-S9			
		Trial 1	Trial 2	Trial 3	
Study performed at SRI International^b					
TA100					
	0	117 \pm 5	107 \pm 3	88 \pm 7	
	33			90 \pm 4	
	100	108 \pm 2	104 \pm 9	84 \pm 4	
	333	128 \pm 4	121 \pm 7	85 \pm 6	
	666		128 \pm 14	90 \pm 7	
	1,000	147 \pm 5	124 \pm 6	92 \pm 6	
	1,666		123 \pm 4	90 \pm 4	
	3,333	167 \pm 10	135 \pm 2	113 \pm 3	
	6,666		49 \pm 23 ^c	124 \pm 4 ^c	
	10,000	76 \pm 6 ^c			
Trial summary		Equivocal	Equivocal	Equivocal	
Positive control ^d		856 \pm 18	845 \pm 11	867 \pm 23	
+ hamster S9					
		10%	30%	30%	30%
TA100 (continued)	0	111 \pm 10	127 \pm 8	110 \pm 6	82 \pm 4.0
	33				101 \pm 7.0
	100	123 \pm 3	124 \pm 8	129 \pm 9	92 \pm 1.0
	333	152 \pm 3	141 \pm 7	152 \pm 8	120 \pm 12.0
	666	157 \pm 2		155 \pm 7	126 \pm 3.0
	1,000	172 \pm 6	179 \pm 24	172 \pm 1	138 \pm 1.0
	1,666	180 \pm 9		172 \pm 3	150 \pm 19.0
	3,333	190 \pm 2	199 \pm 2	194 \pm 10	185 \pm 11.0
	6,666	110 \pm 31 ^c		136 \pm 25 ^c	203 \pm 9.0 ^c
	10,000		66 \pm 28 ^c		
Trial summary		Weakly positive	Weakly positive	Weakly positive	Positive
Positive control		669 \pm 28	693 \pm 15	674 \pm 15	644 \pm 25.0
+ rat S9					
		10%	30%	30%	30%
TA100 (continued)	0	123 \pm 6	123 \pm 2	128 \pm 1	100 \pm 6
	33				101 \pm 5
	100	121 \pm 6	126 \pm 4	143 \pm 9	103 \pm 10
	333	136 \pm 4	140 \pm 8	137 \pm 2	107 \pm 7
	666	136 \pm 2		171 \pm 2	118 \pm 17
	1,000	149 \pm 10	156 \pm 8	169 \pm 9	124 \pm 5
	1,666	156 \pm 7		164 \pm 3	152 \pm 11
	3,333	147 \pm 2	198 \pm 2	151 \pm 9	162 \pm 19
	6,666	101 \pm 2 ^c		77 \pm 12 ^c	138 \pm 18 ^c
	10,000		76 \pm 2 ^c		
Trial summary		Equivocal	Equivocal	Equivocal	Weakly positive
Positive control		632 \pm 15	636 \pm 19	616 \pm 4	576 \pm 10

TABLE E1
Mutagenicity of Bis(2-chloroethoxy)methane in Bacterial Tester Strains

Strain	Dose ($\mu\text{g}/\text{plate}$)	Revertants/Plate		
		-S9		
		Trial 1	Trial 2	
TA1535	0	8 \pm 2	17 \pm 0	
	33		11 \pm 2	
	100	12 \pm 2	13 \pm 3	
	333	14 \pm 1	10 \pm 2	
	666	15 \pm 2	11 \pm 1	
	1,000	19 \pm 1	19 \pm 3	
	1,666	16 \pm 3	11 \pm 1	
	3,333	16 \pm 2	18 \pm 4	
	6,666	13 \pm 2 ^c	24 \pm 4 ^c	
Trial summary		Weakly positive	Negative	
Positive control		873 \pm 18	891 \pm 32	
+ hamster S9				
TA1535 (continued)	0	8 \pm 1	6 \pm 2	8 \pm 0
	33			9 \pm 1
	100	12 \pm 2	14 \pm 2	13 \pm 2
	333	15 \pm 1	16 \pm 2	14 \pm 1
	666	26 \pm 4	20 \pm 5	19 \pm 1
	1,000	25 \pm 5	21 \pm 3	27 \pm 4
	1,666	29 \pm 2	24 \pm 3	28 \pm 2
	3,333	33 \pm 2	31 \pm 2	30 \pm 2
	6,666	11 \pm 2 ^c	16 \pm 7 ^c	7 \pm 0 ^c
Trial summary		Positive	Positive	Positive
Positive control		135 \pm 3	117 \pm 11	127 \pm 11
+ rat S9				
TA1535 (continued)	0	9 \pm 2	7 \pm 2	7 \pm 1
	33			11 \pm 0
	100	14 \pm 1	11 \pm 1	13 \pm 2
	333	10 \pm 4	14 \pm 1	16 \pm 1
	666	13 \pm 1	17 \pm 2	15 \pm 1
	1,000	17 \pm 2	16 \pm 2	21 \pm 1
	1,666	15 \pm 2	19 \pm 1	24 \pm 5
	3,333	16 \pm 1	27 \pm 1	35 \pm 5
	6,666	10 \pm 1 ^c	10 \pm 3 ^c	29 \pm 12 ^c
Trial summary		Equivocal	Positive	Positive
Positive control		115 \pm 11	109 \pm 5	123 \pm 11

TABLE E1
Mutagenicity of Bis(2-chloroethoxy)methane in Bacterial Tester Strains

Strain	Dose ($\mu\text{g}/\text{plate}$)	Revertants/Plate				
		-S9	+ hamster S9	+ rat S9		
		Trial 1	30%	30%		
TA98	0	19 \pm 1	24 \pm 4	20 \pm 1		
	100	24 \pm 1	23 \pm 2	20 \pm 2		
	333	17 \pm 3	16 \pm 2	26 \pm 1		
	1,000	18 \pm 2	27 \pm 2	23 \pm 1		
	3,333	19 \pm 1	20 \pm 2	21 \pm 1		
	10,000	24 \pm 4 ^c	17 \pm 3 ^c	16 \pm 4 ^c		
	Trial summary	Negative	Negative	Negative		
Positive control	424 \pm 5	413 \pm 8	350 \pm 18			
Study performed at SITEK Research Laboratories (lot number B007269977 used in the 2-week and 3-month studies)						
Strain	Dose ($\mu\text{g}/\text{plate}$)	-S9			+ 10% rat S9	
		Trial 1	Trial 2	Trial 3	Trial 1	Trial 2
TA100	0	43 \pm 1	36 \pm 3	59 \pm 15	67 \pm 8	42 \pm 5
	100	41 \pm 3	47 \pm 5	40 \pm 4	67 \pm 2	47 \pm 3
	500	62 \pm 8	48 \pm 5	49 \pm 6	74 \pm 5	50 \pm 6
	1,000	64 \pm 7	42 \pm 3	49 \pm 9	74 \pm 12	66 \pm 2
	5,000	18 \pm 2	42 \pm 5	17 \pm 7	61 \pm 2	83 \pm 2
	7,500	2 \pm 1				
	10,000		Toxic	0 \pm 0	23 \pm 3	72 \pm 2
	Trial summary	Negative	Negative	Negative	Negative	Positive
Positive control	428 \pm 39	403 \pm 12	570 \pm 13	1,121 \pm 88	622 \pm 49	
TA98	0	17 \pm 1	15 \pm 2	15 \pm 1	26 \pm 1	18 \pm 4
	100	18 \pm 3	14 \pm 3	21 \pm 3	24 \pm 3	23 \pm 2
	500	17 \pm 2	13 \pm 3	14 \pm 1	25 \pm 2	25 \pm 1
	1,000	19 \pm 3	19 \pm 3	13 \pm 2	24 \pm 1	19 \pm 3
	2,500		14 \pm 2	10 \pm 2		17 \pm 2
	5,000	15 \pm 2	6 \pm 0	4 \pm 1	18 \pm 2	16 \pm 1
	7,500			1 \pm 1		18 \pm 2
	10,000	Toxic		1 \pm 1	Toxic	11 \pm 2
	Trial summary	Negative	Negative	Negative	Negative	Negative
Positive control	369 \pm 1	506 \pm 17	471 \pm 24	1,119 \pm 53	1,261 \pm 30	
<i>Escherichia coli</i> WP2 <i>uvrA</i>/pKM101 (analogous to TA102)						
	0	231 \pm 54	136 \pm 7		247 \pm 53	178 \pm 3
	100	148 \pm 7	146 \pm 4		223 \pm 14	265 \pm 2
	500	139 \pm 5	161 \pm 7		253 \pm 6	338 \pm 22
	1,000	150 \pm 11	144 \pm 14		357 \pm 32	295 \pm 10
	5,000	110 \pm 24	119 \pm 4		298 \pm 15	270 \pm 13
	10,000	26 \pm 1	84 \pm 6		266 \pm 12	249 \pm 12
Trial summary	Negative	Negative		Negative	Positive	
Positive control	853 \pm 65	806 \pm 76		821 \pm 28	823 \pm 3	

^a Revertants are presented as mean \pm standard error from three plates.

^b The detailed protocol is presented by Zeiger *et al.* (1992). 0 $\mu\text{g}/\text{plate}$ was the solvent control.

^c Slight toxicity

^d The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA*/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Bis(2-chloroethoxy)methane by Gavage^a

Compound	Dose (mg/mg)	Number of Rats with Erythrocytes Scored	Micronucleated PCEs/1,000 PCEs ^b	Pairwise P Value ^c	PCE ^b (%)
Corn oil ^d	0	5	0.60 ± 0.19		46.000 ± 1.76
bis(2-chloroethoxy)methane	16.25	5	1.00 ± 0.27	0.1586	46.800 ± 2.18
	32.5	5	0.90 ± 0.24	0.2192	45.600 ± 3.04
	65	5	0.70 ± 0.20	0.3907	42.200 ± 2.11
	130	2 ^e	0.50 ± 0.00		21.500 ± 5.50
			P=0.696 ^f		
Cyclophosphamide ^g	15	5	14.80 ± 2.33	0.0000	16.600 ± 2.29
	25	3	12.33 ± 3.56	0.0000	6.333 ± 2.19

^a Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al.* (1993).

^b Mean ± standard error

^c Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.008; positive control values are significant at P≤0.05.

^d Vehicle control

^e Statistical tests not performed due to high mortality

^f Significance of micronucleated cells tested by the one-tailed Cochran-Armitage trend test; significant at P≤0.025

^g Positive control

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Dermal Administration of Bis(2-chloroethoxy)methane for 3 Months^a

	Dose (mg/mg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs ^b	Pairwise P Value ^c	PCE ^b (%)
Male					
Ethanol ^d	0	5	0.60 ± 0.37		1.60 ± 0.27
bis(2-chloroethoxy)methane	50	5	0.70 ± 0.25	0.3907	1.96 ± 0.29
	100	5	1.10 ± 0.29	0.1125	1.78 ± 0.13
	200	5	0.80 ± 0.30	0.2964	1.84 ± 0.19
	400	5	0.60 ± 0.29	0.5000	1.88 ± 0.24
	600	5	0.80 ± 0.20	0.2964	1.64 ± 0.35
			P=0.528 ^e		
Female					
Ethanol	0	5	0.90 ± 0.43		1.68 ± 0.14
bis(2-chloroethoxy)methane	50	5	1.30 ± 0.25	0.1968	1.90 ± 0.21
	100	5	1.10 ± 0.37	0.3273	1.78 ± 0.16
	200	5	0.80 ± 0.34	0.5959	1.78 ± 0.12
	400	5	0.80 ± 0.41	0.5959	2.04 ± 0.16
	600	5	1.00 ± 0.27	0.4092	2.08 ± 0.12
			P=0.672		

^a Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990).
NCE=normochromatic erythrocyte; PCE=polychromatic erythrocyte

^b Mean ± standard error

^c Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.005.

^d Vehicle control

^e Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed Cochran-Armitage trend test; significant at P≤0.025

APPENDIX F

CLINICAL PATHOLOGY RESULTS

TABLE F1	Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane	130
TABLE F2	Hematology Data for Mice in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane	135

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane^a

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male						
Hematology						
n						
Day 4	10	10	9	10	10	10
Day 23	10	9	10	10	10	9
Week 14	10	10	10	10	10	0
Hematocrit (%)						
Day 4	40.6 ± 0.6	41.9 ± 0.5	39.7 ± 0.4	40.2 ± 0.5	41.4 ± 0.3	41.2 ± 0.7
Day 23	42.7 ± 0.4	43.0 ± 0.5	42.9 ± 0.2	42.4 ± 0.3	43.2 ± 0.3	42.7 ± 0.3
Week 14	42.9 ± 0.6	42.2 ± 0.6	42.4 ± 0.5	42.0 ± 0.7	41.2 ± 0.5	— ^b
Hemoglobin (g/dL)						
Day 4	13.4 ± 0.2	13.7 ± 0.1	13.0 ± 0.1	13.2 ± 0.2	13.6 ± 0.1	13.5 ± 0.2
Day 23	14.2 ± 0.1	14.3 ± 0.2	14.2 ± 0.1	14.1 ± 0.1	14.2 ± 0.1	14.0 ± 0.1
Week 14	15.1 ± 0.1	15.0 ± 0.2	14.8 ± 0.1	14.8 ± 0.2	14.4 ± 0.1**	—
Erythrocytes (10 ⁶ /μL)						
Day 4	7.24 ± 0.11	7.47 ± 0.08	7.03 ± 0.06	7.15 ± 0.09	7.42 ± 0.05	7.45 ± 0.11
Day 23	7.73 ± 0.08	7.79 ± 0.11	7.78 ± 0.05	7.73 ± 0.06	7.88 ± 0.07	7.79 ± 0.06
Week 14	8.51 ± 0.11	8.42 ± 0.12	8.55 ± 0.09	8.55 ± 0.15	8.33 ± 0.13	—
Reticulocytes (10 ⁶ /μL)						
Day 4	0.36 ± 0.02	0.37 ± 0.03	0.33 ± 0.03	0.34 ± 0.02	0.32 ± 0.02	0.30 ± 0.02
Day 23	0.18 ± 0.01	0.17 ± 0.02	0.17 ± 0.02	0.16 ± 0.01	0.19 ± 0.02	0.16 ± 0.01
Week 14	0.18 ± 0.02	0.17 ± 0.01	0.18 ± 0.02	0.19 ± 0.02	0.19 ± 0.01	—
Nucleated erythrocytes (10 ³ /μL)						
Day 4	0.13 ± 0.04	0.05 ± 0.02	0.07 ± 0.02	0.12 ± 0.03	0.12 ± 0.04	0.06 ± 0.02
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.03 ± 0.03	0.00 ± 0.00 ^c	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.02 ± 0.01	0.01 ± 0.01	0.05 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	—
Mean cell volume (fL)						
Day 4	56.1 ± 0.2	56.1 ± 0.2	56.5 ± 0.3	56.2 ± 0.2	55.8 ± 0.2	55.3 ± 0.2*
Day 23	55.3 ± 0.2	55.2 ± 0.2	55.1 ± 0.3	54.9 ± 0.2	54.9 ± 0.2	54.8 ± 0.2
Week 14	50.4 ± 0.2	50.2 ± 0.2	49.6 ± 0.2**	49.2 ± 0.2**	49.5 ± 0.3**	—
Mean cell hemoglobin (pg)						
Day 4	18.5 ± 0.1	18.3 ± 0.0	18.6 ± 0.1	18.4 ± 0.1	18.3 ± 0.1*	18.2 ± 0.1**
Day 23	18.3 ± 0.1	18.4 ± 0.1	18.3 ± 0.1	18.3 ± 0.1	18.1 ± 0.1*	17.9 ± 0.1**
Week 14	17.8 ± 0.1	17.8 ± 0.1	17.4 ± 0.1	17.3 ± 0.2*	17.3 ± 0.2*	—
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.9 ± 0.1	32.7 ± 0.1	32.8 ± 0.2	32.8 ± 0.1	32.8 ± 0.1	32.8 ± 0.1
Day 23	33.1 ± 0.1	33.3 ± 0.1	33.1 ± 0.1	33.3 ± 0.1	32.9 ± 0.1	32.7 ± 0.1
Week 14	35.3 ± 0.3	35.4 ± 0.2	35.1 ± 0.2	35.2 ± 0.3	35.0 ± 0.2	—
Platelets (10 ³ /μL)						
Day 4	957.7 ± 30.1	996.3 ± 19.0	980.6 ± 28.0	1,005.0 ± 15.8	1,013.4 ± 17.8	949.3 ± 52.2
Day 23	873.4 ± 10.4	855.1 ± 38.8	875.3 ± 19.9	870.5 ± 15.9	842.0 ± 34.8	840.4 ± 20.2
Week 14	549.8 ± 58.7	657.4 ± 29.7	679.1 ± 11.2	669.2 ± 21.6*	670.2 ± 9.2	—
Leukocytes (10 ³ /μL)						
Day 4	6.63 ± 0.44	6.95 ± 0.29	8.22 ± 0.70	6.98 ± 0.41	7.71 ± 0.61	6.15 ± 0.31
Day 23	7.70 ± 0.33	8.13 ± 0.66	8.72 ± 0.47	8.09 ± 0.62	7.66 ± 0.46	8.40 ± 0.42
Week 14	10.49 ± 0.54	9.38 ± 0.42	11.26 ± 0.40	11.07 ± 0.31	9.94 ± 0.33	—
Segmented neutrophils (10 ³ /μL)						
Day 4	0.74 ± 0.04	0.81 ± 0.11	0.76 ± 0.06	0.84 ± 0.12	0.92 ± 0.08	0.73 ± 0.07
Day 23	0.72 ± 0.06	0.61 ± 0.06	0.88 ± 0.13	0.80 ± 0.11 ^c	0.63 ± 0.08	0.66 ± 0.07
Week 14	1.24 ± 0.14	1.14 ± 0.08	1.16 ± 0.13	1.28 ± 0.07	1.17 ± 0.15	—
Bands (10 ³ /μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^c	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male (continued)						
Hematology (continued)						
n						
Day 4	10	10	9	10	10	10
Day 23	10	9	10	10	10	9
Week 14	10	10	10	10	10	0
Lymphocytes (10 ³ /μL)						
Day 4	5.71 ± 0.41	6.04 ± 0.21	7.30 ± 0.68	5.96 ± 0.36	6.62 ± 0.56	5.26 ± 0.29
Day 23	6.87 ± 0.31	7.33 ± 0.57	7.69 ± 0.42	7.11 ± 0.59 ^c	6.84 ± 0.41	7.53 ± 0.36
Week 14	9.09 ± 0.47	8.06 ± 0.37	9.85 ± 0.35	9.53 ± 0.26	8.57 ± 0.42	—
Monocytes (10 ³ /μL)						
Day 4	0.12 ± 0.03	0.11 ± 0.02	0.08 ± 0.02	0.13 ± 0.02	0.14 ± 0.02	0.13 ± 0.02
Day 23	0.15 ± 0.03	0.14 ± 0.05	0.14 ± 0.03	0.14 ± 0.03 ^c	0.19 ± 0.03	0.19 ± 0.04
Week 14	0.05 ± 0.02	0.09 ± 0.02	0.11 ± 0.05	0.13 ± 0.05	0.10 ± 0.04	—
Basophils (10 ³ /μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000 ^c	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	—
Eosinophils (10 ³ /μL)						
Day 4	0.06 ± 0.03	0.02 ± 0.01	0.09 ± 0.03	0.04 ± 0.02	0.05 ± 0.02	0.04 ± 0.02
Day 23	0.03 ± 0.02	0.04 ± 0.02	0.01 ± 0.01	0.06 ± 0.02 ^c	0.01 ± 0.01	0.04 ± 0.02
Week 14	0.09 ± 0.04	0.11 ± 0.03	0.12 ± 0.03	0.10 ± 0.02	0.09 ± 0.02	—
Clinical Chemistry						
n						
Day 4	10	9	9	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	0
Urea nitrogen (mg/dL)						
Day 4	7.9 ± 0.5	9.0 ± 0.4	8.3 ± 0.7	9.4 ± 0.5*	9.3 ± 0.4*	10.2 ± 0.6**
Day 23	11.5 ± 0.5	12.0 ± 0.5	12.5 ± 0.5	11.5 ± 0.4	11.7 ± 0.4	11.7 ± 0.3
Week 14	15.1 ± 0.2	15.5 ± 0.5	14.3 ± 0.4	14.3 ± 0.5	13.6 ± 0.4**	—
Creatinine (mg/dL)						
Day 4	0.44 ± 0.02	0.46 ± 0.02	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.02	0.45 ± 0.02
Day 23	0.50 ± 0.00	0.50 ± 0.00	0.50 ± 0.00	0.52 ± 0.01	0.49 ± 0.01	0.50 ± 0.02
Week 14	0.51 ± 0.01	0.56 ± 0.02	0.53 ± 0.03	0.53 ± 0.02	0.52 ± 0.01	—
Total protein (g/dL)						
Day 4	5.5 ± 0.1	5.7 ± 0.1	5.4 ± 0.0	5.5 ± 0.1	5.6 ± 0.1	5.5 ± 0.1
Day 23	6.0 ± 0.1	5.9 ± 0.0	5.9 ± 0.1	6.0 ± 0.1	6.0 ± 0.0	5.9 ± 0.1
Week 14	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.0	6.7 ± 0.1	6.6 ± 0.1	—
Albumin (g/dL)						
Day 4	3.9 ± 0.1	4.0 ± 0.1	3.8 ± 0.0	3.8 ± 0.1	3.9 ± 0.0	3.9 ± 0.1
Day 23	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.1 ± 0.0
Week 14	4.4 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.2 ± 0.0**	—
Alanine aminotransferase (IU/L)						
Day 4	59 ± 1	57 ± 2	54 ± 2 ^d	55 ± 1	57 ± 2	56 ± 2
Day 23	45 ± 1	43 ± 1	45 ± 1	45 ± 1	42 ± 1*	41 ± 1**
Week 14	75 ± 4	84 ± 9	65 ± 4	68 ± 4	55 ± 2**	—
Alkaline phosphatase (IU/L)						
Day 4	776 ± 17	762 ± 22	721 ± 19	748 ± 18	709 ± 17*	684 ± 17**
Day 23	546 ± 10	531 ± 9	520 ± 15	543 ± 11	550 ± 12	521 ± 15
Week 14	223 ± 5	227 ± 6	221 ± 5	209 ± 6	212 ± 4	—

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male (continued)						
Clinical Chemistry (continued)						
n						
Day 4	10	9	9	10	10	10
Day 23	10	10	10	10	10	9
Week 14	10	10	10	10	10	0
Creatine kinase (IU/L)						
Day 4	303 ± 29	381 ± 30	308 ± 56 ^d	444 ± 111	311 ± 29	344 ± 49
Day 23	240 ± 23	295 ± 61	268 ± 40	250 ± 24	229 ± 36	198 ± 18
Week 14	332 ± 48	263 ± 36	234 ± 32	348 ± 54	260 ± 29	—
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 1	20 ± 1	16 ± 1 ^d	16 ± 1	16 ± 1	14 ± 1
Day 23	17 ± 1	16 ± 1	16 ± 1	17 ± 1	15 ± 1*	14 ± 1**
Week 14	20 ± 1	26 ± 5	18 ± 1	19 ± 1	17 ± 1	—
Bile salts (µmol/L)						
Day 4	26.2 ± 1.7	22.8 ± 1.6	26.1 ± 2.8 ^d	24.8 ± 1.3	22.0 ± 1.9	29.6 ± 3.6
Day 23	25.5 ± 2.3	21.5 ± 1.1	24.8 ± 1.9	21.4 ± 1.2	22.5 ± 1.9	25.7 ± 2.8
Week 14	25.1 ± 3.5	26.1 ± 3.5	21.5 ± 1.9	18.2 ± 1.2	20.4 ± 3.4*	—
Female						
Hematology						
n						
Day 4	10	10	10	9	10	10
Day 23	9	10	10	9	9	8
Week 14	10	10	9	10	8	0
Hematocrit (%)						
Day 4	42.4 ± 0.6	42.2 ± 0.4	42.1 ± 0.6	43.1 ± 0.3	41.8 ± 0.4	43.6 ± 0.4
Day 23	44.6 ± 0.4	44.3 ± 0.4	44.5 ± 0.4	45.2 ± 0.6	44.7 ± 0.4	45.9 ± 0.5
Week 14	41.5 ± 0.5	41.2 ± 0.3	41.2 ± 0.4	41.2 ± 0.4	41.9 ± 0.7	—
Hemoglobin (g/dL)						
Day 4	14.2 ± 0.2	14.0 ± 0.2	14.0 ± 0.2	14.2 ± 0.1	13.9 ± 0.2	14.5 ± 0.1
Day 23	14.9 ± 0.2	14.7 ± 0.1	14.7 ± 0.1	14.9 ± 0.2	14.6 ± 0.1	15.2 ± 0.2
Week 14	14.7 ± 0.3	14.7 ± 0.1	14.7 ± 0.1	14.6 ± 0.2	14.8 ± 0.2	—
Erythrocytes (10 ⁶ /µL)						
Day 4	7.62 ± 0.09	7.54 ± 0.09	7.52 ± 0.12	7.77 ± 0.06	7.49 ± 0.09	7.87 ± 0.09
Day 23	8.05 ± 0.08	7.96 ± 0.09	7.98 ± 0.08	8.19 ± 0.12	8.00 ± 0.08	8.36 ± 0.09
Week 14	7.68 ± 0.09	7.62 ± 0.08	7.66 ± 0.07	7.69 ± 0.08	7.82 ± 0.15	—
Reticulocytes (10 ⁶ /µL)						
Day 4	0.25 ± 0.02	0.23 ± 0.02	0.26 ± 0.02	0.24 ± 0.02	0.22 ± 0.01	0.21 ± 0.02
Day 23	0.04 ± 0.01	0.06 ± 0.01	0.05 ± 0.01 ^c	0.07 ± 0.01	0.04 ± 0.01	0.08 ± 0.02
Week 14	0.12 ± 0.01	0.14 ± 0.01	0.15 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	—
Nucleated erythrocytes (10 ³ /µL)						
Day 4	0.05 ± 0.02	0.02 ± 0.01	0.08 ± 0.03	0.04 ± 0.02	0.06 ± 0.02	0.09 ± 0.04
Day 23	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00 ^c	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.01 ± 0.01	0.03 ± 0.02	0.01 ± 0.01	0.03 ± 0.02	—
Mean cell volume (fL)						
Day 4	55.7 ± 0.2	56.0 ± 0.3	56.0 ± 0.2	55.5 ± 0.3	55.7 ± 0.1	55.4 ± 0.2
Day 23	55.4 ± 0.1	55.7 ± 0.2	55.7 ± 0.1	55.2 ± 0.2	55.9 ± 0.2	54.9 ± 0.1
Week 14	54.0 ± 0.2	54.0 ± 0.2	53.8 ± 0.1	53.6 ± 0.1	53.6 ± 0.3	—

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Female (continued)						
Hematology (continued)						
n						
Day 4	10	10	10	9	10	10
Day 23	9	10	10	9	9	8
Week 14	10	10	9	10	8	0
Mean cell hemoglobin (pg)						
Day 4	18.6 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.3 ± 0.1	18.6 ± 0.1	18.4 ± 0.1
Day 23	18.5 ± 0.1	18.5 ± 0.1	18.4 ± 0.1	18.2 ± 0.1*	18.2 ± 0.1*	18.2 ± 0.1*
Week 14	19.1 ± 0.2	19.3 ± 0.1	19.2 ± 0.1	19.0 ± 0.1	18.9 ± 0.1	—
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.3 ± 0.1	33.2 ± 0.1	33.2 ± 0.1	33.0 ± 0.2	33.4 ± 0.1	33.2 ± 0.1
Day 23	33.3 ± 0.1	33.1 ± 0.1	33.0 ± 0.1	33.0 ± 0.1	32.7 ± 0.1**	33.2 ± 0.2
Week 14	35.5 ± 0.4	35.7 ± 0.2	35.7 ± 0.2	35.5 ± 0.2	35.3 ± 0.2	—
Platelets (10 ³ /μL)						
Day 4	980.4 ± 27.2	986.7 ± 38.3	961.9 ± 16.6	901.6 ± 51.9	897.3 ± 35.5	860.3 ± 58.1
Day 23	834.7 ± 14.3	852.1 ± 11.7	818.9 ± 36.2	811.2 ± 35.5	828.7 ± 36.7	890.5 ± 46.2
Week 14	661.9 ± 16.0 ^c	684.8 ± 13.2	673.0 ± 19.3	652.6 ± 30.8	663.4 ± 21.1	—
Leukocytes (10 ³ /μL)						
Day 4	8.88 ± 0.66	8.16 ± 0.75	9.87 ± 0.57	8.57 ± 0.52	6.85 ± 0.96	8.31 ± 0.57
Day 23	10.78 ± 0.26	9.85 ± 0.50	10.14 ± 0.43	11.03 ± 0.38	10.27 ± 0.22	8.35 ± 0.79*
Week 14	7.67 ± 0.40	9.07 ± 0.48	8.43 ± 0.38	9.06 ± 0.35*	8.64 ± 0.40	—
Segmented neutrophils (10 ³ /μL)						
Day 4	0.82 ± 0.12	0.72 ± 0.11	0.75 ± 0.09	0.63 ± 0.08	0.67 ± 0.08	0.97 ± 0.24
Day 23	0.72 ± 0.05	0.68 ± 0.08	0.76 ± 0.10 ^c	0.90 ± 0.11	0.66 ± 0.07	0.81 ± 0.12
Week 14	0.98 ± 0.07	1.16 ± 0.11	0.90 ± 0.06	1.05 ± 0.05	1.19 ± 0.14	—
Bands (10 ³ /μL)						
Day 4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 23	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 ^c	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	—
Lymphocytes (10 ³ /μL)						
Day 4	7.86 ± 0.54	7.24 ± 0.67	8.81 ± 0.52	7.80 ± 0.51	6.01 ± 0.87	7.18 ± 0.70
Day 23	9.88 ± 0.27	8.91 ± 0.47	9.21 ± 0.45 ^c	10.06 ± 0.33	9.48 ± 0.21	7.40 ± 0.74*
Week 14	6.56 ± 0.34	7.75 ± 0.43	7.36 ± 0.39	7.85 ± 0.37*	7.31 ± 0.32	—
Monocytes (10 ³ /μL)						
Day 4	0.13 ± 0.02	0.15 ± 0.03	0.18 ± 0.03	0.13 ± 0.03	0.12 ± 0.04	0.11 ± 0.03
Day 23	0.12 ± 0.03	0.20 ± 0.06	0.12 ± 0.05 ^c	0.06 ± 0.02	0.10 ± 0.05	0.10 ± 0.02
Week 14	0.06 ± 0.03	0.10 ± 0.03	0.14 ± 0.03	0.12 ± 0.04	0.11 ± 0.04	—
Basophils (10 ³ /μL)						
Day 4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Day 23	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000 ^c	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Week 14	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	—
Eosinophils (10 ³ /μL)						
Day 4	0.07 ± 0.02	0.06 ± 0.02	0.11 ± 0.03	0.03 ± 0.02	0.07 ± 0.03	0.08 ± 0.04
Day 23	0.04 ± 0.02	0.05 ± 0.03	0.02 ± 0.02 ^c	0.02 ± 0.02	0.03 ± 0.02	0.04 ± 0.02
Week 14	0.07 ± 0.03	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.03	0.06 ± 0.03	—

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Female (continued)						
Clinical Chemistry						
n						
Day 4	10	9	10	8	10	10
Day 23	10	10	10	10	10	8
Week 14	10	10	10	10	8	0
Urea nitrogen (mg/dL)						
Day 4	9.3 ± 0.6	9.4 ± 0.6	9.6 ± 0.3	11.1 ± 0.6	11.3 ± 0.5	12.7 ± 2.1
Day 23	14.4 ± 0.4	13.2 ± 0.4	14.2 ± 0.4	15.1 ± 0.5	14.0 ± 0.3	13.0 ± 0.7
Week 14	15.7 ± 0.5	15.0 ± 0.5	15.1 ± 0.5	14.7 ± 0.5	13.8 ± 0.6	—
Creatinine (mg/dL)						
Day 4	0.44 ± 0.02	0.47 ± 0.02	0.44 ± 0.02	0.43 ± 0.02	0.45 ± 0.02	0.45 ± 0.02
Day 23	0.47 ± 0.02	0.49 ± 0.01	0.50 ± 0.00	0.47 ± 0.02	0.45 ± 0.02	0.49 ± 0.01
Week 14	0.53 ± 0.03	0.50 ± 0.03	0.50 ± 0.04	0.48 ± 0.03	0.45 ± 0.02	—
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.6 ± 0.1	5.5 ± 0.1	5.4 ± 0.1
Day 23	6.0 ± 0.1	6.0 ± 0.1	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.0	5.9 ± 0.1
Week 14	6.6 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	—
Albumin (g/dL)						
Day 4	4.0 ± 0.1	4.1 ± 0.0	4.0 ± 0.0	4.1 ± 0.0	4.0 ± 0.0	3.9 ± 0.1
Day 23	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.1 ± 0.0	4.1 ± 0.0
Week 14	4.5 ± 0.1	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.1	4.6 ± 0.1	—
Alanine aminotransferase (IU/L)						
Day 4	47 ± 1	48 ± 1 ^d	49 ± 1	49 ± 1 ^d	50 ± 2	59 ± 5**
Day 23	41 ± 1	40 ± 1	38 ± 1	38 ± 1	37 ± 1	47 ± 3
Week 14	55 ± 4	52 ± 4	49 ± 2	53 ± 4	47 ± 4	—
Alkaline phosphatase (IU/L)						
Day 4	569 ± 10	590 ± 11	575 ± 13	575 ± 17	565 ± 10	518 ± 22
Day 23	410 ± 8	402 ± 7	409 ± 12	417 ± 8	405 ± 6	415 ± 17
Week 14	205 ± 5	210 ± 7	217 ± 7	206 ± 5	199 ± 10	—
Creatine kinase (IU/L)						
Day 4	251 ± 34	293 ± 30 ^d	323 ± 46	286 ± 36 ^d	346 ± 72	335 ± 58
Day 23	229 ± 37	229 ± 85	201 ± 31	191 ± 20	142 ± 9	194 ± 30
Week 14	215 ± 41	220 ± 41	222 ± 68	221 ± 34	438 ± 129	—
Sorbitol dehydrogenase (IU/L)						
Day 4	16 ± 1	18 ± 1 ^d	18 ± 1	16 ± 1 ^d	16 ± 1	14 ± 1
Day 23	14 ± 1	13 ± 1	14 ± 1	14 ± 1	13 ± 0	17 ± 2
Week 14	14 ± 2	13 ± 1	13 ± 1	13 ± 1	14 ± 2	—
Bile salts (µmol/L)						
Day 4	17.6 ± 1.5	17.4 ± 1.2 ^d	18.8 ± 1.2	18.3 ± 1.4 ^d	22.4 ± 2.2	23.6 ± 2.7*
Day 23	17.9 ± 1.7	17.0 ± 1.2	19.4 ± 1.9	15.5 ± 1.0	18.5 ± 1.5	24.1 ± 2.3
Week 14	21.4 ± 2.7	18.8 ± 2.7	18.9 ± 1.5	17.2 ± 1.8	20.9 ± 1.2	—

* Significantly different (P≤0.05) from the vehicle control group by Dunn's or Shirley's test

** P≤0.01

^a Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

^b All 600 mg/kg rats died before the end of the study; no data are available for this group.

^c n=9

^d n=10

TABLE F2
Hematology Data for Mice in the 3-Month Dermal Study of Bis(2-chloroethoxy)methane^a

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (%)	48.3 ± 0.4	48.0 ± 0.5	48.2 ± 0.4	47.4 ± 0.4	46.6 ± 0.4**	45.6 ± 0.6**
Hemoglobin (g/dL)	15.3 ± 0.1	15.2 ± 0.1	14.9 ± 0.4	14.4 ± 0.4*	14.5 ± 0.2**	14.3 ± 0.2**
Erythrocytes (10 ⁶ /μL)	10.34 ± 0.09	10.24 ± 0.11	10.31 ± 0.12	10.03 ± 0.11	9.84 ± 0.10**	9.65 ± 0.13**
Reticulocytes (10 ⁶ /μL)	0.19 ± 0.02	0.19 ± 0.03	0.18 ± 0.02	0.22 ± 0.01	0.22 ± 0.02	0.20 ± 0.02
Nucleated erythrocytes (10 ³ /μL)	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	46.7 ± 0.2	46.9 ± 0.1	46.8 ± 0.2	47.2 ± 0.2	47.4 ± 0.2*	47.3 ± 0.2*
Mean cell hemoglobin (pg)	14.8 ± 0.1	14.9 ± 0.1	14.5 ± 0.3	14.3 ± 0.3	14.7 ± 0.1	14.8 ± 0.0
Mean cell hemoglobin concentration (g/dL)	31.6 ± 0.2	31.8 ± 0.1	30.9 ± 0.7	30.3 ± 0.6*	31.1 ± 0.1*	31.3 ± 0.1*
Platelets (10 ³ /μL)	959.0 ± 30.3	914.2 ± 29.6	921.4 ± 21.3	917.1 ± 30.1	927.8 ± 19.6	929.0 ± 18.0
Leukocytes (10 ³ /μL)	5.35 ± 0.51	5.30 ± 0.46	4.67 ± 0.41	6.29 ± 0.54	6.21 ± 0.34	5.46 ± 0.45
Segmented neutrophils (10 ³ /μL)	0.83 ± 0.15	0.60 ± 0.08	0.53 ± 0.09	0.73 ± 0.10	0.71 ± 0.07	0.71 ± 0.10
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10 ³ /μL)	4.40 ± 0.42	4.55 ± 0.43	4.02 ± 0.37	5.42 ± 0.52	5.30 ± 0.32	4.56 ± 0.41
Monocytes (10 ³ /μL)	0.08 ± 0.04	0.11 ± 0.02	0.06 ± 0.02	0.08 ± 0.03	0.10 ± 0.03	0.14 ± 0.03
Basophils (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10 ³ /μL)	0.07 ± 0.03	0.05 ± 0.02	0.07 ± 0.03	0.08 ± 0.03	0.08 ± 0.03	0.09 ± 0.02
Female						
n	10	10	10	10	10	7
Hematocrit (%)	48.7 ± 0.7	49.2 ± 0.7	49.4 ± 0.5	48.6 ± 0.6	49.5 ± 0.7	46.6 ± 0.5
Hemoglobin (g/dL)	15.6 ± 0.3	16.0 ± 0.2	16.1 ± 0.2	15.8 ± 0.1	16.0 ± 0.2	14.8 ± 0.2
Erythrocytes (10 ⁶ /μL)	10.33 ± 0.17	10.39 ± 0.17	10.53 ± 0.11	10.25 ± 0.17	10.48 ± 0.17	9.64 ± 0.10
Reticulocytes (10 ⁶ /μL)	0.07 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.07 ± 0.02 ^b	0.07 ± 0.01	0.08 ± 0.02
Nucleated erythrocytes (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.2 ± 0.2	47.4 ± 0.2	46.9 ± 0.1	47.5 ± 0.2	47.2 ± 0.2	48.3 ± 0.2**
Mean cell hemoglobin (pg)	15.1 ± 0.2	15.5 ± 0.1	15.3 ± 0.1	15.5 ± 0.1	15.3 ± 0.1	15.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.0 ± 0.4	32.6 ± 0.2	32.6 ± 0.1	32.5 ± 0.2	32.4 ± 0.2	31.7 ± 0.2
Platelets (10 ³ /μL)	829.8 ± 24.5	798.7 ± 38.4	761.4 ± 49.0	872.6 ± 20.9	799.9 ± 40.4	882.0 ± 39.7
Leukocytes (10 ³ /μL)	6.15 ± 0.32	6.13 ± 0.24	5.61 ± 0.59	6.17 ± 0.44	6.06 ± 0.27	5.49 ± 0.41
Segmented neutrophils (10 ³ /μL)	0.64 ± 0.06	0.58 ± 0.05	0.53 ± 0.03	0.80 ± 0.11	0.60 ± 0.06	0.73 ± 0.10
Bands (10 ³ /μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10 ³ /μL)	5.43 ± 0.31	5.42 ± 0.20	4.95 ± 0.57	5.22 ± 0.33	5.29 ± 0.28	4.66 ± 0.42
Monocytes (10 ³ /μL)	0.07 ± 0.02	0.11 ± 0.02	0.10 ± 0.03	0.13 ± 0.02	0.12 ± 0.03	0.10 ± 0.03
Basophils (10 ³ /μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10 ³ /μL)	0.07 ± 0.02	0.03 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.08 ± 0.02	0.01 ± 0.01

* Significantly different (P≤0.05) from the vehicle control group by Shirley's test

** P≤0.01

^a Data are presented as mean ± standard error. Statistical tests were performed on unrounded data.

^b n=9

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

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TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	154 ± 4	153 ± 6	149 ± 4	155 ± 7	152 ± 5	152 ± 5
Heart						
Absolute	0.59 ± 0.01	0.60 ± 0.02	0.62 ± 0.03	0.64 ± 0.02	0.60 ± 0.02	0.61 ± 0.02
Relative	3.842 ± 0.048	3.900 ± 0.061	4.195 ± 0.120*	4.152 ± 0.103	3.937 ± 0.053	3.991 ± 0.119
R. Kidney						
Absolute	0.73 ± 0.02	0.72 ± 0.02	0.68 ± 0.02	0.73 ± 0.04	0.72 ± 0.03	0.72 ± 0.03
Relative	4.767 ± 0.069	4.714 ± 0.072	4.590 ± 0.083	4.678 ± 0.091	4.704 ± 0.030	4.728 ± 0.089
Liver						
Absolute	8.03 ± 0.19	8.06 ± 0.41	8.02 ± 0.18	8.46 ± 0.63	8.18 ± 0.40	8.63 ± 0.43
Relative	52.271 ± 0.959	52.726 ± 0.966	53.987 ± 0.965	54.290 ± 2.125	53.816 ± 1.541	56.630 ± 1.870
Lung						
Absolute	1.17 ± 0.04	1.09 ± 0.04	1.16 ± 0.06	1.33 ± 0.14	1.17 ± 0.05	1.28 ± 0.04
Relative	7.631 ± 0.419	7.196 ± 0.326	7.801 ± 0.495	8.487 ± 0.522	7.694 ± 0.283	8.404 ± 0.078
R. Testis						
Absolute	0.941 ± 0.028	0.904 ± 0.063	0.908 ± 0.032	0.941 ± 0.052	0.922 ± 0.042	0.889 ± 0.053
Relative	6.121 ± 0.126	5.903 ± 0.278	6.101 ± 0.062	6.046 ± 0.099	6.070 ± 0.148	5.830 ± 0.260
Thymus						
Absolute	0.407 ± 0.020	0.386 ± 0.033	0.392 ± 0.009	0.395 ± 0.026	0.382 ± 0.020	0.415 ± 0.023
Relative	2.642 ± 0.093	2.544 ± 0.244	2.640 ± 0.098	2.542 ± 0.108	2.513 ± 0.060	2.722 ± 0.083
Female						
Necropsy body wt	121 ± 4	119 ± 4	117 ± 3	119 ± 3	118 ± 3	119 ± 1
Heart						
Absolute	0.51 ± 0.01	0.52 ± 0.02	0.48 ± 0.01	0.49 ± 0.01	0.51 ± 0.02	0.50 ± 0.01
Relative	4.189 ± 0.054	4.365 ± 0.086	4.116 ± 0.067	4.145 ± 0.050	4.278 ± 0.069	4.214 ± 0.119
R. Kidney						
Absolute	0.61 ± 0.02	0.58 ± 0.02	0.57 ± 0.02	0.63 ± 0.02	0.59 ± 0.03	0.60 ± 0.02
Relative	5.031 ± 0.134	4.891 ± 0.128	4.834 ± 0.072	5.239 ± 0.129	4.996 ± 0.202	5.029 ± 0.135
Liver						
Absolute	6.33 ± 0.21	6.15 ± 0.09	5.66 ± 0.24	5.98 ± 0.13	5.55 ± 0.22*	5.77 ± 0.21
Relative	52.117 ± 0.781	51.669 ± 1.087	48.335 ± 1.131	50.108 ± 0.822	47.012 ± 1.359*	48.685 ± 1.416
Lung						
Absolute	1.06 ± 0.12	0.96 ± 0.07	1.01 ± 0.03	1.01 ± 0.10	0.97 ± 0.02	1.04 ± 0.05
Relative	8.612 ± 0.726	8.040 ± 0.465	8.618 ± 0.341	8.468 ± 1.001	8.239 ± 0.340	8.771 ± 0.530
Thymus						
Absolute	0.387 ± 0.015	0.353 ± 0.014	0.378 ± 0.020	0.336 ± 0.007	0.348 ± 0.022	0.358 ± 0.030
Relative	3.191 ± 0.116	2.977 ± 0.170	3.224 ± 0.105	2.823 ± 0.080	2.959 ± 0.226	3.033 ± 0.277

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^{a,b}

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg
Male					
n	10	10	10	10	10
Necropsy body wt	332 ± 8	322 ± 7	320 ± 8	329 ± 7	307 ± 7
Heart					
Absolute	0.99 ± 0.03	0.99 ± 0.02	0.94 ± 0.02	0.97 ± 0.02	0.95 ± 0.03
Relative	2.965 ± 0.048	3.060 ± 0.039	2.938 ± 0.046	2.962 ± 0.033	3.097 ± 0.055
R. Kidney					
Absolute	0.99 ± 0.02	0.98 ± 0.03	1.00 ± 0.03	1.02 ± 0.02	1.00 ± 0.03
Relative	2.967 ± 0.029	3.052 ± 0.040	3.112 ± 0.044*	3.086 ± 0.037*	3.246 ± 0.047**
Liver					
Absolute	12.72 ± 0.29	12.49 ± 0.28	12.09 ± 0.49	13.18 ± 0.39	12.36 ± 0.33
Relative	38.337 ± 0.525	38.763 ± 0.500	37.660 ± 0.702	40.021 ± 0.768	40.248 ± 0.564
Lung					
Absolute	1.56 ± 0.05	1.53 ± 0.06	1.45 ± 0.05	1.49 ± 0.04	1.39 ± 0.05
Relative	4.700 ± 0.127	4.732 ± 0.150	4.530 ± 0.138	4.534 ± 0.131	4.510 ± 0.078
R. Testis					
Absolute	1.495 ± 0.021	1.460 ± 0.033	1.464 ± 0.026	1.482 ± 0.027	1.432 ± 0.032
Relative	4.516 ± 0.077	4.533 ± 0.075	4.583 ± 0.065	4.509 ± 0.073	4.670 ± 0.080
Thymus					
Absolute	0.328 ± 0.016	0.311 ± 0.014	0.306 ± 0.013	0.302 ± 0.011	0.312 ± 0.013
Relative	0.993 ± 0.053	0.964 ± 0.039	0.962 ± 0.052	0.918 ± 0.035	1.016 ± 0.039
Female					
n	10	10	10	10	8
Necropsy body wt	194 ± 4	193 ± 3	189 ± 3	190 ± 3	195 ± 4
Heart					
Absolute	0.66 ± 0.01	0.67 ± 0.02	0.74 ± 0.08	0.67 ± 0.01	0.67 ± 0.02
Relative	3.388 ± 0.050	3.449 ± 0.055	3.964 ± 0.485	3.506 ± 0.054	3.441 ± 0.061
R. Kidney					
Absolute	0.64 ± 0.01	0.64 ± 0.01	0.62 ± 0.01	0.66 ± 0.02	0.68 ± 0.02
Relative	3.272 ± 0.065	3.314 ± 0.048	3.290 ± 0.052	3.472 ± 0.092	3.473 ± 0.049
Liver					
Absolute	6.67 ± 0.16	6.43 ± 0.10	6.44 ± 0.16	6.84 ± 0.12	6.86 ± 0.22
Relative	34.317 ± 0.474	33.360 ± 0.343	34.059 ± 0.447	35.985 ± 0.471	35.075 ± 0.679
Lung					
Absolute	1.12 ± 0.04	1.10 ± 0.05	1.11 ± 0.05	1.11 ± 0.04	1.02 ± 0.02
Relative	5.796 ± 0.236	5.719 ± 0.198	5.877 ± 0.283	5.865 ± 0.235	5.212 ± 0.120
Thymus					
Absolute	0.275 ± 0.014	0.267 ± 0.007	0.266 ± 0.012	0.266 ± 0.007	0.260 ± 0.014
Relative	1.410 ± 0.061	1.386 ± 0.043	1.408 ± 0.060	1.399 ± 0.043	1.335 ± 0.068

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

^b All 600 mg/kg rats died before the end of the study; no data are available for this group.

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	12.5 mg/kg	25 mg/kg	50 mg/kg	100 mg/kg	200 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	25.0 ± 0.6	25.2 ± 0.4	24.9 ± 0.5	25.3 ± 0.7	24.3 ± 0.7	25.9 ± 0.8
Heart						
Absolute	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.18 ± 0.02	0.13 ± 0.00	0.16 ± 0.01
Relative	6.348 ± 0.484	6.451 ± 0.523	6.349 ± 0.225	6.965 ± 0.517	5.519 ± 0.112	6.098 ± 0.437
R. Kidney						
Absolute	0.26 ± 0.01	0.26 ± 0.02	0.26 ± 0.01	0.27 ± 0.01	0.25 ± 0.01	0.28 ± 0.01
Relative	10.338 ± 0.245	10.197 ± 0.630	10.305 ± 0.184	10.471 ± 0.184	10.506 ± 0.449	10.694 ± 0.209
Liver						
Absolute	1.49 ± 0.05	1.38 ± 0.04	1.39 ± 0.04	1.43 ± 0.07	1.36 ± 0.04	1.52 ± 0.06
Relative	59.497 ± 1.245	54.614 ± 1.288**	55.635 ± 0.442*	56.259 ± 1.046	55.927 ± 0.505	58.596 ± 1.110
Lung						
Absolute	0.19 ± 0.00	0.20 ± 0.01	0.21 ± 0.01	0.22 ± 0.02	0.20 ± 0.01	0.21 ± 0.01
Relative	7.493 ± 0.132	8.088 ± 0.405	8.230 ± 0.229	8.803 ± 0.577	8.029 ± 0.254	8.178 ± 0.277
R. Testis						
Absolute	0.108 ± 0.002	0.108 ± 0.003	0.108 ± 0.003	0.114 ± 0.004	0.105 ± 0.003	0.113 ± 0.002
Relative	4.339 ± 0.079	4.273 ± 0.100	4.343 ± 0.057	4.501 ± 0.193	4.315 ± 0.118	4.389 ± 0.078
Thymus						
Absolute	0.057 ± 0.004	0.045 ± 0.003	0.054 ± 0.005	0.054 ± 0.003	0.050 ± 0.003	0.055 ± 0.001
Relative	2.290 ± 0.152	1.797 ± 0.116*	2.165 ± 0.196	2.125 ± 0.080	2.055 ± 0.073	2.150 ± 0.086
Female						
Necropsy body wt	21.6 ± 0.6	21.5 ± 0.7	21.3 ± 0.4	21.6 ± 0.6	21.2 ± 0.8	22.3 ± 0.7
Heart						
Absolute	0.17 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.00
Relative	7.837 ± 0.334	7.460 ± 0.453	7.378 ± 0.382	7.411 ± 0.209	7.549 ± 0.416	7.239 ± 0.240
R. Kidney						
Absolute	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.00	0.18 ± 0.01	0.19 ± 0.01
Relative	8.462 ± 0.172	8.642 ± 0.139	8.469 ± 0.319	8.389 ± 0.297	8.585 ± 0.198	8.450 ± 0.206
Liver						
Absolute	1.28 ± 0.05	1.21 ± 0.08	1.24 ± 0.04	1.23 ± 0.03	1.24 ± 0.05	1.34 ± 0.06
Relative	59.439 ± 1.141	56.160 ± 1.757	58.235 ± 1.567	56.845 ± 0.729	58.610 ± 1.936	59.957 ± 1.162
Lung						
Absolute	0.20 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.22 ± 0.01	0.22 ± 0.03	0.21 ± 0.01
Relative	9.444 ± 0.213	9.919 ± 0.371	10.823 ± 0.184	10.394 ± 0.404	10.177 ± 0.767	9.176 ± 0.267
Thymus						
Absolute	0.069 ± 0.009	0.070 ± 0.004	0.071 ± 0.003	0.070 ± 0.004	0.077 ± 0.005	0.072 ± 0.003
Relative	3.184 ± 0.316	3.284 ± 0.155	3.339 ± 0.193	3.253 ± 0.279	3.637 ± 0.202	3.250 ± 0.206

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
Male						
n	10	10	10	10	10	10
Necropsy body wt	37.8 ± 0.6	37.8 ± 1.0	38.3 ± 0.6	38.1 ± 1.0	36.9 ± 0.8	37.3 ± 1.1
Heart						
Absolute	0.19 ± 0.01	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.18 ± 0.01
Relative	4.907 ± 0.215	4.936 ± 0.224	5.143 ± 0.202	4.615 ± 0.164	4.765 ± 0.166	4.922 ± 0.240
R. Kidney						
Absolute	0.30 ± 0.01	0.30 ± 0.01	0.31 ± 0.01	0.31 ± 0.01	0.34 ± 0.01**	0.34 ± 0.01**
Relative	7.839 ± 0.176	8.010 ± 0.248	8.167 ± 0.226	8.254 ± 0.192	9.132 ± 0.177**	9.239 ± 0.256**
Liver						
Absolute	1.63 ± 0.06	1.64 ± 0.03	1.76 ± 0.04	1.63 ± 0.03	1.69 ± 0.05	1.62 ± 0.05
Relative	43.093 ± 1.560	43.755 ± 1.244	45.967 ± 1.176	42.869 ± 0.887	45.696 ± 0.937	43.503 ± 1.480
Lung						
Absolute	0.23 ± 0.01	0.24 ± 0.02	0.27 ± 0.01	0.24 ± 0.02	0.23 ± 0.02	0.23 ± 0.01
Relative	6.132 ± 0.275	6.364 ± 0.499	6.933 ± 0.314	6.264 ± 0.589	6.300 ± 0.385	6.073 ± 0.342
R. Testis						
Absolute	0.125 ± 0.003	0.128 ± 0.002	0.127 ± 0.003	0.123 ± 0.002	0.124 ± 0.003	0.126 ± 0.002
Relative	3.298 ± 0.069	3.400 ± 0.093	3.332 ± 0.071	3.250 ± 0.098	3.361 ± 0.080	3.389 ± 0.100
Thymus						
Absolute	0.050 ± 0.002	0.046 ± 0.002	0.050 ± 0.003	0.045 ± 0.002	0.050 ± 0.002	0.050 ± 0.002
Relative	1.310 ± 0.055	1.234 ± 0.067	1.299 ± 0.074	1.187 ± 0.045	1.360 ± 0.068	1.353 ± 0.054
Female						
n	10	10	10	10	10	7
Necropsy body wt	30.5 ± 1.0	32.5 ± 0.7	32.2 ± 0.9	31.9 ± 0.7	32.3 ± 0.9	30.9 ± 1.5
Heart						
Absolute	0.15 ± 0.00	0.15 ± 0.01	0.16 ± 0.00	0.15 ± 0.00	0.15 ± 0.01	0.15 ± 0.00
Relative	4.975 ± 0.220	4.748 ± 0.191	4.918 ± 0.209	4.696 ± 0.127	4.778 ± 0.149	4.995 ± 0.252
R. Kidney						
Absolute	0.19 ± 0.00	0.19 ± 0.00	0.18 ± 0.00	0.19 ± 0.00	0.19 ± 0.00	0.21 ± 0.00**
Relative	6.139 ± 0.210	5.804 ± 0.138	5.718 ± 0.153	5.892 ± 0.136	6.036 ± 0.136	6.873 ± 0.358*
Liver						
Absolute	1.31 ± 0.03	1.44 ± 0.04	1.42 ± 0.04	1.42 ± 0.04	1.48 ± 0.03*	1.47 ± 0.07*
Relative	43.160 ± 0.888	44.370 ± 1.055	44.300 ± 0.797	44.548 ± 0.644	45.949 ± 1.143	48.191 ± 3.390
Lung						
Absolute	0.24 ± 0.02	0.27 ± 0.02	0.26 ± 0.02	0.23 ± 0.01	0.26 ± 0.03	0.23 ± 0.01
Relative	8.126 ± 0.698	8.192 ± 0.570	8.166 ± 0.570	7.380 ± 0.344	8.154 ± 0.729	7.661 ± 0.507
Thymus						
Absolute	0.056 ± 0.002	0.060 ± 0.002	0.060 ± 0.002	0.058 ± 0.003	0.054 ± 0.003	0.055 ± 0.004
Relative	1.838 ± 0.070	1.837 ± 0.069	1.883 ± 0.044	1.808 ± 0.079	1.701 ± 0.115	1.776 ± 0.125

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test

** $P \leq 0.01$

^a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX H

REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

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TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	332 ± 8	320 ± 8	329 ± 7	307 ± 7
L. Cauda epididymis	0.1456 ± 0.0037	0.1388 ± 0.0030	0.1457 ± 0.0036	0.1414 ± 0.0047
L. Epididymis	0.4377 ± 0.0066	0.4215 ± 0.0091	0.4313 ± 0.0082	0.4219 ± 0.0078
L. Testis	1.5681 ± 0.0178	1.5382 ± 0.0220	1.5664 ± 0.0320	1.4756 ± 0.0333
Spermatid measurement				
Spermatid heads (10 ⁶ /g testis)	133.62 ± 4.92	129.31 ± 3.27	140.46 ± 3.31	137.04 ± 3.46
Spermatid heads (10 ⁶ /testis)	179.75 ± 5.77	174.50 ± 5.83	193.88 ± 5.88	181.38 ± 6.26
Epididymal spermatozoal measurements				
Sperm motility (%)	81.87 ± 1.08	80.04 ± 0.87	82.76 ± 1.37	80.44 ± 0.88
Sperm (10 ⁶ /cauda epididymis)	126.7 ± 5.5	119.4 ± 5.4	124.9 ± 7.0	126.2 ± 6.4
Sperm (10 ⁶ /g cauda epididymis)	870 ± 31	861 ± 40	864 ± 55	898 ± 48

^a Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	50 mg/kg	100 mg/kg	200 mg/kg
Number weighed at necropsy				
Necropsy body wt (g)	194 ± 4	193 ± 3	189 ± 3	190 ± 3
Proportion of regular cycling females ^b				
Estrous cycle length (days)	4.9 ± 0.08	5.0 ± 0.00	5.0 ± 0.13	5.2 ± 0.15
Estrous stages (% of cycle)				
Diestrus	56.3	59.2	59.2	57.5
Proestrus	14.3	17.5	15.8	16.7
Estrus	29.4	23.3	24.2	25.8
Metestrus	0.0	0.0	0.8	0.0

^a Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among dose groups and between the vehicle control group and each dosed group indicated dosed females did not have extended estrus or diestrus.

^b Number of females with a regular cycle/number of females cycling

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	200 mg/kg	400 mg/kg	600 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.8 ± 0.6	38.1 ± 1.0	36.9 ± 0.8	37.3 ± 1.1
L. Cauda epididymis	0.0147 ± 0.0006	0.0144 ± 0.0006	0.0140 ± 0.0006	0.0155 ± 0.0015
L. Epididymis	0.0430 ± 0.0005	0.0427 ± 0.0009	0.0438 ± 0.0010	0.0422 ± 0.0022
L. Testis	0.1194 ± 0.0021	0.1177 ± 0.0020	0.1186 ± 0.0011	0.1165 ± 0.0021
Spermatid measurement				
Spermatid heads (10 ⁶ /g testis)	186.16 ± 7.16	182.39 ± 7.62	179.87 ± 7.33	183.44 ± 7.48
Spermatid heads (10 ⁶ /testis)	20.93 ± 0.90	20.71 ± 1.10	20.54 ± 0.79	20.86 ± 0.97
Epididymal spermatozoal measurements				
Sperm motility (%)	90.39 ± 1.20	90.94 ± 0.81	91.05 ± 1.30	91.44 ± 0.79
Sperm (10 ⁶ /cauda epididymis)	23.5 ± 1.7	23.1 ± 1.5	22.7 ± 1.7	20.6 ± 2.1
Sperm (10 ⁶ /g cauda epididymis)	1,600 ± 90	1,619 ± 104	1,632 ± 129	1,419 ± 208

^a Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (spermatid and epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Dermal Study
of Bis(2-chloroethoxy)methane^a

	Vehicle Control	100 mg/kg	200 mg/kg	400 mg/kg
Number weighed at necropsy				
Necropsy body wt (g)	30.5 ± 1.0	32.2 ± 0.9	31.9 ± 0.7	32.3 ± 0.9
Proportion of regular cycling females ^b				
Estrous cycle length (days)	4.1 ± 0.18	4.2 ± 0.19	4.0 ± 0.11	4.1 ± 0.13 ^c
Estrous stages (% of cycle)				
Diestrus	33.3	36.7	31.7	45.0
Proestrus	0.0	0.0	0.8	0.0
Estrus	45.8	44.2	46.7	38.3
Metestrus	20.8	19.2	20.8	16.7

^a Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett's test (body weight) or Dunn's test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages. Tests for equality of transition probability matrices among dose groups and between the vehicle control group and each dosed group indicated dosed females did not have extended estrus or diestrus.

^b Number of females with a regular cycle/number of females cycling

^c Estrous cycle was longer than 12 days or unclear in one of 10 animals.

APPENDIX I

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Bis(2-chloroethoxy)methane

Bis(2-chloroethoxy)methane was obtained from Karl Industries, Inc. (Aurora, OH), in two lots (B007269977 and B004160277). Lot B007269977 was used in the 2-week and 3-month studies, and lot B004160277 was used in the 2-year studies. Identity, purity, and water solubility analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Chemistry Support Services, Columbus, OH). Identity analyses were conducted by the study laboratory at Battelle Columbus Operations (Columbus, OH). Karl Fischer titration and elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the bis(2-chloroethoxy)methane studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a clear, colorless liquid, were identified as bis(2-chloroethoxy)methane by the analytical chemistry laboratory using infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy; in addition, boiling point determinations were performed on lot B004160277. The study laboratory confirmed the identity of both lots of the test article using IR spectroscopy. The infrared spectra were consistent with literature spectra (Sadtler, 1978; Bio-Rad, 2002) of bis(2-chloroethoxy)methane. The proton and carbon-13 NMR spectra were consistent with computer generated spectra and the structure of bis(2-chloroethoxy)methane. Representative IR and NMR spectra are presented in Figures I1, I2, and I3. The boiling point was determined to be 238.8° C, slightly higher than the literature value of 218.1° C at 20 mm Hg (Hawley's, 2001). Solubility of a lot not used in the animal studies was determined to be less than 1,000 ppm in water.

The water content of both lots was determined by Karl Fischer titration. The purity of each lot was determined by Galbraith Laboratories, Inc., using elemental analysis and by the analytical chemistry laboratory using gas chromatography (GC) by system A or a system similar to system A (Table I1). In addition, the analytical chemistry laboratory attempted to identify impurities in lot B0072669977 using GC by system B and determined the purity of lot B004160277 using differential scanning calorimetry (DSC). DSC was performed on a Perkin Elmer DSC-7 calorimeter (Perkin Elmer, Inc., Waltham, MA) scanning from -50° to -32° C at 1° C per minute under a nitrogen atmosphere.

For lot B007269977, Karl Fischer titration indicated 0.06% water. Elemental analyses for carbon, hydrogen, and chlorine were in agreement with the theoretical values for bis(2-chloroethoxy)methane. GC using system A indicated one major peak and two impurities with areas of at least 0.1% of the total peak area. GC using system B tentatively identified the greater impurity as chloromethyl 2-(2-chloroethoxy)ethyl ether and the lesser impurity as a material structurally similar to bis(2-chloroethoxy)ethane. The absence of available standards for these materials prevented a specific assignment of identity. The overall purity of lot B007269977 was determined to be approximately 98.5%.

For lot B004160277, Karl Fischer titration indicated 0.12% water. Elemental analyses for carbon and hydrogen were consistent with the theoretical values for bis(2-chloroethoxy)methane, but the elemental analysis value for chlorine was approximately 6% less than the theoretical value; this low value appeared to be anomalous. DSC indicated a purity of 98.62%. GC by a system similar to system A indicated a major peak and three impurities; the impurities together represented 1.7% of the total peak area. The overall purity of lot B004160277 was determined to be approximately 98.2%.

Lots B007269977 and B004160277 were reanalyzed using GC by a system similar to system A to compare the impurities in the two lots; these analyses indicated that the impurities in the two lots were the same and had approximately the same peak areas.

To ensure stability, the bulk chemical was stored at less than or equal to -20° C, protected from light in glass bottles. Periodic reanalyses of the bulk chemical were performed during the 2-week, 3-month, and 2-year studies by the study laboratory using GC by system C or a system similar to system C. No degradation of the bulk chemical was observed.

Ethanol

USP-grade 95% ethanol was obtained in multiple lots from Spectrum Chemicals and Laboratory Products (Gardena, CA) and used as the vehicle during the 2-week, 3-month, and 2-year studies. Identity was confirmed by the study laboratory using IR spectroscopy, and purity was determined using GC by system D. No impurities with areas of 0.1% or greater relative to the major peak area were found in any of the lots, and benzene was not found in any of the lots.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once during the 2-week studies, four times during the 3-month studies, and approximately every 2 months during the 2-year studies by mixing bis(2-chloroethoxy)methane with 95% ethanol to give the required concentrations (Table I2). The dose formulations were stored at room temperature in amber glass bottles sealed with Teflon[®]-lined lids for up to 42 days.

Stability studies of a 5 mg/mL formulation of a lot not used in the animal studies were performed by the analytical chemistry laboratory using GC by system C (Table I1). Stability was confirmed for at least 42 days for dose formulations stored at room temperature in amber glass bottles sealed with Teflon[®]-lined lids and for at least 3 hours under simulated animal room conditions provided the bottle was kept sealed between the brief periods of formulation removal.

Periodic analyses of the dose formulations of bis(2-chloroethoxy)methane were conducted by the study laboratory using GC by system C or a similar system. During the 2-week studies, the dose formulations were analyzed once; all five formulations for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 10 animal room samples were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed three times; animal room samples of these dose formulations were also analyzed (Table I4). Of the dose formulations analyzed, all 15 for rats and mice were within 10% of the target concentrations; all 30 animal room samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 2 months; animal room samples were also analyzed (Table I5). Of the dose formulations analyzed, all 36 for rats and all 72 for mice were within 10% of the target concentrations; all 36 animal room samples were within 10% of the target concentrations.

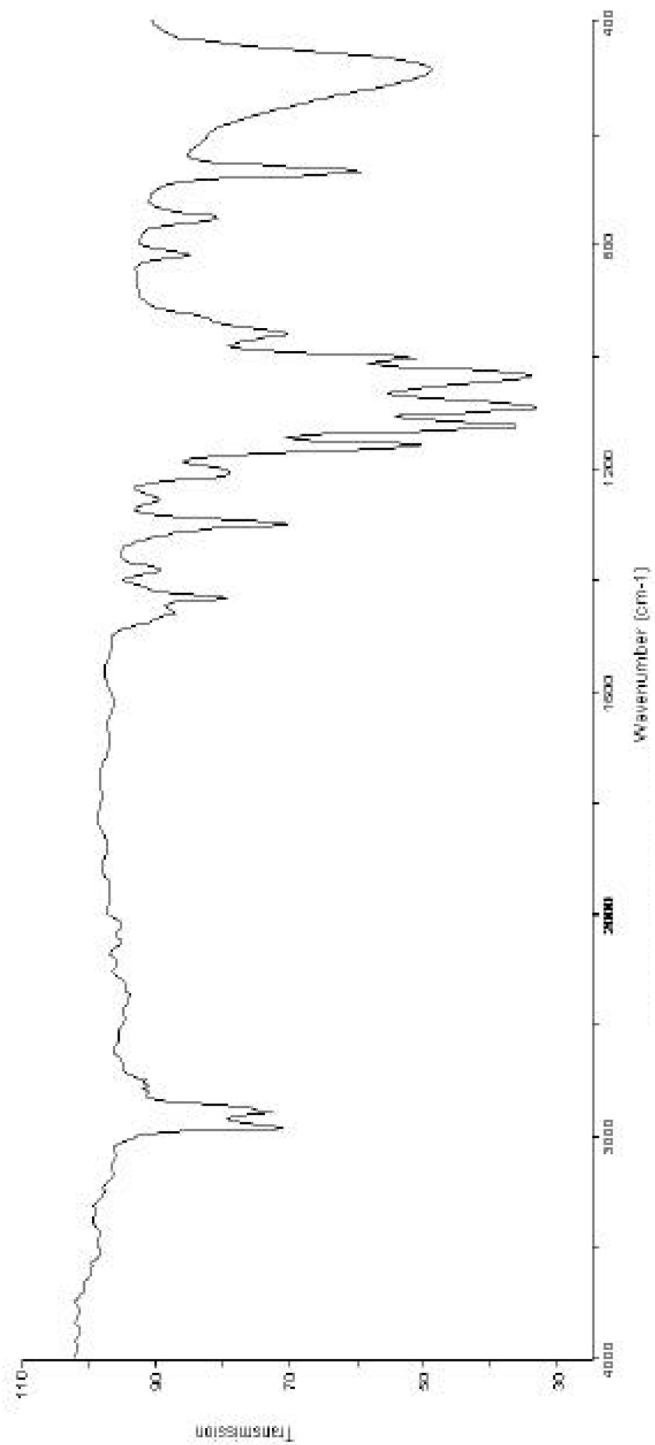


FIGURE II
Infrared Absorption Spectrum of Bis(2-chloroethoxy)methane

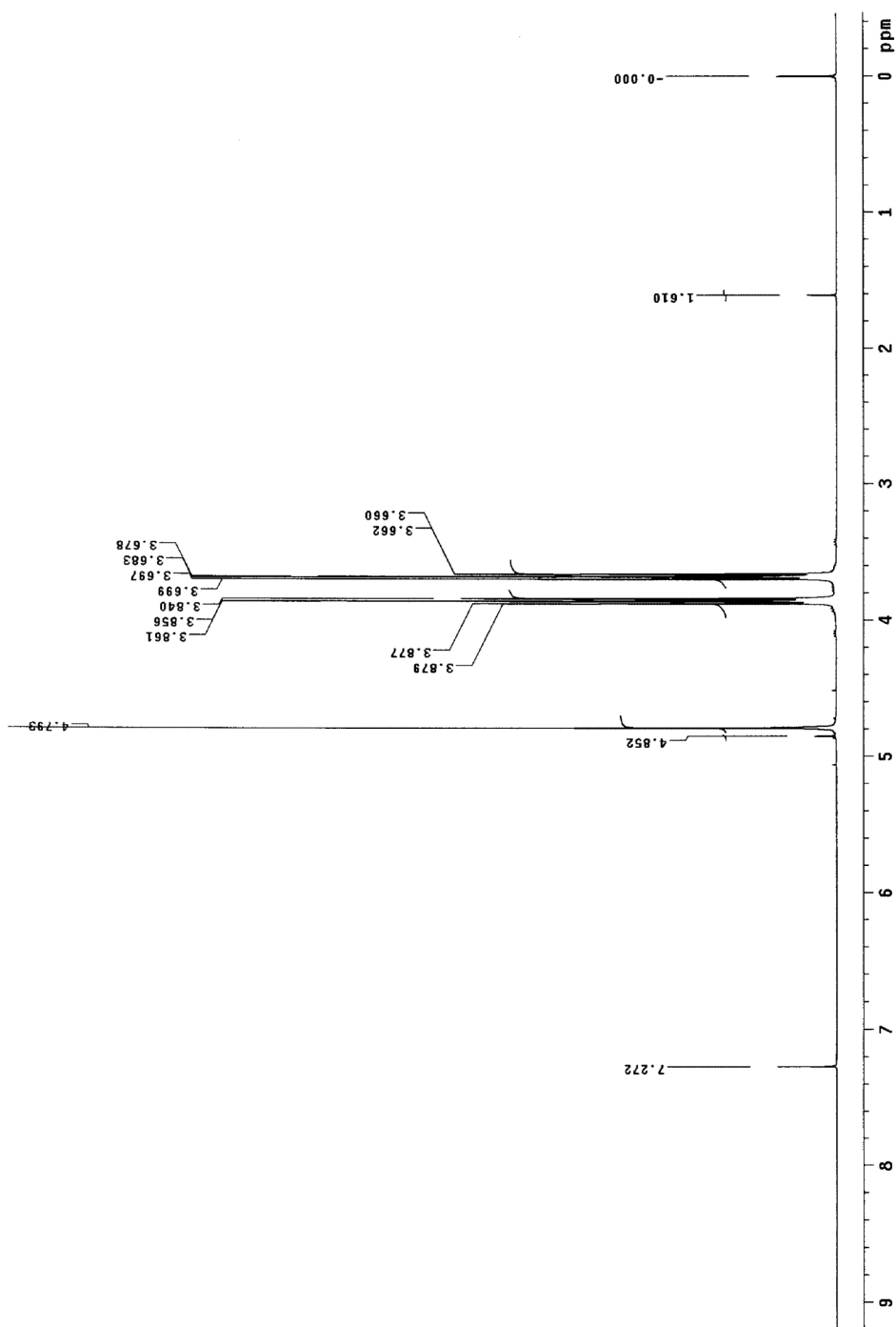


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Bis(2-chloroethoxy)methane

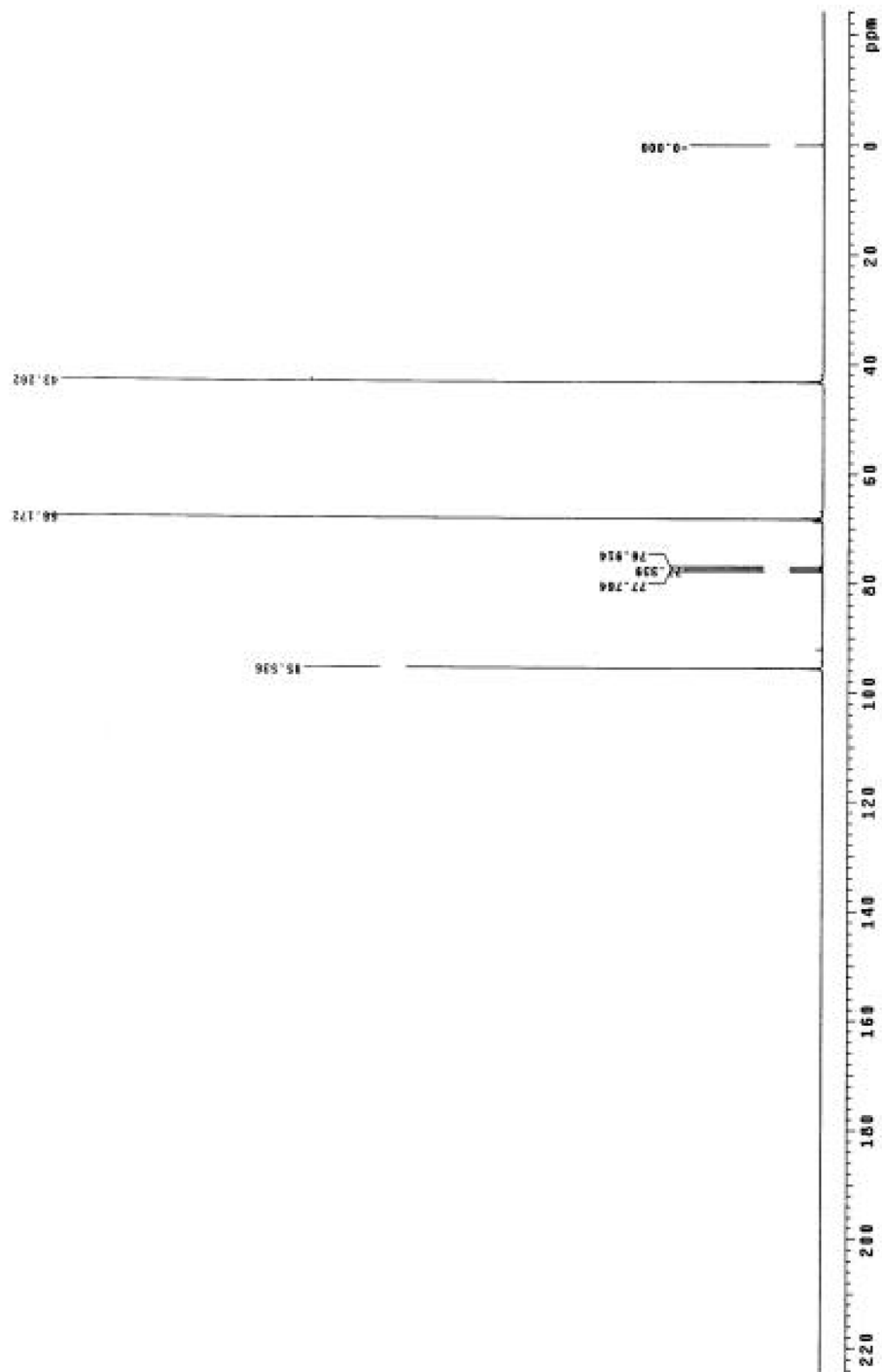


FIGURE I3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Bis(2-chloroethoxy)methane

TABLE II
Gas Chromatography Systems Used in the Dermal Studies of Bis(2-chloroethoxy)methane^a

Detection System	Column	Carrier Gas	Oven Temperature Program
System A Flame ionization	RTX [®] -5, 30 m × 0.25 mm, 0.25- μ m film (Restek, Bellefonte, PA)	Helium at 3 mL/minute	50° C for 2 minutes, then 10° C/minute to 290° C, held for 4 minutes
System B Mass spectrometry with electron impact ionization (10 to 250 amu)	HP-5ms, 30 m × 0.25 mm, 0.25- μ m film (Agilent Technologies, Inc., Santa Clara, CA)	Helium at 1 mL/minute	50° C for 2 minutes, then 7° C/minute to 290° C, held for 4 minutes
System C Flame ionization	RTX [®] -5, 15 m × 0.53 mm, 1.0- μ m film (Restek)	Helium at ~6 mL/minute	100° C for 2 minutes, then 10° C/minute to 160° C, held for 4 minutes
System D Flame ionization	DB-WAX, 30 m × 0.53 mm, 1.0- μ m film (Agilent Technologies, Inc.)	Helium at ~10 mL/minute	80° C for 5 minutes, then 10° C/minute to 220° C, held for 3 minutes

^a The gas chromatographs were manufactured by Agilent Technologies, Inc. (Santa Clara, CA).

TABLE I2
Preparation and Storage of Dose Formulations in the Dermal Studies of Bis(2-chloroethoxy)methane

2-Week Studies	3-Month Studies	2-Year Studies
<p>Preparation The specified weight of bis(2-chloroethoxy)methane was transferred to a volumetric flask of the appropriate capacity using a beaker or transfer pipette. Approximately 100 mL of 95% ethanol was added to the flask, and the flask was shaken and inverted to mix. The contents of the flask were diluted to volume with 95% ethanol, and the flask was again shaken and inverted. The dose formulations were prepared once.</p>	<p>The specified volume of bis(2-chloroethoxy)methane was measured with a graduated cylinder and transferred to a glass mixing bottle calibrated to the appropriate volume. Except for the 1,200 mg/mL rat formulations, the graduated cylinder was rinsed into the glass mixing bottle at least three times with small volumes of 95% ethanol. The contents of the glass mixing bottle were diluted to the specified volume with 95% ethanol, and the bottle was capped, inverted, and shaken. The dose formulations were prepared four times.</p>	<p>The specified volume of bis(2-chloroethoxy)methane was measured with a graduated cylinder and transferred to a glass mixing container calibrated to the appropriate volume. The graduated cylinder was rinsed with 95% ethanol into the glass mixing container. The contents of the glass mixing container were diluted to the specified volume with 95% ethanol, and the container was capped, inverted, and shaken. The dose formulations were prepared approximately every 4 weeks.</p>
<p>Chemical Lot Number B007269977</p>	<p>B007269977</p>	<p>B004160277</p>
<p>Maximum Storage Time 42 days</p>	<p>42 days</p>	<p>42 days</p>
<p>Storage Conditions Stored in amber glass bottles sealed with Teflon[®]-lined lids at room temperature</p>	<p>Stored in amber glass bottles sealed with Teflon[®]-lined lids at room temperature</p>	<p>Stored in amber glass bottles sealed with Teflon[®]-lined lids at room temperature</p>
<p>Study Laboratory Battelle Columbus Operations (Columbus, OH)</p>	<p>Battelle Columbus Operations (Columbus, OH)</p>	<p>Battelle Columbus Operations (Columbus, OH)</p>

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration ^a (mg/mL)	Difference from Target (%)	
Rats					
August 28, 2001	August 29, 2001	25	25.04	0	
		50	49.92	0	
		100	100.2	0	
		200	200.9	0	
		400	393.6	-2	
	October 1-2, 2001 ^b	25	25.53	+2	
		50	51.48	+3	
		100	101.5	+2	
		200	202.2	+1	
		400	409.3	+2	
	Mice				
	August 28, 2001	August 29, 2001	6.25	6.430	+3
			12.5	12.60	+1
			25	25.04	0
			50	49.92	0
100			100.2	0	
October 1-2, 2001 ^b		6.25	6.341	+1	
		12.5	12.57	+1	
		25	24.73	-1	
		50	50.27	+1	
		100	103.0	+3	

^a Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 25 mg/mL=12.5 mg/kg, 50 mg/mL=25 mg/kg, 100 mg/mL=50 mg/kg, 200 mg/mL=100 mg/kg, 400 mg/mL=200 mg/kg. For mice, dosing volume=2 mL/kg; 6.25 mg/mL=12.5 mg/kg, 12.5 mg/mL=25 mg/kg, 25 mg/mL=50 mg/kg, 50 mg/mL=100 mg/kg, 100 mg/mL=200 mg/kg.

^b Animal room samples

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration ^a (mg/mL)	Difference from Target (%)
Rats				
January 25, 2002	January 25, 2002	100	98.73	-1
		200	200.4	0
		400	397.6	-1
		800	798.8	0
		1,200	1,249	+4
	February 27-28, 2002 ^b	100	102.1	+2
		200	203.7	+2
		400	409.3	+2
		800	818.0	+2
		1,200	1,225	+2
February 19, 2002	February 20, 2002	100	101.4	+1
		200	208.5	+4
		400	408.2	+2
		800	836.2	+5
		1,200	1,263	+5
	March 29, 2002 ^b	100	100.4	0
		200	202.7	+1
		400	403.9	+1
		800	826.6	+3
		1,200	1,248	+4
April 9, 2002	April 10, 2002	100	98.56	-1
		200	194.0	-3
		400	389.7	-3
		800	759.8	-5
		1,200	1,239	+3
	May 13, 2002 ^b	100	102.9	+3
		200	204.9	+2
		400	416.8	+4
		800	816.6	+2
		1,200	1,257	+5
Mice				
January 25, 2002	January 25, 2002	25	24.21	-3
		50	49.05	-2
		100	98.73	-1
		200	200.4	0
		300	287.6	-4
	February 27-28, 2002 ^b	25	24.45	-2
		50	50.11	0
		100	102.2	+2
		200	204.7	+2
		300	294.8	-2

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)	
Mice (continued)					
February 19, 2002	February 20, 2002	25	24.82	-1	
		50	49.56	-1	
		100	101.4	+1	
		200	208.5	+4	
		300	300.9	0	
	March 27, 2002 ^b	25	25.20	+1	
		50	50.03	0	
		100	100.2	0	
		200	209.2	+5	
		300	297.4	-1	
	April 9, 2002	April 10, 2002	25	23.67	-5
			50	48.35	-3
			100	98.56	-1
			200	194.0	-3
			300	294.8	-2
May 13, 2002 ^b		25	24.41	-2	
		50	50.28	+1	
		100	100.9	+1	
		200	212.1	+6	
		300	302.4	+1	

^a Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 100 mg/mL=50 mg/kg, 200 mg/mL=100 mg/kg, 400 mg/mL=200 mg/kg, 800 mg/mL=400 mg/kg, 1,200 mg/mL=600 mg/kg. For mice, dosing volume=2 mL/kg; 25 mg/mL=50 mg/kg, 50 mg/mL=100 mg/kg, 100 mg/mL=200 mg/kg, 200 mg/mL=400 mg/kg, 300 mg/mL=600 mg/kg.

^b Animal room samples

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration ^a (mg/mL)	Difference from Target (%)
Rats				
August 30, 2002	September 5, 2002	150	153.1	+2
		300	300.7	0
		600	609.5	+2
	October 17, 2002 ^b	150	155.1	+3
		300	300.6	0
		600	615.2	+3
November 11, 2002	November 12, 2002	150	144.0	-4
		300	286.0	-5
		600	587.6	-2
January 16, 2003	January 16-17, 2003	150	148.8	-1
		300	306.9	+2
		600	615.6	+3
March 25, 2003	March 26, 2003	150	146.4	-2
		300	298.8	0
		600	607.0	+1
	May 12-13, 2003 ^b	150	145.9	-3
		300	301.0	0
		600	618.2	+3
May 30, 2003	June 4, 2003	150	144.0	-4
		300	292.2	-3
		600	611.7	+2
July 29, 2003	July 30-31, 2003	150	142.7	-5
		300	298.8	0
		600	605.7	+1
October 2, 2003	October 3 and 7, 2003	150	150.5	0
		300	299.7	0
		600	629.0 ^c	+5
	November 14, 2003 ^b	150	153.4	+2
		300	313.3	+4
		600	623.1	+4
December 5, 2003	December 8, 2003	150	141.1	-6
		300	292.7	-2
		600	601.7	0
February 3, 2004	February 4, 2004	150	146.3	-2
		300	288.8	-4
		600	599.0	0
April 6, 2004	April 7-8, 2004	150	147.1	-2
		300	290.0	-3
		600	601.3	0
	May 21-22, 2004 ^b	150	152.3	+2
		300	288.9	-4
		600	604.9	+1

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)	
Rats (continued)					
June 10, 2004	June 11, 2004	150	147.7	-2	
		300	301.4	0	
		600	626.0	+4	
August 12, 2004	August 13, 2004	150	146.2	-3	
		300	290.5	-3	
		600	595.4	-1	
Mice					
August 30, 2002	September 5, 2002	50	49.64	-1	
		75	74.55	-1	
		100	97.89	-2	
		150	153.1	+2	
		200	197.7	-1	
		300	300.7	0	
	October 17, 2002 ^b	50	50.47	+1	
		75	76.38	+2	
		100	100.3	0	
		150	153.6	+2	
		200	199.4	0	
		300	307.9	+3	
	November 11, 2002	November 12, 2002	50	48.15	-4
			75	70.86	-6
			100	95.15	-5
150			144.0	-4	
200			194.7	-3	
300			286.0	-5	
January 16, 2003	January 16-17, 2003	50	48.04	-4	
		75	74.91	0	
		100	94.92	-5	
		150	148.8	-1	
		200	203.3	+2	
		300	306.9	+2	
March 25, 2003	March 26, 2003	50	46.30	-7	
		75	70.83	-6	
		100	97.12	-3	
		150	146.4	-2	
		200	199.2	0	
		300	298.8	0	
	May 12-13, 2003 ^b	50	48.65	-3	
		75	73.22	-2	
		100	99.14	-1	
		150	145.5	-3	
		200	200.5	0	
		300	304.6	+2	

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
May 30, 2003	June 4, 2003	50	47.69	-5
		75	72.01	-4
		100	95.41	-5
		150	144.0	-4
		200	191.9	-4
		300	292.2	-3
July 29, 2003	July 30-31, 2003	50	47.82	-4
		75	71.01	-5
		100	94.20	-6
		150	142.7	-5
		200	194.8	-3
		300	298.8	0
October 2, 2003	October 3, 2003	50	50.39	+1
		75	76.53	+2
		100	97.83	-2
		150	150.5	0
		200	201.7	+1
		300	299.7	0
	November 14, 2003 ^b	50	53.24	+6
		75	78.58	+5
		100	99.78	0
		150	153.7	+2
		200	206.7	+3
		300	306.7	+2
December 5, 2003	December 8, 2003	50	48.13	-4
		75	72.56	-3
		100	93.63	-6
		150	141.1	-6
		200	197.5	-1
		300	292.7	-2
February 3, 2004	February 4, 2004	50	47.24	-6
		75	70.97	-5
		100	94.32	-6
		150	146.3	-2
		200	189.1	-5
		300	288.8	-4
April 6, 2004	April 7-8, 2004	50	46.04	-8
		75	72.60 ^c	-3
		100	94.59	-5
		150	147.1	-2
		200	196.6	-2
		300	290.0	-3
	May 21-22, 2004 ^b	50	48.28	-3
		75	72.21	-4
		100	96.19	-4
		150	151.8	+1
		200	200.1	0
		300	289.0	-4

TABLE I5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Dermal Studies
of Bis(2-chloroethoxy)methane

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
June 10, 2004	June 11, 2004	50	48.73	-3
		75	74.61	-1
		100	97.32	-3
		150	147.7	-2
		200	199.0	-1
		300	301.4	0
August 12, 2004	August 13 and 16, 2004	50	49.06 ^d	-2
		75	74.23 ^d	-1
		100	98.46	-2
		150	146.2	-3
		200	193.5	-3
		300	290.5	-3

^a Results of duplicate analyses. For rats, dosing volume=0.5 mL/kg; 150 mg/mL=75 mg/kg, 300 mg/mL=150 mg/kg, 600 mg/mL=300 mg/kg. For mice, dosing volume=2 mL/kg; 50 mg/mL=100 mg/kg, 75 mg/mL=150 mg/kg, 100 mg/mL=200 mg/kg, 150 mg/mL=300 mg/kg, 200 mg/mL=400 mg/kg, 300 mg/mL=600 mg/kg.

^b Animal room samples

^c Results of triplicate analyses

^d Results of quadruplicate analyses

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NTP-2000 RAT AND MOUSE RATION

TABLE J1	Ingredients of NTP-2000 Rat and Mouse Ration	164
TABLE J2	Vitamins and Minerals in NTP-2000 Rat and Mouse Ration	164
TABLE J3	Nutrient Composition of NTP-2000 Rat and Mouse Ration	165
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TABLE J1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix ^a	0.5
Mineral premix ^b	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

^a Wheat middlings as carrier

^b Calcium carbonate as carrier

TABLE J2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration^a

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α -Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
<i>d</i> -Pantothenic acid	10 mg	<i>d</i> -Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B ₁₂	52 μ g	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	<i>d</i> -Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

^a Per kg of finished product

TABLE J3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.46	13.8 – 15.8	25
Crude fat (% by weight)	8.1 ± 0.36	7.4 – 9.0	25
Crude fiber (% by weight)	9.1 ± 0.45	8.2 – 9.9	25
Ash (% by weight)	5.0 ± 0.21	4.4 – 5.3	25
Amino Acids (% of total diet)			
Arginine	0.770 ± 0.070	0.670 – 0.970	18
Cystine	0.225 ± 0.023	0.150 – 0.250	18
Glycine	0.706 ± 0.043	0.620 – 0.800	18
Histidine	0.362 ± 0.082	0.310 – 0.680	18
Isoleucine	0.542 ± 0.046	0.430 – 0.660	18
Leucine	1.087 ± 0.066	0.960 – 1.240	18
Lysine	0.712 ± 0.118	0.310 – 0.840	18
Methionine	0.407 ± 0.051	0.260 – 0.490	18
Phenylalanine	0.626 ± 0.043	0.540 – 0.720	18
Threonine	0.500 ± 0.046	0.430 – 0.610	18
Tryptophan	0.142 ± 0.024	0.110 – 0.200	18
Tyrosine	0.388 ± 0.058	0.280 – 0.540	18
Valine	0.667 ± 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.243	3.49 – 4.54	18
Linolenic	0.30 ± 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	5,060 ± 117	3,400 – 8,900	25
Vitamin D (IU/kg)	1,000 ^a		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm) ^b	8.7 ± 3.65	5.9 – 25.2	25
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm) ^b	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B ₁₂ (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm) ^b	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.961 ± 0.044	0.873 – 1.030	25
Phosphorus (%)	0.584 ± 0.027	0.538 – 0.641	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

^a From formulation

^b As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE J4
Contaminant Levels in NTP-2000 Rat and Mouse Ration

	Mean ± Standard Deviation ^b	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.37 ± 0.160	0.14 – 0.50	25
Cadmium (ppm)	0.07 ± 0.022	0.04 – 0.10	25
Lead (ppm)	0.08 ± 0.025	0.05 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.19 ± 0.027	0.14 – 0.23	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm) ^c	15.1 ± 4.16	10.0 – 24.4	25
Nitrite nitrogen (ppm) ^c	<0.61		25
BHA (ppm) ^d	<1.0		25
BHT (ppm) ^d	<1.0		25
Aerobic plate count (CFU/g)	26 ± 70	10 – 360	25
Coliform (MPN/g)	3.0 ± 0.0	3.0	25
<i>Escherichia coli</i> (MPN/g)	<10		25
<i>Salmonella</i> (MPN/g)	Negative		25
Total nitrosoamines (ppb) ^e	4.2 ± 1.97	2.3 – 8.5	25
<i>N</i> -Nitrosodimethylamine (ppb) ^e	2.5 ± 1.71	1.1 – 6.9	25
<i>N</i> -Nitrosopyrrolidine (ppb) ^e	1.7 ± 0.77	0.9 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.079 ± 0.069	0.020 – 0.259	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.030 ± 0.400	0.020 – 1.850	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

^a All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride

^b For values less than the limit of detection, the detection limit is given as the mean.

^c Sources of contamination: alfalfa, grains, and fish meal

^d Sources of contamination: soy oil and fish meal

^e All values were corrected for percent recovery.

APPENDIX K SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program 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 five male and five female sentinel rats and mice at 1 month and at study termination in the 3-month studies and from five male and five female sentinel rats and mice at 1, 6, 12, and 18 months and five male and five female 300 mg/kg rats, five male 600 mg/kg mice, and five female 400 mg/kg mice at study termination in the 2-year studies. In the 2-year studies at the 18-month collection point, only four male rats and three female mice were available; for the 1 month collection point in female mice, only two were available. Fecal samples were obtained from sentinel male and female mice at 18 months for testing for *Helicobacter*. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance Corporation (Rockville, MD) for determination of antibody titers. The laboratory methods and agents for which testing was performed are tabulated below; the times at which samples were collected during the studies are also listed.

Method and Test

Time of Collection

RATS

3-Month Study

ELISA

Mycoplasma arthritidis

Study termination

Mycoplasma pulmonis

Study termination

PVM (pneumonia virus of mice)

1 month, study termination

RCV/SDA

(rat coronavirus/sialodacryoadenitis virus)

1 month, study termination

Sendai

1 month, study termination

Immunofluorescence Assay

Parvovirus

1 month, study termination

RCV/SDA

1 month

2-Year Study

ELISA

M. arthritidis

Study termination

M. pulmonis

Study termination

PVM

1, 6, 12, and 18 months, study termination

RCV/SDA

1, 6, 12, and 18 months, study termination

Sendai

1, 6, 12, and 18 months, study termination

Immunofluorescence Assay

Parvovirus

1, 6, 12, and 18 months, study termination

PVM

Study termination

RCV/SDA

6 months

Sendai

Study termination

Method and Test**Time of Collection****MICE****3-Month Study**

ELISA

Ectromelia virus

EDIM (epizootic diarrhea of infant mice)

GDVII (mouse encephalomyelitis virus)

LCM (lymphocytic choriomeningitis virus)

Mouse adenoma virus-FL

MHV (mouse hepatitis virus)

*M. arthritidis**M. pulmonis*

PVM

Reovirus 3

Sendai

1 month, study termination

1 month, study termination

1 month

Study termination

Study termination

1 month, study termination

Study termination

Study termination

1 month, study termination

1 month, study termination

1 month, study termination

Immunofluorescence Assay

GDVII

LCM

Mouse adenoma virus-FL

MCMV (mouse cytomegalovirus)

Parvovirus

1 month

Study termination

Study termination

Study termination

1 month, study termination

2-Year Study

ELISA

Ectromelia virus

EDIM

GDVII

LCM

MVM (minute virus of mice)

MAD-FL

MCMV

MHV

*M. arthritidis**M. pulmonis*

PVM

Reovirus 3

Sendai

1, 6, 12, and 18 months, study termination

1, 6, 12, and 18 months, study termination

1, 6, 12, and 18 months, study termination

1, 6, 12, and 18 months, study termination

Study termination

1, 6, 12, and 18 months, study termination

Study termination

1, 6, 12, and 18 months, study termination

Study termination

Study termination

1, 6, 12, and 18 months, study termination

1, 6, 12, and 18 months, study termination

1, 6, 12, and 18 months, study termination

Immunofluorescence Assay

EDIM

LCM

MCMV

MHV

Mouse adenoma virus-FL

Parvovirus

PVM

6 and 12 months

6 months

Study termination

6 months

6 months, study termination

1, 6, 12, and 18 months, study termination

6 months, study termination

Polymerase Chain Reaction

Helicobacter species

18 months

RESULTS

All test results were negative.

APPENDIX L

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

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SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

INTRODUCTION

Single-dose toxicokinetic studies were designed to estimate toxicokinetic parameters for bis(2-chloroethoxy)methane in F344/N rats and B6C3F1 mice and to determine the extent of bioavailability after dermal administration in order to aid in the interpretation of the toxicity study results. Male and female rats received a single intravenous injection of 20 or 40 (male only) mg bis(2-chloroethoxy)methane/kg body weight or a single dermal application of 100, 200, or 400 mg/kg. Mice received a single intravenous injection of 50 or 100 (males only) mg/kg or a single dermal application of 300, 450 (males only), or 600 mg/kg. In addition, groups of male and female rats (20 mg/kg) and male and female mice (50 mg/kg) were administered a single intravenous dose of thiodiglycolic acid, the major metabolite of bis(2-chloroethoxy)methane. Measurements of bis(2-chloroethoxy)methane and thiodiglycolic acid were made in plasma and heart, thymus, and liver tissue, and the results were used to calculate toxicokinetic parameters.

MATERIALS AND METHODS

Bis(2-chloroethoxy)methane was obtained in one lot (B007269977) from Karl Industries, Inc. (Aurora, OH), and was stored at less than or equal to -20°C . The material was identified by infrared and nuclear magnetic resonance spectroscopy. Karl Fisher titration determined that water was present at 0.06%. The purity of lot B007269977 was assessed as described in Appendix I. Two impurities were found. The larger one, present at 1.3% of the total peak area, was tentatively identified as chloromethyl 2-(2-chloroethoxy)ethyl ether using mass spectrometry. The mass spectrum of the smaller peak showed that it was a material structurally similar to bis(2-chloroethoxy)ethane, but in both cases, the lack of authentic standards prevented unequivocal identification of the impurities. Overall, the test article was determined to have a purity of approximately 98.5%.

Thiodiglycolic acid was obtained from Sigma-Aldrich Company (St. Louis, MO) and stored at room temperature. The supplier's certificate of analysis showed a purity of approximately 99.8% by gas chromatography and 99.8% by titration. This value was affirmed by reanalysis using gas chromatography.

Intravenous formulations were prepared at target concentrations in 1:1:8 Cremophor[®]:ethanol:water. Dermal formulations were prepared in 95% ethanol according to procedures described in Appendix I. All formulations were stored at less than or equal to 5°C , analyzed before dosing, and determined to meet acceptance criteria.

F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), and jugular vein catheters were implanted by Hilltop Lab Animals, Inc. (Scottsdale, PA). On the day of dosing, nonfasted male and female F344/N rats were 12 to 14 weeks old and ranged in weight from 221.4 to 306.8 g and 103.2 to 212.2 g, respectively; nonfasted male and female B6C3F1 mice were 11 to 13 weeks old and ranged in weight from 15.5 to 36.1 g and 18.6 to 27.8 g, respectively.

After dosing, animals were anesthetized with approximately 70% CO_2 (30% O_2) and blood was collected from rats via the retroorbital sinus and from mice by cardiac puncture. Three rats and three mice in each dose group were bled at each time point, and up to 2 mL of blood was collected. Rats were bled a second time with a minimum of 1 hour of recovery time. Blood samples were collected into glass tubes containing EDTA anticoagulant and mixed gently; plasma was separated within 60 minutes of collection by centrifugation. The plasma was stored at -70°C until analyzed. Following final blood collection, animals were sacrificed under 100% CO_2 . Table L1 summarizes the design of these studies, and Table L2 presents the blood collection time points for all of the studies.

Following sacrifice, the heart, liver, and thymus were collected from all animals except 40 mg/kg intravenous and 200 mg/kg dermal rats and 100 mg/kg intravenous and 450 mg/kg dermal mice. Samples were placed into plastic vials, flash frozen using liquid nitrogen, and stored at approximately -70°C until analysis with a validated method.

For bis(2-chloroethoxy)methane analysis, samples of 0.2 mL of plasma or 200 mg of tissue mixed with 200 μL of saline were placed in a conical glass tube and 50 μL of 80 ng/mL [$^2\text{H}_8$]-bis(2-chloroethoxy)methane in deionized water were added along with 400 μL of methyl-*t*-butyl ether (MTBE). The preparations were vortexed for 10 to 20 seconds; plasma was tumbled for 30 minutes and tissue was tumbled overnight, and then centrifuged at 1,500 rpm for approximately 5 minutes. The upper (MTBE) layer was transferred to an autosampler vial such that headspace was limited, and the vials were sealed. Single injections were made onto a gas chromatography/mass spectrometry (GC/MS) system (Agilent 6890 Plus GC with 5973N mass spectrometer; Agilent Technologies, Palo Alto, CA) in electron impact ionization mode. Chromatography was performed using a RTX-5 MS column (30 m \times 0.32 mm, 1.0 μm film thickness; Restek, Bellefonte, PA) with an oven program of 90° C for 1 minute, then 8° C/minute to 200° C, then 20° C/minute to 250° C, held for 2 minutes. Ions monitored in selected ion monitoring mode were 93 amu for bis(2-chloroethoxy)methane and 97 amu for [$^2\text{H}_8$]-bis(2-chloroethoxy)methane.

For thiodiglycolic acid analysis, plasma and tissue samples were thawed to room temperature and 0.1 mL of plasma or 100 mg of tissue was combined in a conical glass tube with 0.5 mL of boron trifluoride in methanol. The tubes were capped and heated to approximately 60° C for approximately 1 hour. Two mL of toluene, 5 mL of deionized water, and 25 μL of a 125 $\mu\text{g}/\text{mL}$ solution of ethyl-3-mercaptopropionate in toluene (internal standard) were added to each tube. The tubes were vortexed for 10 to 20 seconds and then rotated end-over-end overnight. The upper (toluene) layer was transferred to an autosampler vial such that headspace was limited, and the vial was sealed. Analysis was conducted with the same analytical system as described for bis(2-chloroethoxy)methane, with an oven program of 80° C for 2 minutes, then 10° C/minute to 300° C, held for 6 minutes. Fragments at 146 amu (analyte) and 134 amu (internal standard) were monitored for detection.

Calibrations for both analyses were performed using 6-point standard curves prepared in rat plasma and linear regression analysis with a $1/x$ weighted quadratic regression equation. The method described was found suitable for samples in the range of 4 to 204 ng of bis(2-chloroethoxy)methane and from 20 to 1,200 ng of thiodiglycolic acid. The method met acceptability criteria for linearity, precision, and accuracy.

TOXICOKINETICS

Toxicokinetic parameter estimates were derived only from those measurements that were above the limits of quantitation [2 ng for bis(2-chloroethoxy)methane in plasma, heart, and thymus and 4 ng in liver; 19.2 ng for thiodiglycolic acid in plasma, heart, thymus, and liver]. Mean tissue and plasma concentration time data sets were used to obtain initial estimates of the primary toxicokinetic parameters for a given noncompartmental model. These initial estimates were obtained because when used with the compartmental modeling algorithm, they facilitated generation of more reliable final parameter estimates. Data were evaluated using a nonlinear least-squares fitting program (WinNonlin, Version 5.0.1, Pharsight Corporation, Mountain View, CA).

Final toxicokinetic parameters for tissues were estimated by noncompartmental models. For plasma data, the following two compartmental models were used for intravenous administration and dermal application, respectively:

$$C_{(t)} = A_0 e^{\alpha t} + B_0 e^{-\beta t}$$

$$C_{(t)} = A e^{\alpha t} + B e^{-\beta t} + C e^{-k_{01} t}$$

$$\text{Where: } \alpha = 0.5 \{ [k_{12} + k_{21} + k_{10}] + [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2} \}$$

$$\beta = 0.5 \{ [k_{12} + k_{21} + k_{10}] - [(k_{12} + k_{21} + k_{10})^2 - 4k_{21}k_{10}]^{1/2} \}$$

$C_{(t)}$ is the plasma concentration at time t ; A_0 and B_0 are the intercepts of the initial and terminal phases with the concentration axis for intravenous data; A , B , and C are the intercepts of the distribution, elimination, and absorption phases with the concentration axis for dermal data; k is a rate constant (subscripts describe the compartment and direction); and α and β are the first-order hybrid rate constants. Half-lives for the absorption and elimination phases were calculated as $0.693/k_{01}$ and $0.693/k_{10}$, respectively.

The area under the curve (AUC_T) was estimated to the last sampling time point (T) using the trapezoidal rule:

$$AUC_T = \sum \frac{C_{n-1} + C_n}{2} \times (t_n - t_{n-1})$$

where C_{n-1} and C_n are the tissue bis(2-chloroethoxy)methane or thiodiglycolic acid concentrations measured at two consecutive time points, t_{n-1} and t_n , respectively. The area under the curve extrapolated to infinity (AUC_∞) was estimated as

$$AUC_\infty = AUC_T + \frac{C_T}{\beta}$$

where C_T is the plasma bis(2-chloroethoxy)methane or thiodiglycolic acid concentration measured at the last time point and β is the first-order hybrid rate constant for the terminal phase.

Absolute bioavailability was expressed as the fraction (F) of the dermal dose that reached systemic circulation and was calculated as

$$F = \frac{Dose_{intravenous} \times AUC_\infty(dermal)}{Dose_{dermal} \times AUC_\infty(intravenous)}$$

Clearance (Cl) was calculated from

$$Cl = \frac{Dose}{AUC_\infty}$$

and the overall volume of distribution (V) and volume of the central compartment (V_1) were calculated as

$$V = \frac{Cl}{\beta} \quad \text{and} \quad V_1 = \frac{Cl}{k_{10}}, \text{ respectively}$$

For the dermal data, clearance and volume of distribution were adjusted for bioavailability (F) and were expressed as $C_{_F}$ and $V_{_F}$, respectively.

RESULTS

Rats

Intravenous Administration of Bis(2-chloroethoxy)methane

Bis(2-chloroethoxy)methane was administered intravenously in a single dose to male and female jugular vein-cannulated rats at 20 or 40 (males only) mg/kg. Bis(2-chloroethoxy)methane in plasma was measurable at the earliest postdose sample collection time (10 minutes) and through at least 300 minutes for all samples. There were two aberrant concentration values in the data for males; however, upon review, there were no findings that could be used to explain the variability, so all data were used in the toxicokinetic analysis. All other relative standard deviation (RSD) values indicated good agreement among samples. Plasma bis(2-chloroethoxy)methane concentration-versus-time profiles following intravenous administration had a biphasic decline and were best fit by a two-compartment model with first-order elimination (Figure L1). Toxicokinetic parameters estimated from these data are presented in Table L3. The maximum bis(2-chloroethoxy)methane plasma concentration, C_{max} (equivalent to C_0 for intravenous administration), occurred close to the earliest sample taken (10 minutes). C_{max} and AUC_∞ increased proportionally with dose. The volume of distribution exceeded the reported total body water volume of 668 mL/kg for F344/N rats (Davies and Morris, 1993), suggesting that bis(2-chloroethoxy)methane might have significant tissue distribution and/or high tissue uptake/binding in rats. The elimination of

bis(2-chloroethoxy)methane from the central compartment was rapid with half-life values between 16.3 and 25.4 minutes. Neither dose- nor sex-related differences were apparent for estimated toxicokinetic parameters.

Bis(2-chloroethoxy)methane levels were determined in heart, thymus, and liver tissue samples from 20 mg/kg males and females. The parent compound was measureable in all tissues from the earliest time point (10 minutes) in both sexes, indicating rapid distribution. Bis(2-chloroethoxy)methane was detectable through at least 300 minutes in male rats with generally good agreement among the samples (RSDs of 30% or less with a few exceptions). In female rats, bis(2-chloroethoxy)methane was measureable through 360 minutes for the heart and 480 minutes for the thymus but only through 60 minutes for the liver. Toxicokinetic parameters estimated from these tissue samples are presented in Table L4. C_{max} values in female rats were generally lower than in male rats, although it may not be significant. The lower levels of bis(2-chloroethoxy)methane observed in the liver compared to the heart and thymus are likely due to the rapid metabolism of the chemical in the liver. The half-life was similar in heart and liver tissues of males and females; the half-life in the thymus was longer in females than in males.

Intravenous Administration of Thiodiglycolic Acid

To gain a better understanding of the thiodiglycolic acid sample data from the dermal studies, the chemical was administered intravenously in a single dose to male and female jugular vein-cannulated rats at 20 mg/kg. Thiodiglycolic acid was measureable in the plasma of males and females at the earliest sample collection time point (10 minutes) and through 240 minutes for males and 320 minutes for females. Two anomalous data points occurred, one in males and one in females; RSDs were 56.7% and 37.7%, respectively, for those time points. Other RSD values were below 30% and indicated good agreement among samples with measureable concentrations of thiodiglycolic acid. Figures L2 and L3 present the data which showed biphasic declines and were best described by two-compartment models with first-order elimination. The curve fit was better for the female data due to the larger number of collection points toward the end of the second elimination phase. Parameter estimates using this model are presented in Table L5. There was no apparent sex-related influence on the toxicokinetic parameters. The maximum thiodiglycolic acid plasma concentration, C_{max} , and AUC_{∞} were higher in females than in males. Thiodiglycolic acid was distributed into tissues in both males and females. The elimination of thiodiglycolic acid from the central compartment was rapid, with half-life values of 9.29 and 30.6 minutes for females and males, respectively.

Thiodiglycolic acid levels were determined in heart, thymus, and liver tissue samples from males and females. The chemical was measureable from the earliest sampling time point (10 minutes) in both sexes, through 320 minutes for males and 480 minutes for females in the heart, through 400 minutes for males and 480 minutes for females in the thymus, and through the final time point (600 minutes) in the liver for both sexes. RSD values were generally under 30% for heart and thymus samples indicating good agreement for all time points. Liver samples agreed less well among samples collected at a given time point with early time points giving RSDs generally less than 30% and the 180 to 600 minute time points agreeing from 33.5% to 79.8%. Noncompartmental analyses of the tissue sample concentrations showed that heart and thymus concentration-time profiles for both males and females had biphasic declines similar to those of the plasma concentration-time profiles. The liver concentration-time profiles for males and females also showed similar biphasic declines, but they first appeared to have a lag time of until the 40-minute time point before the decline phase, which may have been due to the thiodiglycolic acid being sequestered or excessively bound in the liver. Parameter estimates from these tissue data are presented in Table L6. C_{max} was much higher in the liver than in plasma, the heart, or the thymus, indicating that thiodiglycolic acid was being sequestered in the liver. There were no clear sex-related differences in the toxicokinetic parameters except that the liver concentration of thiodiglycolic acid in females was higher than in males.

Dermal Administration of Bis(2-chloroethoxy)methane

Male and female rats received single dermal doses of 100, 200, or 400 mg/kg bis(2-chloroethoxy)methane. The parent compound was measureable in plasma samples from all dosed groups of both sexes from the earliest collection point (5 minutes) through 360 minutes for 100 mg/kg males and females, 600 minutes for 200 mg/kg males, 360 minutes for 200 mg/kg females, and the final time point at 1,800 minutes for 400 mg/kg males and females, except for one animal each in the male and female dosed groups. RSDs were generally higher for all dermal groups than those in the intravenous studies with values typically in the 30% to 45% range, although some were as high as 60% or 70%. The plasma concentration-time profiles for male and female rats exhibited biphasic declines similar to those seen in the intravenous studies, and the curve shapes were best described by a two-

compartment model with first-order input and first-order elimination; Figure L4 shows the data from the 100 mg/kg male and female groups and typifies the overall study findings. Parameters estimated using this model are presented in Table L7. The absorption of bis(2-chloroethoxy)methane was rapid with half-lives less than 11.8 minutes; neither dose- nor sex-related differences were observed. The volume of distribution exceeded the reported body water volume of 668 mL/kg for F344/N rats (Davies and Morris, 1993), suggesting that bis(2-chloroethoxy)methane might have significant tissue distribution and/or high tissue uptake/binding after dermal administration. The plasma C_{max} increased with increasing dose; males showed dose-proportionality, which was not overtly evident in females. AUC_{∞} increased more than proportionally with dose at 400 mg/kg in both sexes, while the elimination half-life of bis(2-chloroethoxy)methane from the central compartment increased two- to fourfold at the same dose, suggesting that perhaps saturation of metabolism began to occur closer to this dose level.

Thiodiglycolic acid plasma concentrations were determined only in samples from 400 mg/kg male and female rats since the detection limits for this analyte were much higher than for bis(2-chloroethoxy)methane. Thiodiglycolic acid was not measurable at the first four time points (5, 10, 15, and 30 minutes) for samples from either males or females. However, thiodiglycolic acid was measurable from 60 minutes through the final time point of 1,800 minutes except for one male rat that was below the experimental limit of quantitation. Noncompartmental analyses of the thiodiglycolic acid plasma concentration-time profiles exhibited biphasic declines similar to those in rats given a single intravenous dose of thiodiglycolic acid. Figure L5 shows the observed and fitted data from plasma analysis of thiodiglycolic acid following dermal administration of bis(2-chloroethoxy)methane, and Table L8 presents the parameter estimates derived from noncompartmental modeling of these data. The elimination of thiodiglycolic acid from plasma appears to be much slower than that of bis(2-chloroethoxy)methane in both sexes as evidenced by a half-life of 322 minutes or greater. The kinetic parameters suggest that there are no apparent sex-related differences.

Bis(2-chloroethoxy)methane levels were determined in heart, thymus, and liver tissue at each collection time point for 100 and 400 mg/kg male and female rats. The chemical was measurable in the heart from the earliest data collection time point (5 minutes) through 240 minutes for 100 mg/kg males and females, 720 minutes for 400 mg/kg males, and 1,800 minutes for 400 mg/kg females. In the thymus, bis(2-chloroethoxy)methane was measurable from the earliest time point until 360 minutes for 100 mg/kg males and females and through the last time point at 1,800 minutes for 400 mg/kg males and females, except for one male at 1,440 minutes. Liver samples contained measurable amounts of bis(2-chloroethoxy)methane from the earliest time point and through 360 minutes for 100 mg/kg males, 120 minutes for 100 mg/kg females, 720 minutes for 400 mg/kg males, and 480 minutes for 400 mg/kg females. Tissue samples generally had higher RSDs than plasma samples, with the 100 mg/kg groups having agreement around 30% RSD and the 400 mg/kg groups typically in the 60% to 70% range. Noncompartmental analyses of these tissue determinations revealed that the heart, thymus, and liver of male and female rats showed biphasic declines similar to those of the plasma concentration profiles following dermal application of bis(2-chloroethoxy)methane and to the tissue analyses following intravenous administration. These biphasic declines were clearer in samples from the 400 mg/kg groups than those from the 100 mg/kg groups. Parameter estimates from the noncompartmental model are provided in Table L9. C_{max} values generally increased with dose and differed between tissues, with the liver generally showing the lowest levels in both sexes. The lower levels of bis(2-chloroethoxy)methane observed in the liver compared to the heart and thymus are likely due to the rapid metabolism of the chemical in the liver. Half-lives in tissues were longer than those observed in plasma, indicating slower elimination of bis(2-chloroethoxy)methane from tissues. No apparent sex-related differences were noted in the toxicokinetic parameters.

Heart, thymus, and liver samples from 400 mg/kg males and females were analyzed for thiodiglycolic acid concentrations. In the heart and thymus, thiodiglycolic acid was not consistently measurable until 60 minutes following dermal administration of bis(2-chloroethoxy)methane but was present at measurable concentrations through the last time point at 1,800 minutes in both males and females. In the liver, thiodiglycolic acid was not measurable at the first time point (5 minutes) but appeared in some of the 10-minute male rat samples and all of the 10-minute female rat samples and was measurable in males and females through the final time point at 1,800 minutes. With few exceptions, heart and liver samples had RSDs of approximately 30%; however, the liver showed more variability with some RSD values of up to 87.7%. Noncompartmental analyses of these data showed the familiar biphasic declines similar to those seen following intravenous administration of thiodiglycolic acid and to those of bis(2-chloroethoxy)methane in these tissues, except that thiodiglycolic acid showed a slower decline in the terminal phase. Table L10 presents the parameter estimates derived from the best-fit noncompartmental model that

had first-order input, first-order output, and uniform weighting. The observed C_{max} in the liver was much higher than in the heart and thymus. The elimination of thiodiglycolic acid in tissues was similar to that in plasma. Based on the kinetic parameters, there were no sex-related differences.

Mice

Intravenous Administration of Bis(2-chloroethoxy)methane

Bis(2-chloroethoxy)methane was administered intravenously in a single dose to male and female jugular vein-cannulated mice at 50 or 100 (males only) mg/kg. The chemical was measureable in plasma at the earliest postdose sample collection time (2 minutes) and through 60 minutes for 50 mg/kg males, 90 minutes for 100 mg/kg males, and 40 minutes for 50 mg/kg females. RSD values of 60% to 70% for the final time points in each data set did not generally show good agreement among samples in any of the dosed groups. All other RSD values indicated good agreement among samples with measureable concentrations. After review for any issues during dosing or sample collection, processing, or analyses, there were no findings that suggested the results were compromised, so all data were included in the toxicokinetic analyses. Plasma bis(2-chloroethoxy)methane concentration-versus-time profiles following intravenous administration had biphasic declines and were best-fit by a two-compartment model with first-order elimination; Figure L6 shows the data from 50 mg/kg male and female mice and 100 mg/kg male mice. Toxicokinetic parameters estimated from these data are presented in Table L11. The lack of measureable time points in the terminal phase of the female mouse study created more uncertainty in these estimates. The maximum bis(2-chloroethoxy)methane plasma concentration, C_{max} (equivalent to C_0 for intravenous administration), occurred close to the earliest sample time point (2 minutes). C_{max} and AUC_{∞} increased proportionally with dose. The volume of distribution exceeded the reported body water volume of 725 mL/kg for B6C3F1 mice (Davies and Morris, 1993), suggesting that bis(2-chloroethoxy)methane might have significant tissue distribution and/or high tissue uptake/binding in mice. The elimination of bis(2-chloroethoxy)methane from the central compartment was rapid with half-life values between 4.86 and 7.01 minutes. Dose- or sex-related differences were not apparent in the toxicokinetic parameters.

Bis(2-chloroethoxy)methane levels were determined in heart, thymus, and liver samples from 50 mg/kg males and females. The parent compound was measureable from the earliest time point (2 minutes) through 90 minutes in the heart and 40 minutes in the thymus and liver of male mice with good RSD values (under 30%) except at the later time points. In female mice, bis(2-chloroethoxy)methane was measureable from the earliest time point through 40 minutes in the heart and thymus and through 30 minutes in the liver with similar variability as seen in the male mice.

The tissue samples appeared to lack a sufficient number of terminal phase time points with which to reduce uncertainty; however, the tissue concentration profiles appeared more monophasic than the plasma bis(2-chloroethoxy)methane concentration profiles. The data were best-fit with a noncompartmental model with bolus input, first-order output, and uniform weighting. Toxicokinetic parameters estimated from these tissue samples are presented in Table L12. In general, no apparent differences were observed between the sexes and the tissues except that C_{max} was much lower in liver tissue compared to heart and thymus tissue which was likely due to the rapid metabolism of bis(2-chloroethoxy)methane in the liver.

Intravenous Administration of Thiodiglycolic Acid

To gain a better understanding of the thiodiglycolic acid sample data from the dermal studies, the chemical was administered intravenously in a single dose to male and female jugular vein-cannulated mice at 50 mg/kg. Thiodiglycolic acid was measureable in the plasma of males and females at the earliest sample collection time point (2 minutes) and through 180 minutes in both sexes. Data were fairly consistent, with RSD values below 30% except at two time points in the male data set. The data exhibited clear biphasic declines and were best described by a two-compartment model with first-order elimination. Figure L7 shows the observed and predicted data, and parameter estimates using this model are presented in Table L13. There did not appear to be any sex-related differences among the toxicokinetic parameters. The estimated V_1 (403 to 451 mL/kg) was slightly lower than the reported volume of total body water of 725 mL/kg for B6C3F1 mice (Davies and Morris, 1993), suggesting that thiodiglycolic acid might be distributed to the total body water or even just to the intra- or extracellular fluid. The elimination of thiodiglycolic acid from the central compartment was rapid with half-life values of 6.23 minutes or less.

Thiodiglycolic acid was measureable in heart tissue of male and female mice following intravenous administration of 50 mg/kg from the earliest time point (2 minutes) through at least 180 minutes for both sexes with good agreement (all RSDs were below 30%). In the thymus, thiodiglycolic acid was measureable from the earliest time point through 90 minutes for male mice and 180 minutes for female mice with the exception of one sample at the

60-minute time point in females. Only one time point in males and two in females had RSDs above 30% (39.2%, 57.6%, and 59.6%, respectively). Thiodiglycolic acid was measureable in the liver from the earliest time point through the final time point (240 minutes) for male and female mice except for the 5-minute time point in male mice. RSD values were generally under 30% for the earlier time points (up to 60 minutes) for heart and thymus samples indicating good agreement for these time points but were higher for some of the later time points (up to 60%). Noncompartmental analyses of liver concentration-time profiles for both male and female mice showed a slow buildup of thiodiglycolic acid peaking in concentration at 15 minutes, while the heart and thymus exhibited maximum concentrations at the earliest time point of 2 minutes. This may have been caused by thiodiglycolic acid being slowly sequestered by the liver or by excessive binding in the liver as seen in the rat study. Parameters from a noncompartmental model with bolus input, first-order output, and uniform weighting are presented in Table L14. C_{max} was higher and the half-life was shorter in the liver than in the heart or thymus in both sexes. No sex-related differences in the toxicokinetic parameters were observed.

Dermal Administration of Bis(2-chloroethoxy)methane

Male and female mice received single dermal doses of 300, 450 (males only), or 600 mg/kg bis(2-chloroethoxy)methane. The parent compound was measureable in plasma samples from the earliest time point (5 minutes) through 120 minutes for 300 mg/kg males and females and 450 mg/kg males and through at least 240 minutes in 600 mg/kg males and females. Similar to rats, bis(2-chloroethoxy)methane measurements from the dermal dose groups were not in as good agreement as the intravenous dose groups with RSDs ranging from 11.5% to 107.9%. The plasma concentration-time profiles for male and female mice exhibited biphasic declines similar to those seen in the intravenous studies, and the curve shape was best described by a two-compartment model with first-order input and first-order elimination; Figure L8 shows the data from 600 mg/kg males and females as a typical example. Parameters estimated using this model are presented in Table L15. Bis(2-chloroethoxy)methane absorption was rapid with half-life values from 5.06 to 7.55 minutes; neither dose- nor sex-related differences were observed. The plasma C_{max} , as well as $AUC_{0-\infty}$, increased proportionally with dose. The volume of distribution exceeded the reported body water volume of 725 mL/kg for B6C3F1 mice (Davies and Morris, 1993), suggesting significant tissue distribution and/or high tissue uptake/binding after dermal administration. The elimination half-life of bis(2-chloroethoxy)methane from the central compartment was less than or equal to 14.3 minutes and was not significantly affected by the dose. Sex-related differences in toxicokinetic parameters were not apparent.

Thiodiglycolic acid plasma concentrations were determined from samples from all dosed groups of male and female mice following dermal administration of bis(2-chloroethoxy)methane. Thiodiglycolic acid was not measureable through the first three time points (5, 10, and 15 minutes) in any dosed group but was measureable from 60 minutes through the final time point of 720 minutes in all dosed groups. The agreement among samples at a given time point was generally good; however, sporadic, anomalous data points drove RSDs much higher. The data exhibited biphasic declines similar to those in mice given a single intravenous dose of thiodiglycolic acid and were best-fit with a noncompartmental model with first-order input, first-order output, and uniform weighting. Parameter estimates from this model are presented in Table L16. C_{max} increased proportionally with dose in both male and female mice. The elimination of thiodiglycolic acid from plasma appeared to be much slower than that of bis(2-chloroethoxy)methane as evidenced from the half-lives. The kinetic parameters suggested that there were no sex-related differences in thiodiglycolic acid disposition.

Bis(2-chloroethoxy)methane levels were determined in heart, thymus, and liver tissue at each collection time point for 300 and 600 mg/kg males and females. The parent chemical was measureable in the heart from the earliest sampling time point (5 minutes) through 90 minutes in 300 mg/kg males, 60 minutes in 300 mg/kg females, 240 minutes in 600 mg/kg males, and 180 minutes in 600 mg/kg females. Agreement among samples of the same dosed group was less than that in the intravenous studies, and RSDs ranged from 10.9% to 109.1%. Bis(2-chloroethoxy)methane was measureable in the thymus from the earliest time point through 60 minutes in 300 mg/kg males and females, 120 minutes in 600 mg/kg males, and 90 minutes in 600 mg/kg females. RSDs ranged from 7.7% to 144.2%. Bis(2-chloroethoxy)methane was measureable in the liver from the earliest time point through 120 minutes in 300 mg/kg males and females, 360 minutes in 600 mg/kg males, and 180 minutes in 600 mg/kg females. RSDs ranged from 6.0% to 124.8%. Noncompartmental analyses of these tissue determinations revealed that the heart, thymus, and liver of male and female mice showed biphasic declines similar to those seen in the plasma concentration profiles following dermal administration of bis(2-chloroethoxy)methane and to the tissue analyses following intravenous administration of bis(2-chloroethoxy)methane. Parameter estimates from the noncompartmental model are presented in Table L17. C_{max} values were generally similar among the tissues

and generally increased proportionally with dose. Although there were no overall sex-related effects, observed heart tissue values were slightly higher in females than in males. Half-lives were slightly higher in the tissues than in the plasma, showing slower elimination in the tissues. Other than the higher C_{max} in female heart tissue, no apparent sex-related differences were noted in the toxicokinetic parameters.

Heart, thymus, and liver samples from 300 and 600 mg/kg males and females were analyzed for thiodiglycolic acid concentrations. In heart samples, thiodiglycolic acid was not consistently measureable until 30 minutes following dermal administration of bis(2-chloroethoxy)methane but was present at measureable concentrations through 240 minutes in 300 mg/kg males and females and through the final time point of 720 minutes in 600 mg/kg males and females. Thiodiglycolic acid was not measureable in the thymus until the 60-minute time point in 300 mg/kg males and females but remained measurable through all the later time points; it was measurably present at 60 minutes and through the final time point of 720 minutes in 600 mg/kg males and females. Thiodiglycolic acid was not reliably measureable in the liver until the 15-minute time point but remained measurable through the final time point of 720 minutes in 300 and 600 mg/kg males and females. RSDs for thiodiglycolic acid measured in these tissues were generally good (approximately 30%) with some exceptions, especially in the liver. Noncompartmental analyses of these data showed the familiar biphasic declines similar to those seen following intravenous administration of thiodiglycolic acid and to those of bis(2-chloroethoxy)methane in these tissues except that thiodiglycolic acid showed a slower decline in the terminal phase. Table L18 presents the parameter estimates derived from the best-fit noncompartmental model which had first-order input, first-order output, and uniform weighting. C_{max} increased proportionally with dose. Levels were higher in the liver than in the heart and thymus, likely due to the metabolism of bis(2-chloroethoxy)methane to thiodiglycolic acid in the liver. The elimination of thiodiglycolic acid from tissues was similar to that from plasma. The kinetic parameters did not suggest that there were any sex- or dose related differences.

DISCUSSION AND CONCLUSIONS

Bis(2-chloroethoxy)methane was rapidly distributed into the peripheral compartment and eliminated from the central compartment following intravenous administration in rats and mice. Absorption of bis(2-chloroethoxy)methane following dermal administration was rapid in both species. In general, dose-proportional increases in C_{max} and AUC were observed in rats and mice following both intravenous and dermal administration, although at the dermal dose of 400 mg/kg in rats, saturation of metabolism was apparent. The AUC_{∞} (predicted) after dermal application was compared with the AUC_{∞} (predicted) after intravenous administration of bis(2-chloroethoxy)methane with respect to dose in order to estimate bioavailability after dermal application. Since there was a potential for saturation at the high dermal doses (400 mg/kg in rats and 650 mg/kg in mice), only the lower dermal doses were used. Estimated bioavailability was low in both species but was higher in rats (11.2% to 17.9% in males and 20.0% to 28.7% in females) than in mice (5.09% to 6.86% in males and 11.9% in females). In a previous study conducted by the NTP in which 0.1 and 10 mg/kg [^{14}C]-bis(2-chloroethoxy)methane was applied under similar conditions to male F344 rats and male B6C3F1 mice, dermal bioavailability 24 hours after the application was estimated to be 33% to 55% in rats and 17% to 25% in mice (RTI, 2002). Factors such as hepatic first-pass metabolism of the parent chemical, differences in the applied doses, and differences in the methodologies used in the estimations of bioavailability (parent chemical plasma levels versus total radioactivity in excreta and tissues) may have played a role in the observed differences in bioavailability between the two studies.

Elimination of bis(2-chloroethoxy)methane following both intravenous administration and dermal application was rapid in both species. However, the overall elimination was more rapid in mice than rats. Although there was no dose-dependent elimination in mice following dermal application, in rats, the elimination half-life increased by two- to fivefold at the highest dose of 400 mg/kg. Tissue measurements showed that bis(2-chloroethoxy)methane was rapidly distributed to the heart, thymus, and liver. The lower levels of bis(2-chloroethoxy)methane in the liver compared to the heart and thymus were likely due to the rapid metabolism of bis(2-chloroethoxy)methane to thiodiglycolic acid in the liver as later evidenced by the high amounts of thiodiglycolic acid measured in the liver after dermal application of bis(2-chloroethoxy)methane. There were no apparent gender-related differences in toxicokinetic parameters of bis(2-chloroethoxy)methane or thiodiglycolic acid. The only species differences were seen in elimination and bioavailability.

These studies indicated that bis(2-chloroethoxy)methane is quickly absorbed after dermal application, distributed from the central compartment, and metabolized to thiodiglycolic acid, which is cleared fairly slowly and is highly concentrated in the liver.

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Davies, B., and Morris, T. (1993). Physiological parameters in laboratory animals and humans. *Pharm. Res.* **10**, 1093-1095.

RTI International (RTI) (2002). Bis(2-chloroethoxy)methane: Comparative Metabolism and Excretion in Rats and Mice. RTI Report No. RTI/64U-6855/14P. NIEHS Contract No. N01-ES-75407. RTI International, Research Triangle Park, NC.

TABLE L1
Experimental Design of the Single-Dose Toxicokinetic Studies of Bis(2-chloroethoxy)methane

	Bis(2-chloroethoxy)methane		Thiodiglycolic Acid
	Intravenous	Dermal	Intravenous
Rats			
Dose (mg/kg)	20 or 40 (males only)	100, 200, or 400	20
Dosing volume (mL/kg)	2	0.5	2
Concentration (mg/mL)	10 or 20	200, 400, or 800	10
Mice			
Dose (mg/kg)	50 or 100 (males only)	300, 450 (males only), or 600	50
Dosing volume (mL/kg)	4	2	4
Concentration (mg/mL)	12.5 or 25	150, 225, or 300	12.5

TABLE L2
Target Blood Collection Time Points in the Single-Dose Toxicokinetic Studies of Bis(2-chloroethoxy)methane^a

Route of Administration	Blood Collection Time Points (minutes postdosing)	
	Bis(2-chloroethoxy)methane	Thiodiglycolic Acid
Rats		
Intravenous	10, 20, 40, 60, 120, 180, 240, 300 (20 mg/kg only), 320 (40 mg/kg only), 360 (20 mg/kg only), 400 (40 mg/kg only), 420 (20 mg/kg only), 480, 540 (20 mg/kg only), 580 (40 mg/kg only), 600 (20 mg/kg only), 660 (20 mg/kg only), 680, 780, and 900 (40 mg/kg only)	10, 20, 40, 60, 120, 180, 240, 320, 400, 480, and 600
Dermal	5, 10, 15, 30, 60, 120 (100 mg/kg only), 180 (200 and 400 mg/kg only), 240 (100 mg/kg only), 360, 480 (100 and 400 mg/kg only), 600 (100 and 200 mg/kg only), 720 (100 and 400 mg/kg only), 960 (200 and 400 mg/kg only), 1,440 (200 and 400 mg/kg only), and 1800 (400 mg/kg only)	
Mice		
Intravenous	2 (50 mg/kg only), 5, 10, 15, 20 (50 mg/kg only), 30, 40 (50 mg/kg only), 45 (100 mg/kg only), 50 (50 mg/kg only), 60, 90, 120 (100 mg/kg only), 180 (100 mg/kg only), 240 (100 mg/kg only), and 360 (100 mg/kg only)	2, 5, 10, 15, 30, 45, 60, 90, 120, 180, and 240
Dermal	5, 10, 15, 30, 60, 90, 120, 180, 240, 360 (600 mg/kg only), 480, 600, and 720	

^a Three blood samples were collected at each time point.

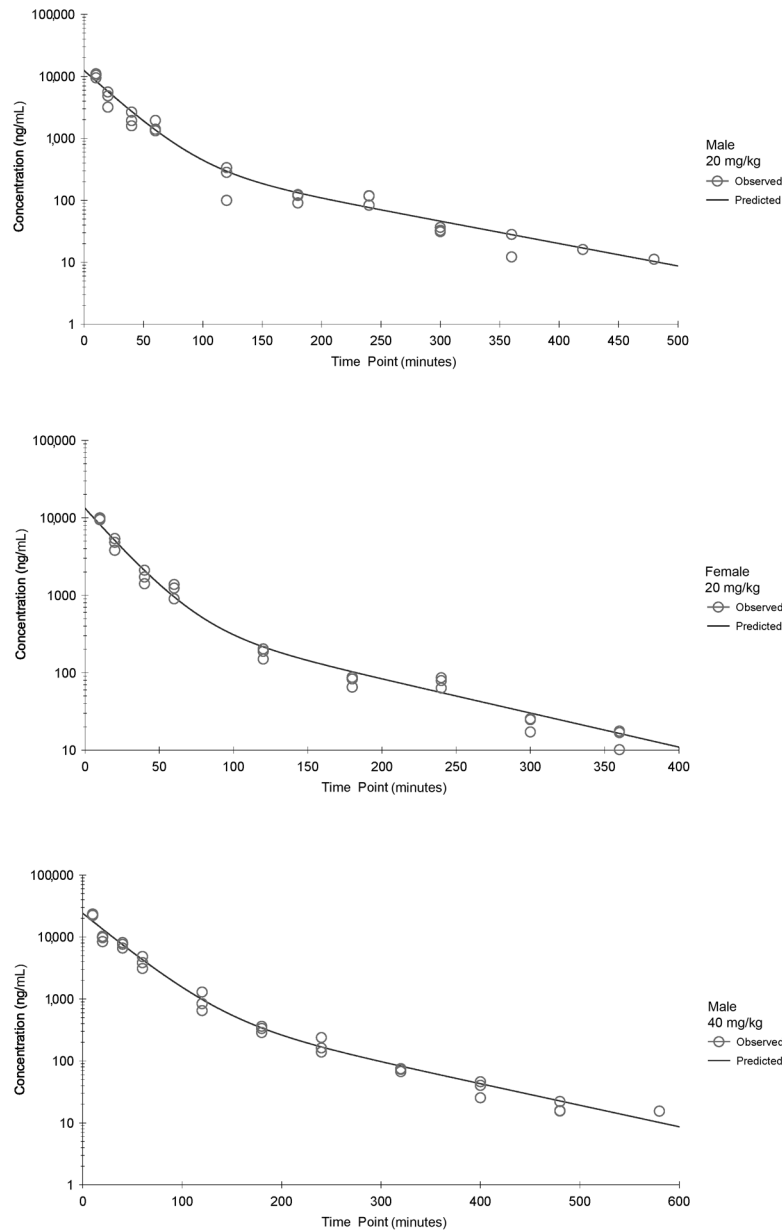


FIGURE L1
Bis(2-chloroethoxy)methane Plasma Concentration-Time Profiles
from a Two-Compartment Model with $1/Y_{hat}^2$ Weighting for F344/N Rats
Following a Single Intravenous Administration of Bis(2-chloroethoxy)methane
 $n \leq$ three plasma samples per time point

TABLE L3
Plasma Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Intravenous Administration of Bis(2-chloroethoxy)methane^a

Parameter	20 mg/kg	40 mg/kg
Male		
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	12.4 \pm 2.2	24.1 \pm 2.8
$t_{1/2\alpha}$ (minute)	17.0 \pm 2.3	22.6 \pm 2.0
$t_{1/2\beta}$ (minute)	83.4 \pm 10.3	86.7 \pm 7.9
k_{10} (minute^{-1})	0.0346 \pm 0.0041	0.0273 \pm 0.0020
k_{10} half-life (minute)	20.0 \pm 2.4	25.4 \pm 1.9
k_{12} (minute^{-1})	0.00460 \pm 0.00137	0.00238 \pm 0.00063
k_{21} (minute^{-1})	0.00976 \pm 0.00149	0.00898 \pm 0.00102
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	357 \pm 32	882 \pm 58
Cl ($\text{mL minute}^{-1} \text{ kg}^{-1}$)	55.9 \pm 5.1	45.4 \pm 3.0
MRT (minute)	42.5 \pm 3.3	46.4 \pm 2.3
V_1 (mL/kg)	1,620 \pm 290	1,660 \pm 190
V_2 (mL/kg)	762 \pm 143	440 \pm 71
Female		
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	13.2 \pm 2.3	
$t_{1/2\alpha}$ (minute)	13.7 \pm 1.7	
$t_{1/2\beta}$ (minute)	68.5 \pm 6.9	
k_{10} (minute^{-1})	0.0424 \pm 0.0046	
k_{10} half-life (minute)	16.3 \pm 1.8	
k_{12} (minute^{-1})	0.00611 \pm 0.00156	
k_{21} (minute^{-1})	0.0120 \pm 0.0015	
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	312 \pm 27	
Cl ($\text{mL minute}^{-1} \text{ kg}^{-1}$)	64.1 \pm 5.5	
MRT (minute)	35.5 \pm 2.6	
V_1 (mL/kg)	1,510 \pm 260	
V_2 (mL/kg)	767 \pm 126	

^a Based on a two-compartment model with bolus input, first-order output, and $1/Y_{\text{hat}}^2$ weighting; MRT =mean residence time. Estimate \pm standard error

^b C_{max} (predicted) based on the model prediction at time=0 minute

TABLE L4
Tissue Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for F344/N Rat
Following a Single Intravenous Administration of 20 mg/kg Bis(2-chloroethoxy)methane^a

Parameter	Heart	Thymus	Liver
Male			
C_{max} (observed) ($\mu\text{g/g}$)	12.6	48.7	3.89
T_{max} (observed) (minute)	14.9	14.8	43.7
Half-life (minute)	69.9	80.5	39.1
Female			
C_{max} (observed) ($\mu\text{g/g}$)	11.6	42.7	1.70
T_{max} (observed) (minute)	15.0	14.9	14.8
Half-life (minute)	68.1 ^b	164	25.7

^a Based on a noncompartmental model with bolus input, first-order output, and $1/Y^2$ weighting.

^b User-defined value used for half-life; noncompartmental analysis gave poor visual fit of terminal phase.

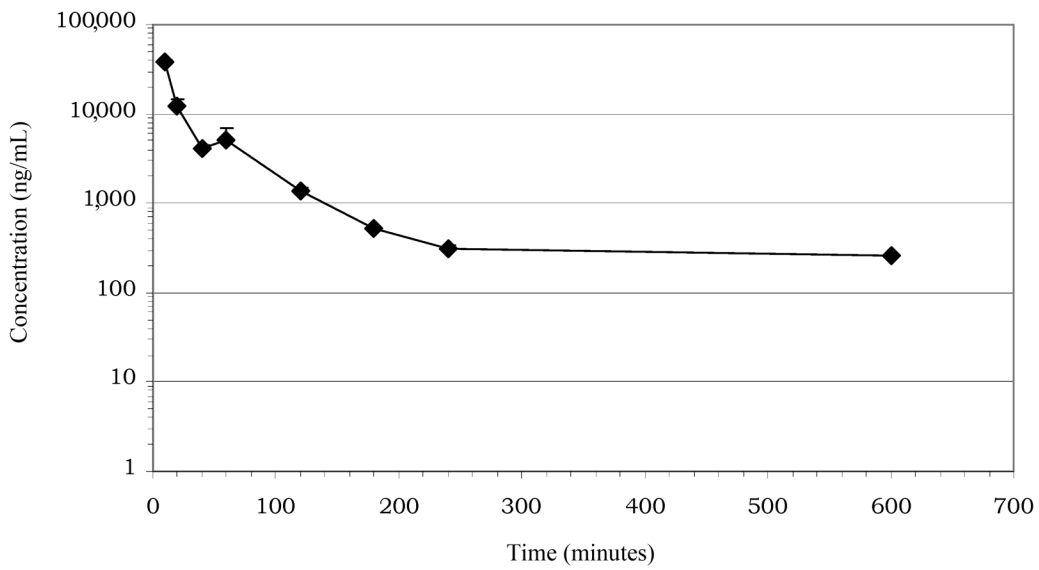
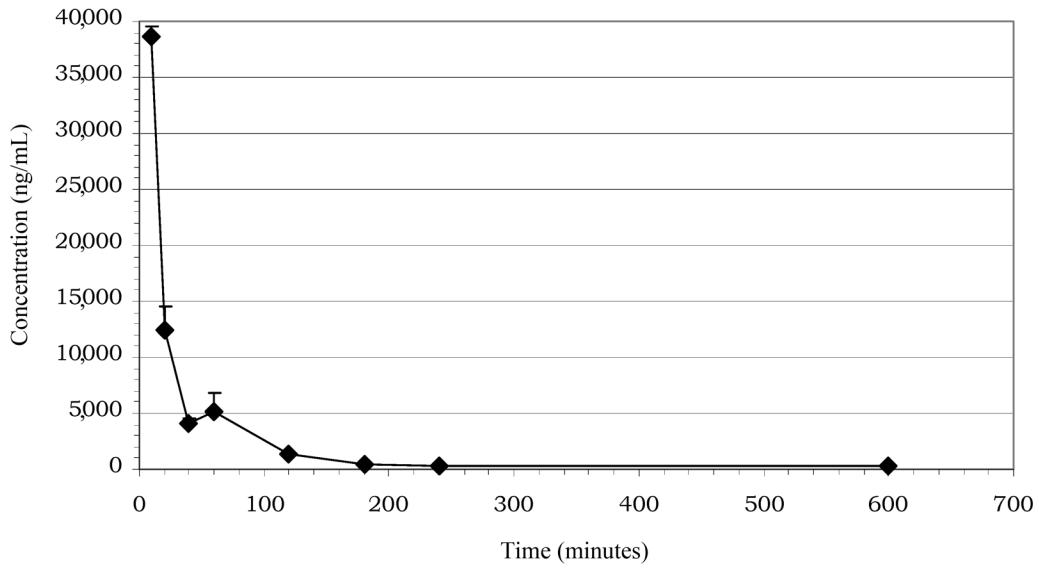


FIGURE L2
Plasma Concentrations of Thiodiglycolic Acid in Male F344/N Rats
Following a Single Intravenous Administration of 20 mg/kg Thiodiglycolic Acid
 $n \leq$ three plasma samples per time point

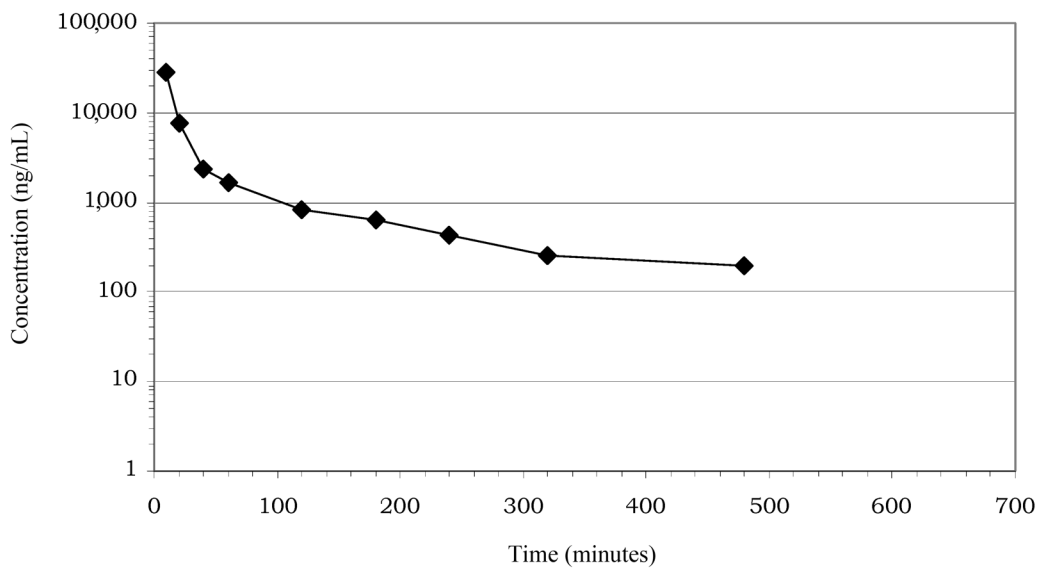
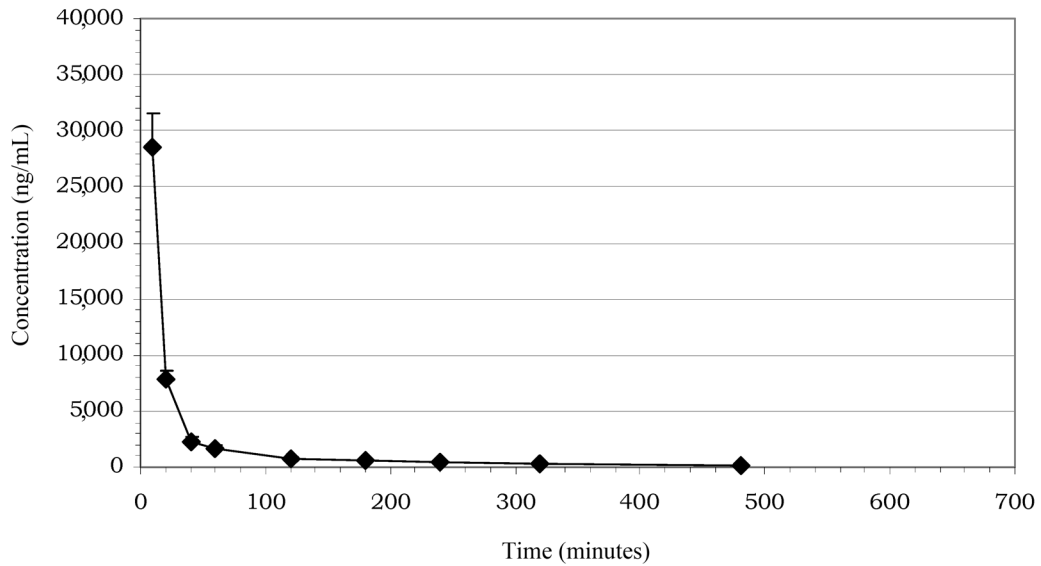


FIGURE L3
Plasma Concentrations of Thiodiglycolic Acid in Female F344/N Rats
Following a Single Intravenous Administration of 20 mg/kg Thiodiglycolic Acid
 $n \leq$ three plasma samples per time point

TABLE L5
Plasma Thiodiglycolic Acid Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Intravenous Administration of 20 mg/kg Thiodiglycolic Acid^a

Parameter	Male	Female
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	32.3 \pm 6.4	71.9 \pm 19.0
$t_{1/2\alpha}$ (minute)	22.0 \pm 3.0	6.35 \pm 0.90
$t_{1/2\beta}$ (minute)	649 \pm 628	129 \pm 15
k_{10} (minute^{-1})	0.0227 \pm 0.0059	0.0746 \pm 0.0125
k_{10} half-life (minute)	30.6 \pm 8.0	9.29 \pm 1.55
k_{12} (minute^{-1})	0.00849 \pm 0.00397	0.0320 \pm 0.0046
k_{21} (minute^{-1})	0.00149 \pm 0.00119	0.00786 \pm 0.00102
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	1,420 \pm 296	963 \pm 107
Cl (mL minute^{-1} kg^{-1})	14.0 \pm 2.9	20.8 \pm 2.3
MRT (minute)	296 \pm 374	67.9 \pm 10.4
V_1 (mL/kg)	619 \pm 123	278 \pm 74
V_2 (mL/kg)	3,530 \pm 4,490	1,130 \pm 275

^a Based on a two-compartment model with bolus input, first-order output, and $1/Y^2$ weighting; MRT =mean residence time. Estimate \pm standard error

^b C_{max} (predicted) based on the model prediction at time=0 minute

TABLE L6
Tissue Thiodiglycolic Acid Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Intravenous Administration of 20 mg/kg Thiodiglycolic Acid^a

Parameter	Heart	Thymus	Liver
Male			
C_{max} (observed) ($\mu\text{g/g}$)	8.93	8.70	83.0
T_{max} (observed) (minute)	15.5	15.7	25.2
Half-life (minute)	129	109	64.6
Female			
C_{max} (observed) ($\mu\text{g/g}$)	7.78	8.78	183
T_{max} (observed) (minute)	14.7	15.0	44.0
Half-life (minute)	147	149	125

^a Based on a noncompartmental model with bolus input, first-order output, and $1/Y^2$ weighting.

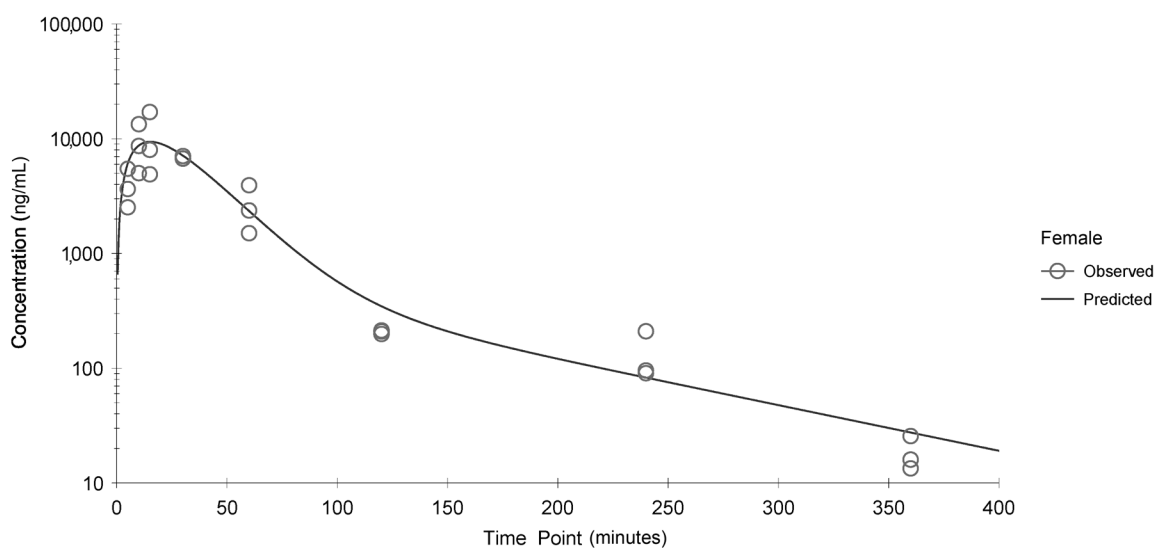
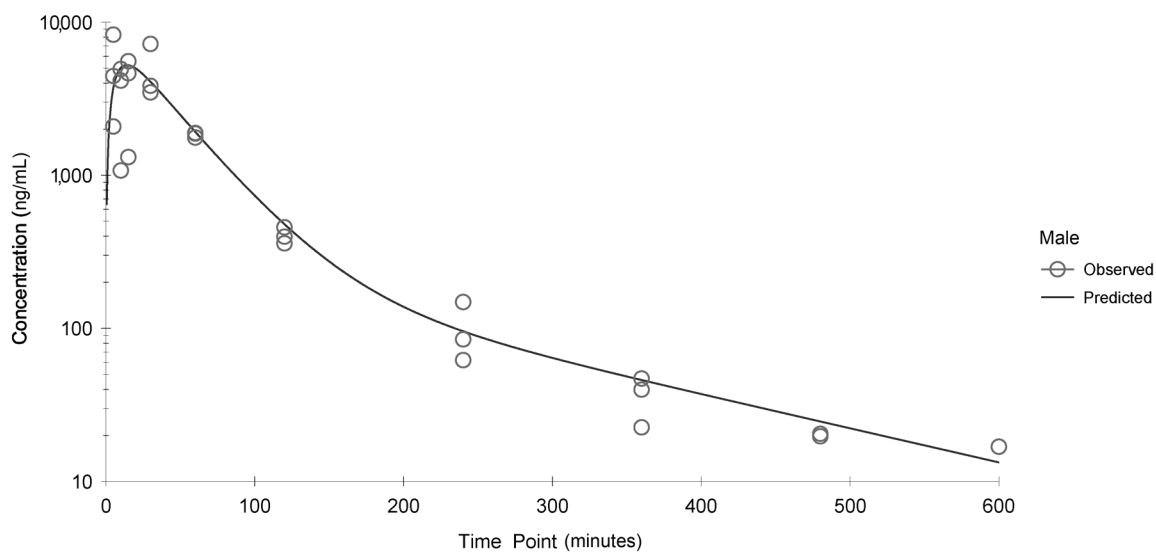


FIGURE L4
Bis(2-chloroethoxy)methane Plasma Concentration-Time Profiles
from a Two-Compartment Model with $1/Y_{hat}^2$ Weighting for F344/N Rats
Following a Single Dermal Application of 100 mg/kg Bis(2-chloroethoxy)methane
 $n \leq$ three plasma samples per time point

TABLE L7
Plasma Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	100 mg/kg	200 mg/kg	400 mg/kg
Male			
Absorption rate (minute ⁻¹) (k_{01})	0.148 ± 0.075	0.136 ± 0.050	0.0686 ± 0.0260
Absorption half-life (minute)	4.67 ± 2.37	5.10 ± 1.89	10.1 ± 3.8
C_{max} (predicted) (µg/mL)	5.26 ± 0.68	7.92 ± 0.87	12.2 ± 1.7
T_{max} (predicted) (minute)	14.1 ± 3.8	15.0 ± 3.2	31.7 ± 6.9
V_{1F} (mL/kg)	13,000 ± 3,100	17,000 ± 2,900	23,100 ± 5,000
V_{2F} (mL/kg)	6,630 ± 1,930	44,300 ± 30,100	21,700 ± 14,900
Cl_F (mL minute ⁻¹ kg ⁻¹)	312 ± 34	403 ± 42	239 ± 33
k_{12} (minute ⁻¹)	0.00303 ± 0.00149	0.00270 ± 0.00073	0.000722 ± 0.000161
k_{21} (minute ⁻¹)	0.00595 ± 0.00203	0.00104 ± 0.00046	0.000766 ± 0.000391
k_{10} (minute ⁻¹)	0.0240 ± 0.0047	0.0237 ± 0.0026	0.0104 ± 0.0012
k_{10} half-life (minute)	28.9 ± 5.6	29.2 ± 3.2	66.8 ± 7.7
$t_{1/2\alpha}$ (minute)	24.9 ± 5.6	26.1 ± 2.7	62.2 ± 6.8
$t_{1/2\beta}$ (minute)	135 ± 40	749 ± 348	973 ± 501
AUC_{∞} (predicted) (µg mL ⁻¹ minute)	320 ± 35	496 ± 52	1,670 ± 230
AUC_{∞} (predicted)/Dose (µg minute mL ⁻¹ kg mg ⁻¹)	3.20 ± 0.35	2.48 ± 0.26	4.2 ± 0.6
Female			
Absorption rate (minute ⁻¹) (k_{01})	0.0860 ± 0.0852	0.0586 ± 0.0496	0.0924 ± 0.0380
Absorption half-life (minute)	8.06 ± 7.98	11.8 ± 10.0	7.50 ± 3.08
C_{max} (predicted) (µg/mL)	9.41 ± 1.48	9.97 ± 1.24	15.0 ± 2.2
T_{max} (predicted) (minute)	15.2 ± 3.4	20.9 ± 3.4	27.2 ± 6.9
V_{1F} (mL/kg)	5,020 ± 3,900	8,950 ± 6,460	20,500 ± 4,300
V_{2F} (mL/kg)	2,590 ± 1,170	4,070 ± 1,840	9,520 ± 3,710
Cl_F (mL minute ⁻¹ kg ⁻¹)	223 ± 31	321 ± 43	188 ± 25
k_{12} (minute ⁻¹)	0.00544 ± 0.00789	0.00304 ± 0.00401	0.000608 ± 0.000179
k_{21} (minute ⁻¹)	0.0106 ± 0.0051	0.00670 ± 0.00272	0.00131 ± 0.00049
k_{10} (minute ⁻¹)	0.0445 ± 0.0323	0.0358 ± 0.0250	0.00914 ± 0.00106
k_{10} half-life (minute)	15.6 ± 11.3	19.4 ± 13.5	75.8 ± 8.8
$t_{1/2\alpha}$ (minute)	13.5 ± 10.8	17.6 ± 13.0	70.4 ± 8.5
$t_{1/2\beta}$ (minute)	75.7 ± 29.3	114 ± 40	570 ± 205
AUC_{∞} (predicted) (µg mL ⁻¹ minute)	448 ± 63	624 ± 84	2,130 ± 280
AUC_{∞} (predicted)/Dose (µg minute mL ⁻¹ kg mg ⁻¹)	4.48 ± 0.63	3.12 ± 0.42	5.3 ± 0.7

^a Based on a two-compartment model with first-order input, first-order output, and 1/Yhat² weighting. Estimate ± standard error

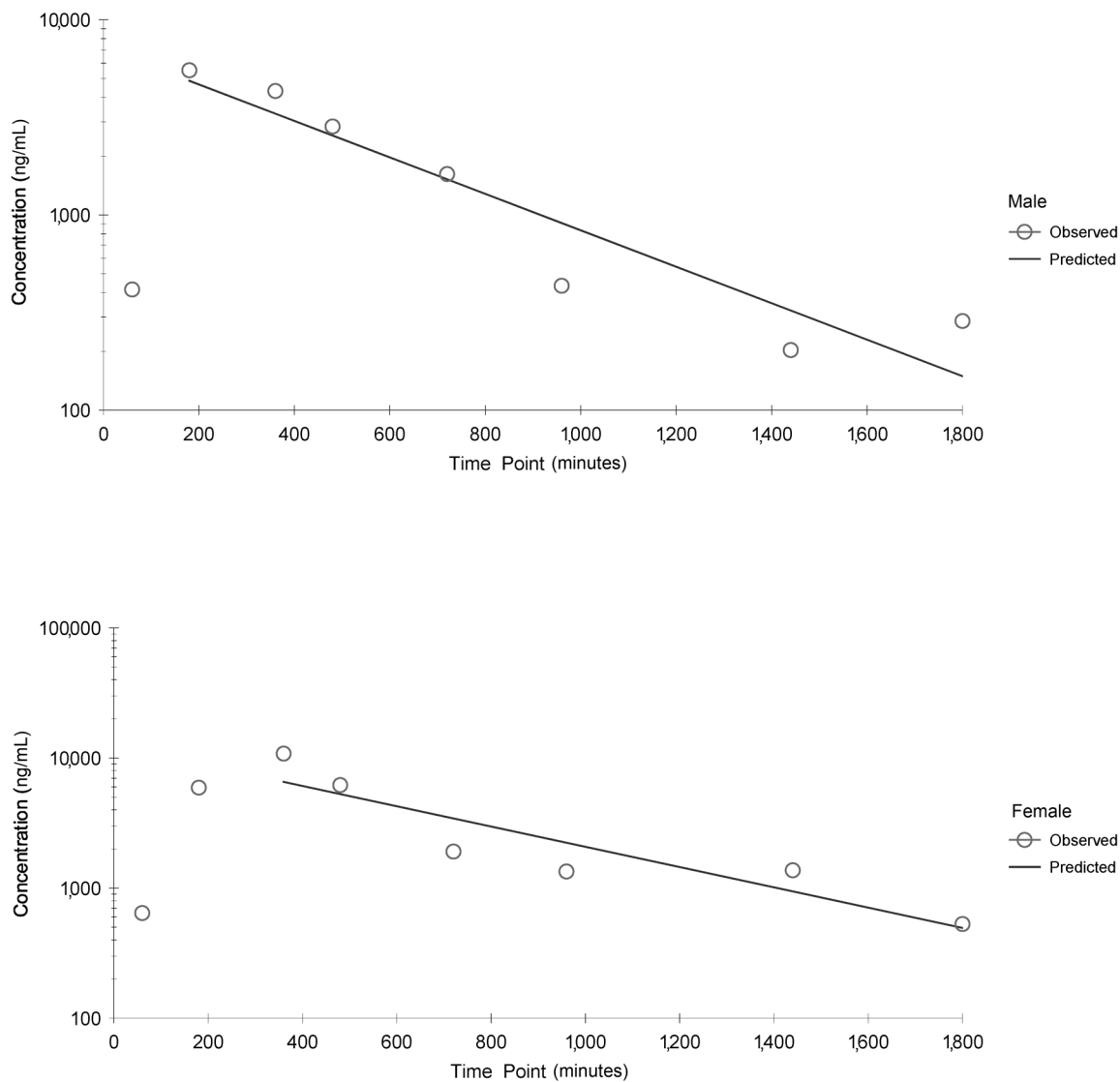


FIGURE L5
Thiodiglycolic Acid Plasma Concentration-Time Profiles from a Noncompartmental Model with Uniform Weighting for F344/N Rats Following a Single Dermal Application of 400 mg/kg Bis(2-chloroethoxy)methane
n ≤ three plasma samples per time point

TABLE L8
Plasma Thiodiglycolic Acid Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Dermal Application of 400 mg/kg Bis(2-chloroethoxy)methane^a

Parameter	Male	Female
C_{max} (observed) ($\mu\text{g/mL}$)	5.51	10.8
T_{max} (observed) (minute)	180	360
Half-life (minute)	322	386

^a Based on a noncompartmental model with first-order input, first-order output, and uniform weighting.

TABLE L9
Tissue Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	Heart		Thymus		Liver	
	100 mg/kg	400 mg/kg	100 mg/kg	400 mg/kg	100 mg/kg	400 mg/kg
Male						
C_{max} (observed) ($\mu\text{g/g}$)	2.95	18.2	6.07	28.7	1.66	11.1
T_{max} (observed) (minute)	34.0	34.7	34.0	34.7	34.0	64.3
Half-life (minute)	71.5	91.0	65.5	175	58.4	86.1
Female						
C_{max} (observed) ($\mu\text{g/g}$)	8.87	20.4	8.37	27.6	2.26	14.5
T_{max} (observed) (minute)	15.5	34.7	15.6	34.3	34.3	64.0
Half-life (minute)	63.0	387	43.8	187	46.7	199

^a Based on a noncompartmental model with first-order input, first order-output, and uniform weighting.

TABLE L10
Tissue Thiodiglycolic Acid Toxicokinetic Parameter Estimates for F344/N Rats
Following a Single Dermal Application of 400 mg/kg Bis(2-chloroethoxy)methane^a

Parameter	Heart	Thymus	Liver
Male			
C_{max} (observed) ($\mu\text{g/g}$)	3.35	5.47	84.4
T_{max} (observed) (minute)	360	480	360
Half-life (minute)	422	853	296
Female			
C_{max} (observed) ($\mu\text{g/g}$)	6.80	11.0	123
T_{max} (observed) (minute)	360	480	480
Half-life (minute)	548	652	373

^a Based on a noncompartmental model with first-order input, first-order output, and uniform weighting.

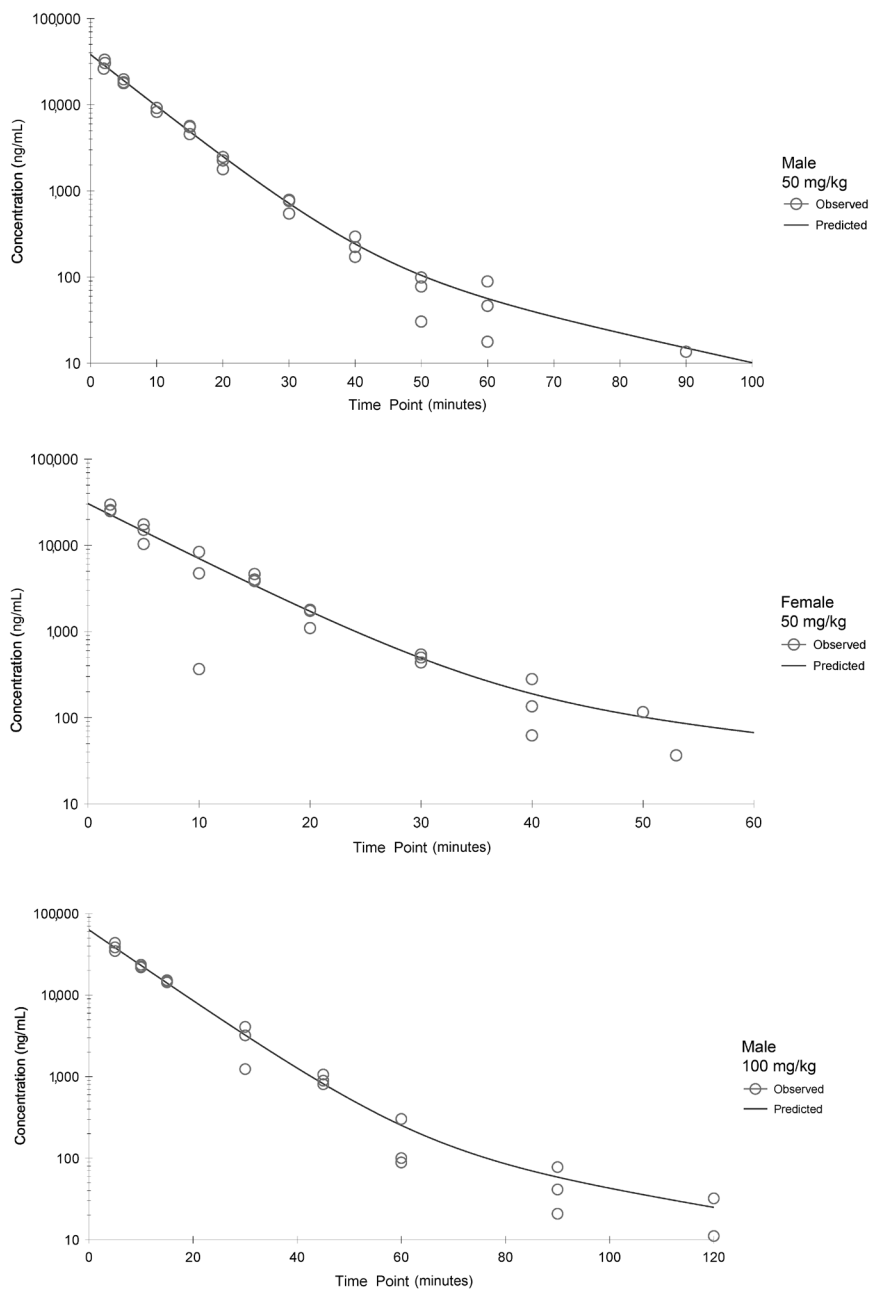


FIGURE L6
Bis(2-chloroethoxy)methane Plasma Concentration-Time Profiles
from a Two-Compartment Model with $1/Y_{hat}^2$ Weighting
for B6C3F1 Mice Following a Single Intravenous Administration
of 50 mg/kg Bis(2-chloroethoxy)methane
 $n \leq$ three plasma samples per time point

TABLE L11
Plasma Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Intravenous Administration of Bis(2-chloroethoxy)methane^a

Parameter	50 mg/kg	100 mg/kg
Male		
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	38.2 \pm 4.7	63.1 \pm 10.5
$t_{1/2\alpha}$ (minute)	4.94 \pm 0.34	6.85 \pm 0.58
$t_{1/2\beta}$ (minute)	17.8 \pm 5.0	28.0 \pm 13.2
k_{10} (minute ⁻¹)	0.136 \pm 0.008	0.0989 \pm 0.0074
k_{10} half-life (minute)	5.11 \pm 0.30	7.01 \pm 0.52
k_{12} (minute ⁻¹)	0.00327 \pm 0.00130	0.00174 \pm 0.00102
k_{21} (minute ⁻¹)	0.0402 \pm 0.0118	0.0253 \pm 0.0123
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	282 \pm 23	638 \pm 72
Cl (mL minute^{-1} kg^{-1})	178 \pm 14	157 \pm 18
MRT (minute)	7.97 \pm 0.41	10.8 \pm 0.7
V_1 (mL/kg)	1,310 \pm 160	1,590 \pm 260
V_2 (mL/kg)	106 \pm 23	109 \pm 28
Female		
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	30.4 \pm 5.8	
$t_{1/2\alpha}$ (minute)	4.63 \pm 0.72	
$t_{1/2\beta}$ (minute)	23.7 \pm 47.7	
k_{10} (minute ⁻¹)	0.142 \pm 0.018	
k_{10} half-life (minute)	4.86 \pm 0.62	
k_{12} (minute ⁻¹)	0.00561 \pm 0.00248	
k_{21} (minute ⁻¹)	0.0307 \pm 0.0628	
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	214 \pm 23	
Cl (mL minute^{-1} kg^{-1})	234 \pm 25	
MRT (minute)	8.30 \pm 3.21	
V_1 (mL/kg)	1,640 \pm 320	
V_2 (mL/kg)	300 \pm 585	

^a Based on a two-compartment model with bolus input, first-order output, and $1/Y_{\text{hat}}^2$ weighting; MRT =mean residence time. Estimate \pm standard error

^b C_{max} (predicted) based on the model prediction at time=0 minute

TABLE L12
Tissue Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Intravenous Administration of 50 mg/kg Bis(2-chloroethoxy)methane^a

Parameter	Heart	Thymus	Liver
Male			
C_{max} (observed) ($\mu\text{g/g}$)	39.3	32.8	1.65
T_{max} (observed) (minute)	3.90	3.90	6.96
Half-life (minute)	8.88	7.91	7.66
Female			
C_{max} (observed) ($\mu\text{g/g}$)	34.6	30.3	0.164
T_{max} (observed) (minute)	4.19	4.19	3.52
Half-life (minute)	6.98	5.74	15.2

^a Based on a noncompartmental model with bolus input, first-order output, and uniform weighting.

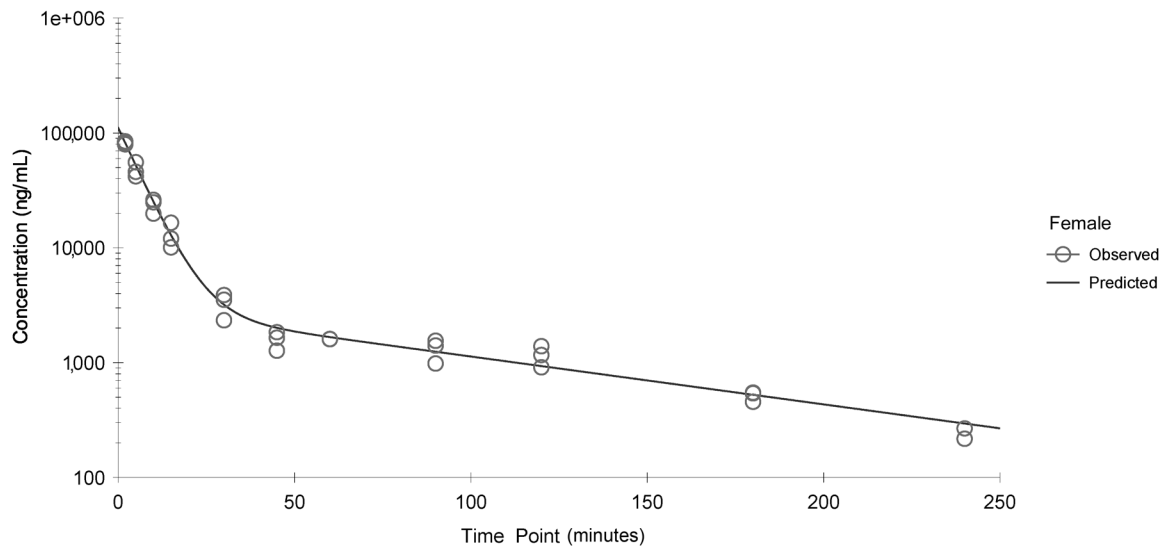
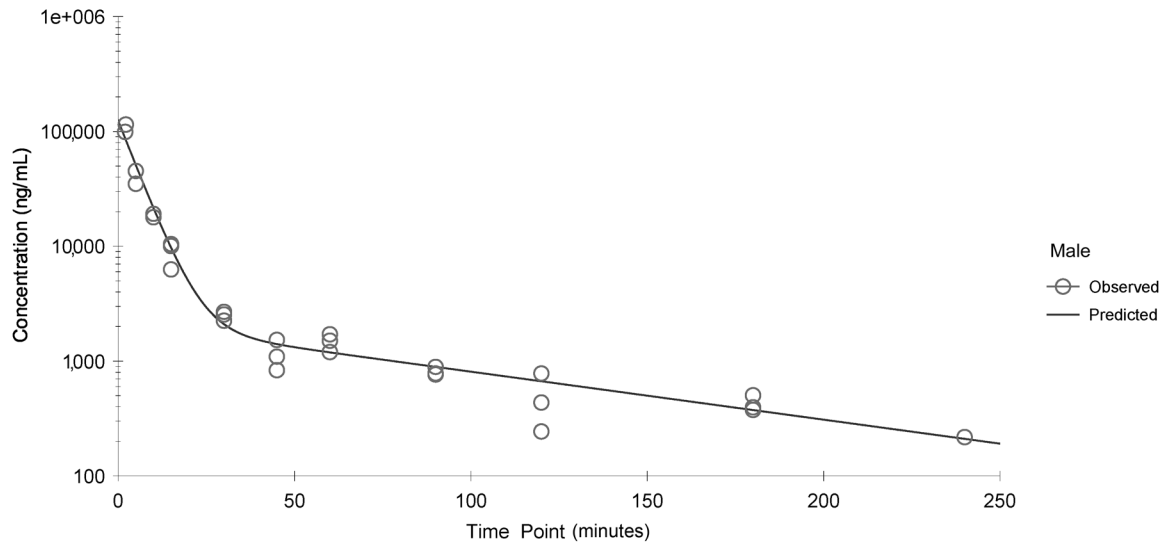


FIGURE L7
Thiodiglycolic Acid Plasma Concentration-Time Profiles from a Two-Compartment Model with $1/Y_{\text{hat}}^2$ Weighting for B6C3F1 Mice Following a Single Intravenous Administration of 50 mg/kg Thiodiglycolic Acid
 $n \leq$ three plasma samples per time point

TABLE L13
Plasma Thiodiglycolic Acid Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Intravenous Administration of 50 mg/kg Thiodiglycolic Acid^a

Parameter	Male	Female
C_{max} (predicted) ($\mu\text{g/mL}$) ^b	124 \pm 18	111 \pm 11
$t_{1/2\alpha}$ (minute)	3.80 \pm 0.33	4.43 \pm 0.32
$t_{1/2\beta}$ (minute)	72.1 \pm 8.1	72.2 \pm 5.7
k_{10} (minute^{-1})	0.140 \pm 0.013	0.111 \pm 0.008
k_{10} half-life (minute)	4.97 \pm 0.46	6.23 \pm 0.45
k_{12} (minute^{-1})	0.0399 \pm 0.0045	0.0414 \pm 0.0041
k_{21} (minute^{-1})	0.0126 \pm 0.0014	0.0135 \pm 0.0012
AUC_{∞} (predicted) ($\mu\text{g mL}^{-1}$ minute)	888 \pm 63	997 \pm 46
Cl (mL minute^{-1} kg^{-1})	56.3 \pm 4.0	50.2 \pm 2.3
MRT (minute)	29.9 \pm 3.6	36.6 \pm 2.9
V_1 (mL/kg)	403 \pm 58	451 \pm 46
V_2 (mL/kg)	1,280 \pm 230	1,380 \pm 160

^a Based on a two-compartment model with bolus input, first-order output, and $1/Y_{\text{hat}}^2$ weighting; MRT =mean residence time.
 Estimate \pm standard error

^b C_{max} (predicted) based on the model prediction at time=0 minute

TABLE L14
Tissue Thiodiglycolic Acid Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Intravenous Administration of 50 mg/kg Thiodiglycolic Acid^a

Parameter	Heart	Thymus	Liver
Male			
C_{max} (observed) ($\mu\text{g/g}$)	40.4	23.9	78.3
T_{max} (observed) (minute)	3.76	6.93	16.3
Half-life (minute)	92.8	128	51.1
Female			
C_{max} (observed) ($\mu\text{g/g}$)	37.9	38.9	96.6
T_{max} (observed) (minute)	3.89	3.89	16.6
Half-life (minute)	73.4	181	52.0

^a Based on a noncompartmental model with bolus input, first-order output, and uniform weighting.

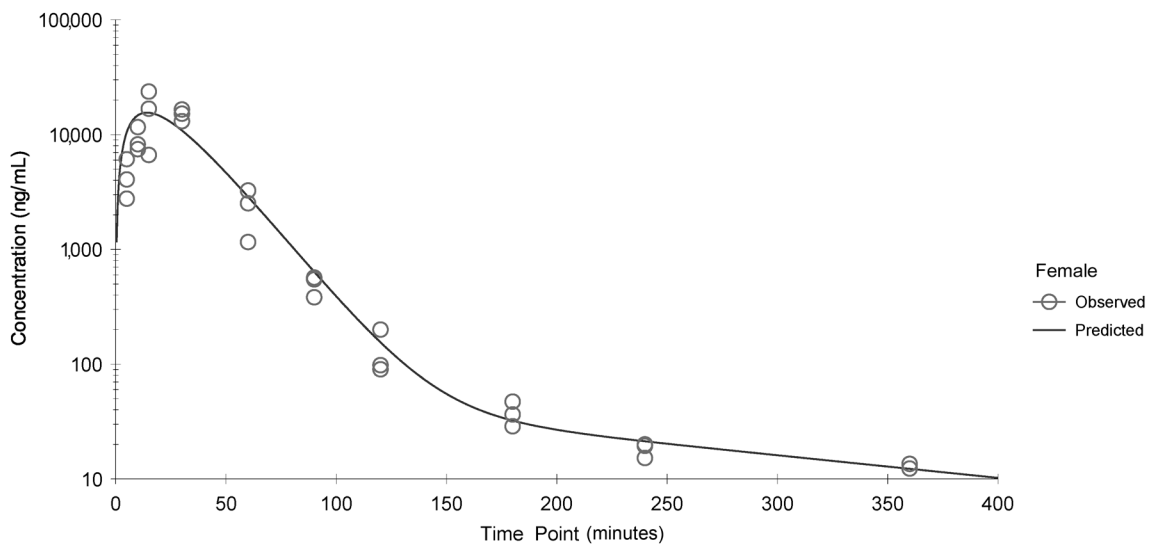
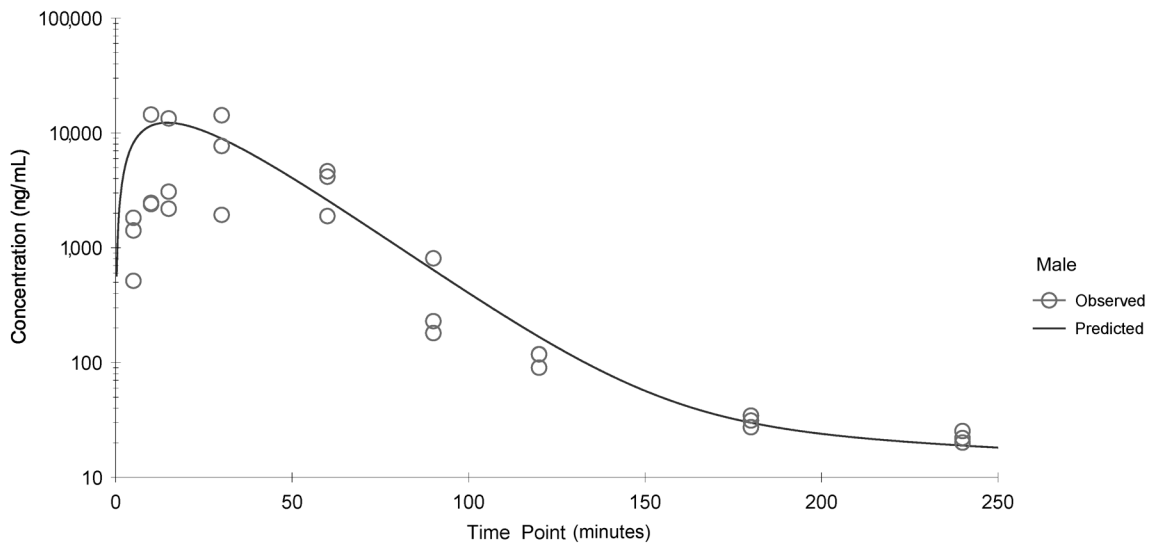


FIGURE L8
Bis(2-chloroethoxy)methane Plasma Concentration-Time Profiles
from a Two-Compartment Model with $1/\hat{Y}^2$ Weighting for B6C3F1 Mice
Following a Single Dermal Application of 600 mg/kg Bis(2-chloroethoxy)methane
 $n \leq$ three plasma samples per time point

TABLE L15
Plasma Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	300 mg/kg	450 mg/kg	600 mg/kg
Male			
Absorption rate (minute ⁻¹) (k_{01})	0.114 ± 0.114	0.0991 ± 0.0505	0.0918 ± 0.0555
Absorption half-life (minute)	6.07 ± 6.05	7.00 ± 3.56	7.55 ± 4.56
C_{max} (predicted) (µg/mL)	2.42 ± 0.50	4.11 ± 0.50	12.3 ± 2.1
T_{max} (predicted) (minute)	11.9 ± 3.7	13.7 ± 2.3	14.6 ± 3.1
V_{1F} (mL/kg)	60,700 ± 40,400	53,500 ± 18,300	23,700 ± 9,400
V_{2F} (mL/kg)	154,000 ± 612,000	80,600 ± 427,000	6,470 ± 33,200
Cl_F (mL minute ⁻¹ kg ⁻¹)	3,080 ± 970	2,590 ± 480	1,150 ± 180
k_{12} (minute ⁻¹)	0.00941 ± 0.01064	0.00399 ± 0.00679	0.000979 ± 0.001271
k_{21} (minute ⁻¹)	0.00370 ± 0.00978	0.00265 ± 0.00903	0.00358 ± 0.01320
k_{10} (minute ⁻¹)	0.0507 ± 0.0398	0.0484 ± 0.0204	0.0488 ± 0.0157
k_{10} half-life (minute)	13.7 ± 10.7	14.3 ± 6.0	14.2 ± 4.6
$t_{1/2\alpha}$ (minute)	11.4 ± 7.0	13.2 ± 3.9	13.9 ± 4.2
$t_{1/2\beta}$ (minute)	224 ± 645	284 ± 1,010	198 ± 734
AUC_{∞} (predicted) (µg mL ⁻¹ minute)	97.5 ± 30.6	174 ± 32	520 ± 80
AUC_{∞} (predicted)/Dose (µg minute mL ⁻¹ kg mg ⁻¹)	0.325 ± 0.102	0.387 ± 0.071	0.867 ± 0.133
Female			
Absorption rate (minute ⁻¹) (k_{01})	0.137 ± 0.094		0.0900 ± 0.0342
Absorption half-life (minute)	5.06 ± 3.46		7.71 ± 2.93
C_{max} (predicted) (µg/mL)	5.58 ± 0.92		15.5 ± 1.8
T_{max} (predicted) (minute)	9.36 ± 2.01		14.1 ± 1.8
V_{1F} (mL/kg)	25,000 ± 10,800		17,900 ± 4,600
V_{2F} (mL/kg)	10,600 ± 5,100		3,730 ± 2,210
Cl_F (mL minute ⁻¹ kg ⁻¹)	1,960 ± 260		959 ± 98
k_{12} (minute ⁻¹)	0.00377 ± 0.00213		0.000965 ± 0.000264
k_{21} (minute ⁻¹)	0.00886 ± 0.00435		0.00462 ± 0.00240
k_{10} (minute ⁻¹)	0.0782 ± 0.0291		0.0536 ± 0.0103
k_{10} half-life (minute)	8.87 ± 3.30		12.9 ± 2.5
$t_{1/2\alpha}$ (minute)	8.41 ± 3.18		12.7 ± 2.4
$t_{1/2\beta}$ (minute)	82.4 ± 39.7		153 ± 79
AUC_{∞} (predicted) (µg mL ⁻¹ minute)	153 ± 20		626 ± 64
AUC_{∞} (predicted)/dose (µg minute mL ⁻¹ kg mg ⁻¹)	0.510 ± 0.067		1.04 ± 0.11

^a Based on a two-compartment model with first-order input, first-order output, and 1/Yhat² weighting. Estimate ± standard error

TABLE L16
Plasma Thiodiglycolic Acid Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	300 mg/kg	450 mg/kg	600 mg/kg
Male			
C_{max} (observed) ($\mu\text{g/mL}$)	2.32	5.82	7.78
T_{max} (observed) (minute)	240	90	240
Half-life (minute)	188	195	205
Female			
C_{max} (observed) ($\mu\text{g/mL}$)	1.95		4.33
T_{max} (observed) (minute)	90		90
Half-life (minute)	185		214

^a Based on a noncompartmental model with first-order input, first-order output, and uniform weighting

TABLE L17
Tissue Bis(2-chloroethoxy)methane Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	Heart		Thymus		Liver	
	300 mg/kg	600 mg/kg	300 mg/kg	600 mg/kg	300 mg/kg	600 mg/kg
Male						
C_{max} (observed) ($\mu\text{g/g}$)	2.51	9.95	3.00	14.0	3.15	13.2
T_{max} (observed) (minute)	18.0	14.0	13.2	13.9	17.6	32.3
Half-life (minute)	23.5	25.5	14.7	14.5	26.4	44.3
Female						
C_{max} (observed) ($\mu\text{g/g}$)	6.63	17.5	5.88	13.7	3.49	15.6
T_{max} (observed) (minute)	14.5	33.0	14.4	18.6	14.1	32.3
Half-life (minute)	10.8	61.9	14.3	16.7	24.0	30.7

^a Based on a noncompartmental model with first-order input, first-order output, and uniform weighting

TABLE L18
Tissue Thiodiglycolic Acid Toxicokinetic Parameter Estimates for B6C3F1 Mice
Following a Single Dermal Application of Bis(2-chloroethoxy)methane^a

Parameter	Heart		Thymus		Liver	
	300 mg/kg	600 mg/kg	300 mg/kg	600 mg/kg	300 mg/kg	600 mg/kg
Male						
C_{max} (observed) (µg/g)	0.731	3.12	2.35	10.0	48.2	88.2
T_{max} (observed) (minute)	180	240	180	480	90	120
Half-life (minute)	448	169	746	542	140	145
Female						
C_{max} (observed) (µg/g)	0.833	2.64	2.94	6.76	56.9	93.4
T_{max} (observed) (minute)	240	240	240	480	90	120
Half-life (minute)	340	218	447	300	133	140

^a Based on a noncompartmental model with first-order input, first-order output, and uniform weighting.



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