NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Bromodichloromethane (CAS No. 75-27-4) in Male F344/N Rats and Female B6C3F$_1$ Mice (Drinking Water Studies)

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FEBRUARY 2006

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF BROMODICHLOROMETHANE

(CAS NO. 75-27-4)

IN MALE F344/N RATS AND FEMALE B6C3F$_1$ MICE

(DRINKING WATER STUDIES)

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

February 2006

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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 Food and Drug Administration (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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CONTENTS

SUMMARY

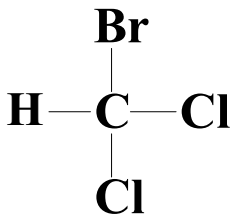
Background
Bromodichloromethane occurs as a by-product of the chlorination of drinking water. In earlier studies, bromodichloromethane caused cancer of the intestine and kidney in rats and of the liver and kidney in mice when doses of the chemical dissolved in corn oil were deposited in the stomachs of the animals. We studied the effects of bromodichloromethane in drinking water on male rats and female mice to see if the same effects occurred.

Methods
We gave drinking water containing 175, 350, or 700 mg of bromodichlomethane per liter of water to groups of 50 male rats and female mice for two years. Control animals received the same tap water with no chemical added. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
Body weights and survival of animals receiving bromodichloromethane were similar to those of the control animals. No cancers or nonneoplastic lesions occurred more frequently as a result of exposure to bromodichloromethane in drinking water.

Conclusions
We conclude that bromodichloromethane in the drinking water did not cause cancer in male rats or female mice.

ABSTRACT



BROMODICHLOROMETHANE

CAS No. 75-27-4

Chemical Formula: $CHBrCl_2$ Molecular Weight: 163.83

Synonym: Dichlorobromomethane

Bromodichloromethane is a by-product of the chlorination of drinking water. It is formed by the halogen substitution and oxidation reactions of chlorine with naturally occurring organic matter (e.g., humic or fulvic acids) in water containing bromide. Bromodichloromethane has been shown to be carcinogenic at multiple sites in rats (large intestine and kidney) and in mice (liver and kidney) after administration by gavage in corn oil. To further characterize its dose-response relationships for evaluations of human risk, bromodichloromethane was nominated to the NTP by the United States Environmental Protection Agency for toxicity and carcinogenicity studies in rats and mice by drinking water exposure. Male F344/N rats and female $B6C3F_1$ mice were exposed to bromodichloromethane (greater than 98% pure) in drinking water for 3 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium,* L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, mouse bone marrow cells, and mouse peripheral blood erythrocytes.

3-Week Study in Rats

Groups of 10 male F344/N rats were exposed to target concentrations of 0, 43.7, 87.5, 175, 350, or 700 mg/L bromodichloromethane (equivalent to average daily doses of approximately 0, 6, 12, 20, 38, or 71 mg bromodichloromethane/kg body weight) in drinking water for 3 weeks. All rats survived to the end of the study. The mean body weight gains of 350 and 700 mg/L rats were significantly less than that of the controls. Concentration-related decreases in water consumption were evident during the first week on study. Relative kidney weights of rats in the 175, 350, and 700 mg/L groups were significantly greater than that of the controls. There were no significant chemical-related histopathological changes.

3-Week Study in Mice

Groups of 10 female $B6C3F_1$ mice were exposed to target concentrations of 0, 43.7, 87.5, 175, 350, or 700 mg/L bromodichloromethane (equivalent to average daily doses of approximately 0, 6, 10, 16, 29 or 51 mg/kg) in drinking water for 3 weeks. All mice survived to the end of the study. Final mean body weights of the 175, 350, and 700 mg/L mice and mean body weight gains of 350 and 700 mg/L mice were significantly less than those of the controls. These decreases were attributed to decreased water consumption. There

were significant concentration-related decreases in water consumption by groups exposed to 87.5 mg/L or greater throughout the study; these decreases were attributed to poor palatability of the dosed water. Relative liver, kidney, and thymus weights of mice in the 350 and 700 mg/L groups were significantly greater than those of the controls. Absolute lung weights of mice in the 350 and 750 mg/L groups were significantly less than that of the controls. There were no significant chemical-related histopathological changes.

2-Year Study in Rats

Groups of 50 male F344/N rats were exposed to target concentrations of 0, 175, 350, or 700 mg/L bromodichloromethane (equivalent to average daily doses of approximately 0, 6, 12, or 25 mg/kg) in drinking water for 2 years. Survival of exposed groups was similar to that of the controls. Mean body weights of all exposed groups were generally similar to those of the controls throughout the study. Water consumption by exposed rats was less than that by the controls throughout the study; the decreases were attributed to poor palatability of the dosed water.

There were no increased incidences of neoplasms that were attributed to bromodichloromethane. The incidences of chronic inflammation in the liver of the 350 and 700 mg/L groups were significantly greater than that in the controls; however, the biological significance of these increases is uncertain.

2-Year Study in Mice

Groups of 50 female B6C3F$_1$ mice were exposed to target concentrations of 0, 175, 350, 700 mg/L bromodichloromethane (equivalent to average daily doses of approximately 9, 18, or 36 mg/kg) in drinking water for 2 years. Survival of exposed groups was similar to that of the controls. Mean body weights of all exposed groups were generally less than those of the controls from week 4 through the end of the study. Water consumption by exposed mice was less than that by the controls throughout the study; the decreases were attributed to poor palatability of the dosed water.

The incidences of hepatocellular adenoma or carcinoma (combined) occurred with a negative trend, and the incidence in the 700 mg/L group was significantly decreased relative to the control group. The incidence of hemangiosarcoma in all organs was significantly decreased in the 350 mg/L group.

Genetic Toxicology

The results of *in vitro* mutagenicity tests with bromodichloromethane were mixed. Bromodichloromethane did not induce mutations in any of several tester strains of *Salmonella typhimurium*, with or without exogenous metabolic activation (S9 liver enzymes). In contrast to the negative results in *Salmonella*, tests for mutation induction in mouse lymphoma L5178Y/tk$^{+/-}$ cells were positive in the presence of induced rat liver S9; no mutagenic activity occurred in tests conducted without S9. In cytogenetic tests with cultured Chinese hamster ovary cells, bromodichloromethane induced a small increase in sister chromatid exchanges (SCEs) in one of four trials conducted in the presence of induced rat liver S9 enzymes; no significant increase in SCEs occurred without S9, and no induction of chromosomal aberrations occurred in bromodichloromethane-treated Chinese hamster ovary cells, with or without S9.

Results of *in vivo* tests for chromosomal damage were negative. No increases in the frequency of micronucleated erythrocytes were seen in bone marrow of male B6C3F$_1$ mice administered bromodichloromethane by intraperitoneal injection for 3 days. In addition, no induction of micronuclei was observed in circulating erythrocytes of female B6C3F$_1$ mice administered up to 700 mg/L bromodichloromethane in drinking water for 3 weeks.

Conclusions

Under the conditions of this 2-year drinking water study, there was *no evidence of carcinogenic activity** of bromodichloromethane in male F344/N rats exposed to target concentrations of 175, 350, or 700 mg/L. There was *no evidence of carcinogenic activity* of bromodichloromethane in female B6C3F$_1$ mice exposed to target concentrations of 175, 350, or 700 mg/L.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Report Review Subcommittee comments and public discussion on this technical report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Bromodichloromethane

	Male F344/N Rats	Female B6C3F$_1$ Mice
Concentrations in drinking water	0, 175, 350, or 700 mg/L	0, 175, 350, or 700 mg/L
Body weights	Exposed groups similar to the control group	Exposed groups less than the control group
Survival rates	29/50, 28/50, 29/50, 26/50	36/50, 36/50, 33/50, 39/50
Nonneoplastic effects	None	None
Neoplastic effects	None	None
Equivocal findings	None	None
Decreased incidences	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence
Genetic toxicology		
Salmonella typhimurium gene mutations:	Experiment 1: negative in TA100, TA1535, TA1537, and TA98 with and without S9 Experiment 2: negative in TA100, TA1535, TA97, and TA98 with and without S9	
Mouse lymphoma gene mutations:	Negative without S9, positive with S9	
Sister chromatid exchanges		
Cultured Chinese hamster ovary cells *in vitro:*	Negative without S9, equivocal with S9	
Chromosomal aberrations		
Cultured Chinese hamster ovary cells *in vitro:*	Negative with and without S9	
Micronucleated erythrocytes		
Mouse bone marrow *in vivo:*	Negative	
Mouse peripheral blood *in vivo:*	Negative	

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on bromodichloromethane on December 9, 2004, 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 December 9, 2004, the draft Technical Report on the toxicology and carcinogenesis studies of bromodichloromethane received public review by the National Toxicology Program's Board of Scientific Counselor's Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of bromodichloromethane by describing its occurrence in drinking water as a by-product of disinfection. He described a previous NTP study where bromodichloromethane given by gavage was carcinogenic at multiple sites in rats and mice. He described the design of subsequent drinking water studies and presented results of several physiologically based pharmacokinetic models of dose response. He attributed the difference in tumor response by the two routes to a variety of factors, including differences in organ dosimetry, diet, and body weight. The proposed conclusions were *no evidence of carcinogenic activity* in male F344/N rats and female B6C3F$_1$ mice exposed to 175, 350, or 700 mg/L bromodichloromethane.

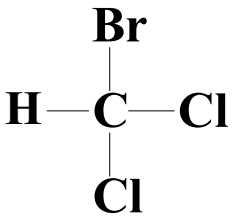
Dr. Vore, the first principal reviewer, thought the study was well designed and she had no scientific criticism.

Dr. Klaunig, the second principal reviewer, thought the study was well designed and said the discussion about the differences between the two routes of administration was good.

Dr. Birt, the third principal reviewer, agreed with the conclusions and offered some suggestions for clarifying the dose selection rationale and the in-life results.

Dr. Vore moved and Dr. Klaunig seconded that the conclusions be accepted as written. The motion was carried unanimously with nine votes.

INTRODUCTION



BROMODICHLOROMETHANE

CAS No. 75-27-4

Chemical Formula: $CHBrCl_2$ Molecular Weight: 163.83

Synonym: Dichlorobromomethane

Chemical and Physical Properties

Bromodichloromethane, a member of the trihalomethane family, is a volatile colorless liquid with a melting point of –57° C, a boiling point of 90° C at 760 mm Hg, vapor pressure of 50 mm Hg at 20° C, and a density of 1.980 g/mL at 20° C (IARC, 1999).

Production, Use, and Human Exposure

Bromodichloromethane is not manufactured for commercial use, but is formed in chlorinated drinking water as a result of the halogen substitution and oxidation reactions of chlorine with naturally occurring organic matter (e.g., humic or fulvic acids) in the presence of bromide (Rook, 1974; Williams, 1985). Trihalomethanes are the most common disinfection by-products found in surface drinking water supplies. In addition to their occurrence in potable water supplies, trihalomethanes have been measured in municipal swimming pools (Stack *et al.,* 2000). The ratios of the various brominated trihalomethanes reflect the bromide concentration of the source water. The rate of formation of trihalomethanes in water supplies is dependent on the type and amount of disinfectant used, concentrations of chlorine and bromide ion, total organic carbon, pH, and temperature (Williams, 1985). Levels of trihalomethanes in tap water samples are higher in the summer than in other seasons (Symanski *et al.,* 2004). Trihalomethanes may also be produced by the decomposition of the corresponding trihaloacetic acids (e.g., bromodichloromethane from bromodichloroacetic acid) (Zhang and Minear, 2002). Levels of trihalomethanes in chlorinated water may be lowered by filtration through granular activated carbon and by reduction of organic precursors prior to chlorination. Because of the large number of by-products that may be formed during water disinfection, seasonal variability, spatial variability in distribution systems, and differences in individual water-use behavior (e.g., consumption of tap water, use of carbon filters, and exposure through showering and bathing), accurate exposure

assessments in epidemiological studies would benefit from measurements of different classes of disinfection by-products, household water sampling rather than distribution system sampling, and adjustments for differences in subject water use (King *et al.,* 2004).

Concentrations of bromodichloromethane in finished drinking water typically occur at concentrations ranging from 6 to 17 µg/L, but levels as high as 183 µg/L have been measured (Krasner *et al.,* 1989; USEPA, 1998). The United States Environmental Protection Agency (*Fed. Regist.,* 1998) has set a maximum contaminant level of 80 µg/L for total trihalomethanes in drinking water. In addition to oral exposure by ingestion of tap water or contaminated foods, human exposure can occur by inhalation during showering and by dermal penetration during bathing and swimming. In one study, showering accounted for 60% of total trihalomethane exposure, while tap water consumption at home accounted for 24% of the daily mean exposure (King *et al.,* 2004). Blood levels of trihalomethanes were increased more in people who took 10-minute showers than in people who drank one liter of the same tap water source in 10 minutes (Backer *et al.,* 2000). Part of this difference was attributed to more rapid metabolism following oral exposure; hence, greater systemic distribution of the parent compound may result from dermal and inhalation exposures.

ABSORPTION, DISTRIBUTION, METABOLISM, AND ELIMINATION

Experimental Animals

[^{14}C]-labeled trihalomethanes are rapidly absorbed after oral administration and extensively metabolized (80% to 90% within 24 hours after dosing); carbon dioxide is the main elimination product in B6C3F$_1$ mice (Mink *et al.,* 1986) and in F344 rats (Mathews *et al.,* 1990). Peak concentrations of bromodichloromethane in blood of F344 rats are reached within about 30 minutes after administration by oral gavage (Lilly *et al.,* 1998). Of an administered dose ranging from 1 to 100 mg/kg, 70% to 80% was exhaled as carbon dioxide and 3% to 5% as carbon monoxide; elimination in urine and feces accounted for only 4% to 5% and 1% to 3% of the dose, respectively (Mathews *et al.,* 1990). The extent of elimination of parent compound via exhalation through the lungs increases as the administered dose approaches metabolic saturation levels. A slower rate of production of carbon dioxide in rats dosed with 100 mg/kg compared to lower doses was indicative of metabolic saturation at this dose. Because repeated oral exposure to bromodichloromethane resulted in an increased rate of carbon dioxide production, it was suggested that bromodichloromethane might induce its own metabolism. The highest tissue-to-blood ratios of radioactivity in rats at 10 days after oral administration [^{14}C]-bromodichloromethane were found in the liver and kidney. The blood half-life of bromodichloromethane in mice is approximately 1.5 hours (Mink *et al.,* 1986).

The rate and extent of chloroform absorption (time to peak blood concentrations and area under the blood concentration-time curve) in Wistar rats were greater when doses were given in aqueous solutions than in a corn oil vehicle (Withey *et al.,* 1983). The same is true for bromodichloromethane (Lilly *et al.,* 1994). It was suggested that corn oil might affect the segment of the gastrointestinal tract where absorption occurs by retaining the agent in immiscible globules and thereby reducing immediate contact with the gastric mucosa. Dix *et al.* (1997) saw minimal effects of the gavage vehicle (2 mL/kg of corn oil, water, or aqueous 3% Emulphor®) on blood, liver, and kidney chloroform concentration-time curves in F344 rats; however, in B6C3F$_1$ mice dosed with 10 mL/kg, tissue concentrations of chloroform were greater with the aqueous vehicle than the corn oil vehicle. The species difference in response may have also been due to differences in dose volumes.

The first step in the oxidative metabolism of trihalomethanes involves an oxygen insertion at the carbon-hydrogen bond, catalyzed by a cytochrome P450-dependent mixed function oxidase system (Figure 1; Stevens and Anders, 1979). In the next step, nonenzymatic loss of a hydrogen halide results in the formation of the highly reactive dihalocarbonyl, e.g., dichlorocarbonyl (phosgene) from chloroform. For bromodichloromethane, this intermediate would be either bromochlorocarbonyl or dichlorocarbonyl. Hydrolysis of this intermediate produces carbon dioxide while reaction with reduced glutathione (GSH) produces carbon monoxide. Because bromine is a better leaving group than chlorine, the major reactive intermediate of bromodichloromethane oxidative metabolism would be the same intermediate as that formed from the oxidative metabolism of chloroform. The rate of metabolism of trihalomethanes to carbon monoxide *in vivo* (Anders *et al.,* 1978) or by rat liver microsomes (Ahmed *et al.,* 1977) followed the halide order: tribromomethane >> chlorodibromomethane > bromodichloromethane ≈ chloroform.

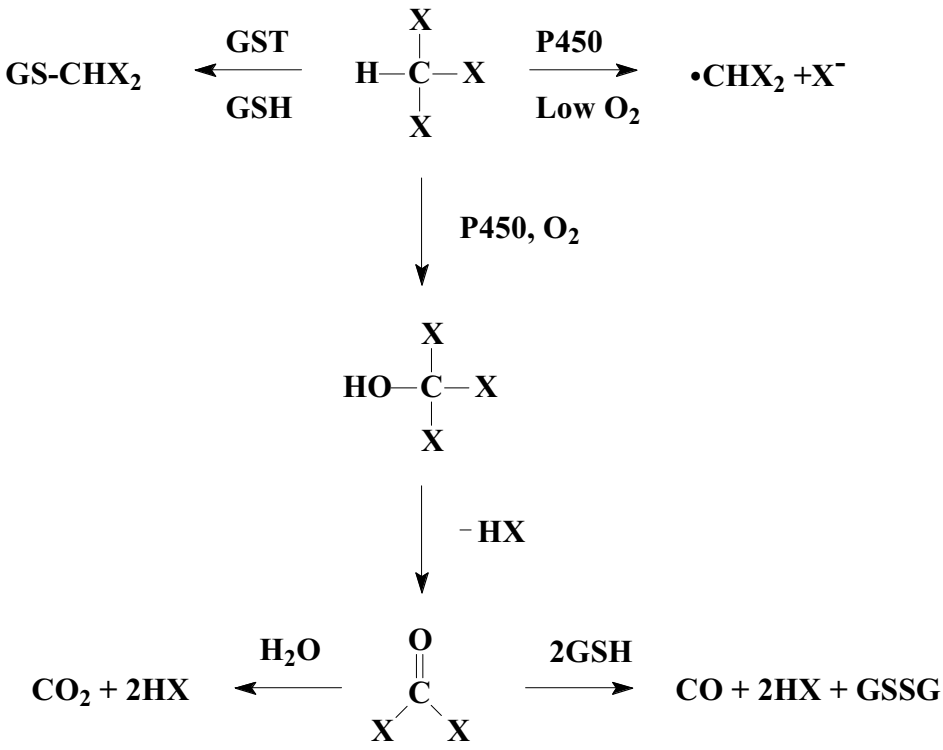


FIGURE 1
Metabolic Scheme for the Biotransformation of Trihalomethanes
X=halogen atom; GSH=reduced glutathione; GSSG=oxidized glutathione; GST=glutathione-*S*-transferase

Tomasi *et al.* (1985) suggested that trihalomethanes might also undergo reductive metabolism mediated by cytochrome P450, since free radical intermediates (e.g., dichloromethyl radical) were detected by the electron spin resonance spin-trapping technique (spin trap: phenyl-*t*-butyl nitrone) in isolated hepatocytes incubated under nitrogen and in the liver of phenobarbital-induced Wistar rats administered chloroform, bromodichloromethane, tribromomethane, or triiodomethane. In hepatocytes, the intensity of the electron spin resonance signal was reduced by exposure to oxygen or by the addition of cytochrome P450 inhibitors (SKF-525A, metyrapone, and carbon monoxide).

A third potential metabolic pathway for bromodichloromethane and other trihalomethanes is glutathione-*S*-transferase (GST)-catalyzed conjugation with glutathione, primarily by the GST *theta*-1-1 isoenzyme, forming DNA-reactive *S*-dihalomethyl metabolites analogous to those formed with dihalomethanes (Pegram *et al.,* 1997; Ross and Pegram, 2003). The initial conjugate formed with bromodichloromethane, $GSCHCl_2$, is reactive and degrades in water to form $GSCH_2OH$, S-formyl-GSH, and formate (Ross and Pegram, 2003). The catalytic efficiency of GST-mediated conjugation of bromodichloromethane with GSH (V_{max}/K_m expressed as nmol/minute/mg protein/mM) is 0.68, 0.038, and 0.22 in liver cytosol from $B6C3F_1$ mice, F344 rats, and humans, respectively. These values are much smaller than the catalytic efficiency for liver CYP2E1-mediated oxidation of bromodichloromethane. However, the opportunity for GSH conjugation is more likely in tissues where GST *theta*-1-1 is expressed and low levels of CYP2E1 are present.

CYP2E1 was suggested to be the predominant P450 isoform involved in the metabolism of bromodichloromethane, because pretreatment of rats with *theta*-dichloroethylene, an inhibitor of this enzyme, resulted in a 450-fold increase in the K_m for bromodichloromethane metabolism in F344 rats (Lilly *et al.,* 1997). CYP1A2 may also be involved in bromodichloromethane metabolism, since induction of this enzyme by 2,3,7,8-tetrachlorodibenzo-*p*-dioxin increased the hepatotoxicity and rate of metabolism of bromodichloromethane in the liver of F344 rats while inhibition of this enzyme by treatment with isosafrole reduced the metabolism and toxicity of bromodichloromethane (Allis *et al.,* 2002). Bromodichloromethane was metabolized by CYP2E1, CYP1A2 and CYP3A4 in human liver microsomes (Zhao and Allis, 2002). Using selective inhibitory antibodies of each P450 isoenzyme, K_m values were found to be 3 μM for CYP2E1 and about 60 μM for CYP1A2 and CYP3A4. Therefore, CYP2E1 should dominate bromodichloromethane metabolism at drinking water concentrations of trihalomethanes.

A physiologically based pharmacokinetic model was created to characterize the oral absorption, tissue dosimetry, and tissue specific rates of metabolism of bromodichloromethane in F344 rats (Lilly *et al.,* 1998). Oral absorption rate constants were estimated by fitting the model to concentration-time curves of blood and organ bromodichloromethane, plasma bromide ion, and exhaled breath chamber levels of bromodichloromethane that were obtained from male F344 rats administered bromodichloromethane by gavage in corn oil or in 10% Emulphor. A multicompartment gastrointestinal tract model provided an improved fit to the multiple peaks of blood bromodichloromethane concentrations observed in oral uptake studies compared to a model based on a first order process for bromodichloromethane absorption. Unfortunately, bromodichloromethane metabolism was modeled as occurring only in the liver (95%) and in the kidney (5%) by a single oxidative pathway; reduction by microsomal enzymes and conjugation with glutathione were ignored. Michaelis-Menten kinetic constants for bromodichloromethane metabolism in F344 rats (V_{max} = 12.8 mg/hour/kg, K_m = 0.5 mg/L) were obtained from previous gas uptake studies, which included measurements of chamber bromodichloromethane levels over a 4-hour period and postexposure measurements of plasma bromide concentrations (Lilly *et al.,* 1997). Gavage administration of bromodichloromethane in an aqueous vehicle (10% Emulphor) resulted in more rapid uptake, greater percent of the dose absorbed, larger percent of dose exhaled, and greater concentrations of parent compound in the kidney compared to administration in corn oil. Model simulations overestimated liver concentrations of bromodichloromethane. Plasma bromide concentration-time curves, which reflect total bromodichloromethane metabolism, were not significantly different between studies using aqueous vehicles and those using oil vehicles. Because oral absorption of bromodichloromethane is more rapid after gavage administration in an aqueous vehicle, first-pass metabolism of bromodichloromethane in the liver is likely to be more limited from aqueous administration at the doses (50 and 100 mg/kg) used to generate the plasma bromide

concentration-time curves. Based on determinations of organ-specific kinetic parameters (V_{max} and K_m) for bromodichloromethane in the F344 rat, Ross and Pegram (2004) suggested that metabolism of bromodichloromethane would elicit greater relative flux through the GST pathway versus the CYP-mediated oxidation pathway in the kidney and large intestine compared to the liver.

A drinking water study of bromodichloromethane in pregnant New Zealand white rabbits indicated that this chemical can cross the placenta and be taken up by fetal tissues (Christian *et al.,* 2001a). With milk-to-blood partition coefficients ranging from 1.3 to 2.9, lactational transfer from mother to infant is another potential pathway of exposure to trihalomethanes (Batterman *et al.,* 2002).

Humans

Prah *et al.* (2002) demonstrated the dermal absorption of bromodichloromethane in healthy male volunteers whose hands and forearms were exposed to ambient levels of bromodichloromethane (18.2 ± 8.0 µg/L) for 1 hour.

TOXICITY

Experimental Animals

The LD_{50} for bromodichloromethane in Sprague-Dawley rats was reported to be 916 mg/kg in males and 969 mg/kg in females (Chu *et al.,* 1980); the LD_{50} for bromodichloromethane in ICR Swiss mice is 450 mg/kg in males and 900 mg/kg in females (Bowman *et al.,* 1978). Liver and thyroid were identified as target organs of bromodichloromethane-induced toxicity in Sprague-Dawley rats exposed to concentrations up to 2,500 ppm in drinking water for 90 days (Chu *et al.,* 1982). Gavage treatment of CD-1 mice for 14 days with bromodichloromethane at doses of 74 or 148 mg/kg caused reduction in renal slice uptake of *p*-aminohippurate and histopathologic changes in the liver and kidney (Condie *et al.,* 1983). In mice treated with 50 to 250 mg/kg bromodichloromethane by gavage for 14 days, increases in serum glutamate oxaloacetate transaminase activity, serum glutamate pyruvate transaminase activity, and blood urea nitrogen levels were indicative of hepatic and renal effects of this agent (Munson *et al.,* 1982). Microencapsulated bromodichloromethane (in gelatin-starch capsules) was administered in the feed to Wistar rats for one month at concentrations ranging from 0.024% to 0.215% (equivalent to mean daily doses of approximately 20 to 200 mg/kg); liver lesions including vacuolization, swelling of hepatocytes, and single cell necrosis were observed (Aida *et al.,* 1992a).

In a 90-day gavage study of bromodichloromethane in F344 rats, compound-related changes in the liver (centrilobular degeneration) and kidney (degeneration of the proximal tubular epithelium) were observed at 300 mg/kg, but not at 150 mg/kg or lower doses (NTP, 1987). Decreases in body weight were observed at 150 mg/kg. In the 90-day gavage study in $B6C3F_1$ mice, liver lesions (cytoplasmic vacuolization) were observed in 200 mg/kg females and kidney lesions (focal necrosis of the proximal renal tubular epithelium) were observed in 100 mg/kg males (NTP, 1987). These results provided the basis for the gavage doses selected for the NTP 2-year toxicology and carcinogenicity studies of bromodichloromethane (50 and 100 mg/kg in male and female rats, 25 and 50 mg/kg in male mice, and 75 and 150 mg/kg in female mice).

Hepatotoxicity and renal toxicity were observed in female F344 rats that were administered bromodichloromethane in 10% Emulphor® solution by gavage for 5 days at daily doses of 150 or 300 mg/kg (Thornton-Manning *et al.,* 1994). Liver effects were characterized by increases in serum lactate dehydrogenase, sorbitol dehydrogenase, aspartate aminotransferase activities, and centrilobular vacuolar degeneration. Kidney toxicity was characterized by increases in blood urea nitrogen levels and by renal tubular vacuolar degeneration and necrosis. In female C57BL/6J mice, the only indicators of hepatotoxicity were increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in the 150 mg/kg group; no toxic effects were observed in rats or mice that received 75 mg/kg.

Some of the toxicity of bromodichloromethane is related to tissue glutathione content. Glutathione may react with free radicals or electrophiles (e.g., dihalocarbonyl) formed during bromodichloromethane metabolism. Pretreatment of F344 rats with butathione sulfoximine, a glutathione synthesis inhibitor, followed by oral dosing with 400 mg/kg bromodichloromethane resulted in increased levels of serum indicators of hepatotoxicity (sorbitol dehydrogenase, lactate dehydrogenase, aspartate aminotransferase, and alanine aminotransferase), increased levels of serum and urinary indicators of nephrotoxicity (blood urea nitrogen and urinary lactate dehydrogenase and alanine aminotransferase), and more

severe hepatocellular and renal tubule necrosis compared with animals treated only with bromodichloromethane (Gao *et al.,* 1996). In addition, protein and lipid binding of [^{14}C]-bromodichloromethane in hepatic microsomal and S9 fractions decreased with the addition of glutathione to the incubation media.

Exposure of female B6C3F$_1$ mice to 75 and 150 mg/kg bromodichloromethane (doses that were used in the NTP carcinogenicity study) for 3 weeks produced minimal to no significant changes in liver weight, serum alanine aminotransferase and sorbitol dehydrogenase activities, and hepatocyte labeling index (Melnick *et al.,* 1998); these parameters were markedly increased with a dose of 326 mg/kg, which is higher than any of the doses in the NTP bioassay. Hepatocellular hydropic degeneration was minimal to mild at 150 mg/kg and mild to moderate at 326 mg/kg. If expressed on a mmol/kg basis, the latter dose was similar to the low dose of chloroform that had been used in the National Cancer Institute carcinogenicity study of that agent (Dunnick and Melnick, 1993). The severity of hepatotoxicity, hepatocyte labeling index, and extent of decreased methylation of the *c-myc* gene in female B6C3F$_1$ mice exposed for 11 days to bromodichloromethane was similar at a gavage dose of 150 mg/kg and a drinking water concentration of 1,000 ppm, a concentration that was reported to provide an equivalent daily dose of 0.85 mmol/kg or 139 mg/kg (Coffin *et al.,* 2000). This study did not include the low dose used in the NTP bioassay, which was associated with a 38% liver tumor incidence.

In male F344 rats exposed to 100 mg/kg bromodichloromethane for four weeks by gavage in corn oil or water vehicle, slight increases in DNA synthesis were detected in proximal tubule epithelial cells (Lipsky *et al.,* 1993), however, no histopathological lesions were observed in the kidneys of treated rats.

Several studies have examined the relative toxicity of trihalomethanes administered in corn oil versus aqueous vehicles. The hepatotoxicity of chloroform in B6C3F$_1$ mice was more marked when administered for 90 days by gavage in corn oil compared to an aqueous vehicle of 2% Emulphor® (Bull *et al.,* 1986). In an acute toxicity study, administration of 400 mg/kg bromodichloromethane to male F344 rats induced higher activities of serum enzymes indicative of hepatotoxicity (alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase, and alkaline phosphatase) and greater severity of hepatocellular lesions (vacuolar degeneration and necrosis) when the agent was given in corn oil versus aqueous 10% Emulphor® solution and measured at 48 hours after dosing (Lilly *et al.,* 1994). The severity and incidence of renal tubule degeneration and necrosis was similar for bromodichloromethane administered in the aqueous and corn oil vehicles.

In mice exposed to bromodichloromethane by inhalation 6 hours per day for 1 week, the kidney was more sensitive than the liver to the cytotoxic effects of this chemical (Torti *et al.,* 2001). Renal tubule degeneration was observed at concentrations as low as 10 ppm, whereas hepatocellular degeneration was observed at concentrations of 30 ppm and higher.

In CD-1 mice given 50 to 250 mg/kg bromodichloromethane in 10% Emulphor® by gavage for 14 days, suppression of the humoral immune system was evidenced by decreases in antibody-forming cells in the spleen and decreases in antibody titers to sheep red blood cells (Munson *et al.,* 1982). In contrast, administration of bromodichloromethane in drinking water to C57BL/6J mice (0.05 to 0.5 g/L) for 2 to 4 weeks or by gavage at doses of 50 to 250 mg/kg for 16 days did not affect antibody response to sheep red blood cells or proliferative responses of splenic and mesenteric lymph node lymphocytes to T-cell and B-cell mitogens. Administration of bromodichloromethane in drinking water to F344 rats (0.07 or 0.7 g/L) for up to 26 weeks or by gavage (75 to 300 mg/kg) for 5 days did not produce decreases in the number of antibody forming cells or in antibody titers; decreases in proliferative responses to T-cell mitogens but not to B-cell mitogens were detected (French *et al.,* 1999). The authors concluded that the immune system is not a particularly sensitive target of bromodichloromethane toxicity.

Humans

No toxicity studies in humans were found in the literature.

CARCINOGENICITY

Experimental Animals

In a previous NTP study, administration of bromodichloromethane by gavage in corn oil for 2 years induced neoplasms at multiple sites in F344 rats and B6C3F$_1$ mice (NTP, 1987; Dunnick *et al.,* 1987). The doses used in these studies were 0, 50, and 100 mg/kg in rats, 0, 25, and 50 mg/kg in male mice, and 0, 75, and

150 mg/kg in female mice. Bromodichloromethane induced increased incidences of tubular cell adenomas and adenocarcinomas in the kidney of male rats (control: 0%, low dose: 2%, high dose: 26%), female rats (0%, 2%, 30%), and male mice (2%, 4%, 18%); adenocarcinomas and adenomatous polyps in the large intestine of male rats (0%, 26%, 90%) and female rats (0%, 0%, 26%); and hepatocellular adenomas and carcinomas in female mice (6%, 38%, 58%). Other trihalomethanes were also carcinogenic to mouse liver (chloroform and chlorodibromomethane), rat kidney (chloroform), and rat large intestine (bromoform) after administration by gavage in corn oil (Dunnick and Melnick, 1993).

In contrast to the above findings, Tumasonis *et al.* (1987) observed increased incidences of neoplastic nodules and adenofibrosis (proliferative lesion of the bile ducts) of the liver when chloroform or bromodichloromethane was administered in drinking water to male and female Wistar rats for their lifetimes at concentrations of 2.9 and 2.4 g/L, respectively, for 72 weeks (after which the concentrations of these trihalomethanes were halved). In a 2-year drinking water study of bromodichloromethane in male F344 rats and male B6C3F$_1$ mice at concentrations ranging from 0.06 to 0.6 g/L (in 0.25% Emulphor®), the only neoplastic effect was an increased incidence and multiplicity of hepatocellular adenomas in the low- and mid-dose rat groups (George *et al.,* 2002). The time-weighted mean daily doses of bromodichloromethane were estimated to be 4, 21, and 36 mg/kg in rats and 8, 27, and 43 mg/kg in mice. The addition of microencapsulated bromodichloromethane (gelatin-starch microcapsules) in the diet of Wistar rats for 2 years at concentrations ranging from 140 to 2,200 ppm (equivalent to 6 to 168 mg/kg per day) did not increase the incidence of tumors at any organ site (Aida *et al.,* 1992b). There was no indication that the dosed diets were monitored for stability of bromodichloromethane during the study. Incidences of bile duct proliferation and cholangiofibrosis were increased in the highest dose groups of males and females, and cholangiocarcinomas, which are rare in untreated Wistar rats, were diagnosed in one high-dose male and three high-dose females. It was noted that a maximum of only 24 rats per sex per treatment group were permitted to remain in this study beyond 18 months (IARC, 1999). Finally, a 2-year drinking water study of chloroform at daily doses comparable to those used in the corn oil gavage study confirmed the kidney tumor response in male rats, but did not show increases in liver tumors in female mice (Jorgenson *et al.,* 1985).

Although bromodichloromethane did not induce colorectal tumors in rats after 2 years of drinking water exposure, administration of bromodichloromethane in drinking water (0.7 g/L) for 13 weeks did induce an increased incidence and multiplicity of aberrant crypt foci (putative preneoplastic lesions of colon neoplasia, Pretlow *et al.,* 1992) in the rectal segment of the colon of male F344 rats (DeAngelo *et al.,* 2002). The authors suggest that drinking water treatment alone was not adequate to promote these lesions to neoplasms. Administration of bromodichloromethane in drinking water (0.7 g/L) or by gavage in corn oil (50 mg/kg per day) for 26 weeks produced a similar increase in the number of aberrant crypt foci per colon in male F344 rats (Geter *et al.,* 2004a). Thus, formation of aberrant crypt foci by bromodichloromethane appears to be independent of the vehicle and mode of oral exposure. In contrast to these findings, corn oil administration increased the number and size of aberrant crypt foci in male rats exposed to the colon carcinogen, azoxymethane. Exposure to bromodichloromethane in drinking water did not induce aberrant crypt foci in the colons of B6C3F$_1$ or A/J mice. Follow-up studies examined whether dietary animal fat promotes colon neoplasia in male F344 rats exposed to trihalomethanes in the drinking water (Geter *et al.,* 2004b). No differences in aberrant crypt foci number or size were observed in animals exposed for 26 weeks to 0.7 g/L bromodichloromethane, 0.9 g/L dibromochloromethane, or 0.5 g/L chloroform and fed a low-fat diet (4.5%) or high-fat diet (19% animal fat); a two-fold increase in foci was observed in rats exposed to 1.1 g/L bromoform and fed the high-fat diet. Thus, high animal fat intake did not influence the early development of aberrant crypt foci by bromodichloromethane in rats. DNA hypomethylation was induced in the colon of male F344 rats, but not in male B6C3F$_1$ mice exposed to bromodichloromethane by gavage or in drinking water for 28 days (Pereira *et al.,* 2004). Decreased DNA methylation in rats was greater and more rapid when bromodichloromethane was administered by gavage in corn oil at doses of 50 or 100 mg/kg than when administered in drinking water at concentrations of 350 or 700 mg/L. DNA hypomethylation was also observed after dietary administration of agents (e.g., bile acids, rutin) that promote colon cancer in rats.

Exposure of Eker rats to 0.07 or 0.7 g/L bromodichloromethane in drinking water for 4 or 10 months produced increases in the numbers of atypical renal tubules and atypical hyperplasias in males and females

(McDorman *et al.,* 2003a) and a nonsignificant but dose-related increase in renal tumors in males (Hooth *et al.,* 2002). This rat model carries a mutation in the tuberous sclerosis 2 tumor suppressor gene that predisposes these animals to develop multiple spontaneous renal tumors. Aberrant crypt foci were also induced in Eker rats exposed to 0.07 or 0.7 g/L bromodichloromethane in their drinking water for 10 months (McDorman *et al.,* 2003b).

Bromodichloromethane was not carcinogenic to Japanese medaka after 9 months exposure to concentrations up to 1.424 mg/mL; hyperplasia of bile ducts and the gallbladder epithelium were observed in that study (Toussaint *et al.,* 2001).

Humans

Bromodichloromethane is listed by the International Agency for Research on Cancer as "possibly carcinogenic to humans (Group 2B)" based on sufficient evidence of carcinogenicity in experimental animals (IARC, 1999). Consumption of chlorinated drinking water containing trihalomethanes has been linked to cancers of the bladder, colon, and rectum in several epidemiological studies. A meta-analysis of epidemiology studies published before 1989 on cancer and chlorination by-products in drinking water yielded a relative risk estimate of 1.21 (95% confidence interval (CI): 1.09-1.34) for bladder cancer and 1.38 (95% CI: 1.01-1.87) for rectal cancer (Morris *et al.,* 1992). In a study performed in Iowa, an increase in colon cancer risk was associated with exposure to chlorination by-products in drinking water, particularly chloroform, among postmenopausal women (Doyle *et al.,* 1997).

In population-based case-control studies, risk of bladder cancer was increased with both duration and concentration of exposure to trihalomethanes in chlorinated water sources in Ontario, Canada (King and Marrett, 1996). Increased colon cancer risk was associated with duration and cumulative exposure to trihalomethanes in males, but not in females; no relationship was observed between rectal cancer risk and exposure to trihalomethanes (King *et al.,* 2000a). A population-based case-control study in Colorado also found an association between prolonged exposure to chlorinated surface water and increased bladder cancer risk in men and women for both smokers and nonsmokers (McGeehin *et al.,* 1993). Population-based case-control studies in Iowa found increased bladder cancer risk (Cantor *et al.,* 1998) and rectal cancer risk (Hildesheim *et al.,* 1998) associated with exposure to trihalomethanes and duration of chlorinated surface water use; no increase in colon cancer risk was detected in the latter study. A pooled analysis of six case-control studies of bladder cancer and long-term exposure to trihalomethanes found significant increases in the odds ratio with increasing exposure in men, but not in women; for example the odds ratio was 1.44 (95% CI: 1.20 to 1.73) for exposures higher than 50 µg/L (Villanueva *et al.,* 2004). Thus, bladder cancer risk appears to be significantly elevated at trihalomethane levels observed in United States drinking water sources. An elevation in brain cancer risk was also associated with exposure to chlorinated surface water (Cantor *et al.,* 1999).

In a North Carolina ecologic study, no association was observed between breast cancer incidence and total trihalomethane levels in public water supplies (Marcus *et al.,* 1998).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

A decrease in sperm motility was observed in rats exposed to 0.7 g bromodichloromethane/L drinking water (equivalent to 39 mg/kg) for 52 weeks (Klinefelter *et al.,* 1995). Administration of bromodichloromethane by gavage in corn oil or in an aqueous vehicle containing 10% Emulphor® to pregnant F344 rats on gestation days 6 to 15 caused full litter resorptions at doses of 50 and 75 mg/kg (Narotsky *et al.,* 1997). This effect required exposure of rats on gestation days 6 to 10, the luteinizing hormone-dependent period of pregnancy; bromodichloromethane had no effect on pregnancy loss when exposure occurred on gestation days 11 to 15, the period when pregnancy is maintained by placental lactogens (Bielmeier *et al.,* 2001). Sprague-Dawley rats maintained their litters even after exposure to 100 mg/kg bromodichloromethane on gestation days 6 to 10. Because exposure of F344 rats to bromodichloromethane on gestation days 8 or 9 was associated with reduced serum progesterone levels and no effect on luteinizing hormone levels, Bielmeier *et al.* (2001) suggested that pregnancy loss by bromo-dichloromethane occurred due to disruption of corpora luteal responsiveness to luteinizing hormone, which led to a decrease in serum progesterone levels. Follow-up studies demonstrated that bromodichloromethane did cause a reduction in luteinizing hormone that preceded the decrease in

progesterone, and that concurrent treatment with progesterone or with human chorionic gonadotropin, a luteinizing hormone agonist, prevented bromodichloromethane-induced pregnancy loss (Bielmeier *et al.*, 2004). Therefore, the authors suggested that pregnancy loss by bromodichloromethane in F344 rats is due to disruption of luteinizing hormone secretion.

Teratogenic effects were not observed in Sprague-Dawley rats after pregnant dams were administered bromodichloromethane in corn oil at daily doses up to 200 mg/kg on gestation days 6 to 15 (Ruddick *et al.,* 1983). In drinking water studies of bromodichloromethane in Sprague-Dawley rats and New Zealand white rabbits, no effects on reproductive performance or development of offspring were observed except for delays in sexual maturation in F_1 rats and delays in skeletal ossification in rat fetuses (Christian *et al.,* 2001a,b, 2002). All toxic effects (e.g., decreases in parental and pup body weights) were attributed to reduced water and feed consumption. Drinking water concentrations of bromodichloromethane extended to 1,350 ppm in the range finding studies (Christian *et al.,* 2001a), to 900 ppm in the developmental toxicity studies (Christian *et al.,* 2001b), and to 450 ppm in a two-generation reproductive toxicity study in rats (Christian *et al.,* 2002). Exposure of rats in the reproductive toxicity studies began before cohabitation, continued through gestation, lactation, and 9 to 11 weeks postweaning; exposures in the developmental toxicity studies were on gestational days 6 to 21 in rats and gestational days 6 to 29 in rabbits.

Humans

Epidemiology studies have found associations between trihalomethanes in drinking water and adverse effects on pregnancy outcome in humans. An increased risk of spontaneous abortions was observed in women who drank five glasses or more per day of tap water that contained 75 µg/L or more total trihalomethanes (odds ratio of 1.8; 95% CI: 1.1-3.0), with the strongest association observed with exposure to tap water containing 18 µg/L or more of bromodichloromethane (odds ratio of 2.0; 95% CI: 1.2 to 3.5) (Waller *et al.,* 1998). An increased risk for stillbirths in Nova Scotia, Canada, was associated with exposure to trihalomethanes in public water supplies (King *et al.,* 2000b). Of the individual trihalomethanes, the strongest association was observed for exposure to bromodichloromethane (relative risk of 2.0, 95% CI 1.2 to 3.5 for exposure to greater than or equal to 20 µg/L compared to less than 5 µg/L). Also, bromodichloromethane concentrations of 20 µg/L or more in municipal water supplies were associated with an increased risk of neural tube defects in infants delivered among residents of Nova Scotia between 1988 and 1995 (relative risk of 2.5; 95% CI: 1.2 to 5.1) (Dodds and King, 2001). Elevated maternal exposures to trihalomethanes (greater than 74 µg/L versus less than or equal to 33 µg/L) were associated with reductions in birth weight, increased risk of reduced body weight for gestational age, longer gestational periods, and reduced risk of preterm delivery (Wright *et al.,* 2004). A decrease in normal sperm morphology with an accompanying increase in sperm head defects in healthy men (mean age of 33 years) was associated with increasing trihalomethane ingestion (Fenster *et al.,* 2003).

Bromodichloromethane disrupted differentiation and reduced chorionic gonadotropin secretion in primary cultures of human placental trophoblasts, suggesting that this disinfection by-product may induce spontaneous abortions in humans by a direct effect on placental production and secretion of hormones critical for maintaining a healthy pregnancy (Chen *et al.,* 2003).

GENETIC TOXICITY

The mutagenicity data for bromodichloromethane were reviewed by IARC (1999). The data show mixed results which may, in some cases, be directly related to inadequate exposures to this volatile chemical. An updated summary of the most significant observations follows.

Bromodichloromethane was mutagenic in *Salmonella typhimurium* strain TA100, inducing base-substitution revertants, when tested in a closed environment of a desiccator in the absence of exogenous metabolic activation (Simmon *et al.,* 1977). Bromodichloromethane was not mutagenic in *S. typhimurium* strain TA100 or additional tester strains with or without metabolic activation in studies that did not control for volatility of this chemical (Mortelmans *et al.,* 1986; Le Curieux *et al.,* 1995). The mutagenic potency of bromodichloromethane was markedly increased in a modified *S. typhimurium* TA1535 strain that had been transfected with rat GST T1-1 (strain RSJ100) compared to the standard strain TA1535 (Pegram *et al.,* 1997); bromodichloromethane was not mutagenic in the control strain that was

transfected with the same cDNA inserted in the opposite direction (strain TPT100 with a nonfunctional GST T1-1 gene). The majority of mutations induced in the transfected strain RSJ1000 were GC→AT transitions (DeMarini *et al.,* 1997).

Bromodichloromethane (maximum dose, 1 mmol/kg) induced chromosome aberrations in bone marrow cells of Long-Evans rats 12 hours after a single intraperitoneal injection, but not after 5 days of oral administration (Fujie *et al.,* 1990). An *in vitro* test for induction of chromosomal aberrations in Chinese hamster ovary cells demonstrated no activity by bromodichloromethane, with or without rat liver S9, in a standard protocol using loosely capped tubes (Anderson *et al.,* 1990). However, bromodichloromethane did induce chromosomal aberrations in cultured Chinese hamster lung fibroblasts incubated for 48 hours in tightly capped flasks, in the presence or absence of rat liver S9 (Matsuoka *et al.,* 1996). Bromodichloromethane induced dose-dependent increases in the frequency of sister chromatid exchanges in cultured human lymphocytes, with and without induced rat liver S9 enzymes, in cultured rat erythroblastic cells in the absence of S9, and in bone marrow cells of male ICR/SJ mice treated by oral gavage once a day for 4 days with doses of 25, 50, or 100 mg/kg bromodichloromethane (Morimoto and Koizumi, 1983; Fujie *et al.,* 1993). Bromodichloromethane did not induce micronuclei in bone marrow cells of male strain ddy mice given one or four daily intraperitoneal injections (up to 500 mg/kg or 200 mg/kg for single or multiple injections, respectively) (Hayashi *et al.,* 1988), but did induce micronuclei in newt larva erythrocytes (Le Curieux *et al.,* 1995) and in C57BL/6 and FVB/N p53 heterozygous mice exposed to 15 ppm bromodichloromethane for 13 weeks by inhalation (Torti *et al.,* 2002). Oral administration of bromodichloromethane did not induce unscheduled DNA synthesis in the livers of Sprague-Dawley rats after single doses of 135 or 450 mg/kg (Stocker *et al.,* 1997), or induce DNA strand breaks in kidney cells of F344 rats after 7 days of exposure to 0.75 or 1.5 mmol/kg (Potter *et al.,* 1996). Bromodichloromethane did induce a dose-related increase in DNA damage in *Escherichia coli* that was enhanced markedly with rat liver S9 (Le Curieux *et al.,*1995). Bromodichloromethane was more potent than other trihalomethanes as well as methylene chloride at inducing DNA strand breaks in cultured human lung epithelial cells (Landi *et al.,* 2003). Bromodichloromethane was not mutagenic at the tk locus in mouse lymphoma L5178Y cells treated without S9 activation, but with induced rat liver S9, a highly significant dose-related induction of mutant colonies was seen (McGregor *et al.,* 1988). DNA strand breaks were induced in human lymphoblastic leukemia cells incubated for 2 hours with 5 or 10 mM bromodichloromethane or other brominated trihalomethanes; in contrast, no increases in DNA strand breaks were detected in liver, kidney, or duodenum epithelial cells of F344 rats exposed to 0.6, 1.2 or 2.4 g/L bromodichloromethane in drinking water for 2 or 5 weeks (Geter *et al.,* 2004c). The effects of trihalomethanes in the human cell line occurred independently of GST T1-1 activity.

Incubation of F344 rat hepatic cytosol with [^{14}C]-bromodichloromethane, glutathione, and calf thymus DNA resulted in a threefold increase in radioactivity associated with DNA compared to incubations not containing rat cytosol; a similar experiment with B6C3F$_1$ mouse cytosol produced a sevenfold increase (Ross and Pegram, 2004). This difference correlated with the greater level of GST T1-1 activity in mouse liver compared to rat liver cytosol.

STUDY RATIONALE

Bromodichloromethane, a water disinfection byproduct, has been shown to be carcinogenic at multiple sites in rats (large intestine and kidney) and in mice (liver and kidney) after administration by gavage in corn oil. To further characterize its dose response relationships for evaluations of human risk, bromodichloromethane was nominated to the NTP by the USEPA for toxicity and carcinogenicity studies in rats and mice by drinking water exposure. The drinking water studies were limited to male rats and female mice because a greater increase in the incidence of large intestine neoplasms was observed in male rats than in female rats in the corn oil gavage study and because increases in the incidence of hepatocellular neoplasms were observed previously in female mice but not in male mice. Attempts were also made to provide doses in the drinking water studies that approached or overlapped those used in the gavage studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BROMODICHLOROMETHANE

A single lot of bromodichloromethane (02107TG) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH), and provided to the study laboratory (Southern Research Institute, Birmingham, AL) for use in the 3-week and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory. Reports on analyses performed in support of the bromodichloromethane studies are on file at the National Institute of Environmental Health Sciences.

Lot 02107TG of the chemical, a clear colorless liquid, was received in 100 g ampules. Since this material is sensitive to air, one of the ampules was divided into smaller aliquots and reampuled for individual purity and identity analyses; some of the samples turned yellow during the process. The colored samples were not used for frozen reference and the purity results reported are for uncolored samples. The material was identified as bromodichloromethane by the analytical chemistry laboratory using infrared (IR), ultraviolet/visible (UV/Vis), and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR and proton NMR. All the IR and NMR spectra were consistent with the literature spectra (*Aldrich,* 1981, 1992) of bromodichloromethane; however, the proton NMR spectrum observed by the analytical chemistry laboratory contained a singlet at 2.17 ppm that was not seen in the reference spectrum. The UV/Vis spectrum indicated no substantial absorption over the range of 200 to 800 nm relative to the blank spectrum. The IR, proton NMR, and carbon-13 NMR spectra are presented in Figures F1, F2, and F3, respectively.

The moisture content of lot 02107TG was determined by Galbraith Laboratories (Knoxville, TN) using Karl Fischer titration. The purity of this lot was determined by Galbraith Laboratories using elemental analysis and by the analytical laboratory and the study laboratory using gas chromatography (GC). Karl Fischer titration indicated a moisture content of less than 0.24%. Elemental analyses for carbon, bromine, and chlorine were in agreement with the theoretical values for bromodichloromethane; the hydrogen content was approximately 10% below theoretical. Purity analysis at the analytical laboratory showed two volatile impurities with peak areas 0.48% and 0.82% of the total peak area.

At the study laboratory, the purity profile of the test chemical dissolved in ethyl acetate obtained using GC indicated a relative purity of 98.2% compared to a frozen reference sample supplied by the analytical chemistry laboratory, and a calculated peak area percent purity of 99.8%. The overall purity of lot 02107TG was determined to be 98% or greater.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC. These studies indicated that bromodichloromethane was stable as a bulk chemical for 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored refrigerated, protected from light, in heat-sealed glass ampules. Stability was monitored by the study laboratory during the 3-week and 2-year studies using GC. No degradation of the bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once prior to the 3-week studies and approximately every 4 weeks during the 2-year studies by mixing bromodichloromethane with tap water (Table F2). Formulations were stored refrigerated in amber glass bottles, protected from air for up to 35 days.

Homogeneity studies of the 43.7 and 700 mg/L dose formulations were performed by the study laboratory using GC. Stability studies of 1 and 20 µg/mL formulations of bromodichloromethane in tap water were performed by

the analytical chemistry laboratory using GC. Homogeneity was confirmed, and stability was confirmed for at least 35 days for the 20 µg/mL formulation stored in amber glass bottles at 5° C, and for at least 7 days in amber drinking water bottles under simulated animal room conditions if a loss of approximately 5% of the test chemical was acceptable.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC. During the 3-week studies, the dose formulations were analyzed once; five of seven dose formulations for rats and mice were within 10% of the target concentrations and all were within 12% of the target concentrations (Table F3). Animal room samples of these dose formulations were also analyzed; one of the ten animal room samples was within 10%, and all were within 30% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks (Table F4). Of the dose formulations analyzed and used in the 2-year studies, 71 of 73 were within 10% of the target concentrations; all were within 12% of the target concentration. The two formulations that were within 12% of the target concentration were used for dosing with permission of the NTP. None of the 24 animal room samples were within 10% of the target concentrations; all rat samples were within 25% of target, and mouse samples ranged from 14% to 62% of target. Water bottles were changed twice weekly.

3-Week Studies

Male F344/N rats and female B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY). On receipt, the rats and mice were 3 to 4 weeks old. Animals were quarantined for 14 (mice) or 15 (rats) days and were 6 weeks old on the first day of the studies. Groups of 10 male rats and 10 female mice were exposed to bromodichloromethane in drinking water at target concentrations of 0, 43.7, 87.5, 175, 350, or 700 mg/L for 22 days. Feed and water were available *ad libitum*. Rats and mice were housed five per cage. Clinical findings were recorded weekly beginning on day 5. Water consumption was recorded twice weekly by cage when the water bottles were changed. The 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.

At the end of the 3-week studies, blood was collected from from the retroorbital sinus under CO_2/O_2 anesthesia for hematology and clinical chemistry (rats) analyses. For hematology analysis, blood was collected into tubes containing EDTA. Hematology parameters were evaluated within 6 hours of sample collection using a Technicon H-1™ with reagents manufactured by R&D Systems, Inc. (Minneapolis, MN), Bayer, Inc. (Tustin, CA), and Fisher Scientific (Norcross, GA). Reticulocyte counts were conducted on the day of sample collection using a Coulter Elite Flow Cytometer with reagents manufactured by Coulter Corp. (Miami, FL) and Molecular Probes (Eugene, OR). The parameters measured are listed in Table 1. For clinical chemistry analysis, blood was collected into tubes containing no anticoagulant. Clinical chemistry parameters (except sorbitol dehydrogenase) were measured using a Hitachi 911 Clinical Chemistry Analyzer using reagents manufactured by Boehringer Mannheim Biochemicals (Indianapolis, IN) and Sigma Chemical Co. (St. Louis, MO). Sorbitol dehydrogenase measurements were conducted with a Cobas Fara chemistry analyzer using reagents manufactured by Sigma Chemical Co. The parameters measured are listed 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 control and 700 mg/L rats and mice. Tissues identified as target organs were examined histopathologically to a no-effect level. Table 1 lists the tissues and organs examined.

2-Year Studies

Study Design

Groups of 50 male rats and 50 female mice were exposed to bromodichloromethane at target concentrations of 0, 175, 350, or 700 mg/L in drinking water for 105 weeks.

Source and Specification of Animals

Male F344/N rats and male and female B6C3F$_1$ mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 12 days before the beginning of the studies. Five male rats and five male and five female mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 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 using male rats and male mice (Appendix I).

Animal Maintenance

Rats were housed up to three per cage and female mice were housed five per cage. Feed and water were available *ad libitum*. Water consumption was measured by cage every 4 weeks for 7 days each time water bottles were changed (twice in the 7-day period). Cages and 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 H.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded at 4-week intervals throughout the studies. Body weights were recorded on day one and then at 4-week intervals throughout the studies.

Complete necropsies and microscopic examinations were performed on all rats and female 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. 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 slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the kidney and liver of male rats and female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, 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 chairperson 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 or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(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 Drinking Water Studies of Bromodichloromethane

3-Week Studies	2-Year Studies
Study Laboratory Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species F344/N rats $B6C3F_1$ mice	F344/N rats $B6C3F_1$ mice
Animal Source Taconic Laboratory Animals and Services (Germantown, NY)	Taconic Laboratory Animals and Services (Germantown, NY)
Time Held Before Studies Rats: 15 days Mice: 14 days	12 days
Average Age When Studies Began 6 weeks	6 weeks
Date of First Exposure Rats: August 7, 1998 Mice: August 6, 1998	December 15, 1998
Duration of Exposure 22 days	105 weeks
Date of Last Exposure Rats: August 28, 1998 Mice: August 27, 1998	December 18, 2000
Necropsy Dates Rats: August 28, 1998 Mice: August 27, 1998	December 12 to 18, 2000
Average Age at Necropsy 9 weeks	110-111 weeks
Size of Study Groups Rats: 10 males Mice: 10 females	Rats: 50 males Mice: 50 females
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 3-week studies
Animals per Cage 5	Rats: 3 Mice: 5
Method of Animal Identification Tail tattoo	Same as 3-week studies

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Bromodichloromethane

3-Week Studies	2-Year Studies
Diet	
Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as 3-week studies, changed weekly
Water	
Tap water (Birmingham municipal supply) via amber glass bottles (Kerr Glass Manufacturing Company, Plainfield, IL) with stainless steel, double-ball sipper tubes (Probes Unlimited, Warminster, PA), available *ad libitum*, changed twice weekly	Same as 3-week studies
Cages	
Solid-bottom polycarbonate (Lab Products, Maywood, NJ), changed twice weekly	Same as 3-week studies
Bedding	
Irradiated hardwood chip (P.J. Murphy Forest Products, Inc., Montville, NJ), changed twice weekly	Same as 3-week studies
Rack Filters	
Reemay® spun-bonded polyester (Andico, Birmingham, AL), changed every 2 weeks	Same as 3-week studies
Racks	
Stainless steel (Lab Products, Maywood, NJ), changed and rotated every two weeks	Same as 3-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
Exposure Concentrations	
0, 43.7, 87.5, 175, 350, or 700 mg/L in drinking water available *ad libitum*	0, 175, 350, or 700 mg/L in drinking water available *ad libitum*
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, weekly and at the end of the studies; clinical findings were recorded weekly beginning day 4 (rats) or 5 (mice). Water consumption was recorded weekly.	Observed twice daily; animals were weighed initially, every 4 weeks, and at the end of the studies. Clinical findings were recorded every 4 weeks. Water consumption was recorded by cage for 1 week every 4 weeks, each time the water bottles were changed (twice during the week of recording).
Method of Sacrifice	
CO_2 asphyxiation	Same as 3-week studies
Necropsy	
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis (rats), and thymus.	Necropsies were performed on all animals.

TABLE 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Bromodichloromethane

3-Week Studies	2-Year Studies
Clinical Pathology	
Blood was collected from the retroorbital sinus of all animals surviving to 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.	None
Histopathology	
Complete histopathology was performed on 0 and 700 mg/L rats and mice. Tissues identified in 700 mg/L animals as target organs were examined in lower exposure groups to a no-effect level. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.	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, eye, gallbladder (mice), harderian gland, heart with aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung and mainstem bronchi, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, urinary bladder, and uterus.

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 or missing were censored from the survival analyses; animals dying from natural causes were not censored. Statistical analyses for possible dose-related effects on survival used Cox's (1972) method for testing two groups for equality and Tarone's (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, and B4 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 A3 and B3) 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., harderian gland, intestine, mammary gland, and skin) before microscopic evaluation, or 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 A3 and B3 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 B6C3F_1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require 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 exposed group with controls and a test for an overall exposure-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 and clinical chemistry 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).

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 22 studies that use the NTP-2000 diet with histopathological findings completed up to the present. 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.

Quality Assurance Methods

The 3-week 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 bromodichloromethane was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium,* mutations in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, micronucleated erythrocytes in mouse bone marrow, and the frequency of micronucleated erythrocytes in mouse peripheral blood. The protocols for these studies and the results are given in Appendix C.

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

RESULTS

RATS

3-WEEK STUDY

All rats survived to the end of the study (Table 2). Final mean body weights of exposed rats were not significantly different from those of the controls. The mean body weight gains of 350 and 700 mg/L rats were significantly less than that of the controls. Concentration-related decreases in water consumption were evident during the first week on study; mean water consumption by 43.7, 87.5, 175, 350, and 700 mg/L groups were 93%, 90%, 80%, 72% and 61% of that by controls, respectively (Table 2). These decreases were attributed to poor palatability of the dosed water and reduced feed consumption. Drinking water concentrations of 43.7, 87.5, 175, 350, or 700 mg/L resulted in average daily doses of approximately 6, 12, 20, 38, or 71 mg bromodichloromethane/kg body weight, respectively. There were no clinical findings related to bromodichloromethane exposure.

The hematology and clinical chemistry data for male rats in the 3-week toxicity study of bromodichloromethane

TABLE 2
Survival, Body Weights, and Water Consumption of Male Rats in the 3-Week Drinking Water Study of Bromodichloromethane

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]		
		Initial	Final	Change		Week 1	Week 2	Week 3
0	10/10	101 ± 3	165 ± 5	65 ± 2	—	16.8	16.1	18.2
43.7	10/10	97 ± 3	161 ± 5	63 ± 3	97	15.7	15.9	17.5
87.5	10/10	98 ± 3	160 ± 4	63 ± 2	97	15.2	16.8	17.7
175	10/10	97 ± 3	157 ± 5	59 ± 2	95	13.5	14.4	15.0
350	10/10	99 ± 3	156 ± 5	57 ± 3*	94	12.1	13.2	14.8
700	10/10	96 ± 3	150 ± 4	54 ± 2**	91	10.3	12.6	13.2

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Number of animals surviving at 3 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

are listed in Table D1. In general, the few hematology or clinical chemistry alterations that were identified statistically were not considered toxicologically relevant.

Relative kidney weights of rats in the 175, 350, and 700 mg/L groups were significantly greater than that of the controls (Table E1).

There were no significant chemical-related histopathological changes. Rats in the 700 mg/L group had a slight increase in the incidence of minimal hepatocyte centrilobular cytoplasmic vacuolization (0 mg/L, 4/10; 43.7 mg/L, not examined; 87.5 mg/L, not examined; 175 mg/L, 1/10; 350 mg/L, 5/10; 700 mg/L, 7/10).

Dose Selection Rationale

The three highest concentrations used in the 3-week study were selected for the 2-year study in order to achieve average daily doses as near as possible to the low dose used in the previous 2-year gavage study in male rats (50 mg/kg per day). It was anticipated that at these drinking water concentrations, the average daily doses of bromodichloromethane would be less in the 2-year study than in the 3-week study.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male rats are shown in Table 3 and in the Kaplan-Meier survival curves (Figure 2). Survival of exposed groups was similar to that of the controls.

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of all exposed groups of male rats were generally similar to those of the controls throughout the study (Table 4 and Figure 3). Water consumption by exposed rats was less than that by the controls throughout the study (Table G1); the decreases were attributed to poor palatability of the dosed water and reduced feed consumption. Decreases were most evident during the first 13 weeks of the study, during which mean water consumption by the 175, 350, and 700 mg/L groups was approximately 9%, 10% and 13% less than that of the controls. From week 53 until the end of the study, mean water consumption by the 175, 350, and 700 mg/L groups was 6%, 8% and 7% less than that by the controls. Drinking water concentrations of 175, 350, and 700 mg/L resulted in average daily doses of approximately 6, 12, or 25 mg/kg. Based on body weight, water consumption, and exposure concentration of bromodichloromethane, average daily doses for all exposed groups were proportional throughout the study. There were no clinical findings related to bromodichloromethane exposure.

TABLE 3
Survival of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	16	19	14	15
Natural deaths	5	2	7	9
Animals surviving to study termination	29[e]	28[e]	29	26
Percent probability of survival at end of study[b]	58	57	58	52
Mean survival (days)[c]	682	686	694	684
Survival analysis[d]	P=0.547	P=1.000	P=1.000N	P=0.630

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

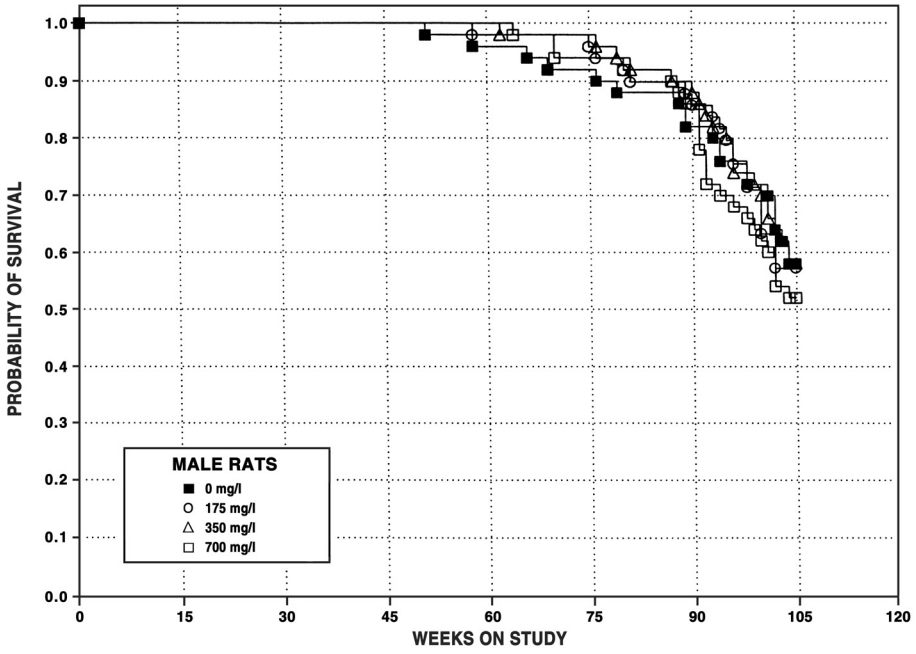


FIGURE 2
Kaplan-Meier Survival Curves for Male Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years

TABLE 4
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	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	104	98	50	105	100	50
4	201	50	195	97	50	194	97	50	193	96	50
8	278	50	269	97	50	272	98	50	269	97	50
12	327	50	318	97	50	319	98	50	314	96	50
16	360	50	348	97	50	347	96	50	345	96	50
20	387	50	376	97	50	375	97	50	371	96	50
24	405	50	393	97	50	392	97	50	391	97	50
28	429	50	415	97	50	414	97	50	410	96	50
32	444	50	430	97	50	428	97	50	425	96	50
36	458	50	444	97	50	444	97	50	438	96	50
40	468	50	455	97	50	454	97	50	449	96	50
44	481	50	468	97	50	468	97	50	462	96	50
48	481	50	470	98	50	470	98	50	465	97	50
52	492	49	479	97	50	479	97	50	472	96	50
56	498	49	484	97	50	486	98	50	476	96	50
60	505	48	493	98	49	492	97	50	486	96	50
64	506	48	495	98	49	495	98	49	487	96	50
68	511	47	497	97	49	497	97	49	488	96	49
72	512	46	500	98	49	497	97	49	487	95	47
76	510	46	503	99	47	495	97	49	490	96	47[a]
80	509	44	503	99	46	497	98	47	491	96	47
81	511	44	504	99	45	494	97	47	489	96	46
84	508	44	497	98	44	495	98	46	482	95	46
88	506	44	500	99	44	496	98	45	483	96	45
92	511	41	498	98	42	492	96	43	478	94	39
96	511	38	483	95	39	489	96	40	483	95	35
100	506	36	474	94	35	488	97	36	477	94	32
104	491	31	482	98	28	481	98	31	473	96	27
Mean for weeks											
1-13	228		222	98		222	98		220	97	
14-52	441		428	97		427	97		423	96	
53-104	507		494	98		492	97		484	96	

[a] The number of animals weighed for this week was less than the number surviving.

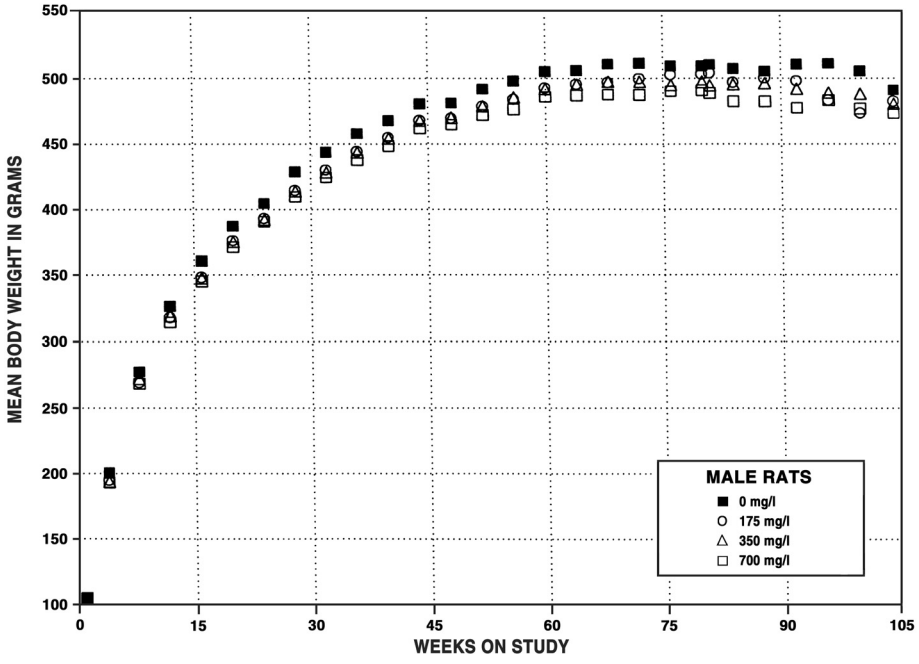


FIGURE 3
Growth Curves for Male Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver, brain, pancreas, and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, 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.

There were no increased incidences of neoplasms that were attributed to exposure to bromodichloromethane.

Liver: The incidences of chronic inflammation in the liver of 350 and 700 mg/L rats were significantly greater than that in the controls (Tables 5 and A4). Chronic inflammation occurred as small, randomly distributed clusters of small macrophages and lymphocytes mixed with lesser numbers of neutrophils. This change is considered morphologically consistent with the spontaneous inflammatory foci that are commonly observed in aged rats, and which are considered to result from bacterial showering from the intestinal tract. The biological significance of these increases is uncertain. The incidences of basophilic foci and clear cell foci were significantly decreased in the 350 and 700 mg/L groups. The incidences of hepatocyte cytoplasmic vacuolization were slightly increased in the 350 and 750 mg/L groups.

Brain: The incidences of focal compression of the brain were significantly increased in the 350 and 700 mg/L groups (Tables 5 and A4). Compression was considered to be related to the presence of neoplasms of the pituitary gland. Neoplasms of the pituitary gland are common background lesions in rats. Large adenomas of the pituitary gland expand dorsally and compress the brain because of the limitation on ventral expansion by the sella tunica. This compression can result in death of the animal. The majority of animals with a microscopic diagnosis of brain compression had pituitary gland masses greater than 5 mm in diameter that caused indentation of the adjacent ventral surface of the brain.

Miscellaneous Lesions: Exposure to bromodichloromethane resulted in significant decreases in the incidences of pancreatic acinus atrophy in all exposed groups and of renal tubule pigmentation in 700 mg/L rats (Tables 5 and A4). Both pancreatic atrophy and renal tubule pigmentation are common spontaneous changes that occur in aged rats. The biological significance of these results is uncertain, but may be related to treatment; however, the incidences in this study are within historical ranges for NTP studies (data not shown).

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L		350 mg/L		700 mg/L	
Liver[a]	50		50		50		50	
Basophilic Focus[b]	33		25		24*		17**	
Clear Cell Focus	28		24		19*		17*	
Inflammation, Chronic	23	(1.3)[c]	29	(1.3)	33*	(1.4)	34*	(1.5)
Hepatocyte, Vacuolization, Cytoplasmic	11	(2.0)	10	(2.0)	19	(1.5)	18	(1.4)
Brain	50		50		50		50	
Compression, Focal	6	(2.5)	8	(2.9)	14*	(3.1)	14*	(2.8)
Pancreas	49		50		50		49	
Acinus, Atrophy, Focal	31	(1.8)	20*	(1.8)	23*	(1.8)	21*	(2.0)
Kidney	49		50		50		49	
Renal Tubule, Pigmentation	10	(2.1)	13	(2.2)	7	(2.3)	3*	(2.3)

* Significantly different ($P \leq 0.05$) from the 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

3-WEEK STUDY

All mice survived to the end of the study (Table 6). Final mean body weights of 175, 350, and 700 mg/L mice and mean body weight gains of 350 and 700 mg/L mice were significantly less than those of the controls; these decreases were attributed to poor palatability of the dosed water. There were significant concentration-related decreases in water consumption by the groups exposed to 87.5 mg/L or greater throughout the study (Table 6); these decreases were attributed to poor palatability of the dosed water and reduced feed consumption. Drinking water concentrations of 43.7, 87.5, 175, 350, or 700 mg/L resulted in average daily doses of approximately 6, 10, 16, 29, or 51 mg/kg. There were no clinical findings related to bromodichloromethane exposure.

The hematology data for female mice in the 3-week toxicity study of bromodichloromethane are listed in Table D2. There appeared to be treatment-related increases in the leukocyte, neutrophil, and lymphocyte counts. Increases in the leukon with increases in neutrophils and lymphocytes have been observed in other species and have been associated with a physiological response related to an endogenous release of epinephrine (i.e., "flight or fright response"). Epinephrine responses are typically of very short duration (less than 6 hours). Because the severity of these changes was not dose-related and the data values were generally within acceptable ranges, it is questionable whether this is truly an epinephrine-type response in the mice at day 21.

Relative liver, kidney, and thymus weights of mice in the 350 and 700 mg/L groups were significantly greater than those of the controls. Absolute lung weights of mice in the 350 and 750 mg/L groups were significantly less than that of the controls.

BrdU labeling of mouse hepatocytes in the 700 mg/L group was similar to that in the control groups (data not shown). BrdU labeling was performed according to the Nyska *et al.* (2002) method.

Dose Selection Rationale

The three highest concentrations used in the 3-week study were selected for the 2-year study in order to achieve average daily doses as near as possible to the low dose used in the previous 2-year gavage study in female mice (75 mg/kg per day). It was anticipated that at these drinking water concentrations, the average daily doses of bromodichloromethane would be less in the 2-year study than in the 3-week study.

TABLE 6
Survival, Body Weights, and Water Consumption of Female Mice in the 3-Week Drinking Water Study of Bromodichloromethane

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]		
		Initial	Final	Change		Week 1	Week 2	Week 3
0	10/10	18.4 ± 0.2	21.3 ± 0.4	2.9 ± 0.4	—	2.7	2.8	2.8
43.7	10/10	18.7 ± 0.2	21.0 ± 0.3	2.3 ± 0.2	98	2.8	2.7	2.8
87.5	10/10	17.8 ± 0.4	21.0 ± 0.3	2.9 ± 0.3	97	2.0	2.2	2.5
175	10/10	17.6 ± 0.4	20.0 ± 0.3**	2.4 ± 0.5	94	1.4	1.9	2.0
350	10/10	18.5 ± 0.3	18.9 ± 0.3**	0.4 ± 0.4**	89	1.1	1.7	1.7
700	10/10	18.3 ± 0.2	19.5 ± 0.3**	1.2 ± 0.2**	91	0.3	1.8	1.8

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 3 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for female mice are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 4). Survival of exposed groups was similar to that of the controls.

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of all exposed groups of mice were generally less than those of the controls from week 4 through the end of the study (Table 8 and Figure 5). The mean body weight of the 700 mg/L group was similar to that of the controls at the end of the study. Water consumption by exposed mice was less than that by the controls throughout the study (Table G2); the decreases were attributed to poor palatability of the dosed water and reduced feed consumption. During the first 13 weeks on study, mean water consumption by the 175, 350, and 700 mg/L groups was 23%, 26%, and 32% less than that by the controls. From week 53 until the end of the study, mean water consumption by the 175, 350, and 700 mg/L groups was 23%, 26%, and 26% less than that by the controls, respectively. Drinking water concentrations of the 175, 350, and 700 mg/L groups resulted in average daily doses of approximately 9, 18, and 36 mg/kg, respectively. Based on body weight, water consumption, and exposure concentration of bromodichloromethane, average daily doses for all exposed groups were proportional throughout the study. There were no clinical findings related to bromodichloromethane exposure.

TABLE 7
Survival of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Animals initially in study	50	50	50	50
Accidental death[a]	0	1	0	0
Moribund	7	6	5	4
Natural deaths	7	7	12	7
Animals surviving to study termination	36	36	33	39
Percent probability of survival at end of study[b]	72	74	66	78
Mean survival (days)[c]	699	683	686	700
Survival analysis[d]	P=0.683N	P=1.000N	P=0.573	P=0.692N

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

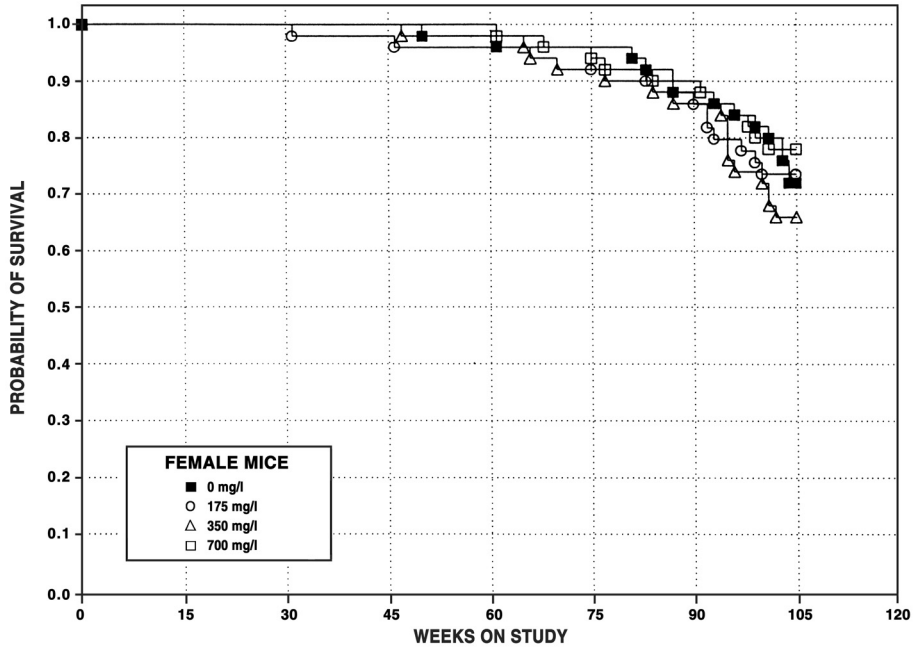


FIGURE 4
Kaplan-Meier Survival Curves for Female Mice Exposed to Bromodichloromethane in Drinking Water for 2 Years

TABLE 8
Mean Body Weights and Survival of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

Weeks on Study	0 ppm		175 ppm			350 ppm			700 ppm		
	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	18.6	50	18.4	99	50	18.6	100	50	18.5	100	50
4	21.7	50	20.8	96	50	20.1	93	50	20.1	93	50
8	26.4	50	24.2	92	50	24.6	93	50	24.4	92	50
12	32.1	50	27.2	85	50	28.2	88	50	27.3	85	50
16	37.9	50	31.8	84	50	33.3	88	50	31.7	84	50
20	42.6	50	34.8	82	50	37.2	87	50	35.5	83	50
24	45.9	50	37.8	82	50	38.5	84	50	37.3	81	50
28	50.0	50	40.1	80	50	41.4	83	50	40.4	81	50
32	51.8	50	43.8	85	49	45.4	88	50	43.7	84	50
36	54.8	50	46.6	85	49	48.2	88	50	47.4	87	50
40	58.1	50	50.6	87	49	52.8	91	50	51.3	88	50
44	59.4	50	54.6	92	49	55.7	94	50	54.3	91	50
48	60.2	50	57.0	95	48	58.4	97	49	56.4	94	50
52	61.2	49	58.1	95	48	58.1	95	49	55.6	91	50
56	62.3	49	57.3	92	48	58.8	94	49	56.3	90	50
60	64.4	49	58.3	91	48	61.2	95	49	58.3	91	50
64	64.7	48	60.0	93	48	60.5	94	49	59.9	93	49
68	65.4	48	60.8	93	48	61.4	94	47	60.3	92	49
72	66.6	48	62.2	93	48	62.7	94	46	61.4	92	48
76	66.6	48	61.3	92	46	61.7	93	46	60.1	90	47
80	65.1	48	59.2	91	45	60.1	92	45	59.1	91	46
84	65.4	46	59.9	92	44	60.7	93	45	59.3	91	46
88	66.1	44	61.2	93	43	60.9	92	43	60.9	92	45
92	64.0	44	59.8	93	42	59.4	93	43	59.6	93	44
96	62.2	43	56.5	91	39	55.8	90	38	57.3	92	43
100	60.4	41	54.7	91	37	52.4	87	36	56.5	94	40
104	56.9	38	53.8	95	36	51.8	91	33	55.3	97	39
Mean for weeks											
1-13	24.7		22.7	93		22.9	94		22.6	93	
14-52	52.2		45.5	87		46.9	90		45.4	86	
53-104	63.9		58.8	92		59.0	93		58.8	92	

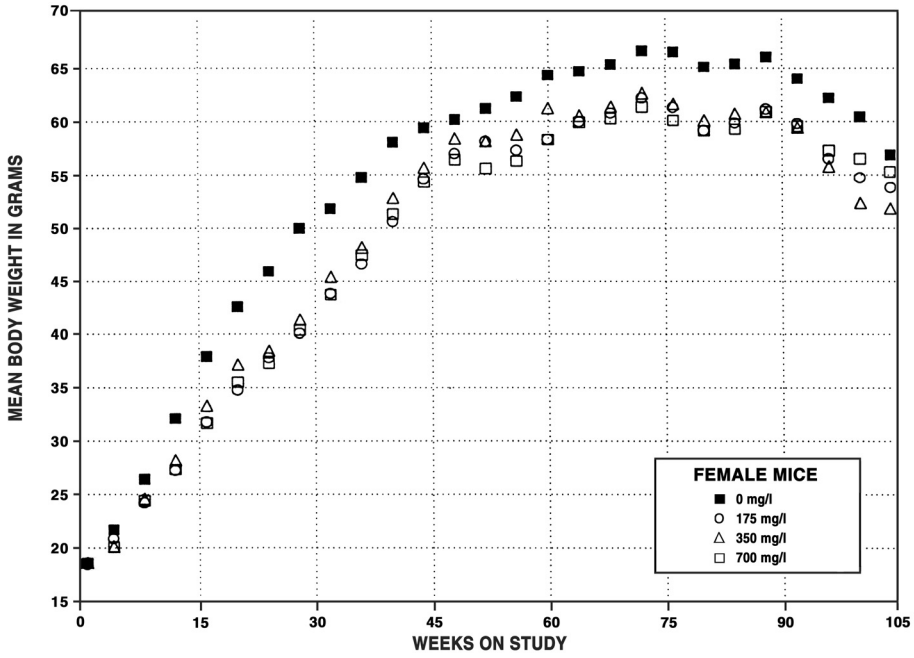


FIGURE 5
Growth Curves for Female Mice Exposed to Bromodichloromethane in Drinking Water for 2 Years

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 liver, spleen, kidney, thyroid gland, bone marrow, and mammary gland, and hemangiosarcoma. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, 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 B.

Liver: The incidences of hepatocellular adenoma or carcinoma (combined) in all exposed groups of female mice were decreased; the incidence in the 700 mg/L group was significantly less than that of the controls (Tables 9 and B3). The incidence in controls was at the upper end of the historical control range for drinking water studies (20% to 63%) and all routes combined (8% to 63%) (Table 9).

All Organs: The incidences of hemangiosarcoma in all organs were decreased in all exposed groups compared to the controls, but the difference was only significant in the 350 mg/L group (0 mg/L, 8/50; 175 mg/L, 2/50, 350 mg/L, 0/50; 700 mg/L, 4/50; Table B3). However, the control incidence was unusually high, exceeding the historical ranges in controls (all routes) given the NTP-2000 feed [33/1,258 (2.9% ± 2.3%), range 0% - 8%]; the highest incidence in previous drinking water studies was 6%.

Miscellaneous Lesions: The incidences of several nonneoplastic lesions were significantly increased or decreased in one or more exposed groups (Tables 10 and B4) The incidences of lymphoid cell hyperplasia of the spleen were significantly increased in 350 and 700 mg/L mice compared to that in the control group. The incidences of hematopoietic cell proliferation of the spleen were significantly decreased in 175, 350, and 700 mg/L mice. The incidences of nephropathy and thyroid gland cystic degeneration were significantly decreased in 350 and 700 mg/L mice. The incidences of bone marrow hyperplasia and mammary gland hyperplasia were significantly decreased in 700 mg/L mice compared to those in the control group. The biological significance of these results is uncertain, but the differences may be related to exposure. However, the incidences in this study are generally within historical ranges for NTP studies (data not shown).

TABLE 9
Incidences of Hepatocellular Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Number Examined Microscopically	50	50	50	50
Hepatocellular Adenoma, Multiple[a]	14	6	8	7
Hepatocellular Adenoma (includes multiple)	24	18	19	16
Hepatocellular Carcinoma, Multiple	4	3	2	1
Hepatocellular Carcinoma (includes multiple)	13	6	12	7
Hepatocellular Adenoma or Carcinoma[b]				
Overall rate[c]	30/50 (60%)	23/50 (46%)	24/50 (48%)	19/50 (38%)
Adjusted rate[d]	64.5%	51.2%	52.4%	41.6%
Terminal rate[e]	24/36 (67%)	20/36 (56%)	16/33 (49%)	17/39 (44%)
First incidence (days)	604	521	539	686
Poly-3 test[f]	P=0.022N	P=0.134N	P=0.162N	P=0.019N

[a] Number of animals with neoplasm
[b] Historical incidence for 2-year drinking water studies with controls given NTP-2000 diet (mean ± standard deviation): 58/149 (27.0% ± 9.9%), range 20%-63%; all routes: 286/1,251 (24.1% ± 12.8%), range 8%-63%
[c] Number of animals with neoplasm per number of animals with liver examined microscopically
[d] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[e] Observed incidence at terminal kill
[f] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or lower incidence in an exposed group is indicated by **N**.

TABLE 10
Incidences of Selected Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L		350 mg/L		700 mg/L	
Spleen[a]	50		49		48		50	
Hematopoietic Cell Proliferation[b]	42	(2.7)[c]	30**	(2.8)	32*	(2.5)	29**	(2.3)
Lymphoid Follicle, Hyperplasia	5	(2.6)	8	(2.6)	13*	(2.7)	14*	(2.5)
Kidney	50		49		50		50	
Nephropathy	16	(1.2)	14	(1.4)	7*	(1.1)	8*	(1.4)
Thyroid Gland	50		49		49		50	
Cystic Degeneration	26	(1.9)	17	(1.7)	12**	(1.8)	11**	(2.1)
Bone Marrow	50		50		50		50	
Hyperplasia	22	(2.6)	24	(2.9)	18	(2.9)	11*	(2.6)
Mammary Gland	50		50		50		50	
Hyperplasia	9	(1.7)	4	(1.3)	4	(2.0)	2*	(2.0)

* 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

GENETIC TOXICOLOGY

The results of *in vitro* mutagenicity tests with bromodichloromethane were mixed and it is possible that the volatility of this chemical was a factor in the outcome of some of the tests (Simmon *et al.,* 1977). Bromodichloromethane was tested for mutagenicity in *Salmonella typhimurium* in two separate experiments at the same laboratory using a total of five tester strains in a standard preincubation assay (Table C1; Mortelmans *et al.,* 1986). No mutagenic activity was observed in any of the strains, with or without induced rat or hamster liver S9 activation enzymes. In contrast to the negative results in bacteria, tests for mutation induction in mouse lymphoma L5178Y/$tk^{+/-}$ cells were positive in the presence of induced rat liver S9; no mutagenic activity occurred in tests conducted without S9 (Table C2; McGregor *et al.,* 1988). In cytogenetic tests with cultured Chinese hamster ovary (CHO) cells, bromodichloromethane induced a small increase in sister chromatid exchanges (SCEs) in one of three trials conducted in the presence of induced rat liver S9 enzymes (Table C3; Anderson *et al.,* 1990); no significant increase in SCEs occurred without S9. Among the three SCE trials conducted with S9, there was variability in the responses and in the levels at which toxicity occurred, which may indicate fluctuating exposures to the volatile chemical. No induction of chromosomal aberrations occurred in CHO cells incubated with bromodichloromethane at concentrations up to 5,000 µg/mL, with or without S9 (Table C4; Anderson *et al.,* 1990).

Results of *in vivo* tests for chromosomal damage in mice were negative. No increases in the frequency of micronucleated polychromatic erythrocytes (PCEs) were seen in bone marrow of male $B6C3F_1$ mice administered bromodichloromethane (200 to 500 mg/kg per day) by intraperitoneal injection for 3 days (Table C5). In the first of two trials in this assay, significant increases in micronucleated PCEs were seen at two of the four dose groups, but the responses were not correlated with dose. In a second trial, no significant increases in micronucleated cells were seen at any of the four dose groups, and the assay was judged to be negative overall. In a second micronucleus test, no induction of micronuclei was observed in peripheral blood normochromatic erythrocytes of female $B6C3F_1$ mice administered up to 700 mg/L bromodichloromethane in drinking water for 3 weeks (Table C6). Although the 3-week exposure time was not long enough to reach equilibrium in the circulating erythrocyte population, which reaches steady state after approximately 35 days of exposure, there was no indication of a response in any of the five exposed groups tested, and the results were concluded to be negative.

DISCUSSION AND CONCLUSIONS

Bromodichloromethane is a trihalomethane disinfection by-product commonly found in chlorinated drinking water supplies and municipal swimming pools; it is not manufactured for commercial use. Based on health effects information, including data on carcinogenicity and reproductive effects in animal models and humans, and on technological feasibility for reducing levels of disinfection by-products in chlorinated water supplies, the United States Environmental Protection Agency has established drinking water standards for trihalomethanes in the United States. The current maximum contaminant level for total trihalomethanes in drinking water is 80 µg/L (*Fed. Regist.*, 1998). Contributing to the health effects database on trihalomethanes were previous NTP studies that demonstrated bromodichloromethane is carcinogenic at multiple organ sites in F344/N rats and $B6C3F_1$ mice after administration in corn oil by gavage for 2 years (Dunnick *et al.,* 1987; NTP, 1987). At daily doses of 50 or 100 mg/kg, bromodichloromethane induced increased incidences of neoplasms of the kidney and large intestine in rats; bromodichloromethane induced neoplasms of the liver in female mice that received 75 or 150 mg/kg, and kidney neoplasms in male mice that received 25 or 50 mg/kg.

Chronic oral studies of bromodichloromethane in drinking water or microencapsulated in feed resulted in different responses than those reported in the NTP studies. Increases in the incidences of liver neoplasms, but not kidney or large intestine neoplasms, were observed in female Wistar rats and male F344 rats administered bromodichloromethane in drinking water (Tumasonis *et al.,* 1987; George *et al.,* 2002); no neoplastic effects were observed in male mice exposed to bromodichloromethane in drinking water (George *et al.,* 2002) or in Wistar rats given microencapsulated bromodichloromethane in feed (Aida *et al.,* 1992b). Factors such as the stability of bromodichloromethane in feed and water, the influence of the vehicle (e.g., corn oil), different rates of absorption and delivery of parent compound to target organs, and different rates of metabolism of bromodichloromethane after gavage and drinking water exposure may have contributed to the contrasting results noted above. For example, the rate of delivery of bromodichloromethane to the large intestine may be important in neoplasm induction at that site, since DNA hypomethylation in the colon of male F344/N rats was greater and more rapid when bromodichloromethane was administered by gavage in corn oil at doses of 50 and 100 mg/kg than when administered in drinking water at concentrations of 350 and 700 mg/L (Pereira *et al.*, 2004). Also relevant to the evaluation of large intestine and kidney neoplasia in rats exposed to bromodichloromethane in drinking water are studies of less than 1 year duration that found increases in putative preneoplastic lesions at these sites; drinking water exposure to bromodichloromethane induced aberrant crypt foci in the colon of F344 rats (DeAngelo *et al.,* 2002) and Eker rats (McDorman *et al.,* 2003b) and atypical tubules and atypical hyperplasias in the kidney of Eker rats (McDorman *et al.,* 2003a).

Additional drinking water carcinogenicity studies on bromodichloromethane were conducted by the NTP to further characterize dose-response relationships for evaluations of human risk. The present studies were limited to male rats and female mice because a greater increase in the incidence of large intestine neoplasms was observed in male rats than in female rats in the NTP (1987) corn oil gavage study and because increases in the incidence of hepatocellular neoplasms were observed previously in female mice but not in male mice. Although a goal of this drinking water study was to include a daily dose that overlapped doses used in the previous gavage study, the concentrations of bromodichloromethane in the dose formulations were limited by the solubility of this chemical in tap water, the palatability of the drinking water solutions, and reduced body weight gains of rats and mice exposed to bromodichloromethane in drinking water. Thus, whereas the gavage doses in the NTP (1987) study in male rats were 50 and 100 mg/kg, the estimated average daily doses of bromodichloromethane in the current drinking water study were 6, 12, and 25 mg/kg. Similarly, in female mice the gavage doses were 75 and 150 mg/kg in the NTP (1987) study, and the estimated average daily

drinking water doses were 9, 18, and 36 mg/kg in the current study. The drinking water doses were calculated based on the target concentrations of bromodichloromethane in the water bottles, monthly measurements of the amount of water consumed per animal per day, and average body weight values measured monthly throughout the studies. These calculated values are recognized to be overestimates of the actual daily doses during the 2-year studies because analyses of animal room samples of the dose formulations found that concentrations of bromodichloromethane in the water bottles decreased by about 15% to 20% in samples taken from water bottles given to rats and by about 20% to 50% in samples taken from water bottles given to mice over the 3- to 4-day period between changes of water bottles with fresh dosing solutions.

In the 3-week preliminary study in male rats, target concentrations of bromodichloromethane in drinking water ranged from 43.7 to 700 mg/L; this was estimated to be equivalent to average daily doses of approximately 6 to 71 mg/kg, assuming target concentrations of bromodichloromethane had been maintained in the water bottles during the exposure periods. Most of the observed effects in this study were attributed to poor palatability of the dosed water solutions; effects included concentration-related decreases in water consumption, decreases in mean body weight gains, and increases in relative (but not absolute) kidney weights. The same target concentrations of bromodichloromethane were given to female mice in the 3-week drinking water study (equivalent to average daily doses of 6 to 51 mg/kg) and produced similar effects as in rats (i.e., concentration-related decreases in water consumption, decreases in mean body weight gains, and increases in relative organ weights that were attributed to poor palatability of the dosed water solutions). The lack of toxic effects at these doses was not unexpected because higher daily doses of bromodichloromethane were required to elicit kidney and liver toxicity in mice in previous subchronic gavage studies (NTP, 1987; Thornton-Manning *et al.,* 1994; Melnick *et al.,* 1998). The three highest concentrations of bromodichloromethane that had been used in the 3-week drinking water study (175, 350, and 700 mg/L) were selected for the 2-year study in order to achieve average daily doses as near as possible to the doses used in the previous 2-year gavage studies and to provide additional doses to characterize dose-response relationships.

In the current study in male rats, survival and mean body weights of exposed groups were similar to those of controls. In female mice, survival in exposed groups was similar to controls; however, due to poor palatability of the dosed water solutions, water consumption was decreased and mean body weights were lower in exposed groups compared to controls throughout most of the study. No exposure-related increases in incidences of neoplasms were observed in rats or mice. The only exposure-related toxic effects in rats were to the liver and these included increased incidences of hepatocyte cytoplasmic vacuolization and chronic inflammation. In mice, incidences of hepatocellular neoplasms and hemangiosarcomas in all organs were decreased in exposed groups compared to controls. Part of this difference may be related to the high incidences of these neoplasms in control mice. For example, in this study the control incidence of hemangiosarcomas in all organs was 16% while the historical control range for this lesion is 0% to 8% for female mice given NTP-2000 diet; likewise, the control incidence of hepatocellular neoplasms was 60% while the mean historical control incidence is 27% for this lesion with a range of 8% to 63% in female mice given NTP-2000 diet.

Results from the previous and present NTP studies on bromodichloromethane raise the question of why this agent was a multiple organ carcinogen in rats and mice after administration in corn oil by gavage but was not carcinogenic in rats or mice after drinking water exposure. The carcinogenic effects of bromodichloromethane in rats and mice are consistent with results of other trihalomethanes that were administered in corn oil by gavage. For example, chloroform induced kidney tumors in rats, bromoform induced large intestine tumors in rats, and chloroform and chlorodibromomethane induced liver tumors in mice (Dunnick and Melnick, 1993). In a 2-year drinking water study of chloroform at daily doses comparable to those used in the corn oil gavage study, kidney tumors were induced in male rats, but there was no increase in liver tumors in female mice (Jorgenson *et al.,* 1985). Drinking water studies of bromodichloromethane and feed studies of microencapsulated bromodichloromethane in rats did not show increases in the incidences of neoplasms of the kidney or large intestine. Differences in organ dosimetry after gavage administration versus drinking water or dietary administration may be important in evaluating the carcinogenic activity of bromodichloromethane. This issue was addressed through toxicokinetic

modeling of the absorption, distribution, metabolism, and elimination of bromodichloromethane and dose-response analyses of the carcinogenic effects of this agent using peak and cumulative rates of metabolism via the GST and CYP450 oxidative pathways in target organs as surrogate dose metrics.

A physiologically based pharmacokinetic (PBPK) model for orally administered bromodichloromethane in F344/N rats was created (Appendix K) and fit to plasma time-course data (Appendix J) obtained from studies of single intravenous administration (dose: 10 mg/kg), single gavage administration in corn oil (at doses of 25, 50, or 100 mg/kg), and single gavage administration in an aqueous vehicle (water:Cremophor®, 9:1; at doses of 25, 50, or 100 mg/kg). The plasma time-course data showed that bromodichloromethane is rapidly absorbed after gavage administration in corn oil or in an aqueous vehicle. In both cases, maximum plasma concentrations were reached within 5 to 15 minutes after gavage administration. Higher plasma concentrations were measured in male rats after administration of equivalent doses in the aqueous vehicle than in corn oil indicating more rapid absorption from the aqueous solution. The PBPK model includes absorption from the gastrointestinal tract lumen and direct passage to the liver capillary space via the portal vein. Absorption occurs in the stomach and in the small and large intestines, and subsequent distribution to other organs occurs by diffusion-limited processes. To estimate the distribution of bromodichloromethane to the kidneys and large intestine with drinking water exposure, the absorption kinetic parameters for bromodichloromethane in an aqueous vehicle were applied to a drinking water pattern in which 90% of daily consumption occurs over the 12-hour dark cycle and the remaining 10% over the 12-hour light cycle (Yuan, 1995).

Bromodichloromethane is metabolized by several pathways, including CYP450 oxidation (Stevens and Anders, 1979), CYP450 reductive metabolism (Tomasi *et al.,* 1985), and GST catalyzed conjugation with glutathione (Pegram *et al.,* 1997; Ross and Pegram, 2003). The GST pathway induces mutations (GC→AT transitions) in *Salmonella* (DeMarini *et al.,* 1997; Pegram *et al.,* 1997). Kinetic parameters used in the PBPK model for cytosolic GST-mediated glutathione conjugation (first order kinetics) and for microsomal CYP450 oxidation (Michaelis-Menten kinetics) of bromodichloromethane in the liver, kidney, and large intestine of male F344 rats were obtained from Ross and Pegram (2004). These parameters were obtained from 14-week old rats; there are no data available on how these parameters may change in aging rats. In contrast to the model developed by Lilly *et al.* (1998), the present model includes metabolism through the GST pathway, distribution to organs that is diffusion-limited rather than flow-limited, metabolic activity in the large intestine, and absorption that is represented as a nonlinear process governed by Michaelis-Menten kinetics with specific rates of transit through the gastrointestinal tract rather than a pulsatile pattern of uptake from several gastrointestinal compartments.

Table 11 presents model-based estimates of maximal blood concentrations and the 24-hour area under the blood concentration time curves (AUCs) for bromodichloromethane in male F344 rats exposed by single intravenous injection, by single gavage in corn oil administration, or in drinking water for 24 hours at the same doses that were used in the 2-year gavage and drinking water studies. The AUCs from the drinking water exposures were smaller than those from the gavage in corn oil exposures; however, the 24-hour AUC for the 50 mg/kg gavage group was only about 60% greater than the 24-hour AUC for the 700 mg/L drinking water group. For both the drinking water and gavage exposures, the bioavailability (i.e., the ratio of the 24-hour AUCs in the gavage or drinking water groups compared to the 24-hour AUC for the intravenous group per unit dose) was only about 11% to 13%. This value is an indication of the percentage of the administered dose that is available systemically (i.e., after first pass liver metabolism) and was used as a dose metric in the dose-response analysis described below. The increase in bioavailability with increasing dose in the gavage groups is indicative of metabolic saturation during the first pass through the liver.

Table 12 presents model-based estimates of the maximal rates and 24-hour cumulative metabolism of bromodichloromethane via GST-mediated conjugation and CYP450-mediated oxidation in the liver, kidney, and large intestine of male rats administered 50 or 100 mg/kg bromodichloromethane by gavage in corn oil. The liver is the major site of metabolism of bromodichloromethane, and this occurs predominantly via the CYP450-mediated oxidation pathway. The 24-hour cumulative metabolism of bromodichloromethane through the GST pathway in the liver at these doses is approximately 77- to 95-fold less than that through the CYP450 pathway. The more than doubling of GST

TABLE 11
Model-based Estimates of Maximum Blood Concentrations and 24-Hour AUCs for Bromodichloromethane in Male F344/N Rats Exposed by Intravenous Injection, Gavage in Corn Oil, or Via Drinking Water[a]

Route	Dose (mg/kg)	Maximum Concentration (mg/L)	24-Hour AUC (mg × hr/L)	Bioavailability[b] (%)
Intravenous	1	116	0.166	
	10	1,161	1.77	
Corn oil gavage	50	0.495	0.928	11
	100	1.29	2.16	13
Drinking water[c]				
175 mg/L	8.46	0.011	0.146	10
350 mg/L	16.9	0.022	0.293	10
700 mg/L	33.8	0.044	0.591	11

[a] AUC = area under the curve
[b] Ratio of 24-hour AUC per 1 mg/kg dose (gavage or drinking water):24-hour AUC for 1 mg/kg intravenous dose × 100
[c] Doses are based on 14.5 mL water consumption/day and 0.3 kg body weight

TABLE 12
Estimated Maximal Rates and 24-Hour Cumulative Metabolism of Bromodichloromethane via GST-Mediated Conjugation with Glutathione or CYP450-Mediated Oxidation in the Male F344/N Rat Liver, Kidney, and Large Intestine After Administration by Gavage in Corn Oil

Dose (mg/kg)	GST Maximal (nmol/min/g tissue)	GST Cumulative Metabolism (nmol/g tissue)	P450 Maximal (nmol/min/g tissue)	P450 Cumulative Metabolism (nmol/g tissue)	P450 Cumulative/ GST Cumulative
Liver					
50	0.690	51.2	52.9	4,850	95
100	1.87	122	92.5	9,370	77
Kidney					
50	0.156	17.5	0.983	120	6.9
100	0.411	40.9	2.07	250	6.1
Large intestine					
50	0.120	89.2	0.656	538	6.0
100	0.225	176	1.06	924	5.3

activity and the less than doubling of P450 metabolism in the liver as the dose was increased from 50 to 100 mg/kg suggests that partial saturation of the P450 pathway at the higher dose results in more bromodichloromethane metabolism occurring through the GST pathway. In the kidney and large intestine the ratio of CYP450 to GST activity is much smaller than that in the liver. Thus, compared to the liver there is greater relative bromodichloromethane metabolism through the GST pathway than through the CYP450 pathway in these organs. Both GST and CYP450 activities increased more than 2-fold in the kidney as the dose was increased from 50 to 100 mg/kg; this is likely due to partial saturation of the first pass CYP450 activity in the liver and greater systemic availability of the parent chemical.

Maximal rates and 24-hour cumulative metabolism of bromodichloromethane via GST-mediated conjugation and CYP450-mediated oxidation in the liver, kidney, and large intestine were also estimated in male rats given bromodichloromethane in drinking water at the concentrations used in the 2-year study (Table 13). Again, CYP450 metabolism in the liver is the predominant pathway of bromodichloromethane metabolism. Under these conditions of exposure, all estimated metabolic rates changed proportionally with increasing concentrations of bromodichloromethane in the drinking water, indicating no metabolic saturation. Consequently, the ratio of CYP450 to GST activity in each organ remained relatively constant as the concentration of bromodichloromethane in drinking water was increased.

The relative bromodichloromethane metabolism through the CYP450 pathway (P450 cumulative/GST cumulative) was greater with exposure in drinking water (Table 13) than by gavage (Table 12), particularly in the liver. Cumulative metabolism through the CYP450 and GST pathways was approximately 30% to 40% lower in rats exposed to 700 mg/L in drinking water (Table 13) compared to rats dosed with 50 mg/kg by gavage (Table 12); however, maximal rates of these activities, which reflect the rate of delivery of bromodichloromethane to metabolizing organs as well as the kinetics of each metabolic pathway, were approximately 2- to 17-fold less in all organs after drinking water exposure (700 mg/L) compared to gavage (50 mg/kg) administration.

Based on this PBPK model, approximately 97% of bromodichloromethane metabolism occurs in the liver after gavage or drinking water exposure; approximately 90% of total metabolism occurs during the first pass clearance by the liver. In the liver, 99% of metabolism occurs via

TABLE 13
Estimated Maximal Rates and 24-Hour Cumulative Metabolism of Bromodichloromethane via GST-Mediated Conjugation with Glutathione or CYP450-Mediated Oxidation in Male F344/N Rat Liver, Kidney, and Large Intestine After Administration in Drinking Water

Dose (mg/L)	GST Maximal (nmol/min/g tissue)	GST Cumulative Metabolism (nmol/g tissue)	P450 Maximal (nmol/min/g tissue)	P450 Cumulative Metabolism (nmol/g tissue)	P450 Cumulative/ GST Cumulative
Liver					
175	0.0098	7.91	1.10	890	113
350	0.0197	15.9	2.20	1,780	112
700	0.0399	32.1	4.39	3,550	111
Kidney					
175	0.0034	2.73	0.025	20.4	7.5
350	0.0068	5.49	0.051	40.8	7.4
700	0.0138	11.1	0.101	81.7	7.4
Large intestine					
175	0.0156	13.4	0.112	97.4	7.3
350	0.0311	26.9	0.215	189	7.0
700	0.0619	53.6	0.397	358	6.7

the CYP450-mediated oxidation pathway; in the kidney and large intestine, 84% to 88% of metabolism occurs via the CYP450 pathway.

Dose-response analyses of the carcinogenic effects of bromodichloromethane in the kidney and large intestine from the corn oil gavage study were conducted using 24-hr blood AUCs for bromodichloromethane (Table 11) and the maximal rates and 24-hour cumulative metabolism via the GST and CYP450 pathways provided in Table 12 as dose metrics for the gavage study. These analyses were performed by fitting the gavage tumor data and the dose metric data to a modified Weibull model as described in Appendix K. Based on those dose-response relationships, predictions of tumor incidence in the drinking water study were made using the dose metrics presented in Table 13.

Table 14 presents the predicted neoplasm incidences in the kidney and large intestine of male F344 rats exposed to target concentrations of 175, 350, or 700 mg/L bromodichloromethane in drinking water. Because the drinking water dose metrics did not adjust for loss of bromodichloromethane from drinking water bottles during the exposures, the values shown in Table 14 are slight overestimates of neoplasm incidence.

This analysis shows that, regardless of the dose metric used, the predicted kidney neoplasm incidence was less than 1% in male F344/N rats exposed to bromodichloromethane in drinking water for 2 years. This prediction is consistent with the observed incidences of 0/50 kidney neoplasms in the three exposed groups. The highest predicted incidences of large intestine neoplasms in the drinking water study were approximately 10% in the 700 mg/L group using the 24-hour blood AUC as the dose metric, 6% using cumulative GST metabolism or cumulative CYP450 metabolism as the dose metric, and 3.5% using maximal GST metabolism or maximal CYP450 metabolism as the dose metric. Thus, if any one of these dose metrics is the determinant of large intestine neoplasia, and if this PBPK model accurately represents metabolic flux through these pathways in the large intestine, then the predicted number of animals with large intestine neoplasms was 2 to 5 in the group of 50 male rats exposed to 700 mg/L for 2 years. A

TABLE 14
Observed and Predicted Incidences of Kidney and Large Intestine Neoplasms in Male F344/N Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years

Exposure Concentration (mg/L)	Observed Neoplasm Incidence (%)	Predicted Neoplasm Incidence (%)[a]				
		24-Hour Blood AUC	GST Maximal	GST Cumulative	P450 Maximal	P450 Cumulative
Kidney						
175	0	<0.1	<0.1	<0.1	<0.1	<0.1
350	0	<0.1	<0.1	<0.1	<0.1	<0.1
700	0	0.5	<0.1	0.6	<0.1	0.6
Large intestine						
175	0	0.3	<0.1	0.1	<0.1	<0.1
350	2	1.9	0.4	0.8	0.3	0.5
700	0	9.7*	3.5	6.3	3.5	6.0

* Significantly different ($P<0.05$) from the observed neoplasm incidence (by the likelihood ratio test)
[a] Predicted neoplasm incidences are based on fitting to a modified Weibull model (Appendix K) the neoplasm incidences in the kidney and large intestine from the gavage study with the dose metric estimates for 24-hour blood AUC for bromodichloromethane and maximal rates and 24-hour cumulative metabolism of bromodichloromethane via GST-mediated conjugation with glutathione or CYP450-mediated oxidation in these organs.

predicted incidence of 5/50 is significantly different than the observed incidence of 0/50, whereas a predicted incidence of 3/50 is not significantly different. If the model adequately predicts the disposition of bromodichloromethane in male rats, then the 24-hour blood AUC may not be a reliable dose metric for predicting large intestine tumors in rats exposed by the drinking water route. The observed incidences of large intestine neoplasms at these drinking water exposure concentrations were 0/49 in controls and 0/49, 1/46 (2%), and 0/46 in the exposed groups. Because these neoplasms are uncommon in untreated male F344/N rats, the finding of one adenomatous polyp in the 350 mg/L group may reflect the expected low incidence at this exposure; the spontaneous rate for neoplasms of the large intestine in male rats given NTP-2000 feed in the NTP historical database is 5/1,159 (0.43%).

If the rates or cumulative metabolism via the GST or CYP450 pathways are the true indicators of the carcinogenic potential of bromodichloromethane, then the liver would be expected to be at greater risk than the kidney or large intestine. In the gavage study, the incidence of liver neoplasms in male rats was 1/50 in vehicle controls, 0/50 at 50 mg/kg, and 4/50 at 100 mg/kg. This response was not identified by NTP (1987) as evidence of carcinogenic activity. Thus, either the liver has a much greater capability of detoxifying intermediates, or repairing damage produced by intermediates of these metabolic pathways or metabolic flux through these pathways may not be the primary determinants of neoplasia induced by bromodichloromethane. For example, the model does not include parameters for the reductive metabolic pathway. In addition, neither the rate nor total metabolic flux through the GST and CYP450 pathways addresses the time-dependent concentration of critical intermediates in target organs. The dihalocarbonyl intermediate of the CYP450 pathway is highly reactive and has a very short half-life, whereas the glutathione conjugate of bromodichloromethane is a stable intermediate. Information on the rate of clearance of DNA-reactive intermediates of the GST pathway in target organs may provide a more reliable indicator of cancer risk.

The induction of neoplasms in the large intestine of F344/N rats exposed to bromodichloromethane may not be due only to the rate and extent of delivery and metabolism of bromodichloromethane at this site; dietary factors may have contributed to differences between the gavage and drinking water studies. For example, colon cancer risk is reduced in populations that consume diets that are high in total fiber and increased in populations that consume high quantities of fat derived from red meat (Lieberman *et al.,* 2003; Slattery *et al.,* 2003). In the drinking water study, rats were fed NTP-2000 diet, which was 9.1% crude fiber by weight (Table H3); whereas, in the gavage study rats were fed NIH-07 diet, which was 3.4% crude fiber by weight (NTP, 1987). Although the historical control rate for large intestine neoplasms is only 0.5% in male F344 rats given either NIH-07 diet or NTP-2000 diet, the impact of this difference in diet on the development of chemically induced large intestine neoplasms in F344 rats is not known. Administration of bromodichloromethane by gavage in corn oil may have had an influence on the promotion of large intestine neoplasms. In male F344/N rats exposed by intraperitoneal injection to the colon carcinogen azoxymethane, the number and size of aberrant crypt foci were increased when animals were also administered corn oil by gavage for 26 weeks (Geter *et al.,* 2004a). However, no differences in aberrant crypt foci were observed in rats administered bromodichloromethane in drinking water versus in corn oil by gavage (Geter *et al.,* 2004a). In addition, no differences in aberrant crypt foci number or size were observed in male F344 rats exposed to 700 mg/L bromodichloromethane in drinking water and fed a low-fat diet (4.5%) or a high-fat diet (19% animal fat) for 26 weeks (Geter *et al.,* 2004b). There are no data available addressing whether a longer exposure period to a high-fat diet or chronic gavage dosing with corn oil influences the development of colon neoplasms in rats exposed to bromodichloromethane.

No increased neoplasm incidences were observed in female mice exposed to bromodichloromethane in drinking water, though a dose-related increase in hepatocellular neoplasms (3/50 in vehicle controls, 18/48 at 75 mg/kg, and 29/50 at 150 mg/kg) was observed in the previous gavage study (Dunnick *et al.,* 1987; NTP, 1987). In the drinking water study, the incidences of hepatocellular neoplasms were less in the exposed groups compared to controls (30/50 in controls, 23/50 at 175 mg/L, 24/50 at 350 mg/L, and 19/50 at 700 mg/L). The estimated mean daily doses in this study (9, 18, and 36 mg/kg) were less than those used in the gavage study and the latter values do not account for loss of bromodichloromethane from water bottles during the exposure periods. An organ dosimetry analysis similar to that done for rats was not performed for mice because no

data are available on metabolic kinetic parameters for GST and CYP450-mediated metabolism of bromodichloromethane in mouse organs; scaling kinetic parameters from rats to mice by body weight is not a reliable approach for characterizing the kinetics of metabolic pathways in different species.

In addition, the large difference in liver neoplasm incidences in control female mice in the gavage and drinking water studies suggests that factors beyond dosimetry of bromodichloromethane may also be involved. A major difference between these studies is animal body weight; at week 52 of the gavage study, the mean body weight of vehicle control female mice was 44.4 grams (NTP, 1987) while at week 52 of the drinking water study the mean body weight of control female mice was 61.2 grams. Haseman *et al.* (1997) found a strong positive correlation between body weight and liver neoplasm incidence in female B6C3F$_1$ mice. Liver neoplasm incidence was also higher in control female mice in drinking water studies compared to gavage studies. Haseman *et al.* (1997) fit a logistic regression model to the liver tumor and body weight data to derive parameters to predict neoplasm incidence in control animals as a function of body weight, age, and route of exposure. Using those parameter values, the expected number of control female mice with a liver neoplasm in the bromodichloromethane drinking water study is 32; the observed number of control female mice with a liver neoplasm in this study was 30. Model-based predictions of the numbers of exposed female mice with liver neoplasms are 27 at 175 mg/L, 27 at 350 mg/L, and 24 at 700 mg/L; these values agree closely with the empirical data (i.e., 23, 24, and 19, in the respective dose groups). In addition, the 95% confidence interval for the survival-adjusted neoplasm incidence is ±14%. Thus, the observed decrease in liver neoplasm incidence in exposed female mice may simply reflect the impact of body weight differences due to reduced fluid and feed intake rather than a direct effect of bromodichloromethane on critical events in liver neoplasm development.

A final issue concerns the evaluation of cancer risk for bromodichloromethane from drinking water exposures. In contrast to the previous gavage study, no increases in neoplasm incidence were observed in male rats or female mice exposed to bromodichloromethane at concentrations that are approximately 10,000 times higher than concentrations that humans are exposed to in chlorinated drinking water. Although drinking water studies in laboratory animals might reflect human risks associated with oral exposure, they may not adequately represent risks from dermal or inhalation exposures. The latter exposures lack first-pass liver metabolism and may result in a relatively greater extrahepatic distribution of bromodichloromethane than from oral exposure alone. Indeed, blood levels of trihalomethanes including bromodichloromethane were four to five times higher in people who took 10-minute showers or bathed for 10 minutes than in people who drank one liter from the same tap water source in 10 minutes (Backer *et al.,* 2000). Thus, evaluations of human risk to bromodichloromethane in tap water need to account for all potential routes of exposure, not just oral exposure.

Previous 2-year gavage studies of bromodichloromethane by the NTP provided clear evidence of carcinogenic activity for male and female F344/N rats (kidney and large intestine neoplasms in both sexes) and for male and female B6C3F$_1$ mice (kidney and liver neoplasms, respectively). The different responses observed in these studies were attributed to differences in organ dosimetry by these routes of exposure and possible influences of dietary factors and differences in body weight on neoplasm development.

CONCLUSIONS

Under the conditions of this 2-year drinking water study, there was *no evidence of carcinogenic activity** of bromodichloromethane in male F344/N rats exposed to target concentrations of 175, 350, or 700 mg/L. There was *no evidence of carcinogenic activity* of bromodichloromethane in female B6C3F$_1$ mice exposed to target concentrations of 175, 350, or 700 mg/L.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Report Review Subcommittee comments and public discussion on this technical report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR DRINKING WATER STUDY OF BROMODICHLOROMETHANE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	16	19	14	15
Natural deaths	5	2	7	9
Survivors				
Died last week of study	1	1		
Terminal sacrifice	28	27	29	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, colon	(48)	(49)	(46)	(44)
Intestine large, rectum	(49)	(49)	(46)	(46)
Polyp adenomatous			1 (2%)	
Intestine large, cecum	(46)	(49)	(44)	(43)
Intestine small, duodenum	(48)	(50)	(48)	(45)
Intestine small, jejunum	(47)	(49)	(46)	(44)
Intestine small, ileum	(46)	(49)	(45)	(42)
Liver	(49)	(50)	(50)	(50)
Hepatocellular adenoma	1 (2%)	2 (4%)	3 (6%)	
Mesentery	(15)	(13)	(14)	(15)
Oral mucosa		(2)	(1)	
Squamous cell papilloma		1 (50%)		
Gingival, squamous cell carcinoma			1 (100%)	
Pancreas	(49)	(50)	(50)	(49)
Acinus, adenoma	3 (6%)	1 (2%)	3 (6%)	4 (8%)
Acinus, adenoma, multiple			1 (2%)	
Salivary glands	(50)	(50)	(50)	(49)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Stomach, glandular	(49)	(50)	(50)	(50)
Fibrosarcoma				1 (2%)
Tongue	(1)	(1)	(1)	(1)
Squamous cell papilloma	1 (100%)			1 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	1 (2%)		1 (2%)	
Myocardium, pericardium, epicardium, mesothelioma malignant	1 (2%)			
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Adenoma			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(49)
Pheochromocytoma malignant	1 (2%)		1 (2%)	2 (4%)
Pheochromocytoma complex	2 (4%)	1 (2%)		
Pheochromocytoma benign	5 (10%)	8 (16%)	10 (20%)	8 (16%)
Bilateral, pheochromocytoma benign	3 (6%)	1 (2%)	2 (4%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Endocrine System (continued)				
Islets, pancreatic	(49)	(50)	(50)	(49)
Adenoma	3 (6%)	1 (2%)	5 (10%)	3 (6%)
Carcinoma		1 (2%)	1 (2%)	1 (2%)
Pituitary gland	(49)	(50)	(50)	(48)
Pars distalis, adenoma	22 (45%)	29 (58%)	28 (56%)	26 (54%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(46)	(48)	(44)	(42)
Bilateral, C-cell, adenoma			1 (2%)	
C-cell, adenoma	8 (17%)	6 (13%)	9 (20%)	7 (17%)
C-cell, carcinoma	1 (2%)	1 (2%)	1 (2%)	4 (10%)
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma		2 (4%)		
General Body System				
Peritoneum	(2)	(2)	(2)	(1)
Tissue NOS	(3)	(4)	(2)	(4)
Schwannoma malignant				1 (25%)
Abdominal, schwannoma malignant		1 (25%)		
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(49)
Adenoma	2 (4%)	2 (4%)	3 (6%)	
Carcinoma	1 (2%)	3 (6%)		1 (2%)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	41 (82%)	40 (80%)	40 (80%)	39 (78%)
Interstitial cell, adenoma	4 (8%)	7 (14%)	1 (2%)	8 (16%)
Hematopoietic System				
Bone marrow	(50)	(45)	(49)	(49)
Lymph node	(25)	(28)	(26)	(26)
Mediastinal, C-cell, carcinoma, metastatic, thyroid gland		1 (4%)		
Lymph node, mandibular		(3)	(1)	
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Spleen	(48)	(49)	(49)	(50)
Hemangiosarcoma	1 (2%)			
Thymus	(48)	(48)	(50)	(47)
Thymoma benign		1 (2%)		
Integumentary System				
Mammary gland	(44)	(45)	(47)	(42)
Adenoma	1 (2%)			1 (2%)
Fibroadenoma	1 (2%)	3 (7%)	2 (4%)	2 (5%)
Fibroadenoma, multiple		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Integumentary System (continued)				
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Basal cell carcinoma	1 (2%)			
Fibroma			2 (4%)	
Keratoacanthoma	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma				1 (2%)
Subcutaneous tissue, basal cell adenoma	1 (2%)		1 (2%)	
Subcutaneous tissue, fibroma	6 (12%)	8 (16%)	6 (12%)	6 (12%)
Subcutaneous tissue, fibroma, multiple			2 (4%)	
Subcutaneous tissue, fibrosarcoma				1 (2%)
Subcutaneous tissue, hemangioma				1 (2%)
Subcutaneous tissue, lipoma			1 (2%)	
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)		
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma			1 (2%)	
Cranium, schwannoma malignant, metastatic, brain			1 (2%)	
Turbinate, sarcoma				1 (2%)
Skeletal muscle	(3)			(2)
Fibrosarcoma	1 (33%)			
Sarcoma	1 (33%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)	1 (2%)		
Granular cell tumor malignant				1 (2%)
Oligodendroglioma benign	1 (2%)			
Cerebrum, schwannoma malignant, metastatic, skin			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		
Alveolar/bronchiolar carcinoma	1 (2%)		2 (4%)	
Carcinoma, metastatic, thyroid gland		1 (2%)		
Neural crest tumor, metastatic, ear	1 (2%)			
Sarcoma, metastatic, skeletal muscle	1 (2%)			
C-cell, carcinoma, metastatic, thyroid gland		1 (2%)	1 (2%)	1 (2%)
Nose	(50)	(50)	(50)	(50)
Sarcoma	1 (2%)			
Pleura	(1)			
Trachea	(50)	(50)	(50)	(50)
C-cell, carcinoma, metastatic, thyroid gland				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Special Senses System				
Ear	(1)		(1)	
Pinna, neural crest tumor	1 (100%)		1 (100%)	
Eye	(49)	(49)	(48)	(47)
Schwannoma malignant, metastatic, brain			1 (2%)	
Harderian gland	(50)	(50)	(50)	(48)
Adenoma	1 (2%)		1 (2%)	
Zymbal's gland	(3)		(1)	(2)
Adenoma			1 (100%)	
Carcinoma	1 (33%)			1 (50%)
Urinary System				
Kidney	(49)	(50)	(50)	(49)
Urinary bladder	(49)	(50)	(50)	(49)
Transitional epithelium, papilloma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	16 (32%)	18 (36%)	16 (32%)	15 (30%)
Lymphoma malignant			1 (2%)	
Mesothelioma malignant	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	50	49
Total primary neoplasms	142	146	159	143
Total animals with benign neoplasms	49	49	50	49
Total benign neoplasms	108	114	130	111
Total animals with malignant neoplasms	28	27	27	27
Total malignant neoplasms	33	32	28	32
Total animals with metastatic neoplasms	2	3	2	1
Total metastatic neoplasms	2	10	4	2
Total animals with uncertain neoplasms-benign or malignant	1		1	
Total uncertain 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 A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	354	405	459	479	526	553	610	620	620	651	654	655	680	683	702	729	729	729	718	722	722	729	729	729	729
Carcass ID Number	045	043	003	007	028	049	002	012	036	024	033	015	011	018	046	013	020	022	008	016	040	001	010	019	021
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	A	+	+	+	+	+	+
Liver	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery					+	+							+		+			+			+	+			
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																			X						
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue										+															
Squamous cell papilloma										X															
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																			X						
Pheochromocytoma complex												X						X							
Pheochromocytoma benign																						X			
Bilateral, pheochromocytoma benign																					X				
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X			X	X					X		X			X	X	X				
Thyroid gland	+	A	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	A	+	+	+	+	+	+
C-cell, adenoma					X			X		X															X
C-cell, carcinoma																							X		
General Body System																									
Peritoneum																						+			
Tissue NOS							+			+															

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	731	731	731	732	732	732	732	732	732	732	732	732	732	732	735	
Carcass ID Number	00114	00125	00226	00227	00229	00230	00331	00332	00341	00442	00404	00505	00006	00009	01107	01223	01334	01535	01737	01839	01940	02247	02448	02550	02744	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatocellular adenoma													X													1
Mesentery			+					+				+	+				+	+	+				+			15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																X					X					3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign																									X	1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma complex																										2
Pheochromocytoma benign					X											X		X				X				5
Bilateral, pheochromocytoma benign			X																X							3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma													X										X			3
Parathyroid gland	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X				X	X	X	X	X		X		X	X				X		X	X			X		22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
C-cell, adenoma																X		X		X	X					8
C-cell, carcinoma																										1
General Body System																										
Peritoneum																				+						2
Tissue NOS																	+									3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	3 5 4	4 0 5	4 5 9	4 7 9	5 2 6	5 5 3	6 1 0	6 2 0	6 2 0	6 5 1	6 5 4	6 5 5	6 8 0	6 8 3	7 0 2	7 0 9	7 0 9	7 0 9	7 1 8	7 2 2	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 4 5	0 4 4 3	0 0 3 3	0 0 3 7	0 0 2 8	0 4 4 9	0 0 1 2	0 1 2 2	0 1 3 6	0 2 4 4	0 3 4 3	0 1 5 5	0 1 1 1	0 1 1 8	0 4 4 6	0 1 3 3	0 2 2 0	0 2 2 2	0 2 0 8	0 1 6 6	0 1 4 0	0 1 0 1	0 1 1 0	0 1 1 9	0 2 1 1
Genital System																									
Epididymis	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma			X																						X
Carcinoma																				X					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma				X			X	X		X		X	X	X	X	X	X	X	X	X		X	X	X	X
Interstitial cell, adenoma		X				X					X										X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+		+	+		+	+			+	+		+	+	+	+	+	+			+				
Lymph node, mandibular	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+
Integumentary System																									
Mammary gland	+	A	+	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+
Adenoma																									
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																				X					
Keratoacanthoma																				X		X			
Subcutaneous tissue, basal cell adenoma																									
Subcutaneous tissue, fibroma																	X								X
Subcutaneous tissue, sarcoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+							+																	
Fibrosarcoma	X																								
Sarcoma								X																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Oligodendroglioma benign																									
Peripheral nerve				+	+																				
Spinal cord				+	+																				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	731	731	731	732	732	732	732	732	732	732	732	732	732	732	735	
Carcass ID Number	01 14	02 45	02 66	02 77	02 99	03 00	03 11	03 22	04 11	04 22	04 04	05 05	05 06	05 09	06 07	06 23	06 34	06 55	06 73	06 88	06 93	07 74	07 84	08 04	08 44	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	41
Interstitial cell, adenoma																										4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+				+		+			+		+			+	+	+	+		+		+		25
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma					X																					1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	44
Adenoma									X																	1
Fibroadenoma	X																									1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																										1
Keratoacanthoma			X																							3
Subcutaneous tissue, basal cell adenoma									X																	1
Subcutaneous tissue, fibroma							X	X						X		X										6
Subcutaneous tissue, sarcoma				X																						1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																	+									3
Fibrosarcoma																										1
Sarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																	X									1
Oligodendroglioma benign													X													1
Peripheral nerve		+																								3
Spinal cord		+																								3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	3 5 4	4 0 5	4 5 9	4 7 9	5 2 6	5 5 3	6 1 0	6 2 0	6 2 0	6 5 1	6 5 4	6 5 5	6 8 0	6 8 3	7 0 2	7 0 9	7 0 9	7 0 9	7 1 8	7 2 2	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 4 5	0 4 3	0 0 3	0 0 7	0 2 8	0 4 9	0 0 2	0 1 2	0 3 6	0 2 4	0 3 3	0 1 5	0 1 1	1 1 8	0 4 6	1 1 3	2 2 0	2 2 2	0 2 8	1 6 6	4 4 0	0 1 1	1 1 0	1 9 9	0 2 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma													X												
Neural crest tumor, metastatic, ear											X														
Sarcoma, metastatic, skeletal muscle								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma		X																							
Pleura																									
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear											+														
Pinna, neural crest tumor											X														
Eye	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Zymbal's gland			+								+														
Carcinoma			X																						
Urinary System																									
Kidney	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X		X	X			X	X		X	X	X	X	X	X			X				
Mesothelioma malignant																						X			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	735	
Carcass ID Number	01 14	02 45	02 56	02 67	02 79	03 01	03 11	03 22	04 11	04 22	00 44	00 05	00 06	00 91	01 72	02 33	03 44	03 55	03 77	03 88	03 99	04 77	04 88	05 00	04 44			Total Tissues/ Tumors
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Alveolar/bronchiolar carcinoma																												1
Neural crest tumor, metastatic, ear																												1
Sarcoma, metastatic, skeletal muscle																												1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Sarcoma																												1
Pleura																				+								1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Special Senses System																												
Ear																												1
Pinna, neural crest tumor																												1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Adenoma													X															1
Zymbal's gland	+																											3
Carcinoma																												1
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			49
Systemic Lesions																												
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			50
Leukemia mononuclear														X			X	X		X								16
Mesothelioma malignant																				X			X					3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	405	498	520	526	556	561	620	624	651	654	660	667	667	681	683	695	697	697	697	709	709	709	729	729	729
Carcass ID Number	078	054	084	057	067	065	075	092	052	061	090	087	089	093	091	095	070	071	086	066	076	094	060	062	085
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma															X										
Serosa, mesothelioma malignant, metastatic, mesentery																									
Mesentery	+											+		+		+	+	+	+					+	
Oral mucosa		+																							+
Squamous cell papilloma																									X
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery																									
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue				+																					
Tooth																									+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Extra adrenal tissue, mesothelioma malignant, metastatic, mesentery																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma complex														X											
Pheochromocytoma benign															X						X	X	X	X	
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma													X												
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X	X	X		X		X	X	X	X	X	X				X				
Pars intermedia, adenoma																						X			
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+
C-cell, adenoma								X																	
C-cell, carcinoma																									
Follicular cell, carcinoma																					X				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 0 7 7	0 0 8 8	0 0 5 8	0 0 5 9	0 0 7 2	0 0 7 3	0 0 7 4	0 0 7 9	0 0 8 0	0 0 5 1	0 0 5 3	0 0 8 1	0 0 8 2	0 0 8 3	0 0 9 9	1 0 0	0 0 5 5	0 0 5 6	0 0 6 3	0 0 6 4	0 0 6 8	0 0 6 9	0 0 9 6	0 0 9 7	0 0 9 8	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Mesothelioma malignant, metastatic, mesentery																				X						1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma														X												2
Serosa, mesothelioma malignant, metastatic, mesentery																				X						1
Mesentery				+										+						+	+		+			13
Oral mucosa																										2
Squamous cell papilloma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																				X						1
Acinus, adenoma																X										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Extra adrenal tissue, mesothelioma malignant, metastatic, mesentery																				X						1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma complex																										1
Pheochromocytoma benign		X				X					X															8
Bilateral, pheochromocytoma benign																	X									1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																								X		1
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X	X	X	X					X	X	X	X		X	X	X	X		X		X	X	X	X		29
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma										X		X						X						X	X	6
C-cell, carcinoma				X																						1
Follicular cell, carcinoma					X																					2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	4 0 5	4 9 8	5 2 0	5 2 6	5 5 6	5 6 1	6 2 0	6 2 4	6 5 1	6 5 4	6 6 0	6 6 7	6 6 7	6 8 1	6 8 3	6 9 5	6 9 7	6 9 7	6 9 7	7 0 9	7 0 9	7 0 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 0 7 8	0 5 4	0 8 4	0 5 7	0 6 7	0 6 5	0 7 5	0 9 2	0 5 2	0 6 1	0 9 0	0 8 7	0 8 9	0 9 3	0 9 1	0 9 5	0 7 0	0 7 1	0 8 6	0 6 6	0 7 6	0 9 4	0 6 0	0 6 2	0 8 5
General Body System																									
Peritoneum														+										+	
Tissue NOS							+											+			+				
Abdominal, schwannoma malignant							X																		
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma					X												X								
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery																									
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesothelioma malignant, metastatic, mesentery																									
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X	X	X	X	X			X	X		X		X			X	X	X	X		X	X	X	X
Interstitial cell, adenoma								X			X		X		X						X				
Hematopoietic System																									
Bone marrow		+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	A
Lymph node	+		+		+		+				+	+				+	+	+	+		+	+		+	+
Mediastinal, C-cell, carcinoma, metastatic, thyroid gland																									
Lymph node, mandibular	M	M	+	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Capsule, mesothelioma malignant, metastatic, mesentery																									
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Fibroadenoma																									
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									X
Subcutaneous tissue, fibroma				X		X			X																
Subcutaneous tissue, sarcoma																									X
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 7 7	0 8 8	0 5 8	0 5 9	0 7 2	0 7 3	0 7 4	0 7 9	0 8 0	0 5 1	0 5 3	0 8 1	0 8 2	0 8 3	0 9 9	1 0 0	0 5 5	0 5 6	0 6 3	0 6 4	0 6 8	0 6 9	0 9 6	0 9 7	0 9 8	Total Tissues/ Tumors
General Body System																										
Peritoneum																										2
Tissue NOS							+																			4
Abdominal, schwannoma malignant																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X						X																	2
Carcinoma																						X				3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																				X						1
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesothelioma malignant, metastatic, mesentery																				X						1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X		X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	40
Interstitial cell, adenoma				X									X													7
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph node	+	+	+	+		+	+		+		+	+			+	+	+					+	+			28
Mediastinal, C-cell, carcinoma, metastatic, thyroid gland				X																						1
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	3
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Capsule, mesothelioma malignant, metastatic, mesentery																				X						1
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymoma benign																									X	1
Integumentary System																										
Mammary gland	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Fibroadenoma								X															X	X		3
Fibroadenoma, multiple																									X	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma																										1
Subcutaneous tissue, fibroma			X		X					X				X				X								8
Subcutaneous tissue, sarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	405	498	520	526	556	561	620	624	651	654	660	667	677	681	683	695	697	697	699	709	709	709	729	729	729
Carcass ID Number	07758	05844	08447	05567	06767	06565	07575	09592	09522	09561	09690	09887	09889	09993	09911	09915	09970	09971	09816	09866	09967	09794	09960	09662	09885
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant								X																	
Peripheral nerve								+																	
Spinal cord								+																	
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X	
Carcinoma, metastatic, thyroid gland																					X				
C-cell, carcinoma, metastatic, thyroid gland																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X		X							X	X			X	X	X	X	X	X	X			X	X	
Mesothelioma malignant													X											X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 0 7 7	0 0 8 8	0 0 5 8	0 0 5 9	0 0 7 2	0 0 7 3	0 0 7 4	0 0 7 9	0 0 8 0	0 0 5 1	0 0 5 3	0 0 8 1	0 0 8 2	0 0 8 3	0 0 9 9	0 1 0 0	0 0 5 5	0 0 5 6	0 0 6 3	0 0 6 4	0 0 6 8	0 0 6 9	0 0 9 6	0 0 9 7	0 0 9 8	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, thyroid gland																										1
C-cell, carcinoma, metastatic, thyroid gland				X																						1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X				X		X		X					X										18
Mesothelioma malignant																				X						3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	430	526	548	561	605	628	633	642	649	655	667	669	669	691	697	702	703	709	721	722	727	729	729	729
Carcass ID Number	12020	11014	11449	11339	11122	11212	11332	11008	11244	11235	11348	11416	11131	11416	11404	11505	11155	11447	11418	11227	11213	11119	12255	12266	12288
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+
Polyp adenomatous																									
Intestine large, cecum	A	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+
Intestine small, ileum	A	+	+	+	+	A	+	+	A	+	+	+	+	A	+	+	+	+	A	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery		+	+	+	+		+			+	+			+	+	+									
Oral mucosa												+													
Gingival, squamous cell carcinoma												X													
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																					X				
Acinus, adenoma, multiple																								X	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																					+				
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma benign																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign									X			X	X				X			X	X		X		
Bilateral, pheochromocytoma benign															X										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X		X				X				
Carcinoma											X														
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X		X				X	X	X		X		X				X	X		X	X	X	X		
Thyroid gland	+	+	A	+	+	A	+	+	A	+	+	+	+	A	+	+	A	+	A	+	+	+	+	+	+
Bilateral, C-cell, adenoma																									
C-cell, adenoma																		X		X				X	X
C-cell, carcinoma																									
Follicular cell, adenoma																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	732	735	735	735	735	735	735	
Carcass ID Number	12309	13090	14909	10202	10023	10707	10909	11010	11011	11313	11443	11455	10505	10606	11222	11233	11934	11936	11611	11714	11337	11838	11440	11441	11442	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Polyp adenomatous			X																							1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma							X	X		X																3
Mesentery			+										+						+	+						14
Oral mucosa																										1
Gingival, squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma	X								X																	3
Acinus, adenoma, multiple																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																						X				1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma benign					X																					1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant	X																									1
Pheochromocytoma benign			X				X																		X	10
Bilateral, pheochromocytoma benign				X																						2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X																X				5
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X		X	X	X	X		X				X	X		X		X		X		X	X	X		X	28
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Bilateral, C-cell, adenoma																								X		1
C-cell, adenoma									X	X					X	X				X						9
C-cell, carcinoma																						X				1
Follicular cell, adenoma																							X			1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	430	526	548	561	605	628	633	642	649	655	667	669	669	691	697	702	703	709	721	722	727	729	729	729	729
Carcass ID Number	12020	11014	11044	11439	11122	11212	11232	11308	12024	12035	11448	11416	11331	11466	11404	11505	11155	11447	11418	12127	12113	11199	12255	12266	12288
General Body System																									
Peritoneum		+																							
Tissue NOS					+									+											
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X		X	X	X			X		X	X		X	X	X		X	X	X	X	X	X	X	X
Interstitial cell, adenoma										X															
Hematopoietic System																									
Bone marrow	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+	+	+					+			+	+	+			+	+	+	+	+		+	
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Fibroadenoma													X												
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma								X																	
Keratoacanthoma																									
Subcutaneous tissue, basal cell adenoma																									
Subcutaneous tissue, fibroma									X			X			X							X			
Subcutaneous tissue, fibroma, multiple																		X							
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, schwannoma malignant																X									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Chordoma						X																			
Cranium, schwannoma malignant, metastatic, brain																X									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	729	729	729	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	732	735	735	735	735	735	735	
Carcass ID Number	12029	13030	14909	10002	10003	10007	10009	11090	11091	13093	14043	14045	10005	10006	12022	12023	13034	13036	11077	11174	13375	13408	14040	14041	14042	Total Tissues/ Tumors
General Body System																										
Peritoneum																			+							2
Tissue NOS																										2
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X									X														3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	40
Interstitial cell, adenoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+		+	+			+				+	+	+	+	+						+			26
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Fibroadenoma																			X							2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma															X											2
Keratoacanthoma	X																						X			2
Subcutaneous tissue, basal cell adenoma	X																									1
Subcutaneous tissue, fibroma								X			X															6
Subcutaneous tissue, fibroma, multiple	X																									2
Subcutaneous tissue, lipoma																	X									1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma																										1
Cranium, schwannoma malignant, metastatic, brain																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	430	526	548	561	605	628	633	642	649	665	666	667	669	699	691	697	702	703	709	721	722	727	729	729	729	729
Carcass ID Number	12020	12001	12044	12039	12012	12021	12032	12038	12044	12035	12048	12016	12031	12046	12040	12050	12115	12047	12118	12127	12113	12119	12125	12126	12128	12128
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cerebrum, schwannoma malignant, metastatic, skin																X										
Peripheral nerve					+																+	+				
Spinal cord					+																+	+				
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma			X																							
C-cell, carcinoma, metastatic, thyroid gland																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																										
Pinna, neural crest tumor																										
Eye	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, brain																X										
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X															
Zymbal's gland																	+									
Adenoma																	X									
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma																										
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X	X					X				X	X				X	X				X		
Lymphoma malignant																										
Mesothelioma malignant		X																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	729	729	729	730	300	300	300	300	300	300	300	300	301	311	311	311	311	311	312	325	335	335	335	335	335	
Carcass ID Number	12029	13030	14049	10202	10003	10007	10009	11010	11011	11013	14043	14045	10055	10066	12022	12023	13034	13036	11067	11074	13037	13038	14040	14041	14042	Total Tissues/ Tumors
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cerebrum, schwannoma malignant, metastatic, skin																										1
Peripheral nerve											+															4
Spinal cord											+															4
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma									X																	2
C-cell, carcinoma, metastatic, thyroid gland																						X				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																									+	1
Pinna, neural crest tumor																									X	1
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Schwannoma malignant, metastatic, brain																										1
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Zymbal's gland																										1
Adenoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma														X												1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X		X		X						X	X		X										16
Lymphoma malignant																				X						1
Mesothelioma malignant				X															X							3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	4 4 8	4 8 5	4 8 6	5 5 4	6 0 5	6 1 6	6 1 9	6 3 3	6 3 3	6 3 6	6 3 7	6 4 2	6 4 2	6 4 4	6 5 4	6 7 2	6 8 6	6 9 3	6 9 5	7 0 5	7 0 9	7 0 9	7 0 9	7 2 2	7 2 9
Carcass ID Number	1 1 6 4	1 9 0	1 9 5	1 8 1	1 8 6	1 8 5	1 6 6	1 8 7	1 9 2	1 5 1	1 5 3	1 5 6	1 8 8	1 9 3	1 8 3	1 7 4	1 6 8	1 7 0	1 6 5	2 0 0	1 5 2	1 5 7	1 6 1	1 9	1 5 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, rectum	+	+	A	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	A	A	A	A	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	A	A	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	A	A	A	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	A	A	A	A	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery					+	+	+		+		+	+	+	+											
Pancreas	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																				X			X		
Salivary glands	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Tongue													+												
Squamous cell papilloma													X												
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																								X	
Pheochromocytoma benign																		X	X			X			
Islets, pancreatic	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X								
Carcinoma																X									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X		X	X			X				X		X	X	X	X	X		X	X	X	X	
Thyroid gland	+	+	A	+	+	A	A	A	A	+	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+
C-cell, adenoma																									X
C-cell, carcinoma													X										X		
General Body System																									
Peritoneum																									
Tissue NOS				+					+			+	+												
Schwannoma malignant				X																					

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	735	735	735	
Carcass ID Number	15505	16909	17701	17808	18000	17505	17606	17707	15808	15909	16000	16202	16303	16707	17202	17303	19909	19101	19404	19606	19707	19808	18202	18404	18809	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery	+			+			+						+					+		+			+			15
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma						X											X									4
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma											X															1
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant			X																							2
Pheochromocytoma benign			X													X					X	X			X	8
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																X		X								3
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma		X			X				X	X		X	X			X		X	X	X			X	X		26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
C-cell, adenoma					X	X		X								X					X				X	7
C-cell, carcinoma			X										X													4
General Body System																										
Peritoneum				+																						1
Tissue NOS																										4
Schwannoma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	448	485	486	545	605	616	619	633	633	636	637	642	642	644	645	647	648	663	695	705	709	709	709	722	729
Carcass ID Number	16404	19005	19905	18811	18816	18855	16666	18867	19072	19511	15513	15636	18808	19333	18343	17474	16868	17010	16505	20020	15252	15577	16171	16799	15454
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma		X		X		X	X			X	X	X	X	X		X	X		X	X	X		X	X	X
Interstitial cell, adenoma	X				X			X	X									X				X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+				+	+	+	+	+	+	+	+	+			+	+	+				+		
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	I	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Adenoma																			X						
Fibroadenoma																									X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Keratoacanthoma												X													
Squamous cell carcinoma												X													
Squamous cell papilloma																X									
Subcutaneous tissue, fibroma																	X		X	X					
Subcutaneous tissue, fibrosarcoma								X																	
Subcutaneous tissue, hemangioma										X															
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Turbinate, sarcoma		X																							
Skeletal muscle																						+		+	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granular cell tumor malignant										X															
Peripheral nerve										+		+		+	+							+			
Spinal cord										+		+		+	+							+			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	732	735	735	735	
Carcass ID Number	15555	16969	16771	17788	18080	17745	17766	17767	15758	15959	16060	16262	16363	16667	17272	17373	17991	19191	19404	19626	19737	19838	19982	18224	18484	18889	Total Tissues/ Tumors
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma					X																						1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	39
Interstitial cell, adenoma			X									X															8
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node		+		+	+		+										+		+	+	+	+			+	+	26
Lymph node, mandibular	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	47
Integumentary System																											
Mammary gland	+	+	M	+	+	M	+	+	M	M	+	+	I	M	+	+	+	+	+	+	+	+	+	+	+	+	42
Adenoma																											1
Fibroadenoma		X																									2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma									X																		1
Keratoacanthoma						X													X								3
Squamous cell carcinoma																											1
Squamous cell papilloma																											1
Subcutaneous tissue, fibroma						X											X						X				6
Subcutaneous tissue, fibrosarcoma																											1
Subcutaneous tissue, hemangioma																											1
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Turbinate, sarcoma																											1
Skeletal muscle																											2
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granular cell tumor malignant																											1
Peripheral nerve																											5
Spinal cord																											5

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	448	485	486	545	605	616	619	633	633	636	637	642	644	644	645	724	726	783	693	695	705	709	709	709	722	729
Carcass ID Number	16404	16905	16995	16981	16886	16885	16666	16887	16892	16951	16953	16956	16988	16993	16983	16974	16768	16970	16765	16900	16752	16757	16761	16779	16754	
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma, metastatic, thyroid gland																							X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, carcinoma, metastatic, thyroid gland																							X			
Special Senses System																										
Eye	+	+	+	+	+	A	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Zymbal's gland											+															
Carcinoma											X															
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X	X		X	X		X	X	X				X								
Mesothelioma malignant																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	729	729	729	729	729	730	730	730	731	731	731	731	731	731	731	731	731	732	732	732	732	732	735	735	735	
Carcass ID Number	15585	16969	17711	17788	18080	17755	17766	17777	15858	15959	16060	16262	16363	16667	17272	17373	17991	19014	19945	19966	19987	19998	18282	18484	18889	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, carcinoma, metastatic, thyroid gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, carcinoma, metastatic, thyroid gland																										1
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Zymbal's gland												+														2
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X											X			X		X	X		X			X		15
Mesothelioma malignant				X																X						2

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	8/50 (16%)	9/50 (18%)	12/50 (24%)	8/49 (16%)
Adjusted rate[b]	18.7%	20.8%	26.6%	19.0%
Terminal rate[c]	7/29 (24%)	6/28 (21%)	5/29 (17%)	5/26 (19%)
First incidence (days)	722	683	649	693
Poly-3 test[d]	P=0.524	P=0.510	P=0.265	P=0.597
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	11/50 (22%)	10/50 (20%)	13/50 (26%)	9/49 (18%)
Adjusted rate	25.5%	23.0%	28.8%	21.3%
Terminal rate	7/29 (24%)	6/28 (21%)	6/29 (21%)	5/26 (19%)
First incidence (days)	655	681	649	693
Poly-3 test	P=0.417N	P=0.494N	P=0.455	P=0.422N
Liver: Hepatocellular Adenoma				
Overall rate	1/49 (2%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	2.4%	4.6%	6.8%	0.0%
Terminal rate	1/29 (3%)	1/28 (4%)	3/29 (10%)	0/26 (0%)
First incidence (days)	729 (T)	683	729 (T)	—[e]
Poly-3 test	P=0.342N	P=0.504	P=0.318	P=0.501N
Mammary Gland: Fibroadenoma				
Overall rate	1/50 (2%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	2.3%	9.3%	4.5%	4.7%
Terminal rate	1/29 (3%)	4/28 (14%)	1/29 (3%)	2/26 (8%)
First incidence (days)	729 (T)	729 (T)	669	729 (T)
Poly-3 test	P=0.566	P=0.179	P=0.513	P=0.497
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate	4.7%	9.3%	4.5%	7.1%
Terminal rate	2/29 (7%)	4/28 (14%)	1/29 (3%)	2/26 (8%)
First incidence (days)	729 (T)	729 (T)	669	695
Poly-3 test	P=0.520	P=0.338	P=0.681N	P=0.498
Pancreas: Adenoma				
Overall rate	3/49 (6%)	1/50 (2%)	4/50 (8%)	4/49 (8%)
Adjusted rate	7.0%	2.3%	9.1%	9.5%
Terminal rate	2/29 (7%)	1/28 (4%)	3/29 (10%)	2/26 (8%)
First incidence (days)	718	729 (T)	727	705
Poly-3 test	P=0.266	P=0.302N	P=0.519	P=0.492
Pancreatic Islets: Adenoma				
Overall rate	3/49 (6%)	1/50 (2%)	5/50 (10%)	3/49 (6%)
Adjusted rate	7.0%	2.3%	11.3%	7.1%
Terminal rate	2/29 (7%)	1/28 (4%)	2/29 (7%)	2/26 (8%)
First incidence (days)	722	729 (T)	697	686
Poly-3 test	P=0.411	P=0.302N	P=0.378	P=0.656

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/49 (6%)	2/50 (4%)	6/50 (12%)	4/49 (8%)
Adjusted rate	7.0%	4.6%	13.5%	9.5%
Terminal rate	2/29 (7%)	1/28 (4%)	2/29 (7%)	2/26 (8%)
First incidence (days)	722	667	667	672
Poly-3 test	P=0.300	P=0.494N	P=0.264	P=0.495
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	22/49 (45%)	29/50 (58%)	28/50 (56%)	26/48 (54%)
Adjusted rate	49.4%	62.6%	59.4%	58.8%
Terminal rate	13/29 (45%)	17/28 (61%)	17/29 (59%)	12/25 (48%)
First incidence (days)	459	526	430	486
Poly-3 test	P=0.300	P=0.140	P=0.222	P=0.245
Preputial Gland: Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	3/50 (6%)	0/49 (0%)
Adjusted rate	4.6%	4.7%	6.8%	0.0%
Terminal rate	1/29 (3%)	2/28 (7%)	3/29 (10%)	0/25 (0%)
First incidence (days)	459	729 (T)	729 (T)	—
Poly-3 test	P=0.213N	P=0.689	P=0.506	P=0.248N
Preputial Gland: Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/49 (2%)
Adjusted rate	2.3%	6.9%	0.0%	2.4%
Terminal rate	0/29 (0%)	1/28 (4%)	0/29 (0%)	1/25 (4%)
First incidence (days)	722	556	—	729 (T)
Poly-3 test	P=0.414N	P=0.312	P=0.494N	P=0.754
Preputial Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	5/50 (10%)	3/50 (6%)	1/49 (2%)
Adjusted rate	6.9%	11.5%	6.8%	2.4%
Terminal rate	1/29 (3%)	3/28 (11%)	3/29 (10%)	1/25 (4%)
First incidence (days)	459	556	729 (T)	729 (T)
Poly-3 test	P=0.172N	P=0.357	P=0.657N	P=0.323N
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	7.0%	2.3%	4.5%	7.0%
Terminal rate	2/29 (7%)	1/28 (4%)	2/29 (7%)	2/26 (8%)
First incidence (days)	722	729 (T)	729 (T)	642
Poly-3 test	P=0.477	P=0.304N	P=0.486N	P=0.662
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	7.0%	2.3%	4.5%	9.3%
Terminal rate	2/29 (7%)	1/28 (4%)	2/29 (7%)	2/26 (8%)
First incidence (days)	722	729 (T)	729 (T)	642
Poly-3 test	P=0.293	P=0.304N	P=0.486N	P=0.502

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	7.0%	2.3%	4.5%	9.3%
Terminal rate	2/29 (7%)	1/28 (4%)	2/29 (7%)	2/26 (8%)
First incidence (days)	722	729 (T)	729 (T)	642
Poly-3 test	P=0.293	P=0.304N	P=0.486N	P=0.502
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate	9.3%	2.3%	4.5%	11.6%
Terminal rate	3/29 (10%)	1/28 (4%)	2/29 (7%)	3/26 (12%)
First incidence (days)	722	729 (T)	729 (T)	642
Poly-3 test	P=0.274	P=0.177N	P=0.323N	P=0.502
Skin: Fibroma				
Overall rate	6/50 (12%)	8/50 (16%)	10/50 (20%)	6/50 (12%)
Adjusted rate	14.0%	18.0%	22.1%	14.0%
Terminal rate	5/29 (17%)	5/28 (18%)	5/29 (17%)	3/26 (12%)
First incidence (days)	709	526	642	686
Poly-3 test	P=0.534N	P=0.413	P=0.238	P=0.621
Skin: Fibroma, Fibrosarcoma, or Sarcoma				
Overall rate	7/50 (14%)	9/50 (18%)	10/50 (20%)	7/50 (14%)
Adjusted rate	16.3%	20.3%	22.1%	16.2%
Terminal rate	6/29 (21%)	6/28 (21%)	5/29 (17%)	3/26 (12%)
First incidence (days)	709	526	642	633
Poly-3 test	P=0.511N	P=0.422	P=0.338	P=0.609N
Testes: Adenoma				
Overall rate	45/50 (90%)	47/50 (94%)	41/50 (82%)	47/50 (94%)
Adjusted rate	95.0%	96.6%	86.1%	95.9%
Terminal rate	28/29 (97%)	28/28 (100%)	26/29 (90%)	25/26 (96%)
First incidence (days)	405	498	526	448
Poly-3 test	P=0.538N	P=0.559	P=0.102N	P=0.629
Thyroid Gland (C-cell): Adenoma				
Overall rate	8/46 (17%)	6/48 (13%)	10/44 (23%)	7/42 (17%)
Adjusted rate	19.4%	14.4%	25.1%	18.9%
Terminal rate	5/29 (17%)	5/28 (18%)	8/29 (28%)	7/26 (27%)
First incidence (days)	526	624	709	729 (T)
Poly-3 test	P=0.462	P=0.379N	P=0.360	P=0.591N
Thyroid Gland (C-cell): Carcinoma				
Overall rate	1/46 (2%)	1/48 (2%)	1/44 (2%)	4/42 (10%)
Adjusted rate	2.5%	2.4%	2.5%	10.7%
Terminal rate	1/29 (3%)	1/28 (4%)	1/29 (3%)	2/26 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	642
Poly-3 test	P=0.054	P=0.754N	P=0.759	P=0.158

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	Vehicle Control	175 mg/L	350 mg/L	700 mg/L
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	9/46 (20%)	7/48 (15%)	11/44 (25%)	11/42 (26%)
Adjusted rate	21.8%	16.8%	27.7%	29.3%
Terminal rate	6/29 (21%)	6/28 (21%)	9/29 (31%)	9/26 (35%)
First incidence (days)	526	624	709	642
Poly-3 test	P=0.164	P=0.384N	P=0.360	P=0.305
All Organs: Mononuclear Cell Leukemia				
Overall rate	16/50 (32%)	18/50 (36%)	16/50 (32%)	15/50 (30%)
Adjusted rate	34.9%	39.3%	35.0%	33.3%
Terminal rate	4/29 (14%)	7/28 (25%)	9/29 (31%)	7/26 (27%)
First incidence (days)	479	405	561	616
Poly-3 test	P=0.413N	P=0.413	P=0.584	P=0.524N
All Organs: Malignant Mesothelioma				
Overall rate	3/50 (6%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	7.0%	7.0%	6.7%	4.7%
Terminal rate	3/29 (10%)	2/28 (7%)	2/29 (7%)	2/26 (8%)
First incidence (days)	729 (T)	681	526	729 (T)
Poly-3 test	P=0.399N	P=0.659N	P=0.642N	P=0.504N
All Organs: Benign Neoplasms				
Overall rate	49/50 (98%)	49/50 (98%)	50/50 (100%)	49/50 (98%)
Adjusted rate	99.8%	99.7%	100.0%	98.0%
Terminal rate	29/29 (100%)	28/28 (100%)	29/29 (100%)	25/26 (96%)
First incidence (days)	405	498	430	448
Poly-3 test	P=0.275N	P=1.000N	P=1.000	P=0.549N
All Organs: Malignant Neoplasms				
Overall rate	28/50 (56%)	27/50 (54%)	27/50 (54%)	27/50 (54%)
Adjusted rate	57.2%	56.8%	56.5%	57.1%
Terminal rate	9/29 (31%)	11/28 (39%)	14/29 (48%)	12/26 (46%)
First incidence (days)	354	405	526	485
Poly-3 test	P=0.538N	P=0.569N	P=0.554N	P=0.581N
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100.0%	100.0%	100.0%	98.0%
Terminal rate	29/29 (100%)	28/28 (100%)	29/29 (100%)	25/26 (96%)
First incidence (days)	354	405	430	448
Poly-3 test	P=0.199N	—[f]	—	P=0.500N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, pancreas, 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 control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	16	19	14	15
Natural deaths	5	2	7	9
Survivors				
Died last week of study	1	1		
Terminal sacrifice	28	27	29	26
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(46)	(49)	(44)	(43)
Edema			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal	4 (8%)	4 (8%)	5 (10%)	2 (4%)
Basophilic focus	33 (66%)	25 (50%)	24 (48%)	17 (34%)
Cholangiofibrosis	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Clear cell focus	28 (56%)	24 (48%)	19 (38%)	17 (34%)
Congestion	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Degeneration, cystic, focal	31 (62%)	24 (48%)	23 (46%)	29 (58%)
Eosinophilic focus		1 (2%)	4 (8%)	
Fibrosis, focal	1 (2%)	1 (2%)	1 (2%)	3 (6%)
Hemorrhage, focal				1 (2%)
Hepatodiaphragmatic nodule	5 (10%)	5 (10%)	12 (24%)	4 (8%)
Hyperplasia, focal, histiocytic			1 (2%)	
Hyperplasia, focal, regenerative				1 (2%)
Infiltration cellular, mixed cell	30 (60%)	31 (62%)	34 (68%)	34 (68%)
Inflammation, chronic	23 (46%)	29 (58%)	33 (66%)	34 (68%)
Mixed cell focus		3 (6%)	10 (20%)	4 (8%)
Necrosis, focal	2 (4%)			1 (2%)
Tension lipidosis			1 (2%)	
Bile duct, hyperplasia	45 (90%)	49 (98%)	49 (98%)	50 (100%)
Hepatocyte, fatty change	36 (72%)	34 (68%)	36 (72%)	40 (80%)
Hepatocyte, hyperplasia, regenerative				1 (2%)
Hepatocyte, hypertrophy		1 (2%)		
Hepatocyte, vacuolization cytoplasmic	11 (22%)	10 (20%)	19 (38%)	18 (36%)
Hepatocyte, centrilobular, necrosis	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Serosa, bile duct, inflammation, chronic, focal				1 (2%)
Mesentery	(15)	(13)	(14)	(15)
Hemorrhage, focal				1 (7%)
Inflammation, chronic	1 (7%)		1 (7%)	
Fat, necrosis, focal	8 (53%)	4 (31%)	9 (64%)	7 (47%)

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

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Alimentary System (continued)				
Pancreas	(49)	(50)	(50)	(49)
Cyst			1 (2%)	
Hyperplasia, focal, histiocytic				1 (2%)
Acinus, atrophy, focal	31 (63%)	20 (40%)	23 (46%)	21 (43%)
Acinus, hyperplasia, focal	1 (2%)			
Duct, cyst	1 (2%)			
Duct, cyst, focal, multiple	16 (33%)	14 (28%)	17 (34%)	11 (22%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	2 (4%)	2 (4%)	3 (6%)	4 (8%)
Erosion				1 (2%)
Inflammation, chronic, diffuse			1 (2%)	
Inflammation, diffuse			2 (4%)	
Inflammation, focal	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Ulcer	1 (2%)	4 (8%)	4 (8%)	4 (8%)
Epithelium, hyperplasia	2 (4%)	5 (10%)	4 (8%)	6 (12%)
Stomach, glandular	(49)	(50)	(50)	(50)
Atypia cellular, focal			1 (2%)	
Edema		1 (2%)		
Erosion	2 (4%)	4 (8%)	1 (2%)	4 (8%)
Fibrosis, focal				1 (2%)
Infarct		1 (2%)		
Inflammation, focal		1 (2%)		
Pigmentation, focal		1 (2%)		1 (2%)
Ulcer	1 (2%)			
Artery, inflammation, chronic		1 (2%)		
Epithelium, hyperplasia				2 (4%)
Epithelium, hyperplasia, focal	1 (2%)			
Glands, ectasia, focal		1 (2%)		1 (2%)
Glands, necrosis, focal		1 (2%)		
Tongue	(1)	(1)	(1)	(1)
Infiltration cellular, focal, mixed cell		1 (100%)		
Epithelium, hyperplasia, focal			1 (100%)	
Tooth		(1)		
Epithelium alveolus, hyperplasia		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	16 (32%)	18 (36%)	21 (42%)	14 (28%)
Fibrosis, focal			1 (2%)	
Infiltration cellular, mixed cell	1 (2%)			
Thrombosis		2 (4%)		2 (4%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(49)
Accessory adrenal cortical nodule	14 (28%)	8 (16%)	8 (16%)	14 (29%)
Angiectasis			1 (2%)	
Cytoplasmic alteration, focal	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Infiltration cellular, mixed cell		1 (2%)		
Necrosis, focal	2 (4%)		1 (2%)	1 (2%)
Vacuolization cytoplasmic, focal	9 (18%)	5 (10%)	8 (16%)	12 (24%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Endocrine System (continued)				
Adrenal medulla	(50)	(50)	(50)	(49)
Cytoplasmic alteration, focal				1 (2%)
Hemorrhage			1 (2%)	
Hyperplasia				1 (2%)
Hyperplasia, focal	11 (22%)	17 (34%)	15 (30%)	13 (27%)
Necrosis, focal	1 (2%)		1 (2%)	
Thrombosis				1 (2%)
Bilateral, hyperplasia, focal		1 (2%)		
Islets, pancreatic	(49)	(50)	(50)	(49)
Hyperplasia		1 (2%)	1 (2%)	1 (2%)
Hyperplasia, focal	2 (4%)			
Parathyroid gland	(48)	(49)	(50)	(50)
Cyst				1 (2%)
Hyperplasia, focal			1 (2%)	
Pituitary gland	(49)	(50)	(50)	(48)
Angiectasis	2 (4%)	2 (4%)		1 (2%)
Craniopharyngeal duct, cyst	1 (2%)			
Pars distalis, cyst	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Pars distalis, cytoplasmic alteration, focal	7 (14%)	6 (12%)	1 (2%)	6 (13%)
Pars distalis, degeneration, cystic, focal	1 (2%)			3 (6%)
Pars distalis, hemorrhage, focal	3 (6%)	5 (10%)	1 (2%)	3 (6%)
Pars distalis, hyperplasia, focal	3 (6%)	2 (4%)	2 (4%)	7 (15%)
Pars distalis, necrosis, focal	1 (2%)			
Pars intermedia, cyst		1 (2%)		
Rathke's cleft, cyst	2 (4%)	2 (4%)		
Rathke's cleft, hemorrhage			1 (2%)	
Thyroid gland	(46)	(48)	(44)	(42)
Hemorrhage			1 (2%)	
C-cell, hyperplasia	44 (96%)	46 (96%)	40 (91%)	41 (98%)
Follicle, degeneration, cystic, focal		2 (4%)		
Follicular cell, hyperplasia				2 (5%)
Follicular cell, hyperplasia, cystic, focal	5 (11%)	4 (8%)	4 (9%)	3 (7%)
General Body System				
None				
Genital System				
Epididymis	(49)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)			
Preputial gland	(50)	(50)	(50)	(49)
Degeneration, cystic	1 (2%)		1 (2%)	2 (4%)
Fibrosis				1 (2%)
Hyperplasia, cystic	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic	32 (64%)	30 (60%)	31 (62%)	37 (76%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	29 (58%)	33 (66%)	35 (70%)	38 (76%)
Mineralization, focal				2 (4%)
Epithelium, hyperplasia, focal	25 (50%)	21 (42%)	24 (48%)	23 (46%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation			1 (2%)	
Inflammation, chronic			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Genital System (continued)				
Testes	(50)	(50)	(50)	(50)
Atrophy	5 (10%)	8 (16%)	8 (16%)	8 (16%)
Necrosis	1 (2%)			
Artery, thrombosis, focal	1 (2%)			
Bilateral, atrophy	1 (2%)		1 (2%)	1 (2%)
Bilateral, interstitial cell, hyperplasia, focal		1 (2%)		
Interstitial cell, hyperplasia			1 (2%)	
Interstitial cell, hyperplasia, focal	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Tunic, inflammation, chronic	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(45)	(49)	(49)
Hyperplasia	2 (4%)	2 (4%)	5 (10%)	2 (4%)
Hyperplasia, focal, histiocytic		1 (2%)		1 (2%)
Hyperplasia, histiocytic		1 (2%)		
Myeloid cell, hyperplasia	2 (4%)		3 (6%)	1 (2%)
Myeloid cell, erythroid cell, hyperplasia	1 (2%)	5 (11%)		5 (10%)
Lymph node	(25)	(28)	(26)	(26)
Bronchial, hyperplasia, plasma cell		1 (4%)		
Deep cervical, ectasia				1 (4%)
Mediastinal, angiectasis	2 (8%)			1 (4%)
Mediastinal, ectasia	3 (12%)	1 (4%)	3 (12%)	4 (15%)
Mediastinal, hemorrhage			1 (4%)	2 (8%)
Mediastinal, hyperplasia		1 (4%)		
Mediastinal, hyperplasia, histiocytic	2 (8%)		1 (4%)	2 (8%)
Mediastinal, hyperplasia, lymphoid	1 (4%)	1 (4%)	1 (4%)	
Mediastinal, hyperplasia, plasma cell	1 (4%)	3 (11%)	6 (23%)	1 (4%)
Mediastinal, inflammation	1 (4%)			
Pancreatic, angiectasis				1 (4%)
Pancreatic, ectasia	3 (12%)	5 (18%)	3 (12%)	5 (19%)
Pancreatic, hemorrhage	1 (4%)		2 (8%)	1 (4%)
Pancreatic, hyperplasia, histiocytic	3 (12%)	7 (25%)		5 (19%)
Pancreatic, pigmentation	1 (4%)		1 (4%)	1 (4%)
Lymph node, mesenteric	(50)	(49)	(50)	(50)
Ectasia		1 (2%)		
Hemorrhage	2 (4%)			1 (2%)
Hyperplasia, histiocytic		2 (4%)		1 (2%)
Spleen	(48)	(49)	(49)	(50)
Accessory spleen	1 (2%)		1 (2%)	1 (2%)
Angiectasis, focal	1 (2%)	2 (4%)		
Congestion		1 (2%)	1 (2%)	
Fibrosis, focal	4 (8%)	3 (6%)	1 (2%)	1 (2%)
Hematopoietic cell proliferation	6 (13%)	7 (14%)	5 (10%)	11 (22%)
Hemorrhage				2 (4%)
Hemorrhage, chronic	1 (2%)			
Hyperplasia, focal, histiocytic		1 (2%)		
Hyperplasia, lymphoid	1 (2%)			
Infarct	2 (4%)	1 (2%)	1 (2%)	
Necrosis, focal				1 (2%)
Pigmentation			1 (2%)	
Artery, thrombosis	1 (2%)			
Capsule, fibrosis, focal	1 (2%)			

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System (continued)				
Thymus	(48)	(48)	(50)	(47)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid			1 (2%)	
Integumentary System				
Mammary gland	(44)	(45)	(47)	(42)
Atypia cellular, focal			1 (2%)	
Dilatation	8 (18%)	18 (40%)	16 (34%)	12 (29%)
Ectasia			1 (2%)	1 (2%)
Fibrosis		1 (2%)		
Fibrosis, focal				1 (2%)
Hyperplasia	2 (5%)	3 (7%)	2 (4%)	1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion				2 (4%)
Hyperkeratosis, focal				1 (2%)
Inflammation, chronic, focal			1 (2%)	
Mineralization, focal			1 (2%)	
Subcutaneous tissue, angiectasis, focal	1 (2%)	1 (2%)		
Subcutaneous tissue, cyst, chronic	1 (2%)			
Subcutaneous tissue, cyst epithelial inclusion			1 (2%)	
Subcutaneous tissue, edema	1 (2%)			
Subcutaneous tissue, hemorrhage, focal	1 (2%)		1 (2%)	1 (2%)
Subcutaneous tissue, inflammation, focal, granulomatous			1 (2%)	
Subcutaneous tissue, metaplasia, focal, osseous	1 (2%)			
Subcutaneous tissue, necrosis, fatty, focal				1 (2%)
Subcutaneous tissue, epidermis, hyperplasia, focal			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		1 (2%)		
Skeletal muscle	(3)			(2)
Hemorrhage, focal				1 (50%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	6 (12%)	8 (16%)	14 (28%)	14 (28%)
Hemorrhage, focal	2 (4%)	4 (8%)	1 (2%)	3 (6%)
Hydrocephalus			2 (4%)	
Necrosis, focal	1 (2%)	2 (4%)		
Cerebellum, hemorrhage, focal			1 (2%)	
Cerebrum, ventricle, hydrocephalus		1 (2%)		
Pineal gland, vacuolization cytoplasmic			1 (2%)	

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	4 (8%)	1 (2%)	3 (6%)	5 (10%)
Fibrosis, focal				1 (2%)
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Hemorrhage, focal	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Hyperplasia, focal, histiocytic	1 (2%)	1 (2%)		
Hyperplasia, histiocytic	1 (2%)	1 (2%)		2 (4%)
Infiltration cellular, mixed cell	2 (4%)			1 (2%)
Inflammation, chronic, focal		2 (4%)	3 (6%)	2 (4%)
Metaplasia, squamous		1 (2%)		
Alveolar epithelium, hyperplasia, focal	7 (14%)	6 (12%)	9 (18%)	5 (10%)
Interstitium, edema	1 (2%)			
Mediastinum, angiectasis, focal				1 (2%)
Nose	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation, suppurative	2 (4%)	3 (6%)	3 (6%)	3 (6%)
Nasolacrimal duct, inflammation			3 (6%)	1 (2%)
Olfactory epithelium, mineralization, focal				1 (2%)
Special Senses System				
Eye	(49)	(49)	(48)	(47)
Cataract	2 (4%)	1 (2%)	7 (15%)	
Inflammation, chronic			1 (2%)	
Retina, degeneration	3 (6%)	1 (2%)	8 (17%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(48)
Hyperplasia, focal, histiocytic			1 (2%)	
Hyperplasia, focal, lymphoid			1 (2%)	
Hyperplasia, lymphoid	1 (2%)			
Inflammation, chronic, focal	1 (2%)		1 (2%)	
Epithelium, hyperplasia, focal			1 (2%)	
Urinary System				
Kidney	(49)	(50)	(50)	(49)
Cyst				1 (2%)
Infarct		3 (6%)	1 (2%)	
Metaplasia, focal, osseous			1 (2%)	
Nephropathy	49 (100%)	50 (100%)	49 (98%)	46 (94%)
Artery, thrombosis		1 (2%)		
Cortex, necrosis, focal				1 (2%)
Pelvis, dilatation			1 (2%)	
Pelvis, transitional epithelium, hyperplasia				1 (2%)
Renal tubule, hyperplasia, atypical, focal				1 (2%)
Renal tubule, pigmentation	10 (20%)	13 (26%)	7 (14%)	3 (6%)
Urinary bladder	(49)	(50)	(50)	(49)
Hemorrhage		1 (2%)	1 (2%)	
Inflammation, chronic			1 (2%)	

APPENDIX B
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR DRINKING WATER STUDY OF BROMODICHLOROMETHANE

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund	7	6	5	4
Natural deaths	7	7	12	7
Survivors				
Terminal sacrifice	36	36	33	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(45)	(48)	(45)
Histiocytic sarcoma			1 (2%)	
Intestine large, colon	(50)	(49)	(49)	(49)
Intestine large, cecum	(49)	(48)	(48)	(49)
Intestine small, duodenum	(48)	(48)	(46)	(46)
Polyp adenomatous			1 (2%)	
Intestine small, jejunum	(46)	(49)	(48)	(48)
Carcinoma		1 (2%)		
Leiomyosarcoma			1 (2%)	
Intestine small, ileum	(45)	(46)	(49)	(49)
Carcinoma	1 (2%)			
Histiocytic sarcoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)		
Hepatoblastoma	1 (2%)			1 (2%)
Hepatocellular carcinoma	9 (18%)	3 (6%)	10 (20%)	6 (12%)
Hepatocellular carcinoma, multiple	4 (8%)	3 (6%)	2 (4%)	1 (2%)
Hepatocellular adenoma	10 (20%)	12 (24%)	11 (22%)	9 (18%)
Hepatocellular adenoma, multiple	14 (28%)	6 (12%)	8 (16%)	7 (14%)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Mesentery	(21)	(17)	(16)	(17)
Hemangioma		1 (6%)		
Hemangiosarcoma	1 (5%)			
Hepatoblastoma, metastatic, liver	1 (5%)			
Hepatocellular carcinoma, metastatic, liver			1 (6%)	
Histiocytic sarcoma				1 (6%)
Leiomyosarcoma, metastatic, stomach, glandular			1 (6%)	
Rhabdomyosarcoma, metastatic, skeletal muscle			1 (6%)	
Sarcoma, metastatic, skin		1 (6%)		
Sarcoma, metastatic, skeletal muscle	1 (5%)			
Pancreas	(50)	(48)	(49)	(49)
Sarcoma, metastatic, skeletal muscle	2 (4%)			
Acinus, rhabdomyosarcoma, metastatic, skeletal muscle			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Sarcoma		1 (2%)		
Stomach, forestomach	(50)	(49)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma		1 (2%)		2 (4%)
Stomach, glandular	(50)	(49)	(49)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)			
Leiomyosarcoma			1 (2%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Histiocytic sarcoma			1 (2%)	
Capsule, adenoma		1 (2%)		
Adrenal medulla	(50)	(48)	(49)	(49)
Pheochromocytoma benign	1 (2%)			
Islets, pancreatic	(49)	(48)	(48)	(49)
Adenoma	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Carcinoma				1 (2%)
Pituitary gland	(49)	(50)	(49)	(49)
Schwannoma malignant, metastatic, brain				1 (2%)
Pars distalis, adenoma	3 (6%)	3 (6%)	1 (2%)	3 (6%)
Pars intermedia, adenoma	2 (4%)			
Thyroid gland	(50)	(49)	(49)	(50)
Follicular cell, adenoma	2 (4%)		1 (2%)	1 (2%)
Follicular cell, carcinoma		1 (2%)	1 (2%)	
General Body System				
None				
Genital System				
Clitoral gland	(48)	(49)	(48)	(49)
Histiocytic sarcoma			1 (2%)	
Ovary	(50)	(49)	(50)	(49)
Cystadenoma	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Granulosa cell tumor benign	1 (2%)		1 (2%)	
Hemangiosarcoma	2 (4%)	1 (2%)		4 (8%)
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Tubulostromal adenoma			1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Deciduoma benign	1 (2%)	1 (2%)		
Hemangioma			1 (2%)	
Hemangiosarcoma	2 (4%)			
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Leiomyosarcoma			1 (2%)	
Polyp stromal		1 (2%)		
Sarcoma stromal	2 (4%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)			
Histiocytic sarcoma		1 (2%)	1 (2%)	1 (2%)
Sarcoma, metastatic, skin			1 (2%)	
Lymph node	(16)	(9)	(7)	(4)
Sarcoma	1 (6%)			
Bronchial, hepatoblastoma, metastatic, liver	1 (6%)			
Iliac, rhabdomyosarcoma, metastatic, skeletal muscle			1 (14%)	
Inguinal, histiocytic sarcoma			1 (14%)	
Mediastinal, hepatocellular carcinoma, metastatic, liver			1 (14%)	
Mediastinal, rhabdomyosarcoma, metastatic, skeletal muscle			1 (14%)	
Mediastinal, sarcoma, metastatic, skeletal muscle	2 (13%)			
Pancreatic, hemangiosarcoma	1 (6%)			
Lymph node, mandibular	(50)	(50)	(50)	(47)
Histiocytic sarcoma			1 (2%)	1 (2%)
Lymph node, mesenteric	(49)	(48)	(46)	(50)
Carcinoma, metastatic, islets, pancreatic				1 (2%)
Hepatoblastoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma			1 (2%)	
Spleen	(50)	(49)	(48)	(50)
Carcinoma, metastatic, islets, pancreatic				1 (2%)
Hemangioma	1 (2%)			
Hemangiosarcoma	4 (8%)			
Thymus	(49)	(49)	(50)	(47)
Histiocytic sarcoma			1 (2%)	
Thymoma malignant	1 (2%)			
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoacanthoma		1 (2%)		
Carcinoma		2 (4%)		1 (2%)
Skin	(50)	(50)	(50)	(50)
Mast cell tumor malignant	1 (2%)			
Subcutaneous tissue, fibrosarcoma	1 (2%)		1 (2%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma	4 (8%)			
Subcutaneous tissue, liposarcoma		1 (2%)		
Subcutaneous tissue, sarcoma	4 (8%)	1 (2%)	3 (6%)	2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma	1 (2%)			
Osteosarcoma			1 (2%)	
Skeletal muscle	(5)	(2)	(4)	(1)
Leiomyosarcoma, metastatic, stomach, glandular			1 (25%)	
Rhabdomyosarcoma			1 (25%)	
Sarcoma	2 (40%)	1 (50%)		
Sarcoma, metastatic, skin	1 (20%)		1 (25%)	

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Nervous System				
Brain	(50)	(50)	(50)	(50)
Cranial nerve, schwannoma malignant				1 (2%)
Spinal cord	(4)	(4)	(2)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	6 (12%)	2 (4%)		3 (6%)
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	2 (4%)	
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Fibrosarcoma, metastatic, skin			1 (2%)	
Hemangiosarcoma, metastatic, skin	1 (2%)			
Hepatoblastoma, metastatic, liver	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	1 (2%)	2 (4%)	3 (6%)	
Histiocytic sarcoma			1 (2%)	1 (2%)
Osteosarcoma, metastatic, bone			1 (2%)	
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Sarcoma, metastatic, skin	1 (2%)		2 (4%)	
Sarcoma, metastatic, skeletal muscle	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(48)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	5 (10%)	2 (4%)	3 (6%)
Carcinoma	1 (2%)	2 (4%)	1 (2%)	
Histiocytic sarcoma			1 (2%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Hepatoblastoma, metastatic, liver	1 (2%)			
Histiocytic sarcoma			1 (2%)	
Urinary bladder	(50)	(49)	(48)	(50)
Hepatocellular carcinoma, metastatic, liver			1 (2%)	
Histiocytic sarcoma		1 (2%)		
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant	29 (58%)	30 (60%)	16 (32%)	23 (46%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	44	37	43
Total primary neoplasms	128	89	71	74
Total animals with benign neoplasms	34	25	24	28
Total benign neoplasms	52	35	29	31
Total animals with malignant neoplasms	43	38	31	35
Total malignant neoplasms	76	54	42	43
Total animals with metastatic neoplasms	5	2	9	2
Total metastatic neoplasms	16	3	20	3
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 B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	350	423	563	581	604	606	649	669	689	702	711	715	723	724	729	729	729	729	729	731	731	731	731	731	731
Carcass ID Number	029	011	039	024	005	019	011	049	040	045	012	011	006	037	031	032	033	034	035	022	023	025	041	042	043
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	A	+	+	+	A	M	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	M	+	+	+	+	M	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	+	M	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									X
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma					X																				
Hepatoblastoma						X																			
Hepatocellular carcinoma									X					X	X	X	X								
Hepatocellular carcinoma, multiple					X						X								X						
Hepatocellular adenoma											X												X		
Hepatocellular adenoma, multiple							X					X		X			X		X		X			X	
Mesentery	+		+	+		+	+	+	+	+	+			+			+	+	+		+				
Hemangiosarcoma																									
Hepatoblastoma, metastatic, liver						X																			
Sarcoma, metastatic, skeletal muscle							X																		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma, metastatic, skeletal muscle							X																		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver						X																			
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									X
Islets, pancreatic	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X					X		
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																				X		X			
Pars intermedia, adenoma																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma					X																				

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	7 3 1	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 4 4	0 1 3	0 1 4	0 1 5	0 0 2	0 0 3	0 0 4	0 0 7	0 0 8	0 0 9	0 1 0	0 1 6	0 1 7	0 1 8	0 2 0	0 2 6	0 2 7	0 2 8	0 3 0	0 3 6	0 3 8	0 4 6	0 4 7	0 4 8	0 5 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Carcinoma																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma						X																				2
Hepatoblastoma																										1
Hepatocellular carcinoma																	X	X		X		X				9
Hepatocellular carcinoma, multiple															X											4
Hepatocellular adenoma			X								X					X		X	X		X		X	X		10
Hepatocellular adenoma, multiple	X				X		X			X							X					X			X	14
Mesentery	+	+				+	+							+	+								+			21
Hemangiosarcoma						X																				1
Hepatoblastoma, metastatic, liver																										1
Sarcoma, metastatic, skeletal muscle																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma, metastatic, skeletal muscle			X																							2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Cardiovascular System																										
Blood vessel		+															+									2
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																	X									3
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma										X																3
Pars intermedia, adenoma															X				X							2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																X										2

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	3 5 0	4 2 3	5 6 3	5 8 1	6 0 4	6 0 6	6 4 9	6 6 9	6 8 9	7 0 2	7 1 5	7 1 5	7 2 3	7 2 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	0 2 9	0 1 1	0 1 9	0 2 4	0 0 5	0 1 9	0 1 1	0 4 9	0 4 0	0 4 5	0 1 2	0 2 1	0 0 6	0 3 7	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 2 2	0 2 3	0 2 5	0 4 1	0 4 2	0 4 3
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma										X	X													X	
Granulosa cell tumor benign																									
Hemangiosarcoma												X													
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign																									
Hemangiosarcoma									X																
Sarcoma stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																					X				
Lymph node		+		+		+	+	+	+		+	+		+						+				+
Sarcoma				X																					
Bronchial, hepatoblastoma,																									
Metastatic, liver						X																			
Mediastinal, sarcoma, metastatic,																									
skeletal muscle							X																		
Pancreatic, hemangiosarcoma																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver						X																			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma					X																				
Hemangiosarcoma																									
Thymus	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant																X									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mast cell tumor malignant																									
Subcutaneous tissue, fibrosarcoma																				X					
Subcutaneous tissue, hemangiosarcoma					X																X				
Subcutaneous tissue, sarcoma			X	X		X																			

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	7 3 1	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 4 4	0 1 3	0 1 4	0 1 5	0 0 2	0 0 3	0 0 4	0 0 7	0 0 8	0 0 9	0 1 0	0 1 6	0 1 7	0 1 8	0 2 0	0 2 6	0 2 7	0 2 8	0 3 0	0 3 6	0 3 8	0 4 6	0 4 7	0 4 8	0 5 0	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma							X																			4
Granulosa cell tumor benign									X																	1
Hemangiosarcoma						X																				2
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deciduoma benign										X																1
Hemangiosarcoma						X																				2
Sarcoma stromal										X		X														2
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma													X													2
Lymph node	+		+								+								+				+			16
Sarcoma																										1
Bronchial, hepatoblastoma, metastatic, liver																										1
Mediastinal, sarcoma, metastatic, skeletal muscle			X																							2
Pancreatic, hemangiosarcoma	X																									1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hepatoblastoma, metastatic, liver																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																										1
Hemangiosarcoma	X					X							X										X			4
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mast cell tumor malignant																X										1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma	X					X																				4
Subcutaneous tissue, sarcoma																			X							4

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	350	423	563	581	604	606	649	669	689	702	715	711	723	724	729	729	729	729	729	731	731	731	731	731	731
Carcass ID Number	02949	01911	03919	02924	00505	01919	01041	04949	04400	04505	01212	02101	00206	03707	03311	03232	03333	03434	03335	02222	02323	02425	04141	04442	04043
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteoma																								X	
Skeletal muscle		+		+			+				+														
Sarcoma							X																		
Sarcoma, metastatic, skin				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+		+					+		+									+					
Spinal cord				+					+		+									+					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X							X						
Alveolar/bronchiolar carcinoma																									
Hemangiosarcoma, metastatic, skin					X																				
Hepatoblastoma, metastatic, liver						X																			
Hepatocellular carcinoma, metastatic, liver					X																				
Sarcoma, metastatic, skin				X																					
Sarcoma, metastatic, skeletal muscle																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	A	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma										X															
Carcinoma																									
Lacrimal gland													+												
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma, metastatic, liver						X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant			X					X	X	X	X			X		X			X				X	X	X

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 0 mg/L

Number of Days on Study	731	732	732	732	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	735	
Carcass ID Number	04 44	01 13	01 14	01 15	00 02	00 03	00 04	00 07	00 08	00 09	01 10	01 16	01 17	01 18	02 00	02 06	02 07	02 08	03 00	03 06	03 08	04 06	04 07	04 08	05 00	Total Tissues/ Tumors
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteoma																										1
Skeletal muscle			+																							5
Sarcoma			X																							2
Sarcoma, metastatic, skin																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										5
Spinal cord																										4
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma								X		X													X	X		6
Alveolar/bronchiolar carcinoma																								X		1
Hemangiosarcoma, metastatic, skin																										1
Hepatoblastoma, metastatic, liver																										1
Hepatocellular carcinoma, metastatic, liver																										1
Sarcoma, metastatic, skin																										1
Sarcoma, metastatic, skeletal muscle			X																							1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X			X											3
Carcinoma																						X				1
Lacrimal gland																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma, metastatic, liver																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant	X	X		X	X		X	X	X	X	X	X	X		X			X	X		X	X	X	X		29

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	217	318	521	521	547	581	604	626	640	641	649	674	689	696	729	729	729	729	729	730	730	730	730	730	730
Carcass ID Number	09993	09999	09975	09991	09995	09970	09958	09990	09885	09898	09669	10040	10959	10551	10776	10777	10778	10779	10856	10956	10970	11081	11082	11083	
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Gallbladder	A	+	+	M	+	+	A	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine large, cecum	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, duodenum	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Carcinoma																									
Intestine small, ileum	M	+	A	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Hemangiosarcoma															X										
Hepatocellular carcinoma					X							X													
Hepatocellular carcinoma, multiple															X	X									X
Hepatocellular adenoma			X																X				X		
Hepatocellular adenoma, multiple												X										X			
Histiocytic sarcoma																									
Mesentery	+					+	+		+		+	+			+							+			
Hemangioma																									
Sarcoma, metastatic, skin															X										
Pancreas	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Sarcoma																									
Stomach, forestomach	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Squamous cell carcinoma																						X			
Squamous cell papilloma						X																			
Stomach, glandular	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Capsule, adenoma																									
Adrenal medulla	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Islets, pancreatic	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pars distalis, adenoma																							X		
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Follicular cell, carcinoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	0 8 4	0 5 2	0 5 3	0 5 4	0 5 5	0 6 1	0 6 2	0 6 3	0 6 4	0 6 5	0 6 6	0 6 7	0 6 8	0 7 1	0 7 2	0 7 3	0 7 4	0 8 6	0 8 7	0 8 8	0 8 9	0 9 2	0 9 4	0 9 6	0 9 7	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																				X						1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Hepatocellular carcinoma									X																	3
Hepatocellular carcinoma, multiple																										3
Hepatocellular adenoma			X					X		X		X					X				X		X	X	X	12
Hepatocellular adenoma, multiple	X					X					X			X												6
Histiocytic sarcoma															X											1
Mesentery	+			+		+				+		+		+		+			+					+		17
Hemangioma				X																						1
Sarcoma, metastatic, skin																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma									X																	1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Squamous cell carcinoma																										1
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Capsule, adenoma													X													1
Adrenal medulla	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																					X					1
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma											X											X				3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49
Follicular cell, carcinoma																	X									1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	2 1 7	3 1 8	5 2 1	5 2 1	5 4 7	5 8 1	6 0 4	6 2 6	6 4 0	6 4 1	6 4 9	6 7 4	6 8 9	6 9 6	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	0 9 9 3	0 9 9 9	0 7 9 5	0 9 1 1	0 9 9 5	0 7 5 0	0 5 9 8	0 9 8 0	0 8 5 5	0 9 8 8	0 6 9 9	0 1 0 0	0 5 9 9	0 5 1 6	0 7 7 6	0 7 7 7	0 7 7 8	0 7 7 9	0 8 0 0	0 5 5 6	0 5 5 7	0 6 7 0	0 8 1 1	0 8 1 2	0 8 2 3
Genital System																									
Clitoral gland	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma												X													
Hemangiosarcoma							X																		
Histiocytic sarcoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign																									
Histiocytic sarcoma																									
Polyp stromal																						X			
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma												X													
Lymph node						+	+	+	+		+		+	+											
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoacanthoma																									
Carcinoma			X		X																				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, liposarcoma													X												
Subcutaneous tissue, sarcoma															X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle														+											
Sarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve		+				+					+			+											
Spinal cord		+				+				+	+														
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																						X			
Alveolar/bronchiolar carcinoma																									
Alveolar/bronchiolar carcinoma, multiple																									
Hepatocellular carcinoma, metastatic, liver															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	730	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	735	735	735	735	
Carcass ID Number	08484	05252	05353	05454	05555	06061	06262	06363	06464	06565	06666	06767	06868	07071	07272	07373	07474	07686	08087	08888	08989	09292	09494	09696	09797	Total Tissues/ Tumors	
Genital System																											
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49	
Cystadenoma																										1	
Hemangiosarcoma																										1	
Histiocytic sarcoma															X											1	
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Deciduoma benign																X										1	
Histiocytic sarcoma															X											1	
Polyp stromal																										1	
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Histiocytic sarcoma																										1	
Lymph node											+								+							9	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Lymph node, mesenteric	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Integumentary System																											
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Adenoacanthoma				X																						1	
Carcinoma																										2	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Subcutaneous tissue, liposarcoma																										1	
Subcutaneous tissue, sarcoma																										1	
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Skeletal muscle																		+								2	
Sarcoma																		X								1	
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Peripheral nerve																										4	
Spinal cord																										4	
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Alveolar/bronchiolar adenoma				X																						2	
Alveolar/bronchiolar carcinoma							X																			1	
Alveolar/bronchiolar carcinoma, multiple																	X									1	
Hepatocellular carcinoma, metastatic, liver									X																	2	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	49	

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	217	318	521	521	547	581	604	626	640	641	649	674	689	696	729	729	729	729	729	730	730	730	730	730	730
Carcass ID Number	093	099	075	091	095	070	058	090	085	098	069	100	059	051	076	077	078	079	080	056	057	060	081	082	083
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X												X							
Carcinoma							X																X		
Urinary System																									
Kidney	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma																									
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma												X													
Lymphoma malignant					X	X		X	X	X	X		X	X	X	X		X		X		X	X		

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 175 mg/L

Number of Days on Study	730	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	732	735	735	735	735	
Carcass ID Number	08484	05212	05353	05354	05355	06061	06062	06063	06064	06065	06066	06067	06068	07071	07072	07073	07074	07086	07087	07088	07089	07092	07094	07096	07097	Total Tissues/ Tumors
Special Senses System																											
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X									X			X											5
Carcinoma																											2
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma															X												1
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma															X												2
Lymphoma malignant	X				X	X	X			X	X	X	X	X		X	X	X	X			X	X		X		30

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	3 2 3	4 5 5	4 6 0	4 8 6	5 3 9	5 8 3	6 0 4	6 5 8	6 5 9	6 6 3	6 6 3	6 6 4	6 6 9	6 9 6	7 0 1	7 0 7	7 1 4	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 2 7	1 2 1	1 0 1	1 0 5	1 3 3	1 2 8	1 4 3	1 4 2	1 4 7	1 1 2	1 2 4	1 4 6	1 4 8	1 4 0	1 3 9	1 3 0	1 2 6	1 3 1	1 3 2	1 3 4	1 3 5	1 0 2	1 0 3	1 0 4	1 3 6
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	A	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	M	A	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Polyp adenomatous																							X		
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																						X			
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma					X	X		X				X										X			
Hepatocellular carcinoma, multiple									X						X										
Hepatocellular adenoma					X			X	X						X			X	X			X			
Hepatocellular adenoma, multiple										X		X				X								X	X
Histiocytic sarcoma													X												
Mesentery								+		+	+	+	+	+		+	+					+			
Hepatocellular carcinoma, metastatic, liver																									
Leiomyosarcoma, metastatic, stomach, glandular												X													
Rhabdomyosarcoma, metastatic, skeletal muscle										X															
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Acinus, rhabdomyosarcoma, metastatic, skeletal muscle										X															
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma												X													
Tongue			+																						
Tooth																								+	
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 3 3 7	1 3 3 8	1 4 0 1	1 4 4 4	1 4 4 5	1 4 4 9	1 5 0 0	1 0 0 6	1 0 0 7	1 0 0 8	1 0 0 9	1 1 1 0	1 1 1 6	1 1 1 7	1 1 1 8	1 1 1 9	1 2 2 0	1 2 2 2	1 2 2 3	1 2 2 5	1 1 1 1	1 1 1 3	1 1 1 4	1 1 1 5	1 2 2 9	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	48
Histiocytic sarcoma																										1
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Polyp adenomatous																										1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyosarcoma																										1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Histiocytic sarcoma																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma					X							X					X	X	X							10
Hepatocellular carcinoma, multiple																										2
Hepatocellular adenoma				X		X									X		X									11
Hepatocellular adenoma, multiple									X									X					X			8
Histiocytic sarcoma																										1
Mesentery								+						+	+		+	+	+				+			16
Hepatocellular carcinoma, metastatic, liver																			X							1
Leiomyosarcoma, metastatic, stomach, glandular																										1
Rhabdomyosarcoma, metastatic, skeletal muscle																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, rhabdomyosarcoma, metastatic, skeletal muscle																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma, metastatic, liver																			X							1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																										1
Tongue																										1
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	3 2 3	4 5 5	4 6 0	4 8 6	5 3 9	5 8 3	6 0 4	6 5 8	6 5 9	6 6 3	6 6 3	6 6 4	6 6 9	6 9 6	7 0 1	7 0 7	7 1 4	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 2 7	1 2 1	1 0 1	1 0 5	1 3 3	1 2 8	1 4 3	1 4 2	1 4 7	1 1 2	1 2 4	1 4 6	1 4 8	1 4 0	1 3 9	1 3 0	1 2 6	1 3 1	1 3 2	1 3 4	1 3 5	1 0 2	1 0 3	1 0 4	1 3 6
Endocrine System																								
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	A	+	M	+	+	+	+	+	+	+	+	+
Adenoma																								
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma							X																	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								
Follicular cell, carcinoma																								
General Body System																								
None																								
Genital System																								
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma									X															
Granulosa cell tumor benign																								
Histiocytic sarcoma													X											
Tubulostromal adenoma																								
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																								
Histiocytic sarcoma													X											
Leiomyosarcoma																								
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Sarcoma, metastatic, skin					X																			
Lymph node										+	+		+	+			+							
Iliac, rhabdomyosarcoma, metastatic, skeletal muscle										X														
Inguinal, histiocytic sarcoma													X											
Mediastinal, hepatocellular carcinoma, metastatic, liver																								
Mediastinal, rhabdomyosarcoma, metastatic, skeletal muscle										X														
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Lymph node, mesenteric	+	+	+	+	+	+	A	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	730	730	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	735	735	735	735	735	
Carcass ID Number	13737	13738	14141	14144	14145	14149	15050	10106	10007	10008	10009	11010	11016	11017	11018	11019	12020	12022	12023	12025	11011	11013	11014	11015	11012	11019	Total Tissues/ Tumors
Endocrine System																											
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		49
Histiocytic sarcoma																											1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Adenoma					X																						1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	I	+	+	+	+	+	+	+		46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Pars distalis, adenoma																											1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		49
Follicular cell, adenoma																						X					1
Follicular cell, carcinoma			X																								1
General Body System																											
None																											
Genital System																											
Clitoral gland	I	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		48
Histiocytic sarcoma																											1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Cystadenoma																											1
Granulosa cell tumor benign																							X				1
Histiocytic sarcoma																											1
Tubulostromal adenoma									X																		1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Hemangioma			X																								1
Histiocytic sarcoma																											1
Leiomyosarcoma																							X				1
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Sarcoma, metastatic, skin																											1
Lymph node																			+			+					7
Iliac, rhabdomyosarcoma, metastatic, skeletal muscle																											1
Inguinal, histiocytic sarcoma																											1
Mediastinal, hepatocellular carcinoma, metastatic, liver																			X								1
Mediastinal, rhabdomyosarcoma, metastatic, skeletal muscle																											1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+		46
Histiocytic sarcoma																											1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+		48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		50
Histiocytic sarcoma																											1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	3 2 3	4 5 5	4 6 0	4 8 6	5 3 9	5 8 3	6 0 4	6 5 8	6 5 9	6 6 3	6 6 3	6 6 4	6 6 9	6 9 6	7 0 1	7 0 7	7 1 4	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	1 2 7	1 2 1	1 0 1	1 0 5	1 3 3	1 2 8	1 4 3	1 4 2	1 4 7	1 1 2	1 2 4	1 4 6	1 4 8	1 4 0	1 3 9	1 3 0	1 2 6	1 3 1	1 3 2	1 3 4	1 3 5	1 0 2	1 0 3	1 0 4	1 3 6
Integumentary System																								
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma																								
Subcutaneous tissue, sarcoma					X																	X		
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma	X																							
Skeletal muscle					+					+		+												
Leiomyosarcoma, metastatic, stomach, glandular												X												
Rhabdomyosarcoma										X														
Sarcoma, metastatic, skin					X																			
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve					+																			
Spinal cord	+				+																			
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																			X					
Fibrosarcoma, metastatic, skin																								
Hepatocellular carcinoma, metastatic, liver									X			X												
Histiocytic sarcoma													X											
Osteosarcoma, metastatic, bone	X																							
Osteosarcoma, metastatic, uncertain primary site				X																				
Sarcoma, metastatic, skin					X																	X		
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X									
Carcinoma																								
Histiocytic sarcoma													X											
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X											
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma, metastatic, liver																								

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	1 3 7	1 3 8	1 4 1	1 4 4	1 4 5	1 4 9	1 5 0	1 0 6	1 0 7	1 0 8	1 0 9	1 1 0	1 1 6	1 1 7	1 1 8	1 1 9	1 2 0	1 2 2	1 2 3	1 2 5	1 1 1	1 1 3	1 1 4	1 1 5	1 2 9	Total Tissues/ Tumors
Integumentary System																												
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma											X																	1
Subcutaneous tissue, sarcoma					X																							3
Musculoskeletal System																												
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																												1
Skeletal muscle					+																							4
Leiomyosarcoma, metastatic, stomach, glandular																												1
Rhabdomyosarcoma																												1
Sarcoma, metastatic, skin																												1
Nervous System																												
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																												1
Spinal cord																												2
Respiratory System																												
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																				X								2
Fibrosarcoma, metastatic, skin											X																	1
Hepatocellular carcinoma, metastatic, liver																			X									3
Histiocytic sarcoma																												1
Osteosarcoma, metastatic, bone																												1
Osteosarcoma, metastatic, uncertain primary site																												1
Sarcoma, metastatic, skin																												2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																												
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																			X									2
Carcinoma																X												1
Histiocytic sarcoma																												1
Urinary System																												
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																												1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+			48
Hepatocellular carcinoma, metastatic, liver																			X									1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	323	455	460	486	539	583	604	658	659	663	663	664	669	696	701	707	714	729	729	729	730	730	730	730
Carcass ID Number	12707	12201	10011	10055	13033	12238	14432	14422	14477	11212	12424	14446	14488	14400	13309	13300	12326	13131	13312	13324	13335	10022	10033	10044	13366
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma													X												
Lymphoma malignant					X						X			X			X		X			X		X	

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 350 mg/L

Number of Days on Study	730	730	731	731	731	731	731	732	732	732	732	732	732	732	732	732	732	732	732	732	732	735	735	735	735	735	
Carcass ID Number	13 7	13 8	14 1	14 4	14 5	14 9	15 0	00 6	00 7	00 8	00 9	01 0	11 6	11 7	11 8	11 9	12 0	22 2	22 3	22 5	11 1	11 3	11 4	11 5	11 9	12 9	Total Tissues/ Tumors
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																											1
Lymphoma malignant		X				X		X	X			X				X			X				X	X			16

Table B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	4	4	5	5	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	2	7	2	3	8	3	4	6	8	9	0	2	2	2	2	2	2	2	3	3	3	3	3	3	3
	4	2	2	5	7	1	7	9	6	3	3	9	9	9	9	9	9	9	0	0	0	0	0	0	0
Carcass ID Number	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
	9	8	6	5	6	7	6	5	5	9	8	8	8	9	9	9	9	9	5	5	5	7	7	7	7
	4	7	7	4	9	8	3	8	1	6	8	6	9	0	1	2	3	5	2	3	5	1	2	3	4
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	A	I	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	I	I	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatoblastoma																									
Hepatocellular carcinoma									X		X														
Hepatocellular carcinoma, multiple																									
Hepatocellular adenoma									X				X			X						X			
Hepatocellular adenoma, multiple											X							X							
Histiocytic sarcoma			X																						
Mesentery			+		+						+		+					+					+		
Histiocytic sarcoma			X																						
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma						X										X									
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel																								+	
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma						X																			
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant, metastatic, brain																								X	
Pars distalis, adenoma														X											
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	730	730	730	730	730	730	731	731	731	731	731	731	731	731	732	732	732	732	735	735	735	735	735	735	735	
Carcass ID Number	17 5	18 1	18 2	18 3	18 4	18 5	16 1	16 2	16 4	16 5	16 6	17 7	17 9	18 0	15 6	15 7	15 9	16 0	16 6	16 8	17 0	19 7	19 8	19 9	20 0	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatoblastoma					X																					1
Hepatocellular carcinoma										X	X													X	X	6
Hepatocellular carcinoma, multiple			X																							1
Hepatocellular adenoma						X						X		X	X		X									9
Hepatocellular adenoma, multiple			X		X						X					X			X							7
Histiocytic sarcoma																										1
Mesentery	+		+	+	+	+				+	+			+					+	+	+					17
Histiocytic sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel																										1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma							X											X								2
Carcinoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	I	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Schwannoma malignant, metastatic, brain																										1
Pars distalis, adenoma																			X		X					3
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																				X						1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	424	447	522	535	587	631	647	669	686	693	703	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	19947	18767	16754	15469	16978	17763	15858	15151	15196	18888	18686	18789	19090	19191	19223	19935	19552	15533	15555	17171	17223	17334	17474
Genital System																									
Clitoral gland	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																									
Hemangiosarcoma									X										X						
Histiocytic sarcoma			X																						
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymph node				+						+															
Lymph node, mandibular	+	+	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic						X																			
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, islets, pancreatic						X																			
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma		X																							
Subcutaneous tissue, fibrous histiocytoma																		X							
Subcutaneous tissue, sarcoma								X								X									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle								+																	
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranial nerve, schwannoma malignant																								X	
Peripheral nerve								+																	
Spinal cord								+																	

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	730	730	730	730	730	730	731	731	731	731	731	731	731	731	732	732	732	732	735	735	735	735	735	735	735	
Carcass ID Number	17575	18181	18182	18183	18184	18185	16161	16162	16164	16165	17776	17777	17779	18180	15556	15557	15559	16060	16668	16818	17070	17997	19898	19899	20000	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	49
Cystadenoma																									X	1
Hemangiosarcoma						X											X									4
Histiocytic sarcoma																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymph node			+			+																				4
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Histiocytic sarcoma																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, islets, pancreatic																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																								X		1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, fibrous histiocytoma																										1
Subcutaneous tissue, sarcoma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranial nerve, schwannoma malignant																										1
Peripheral nerve																										1
Spinal cord																										1

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	424	427	522	535	587	631	647	669	686	693	703	729	729	729	729	729	729	729	730	730	730	730	730	730	730
Carcass ID Number	19 4	18 7	16 7	15 4	16 9	17 8	16 3	15 8	15 1	19 6	18 8	18 6	18 9	19 0	19 1	19 2	19 3	19 5	15 2	15 3	15 5	17 1	17 2	17 3	17 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma	X																						X		
Histiocytic sarcoma			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma													X		X										
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Histiocytic sarcoma			X																						
Lymphoma malignant				X						X		X	X	X		X					X		X		X

TABLE B2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane: 700 mg/L

Number of Days on Study	730	730	730	730	730	730	731	731	731	731	731	731	731	731	732	732	732	732	735	735	735	735	735	735	735	
Carcass ID Number	17575	18181	18182	18183	18184	18185	16161	16162	16164	16165	17676	17677	17679	18080	15556	15557	15559	16060	16668	16670	17097	19798	19799	20000		Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma										X																3
Histiocytic sarcoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																							X			3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Histiocytic sarcoma																										1
Lymphoma malignant		X	X	X	X	X		X		X			X	X	X	X		X				X		X		23

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Harderian Gland: Adenoma				
Overall rate[a]	3/50 (6%)	5/50 (10%)	2/50 (4%)	3/50 (6%)
Adjusted rate[b]	6.6%	11.4%	4.6%	6.6%
Terminal rate[c]	2/36 (6%)	4/36 (11%)	1/33 (3%)	3/39 (8%)
First incidence (days)	702	581	701	729 (T)
Poly-3 test[d]	P=0.445N	P=0.337	P=0.520N	P=0.661
Harderian Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	7/50 (14%)	3/50 (6%)	3/50 (6%)
Adjusted rate	8.8%	15.7%	6.9%	6.6%
Terminal rate	3/36 (8%)	5/36 (14%)	2/33 (6%)	3/39 (8%)
First incidence (days)	702	581	701	729 (T)
Poly-3 test	P=0.262N	P=0.246	P=0.524N	P=0.501N
Liver: Hepatocellular Adenoma				
Overall rate	24/50 (48%)	18/50 (36%)	19/50 (38%)	16/50 (32%)
Adjusted rate	52.3%	40.6%	42.0%	35.0%
Terminal rate	20/36 (56%)	16/36 (44%)	12/33 (36%)	14/39 (36%)
First incidence (days)	649	521	539	686
Poly-3 test	P=0.074N	P=0.179N	P=0.215N	P=0.068N
Liver: Hepatocellular Carcinoma				
Overall rate	13/50 (26%)	6/50 (12%)	12/50 (24%)	7/50 (14%)
Adjusted rate	28.2%	13.5%	26.4%	15.3%
Terminal rate	9/36 (25%)	4/36 (11%)	6/33 (18%)	5/39 (13%)
First incidence (days)	604	547	539	686
Poly-3 test	P=0.170N	P=0.071N	P=0.518N	P=0.106N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	30/50 (60%)	23/50 (46%)	24/50 (48%)	19/50 (38%)
Adjusted rate	64.5%	51.2%	52.4%	41.6%
Terminal rate	24/36 (67%)	20/36 (56%)	16/33 (49%)	17/39 (44%)
First incidence (days)	604	521	539	686
Poly-3 test	P=0.022N	P=0.134N	P=0.162N	P=0.019N
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	14/50 (28%)	6/50 (12%)	12/50 (24%)	8/50 (16%)
Adjusted rate	30.1%	13.5%	26.4%	17.5%
Terminal rate	9/36 (25%)	4/36 (11%)	6/33 (18%)	6/39 (15%)
First incidence (days)	604	547	539	686
Poly-3 test	P=0.194N	P=0.047N	P=0.437N	P=0.119N
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	31/50 (62%)	23/50 (46%)	24/50 (48%)	19/50 (38%)
Adjusted rate	66.1%	51.2%	52.4%	41.6%
Terminal rate	24/36 (67%)	20/36 (56%)	16/33 (49%)	17/39 (44%)
First incidence (days)	604	521	539	686
Poly-3 test	P=0.015N	P=0.102N	P=0.125N	P=0.012N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	2/50 (4%)	0/50 (0%)	3/50 (6%)
Adjusted rate	13.2%	4.6%	0.0%	6.5%
Terminal rate	5/36 (14%)	2/36 (6%)	0/33 (0%)	2/39 (5%)
First incidence (days)	715	729 (T)	—[e]	424
Poly-3 test	P=0.178N	P=0.147N	P=0.018N	P=0.234N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	6/50 (12%)	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rate	13.2%	9.2%	4.6%	6.5%
Terminal rate	5/36 (14%)	4/36 (11%)	2/33 (6%)	2/39 (5%)
First incidence (days)	715	729 (T)	729 (T)	424
Poly-3 test	P=0.162N	P=0.398N	P=0.148N	P=0.234N
Ovary: Cystadenoma				
Overall rate	4/50 (8%)	1/49 (2%)	1/50 (2%)	1/49 (2%)
Adjusted rate	8.8%	2.3%	2.3%	2.3%
Terminal rate	2/36 (6%)	0/35 (0%)	0/33 (0%)	1/38 (3%)
First incidence (days)	702	674	659	729 (T)
Poly-3 test	P=0.134N	P=0.199N	P=0.192N	P=0.187N
Ovary: Hemangiosarcoma				
Overall rate	2/50 (4%)	1/49 (2%)	0/50 (0%)	4/49 (8%)
Adjusted rate	4.4%	2.3%	0.0%	9.0%
Terminal rate	1/36 (3%)	0/35 (0%)	0/33 (0%)	3/38 (8%)
First incidence (days)	715	604	—	686
Poly-3 test	P=0.161	P=0.520N	P=0.248N	P=0.328
Pancreatic Islets: Adenoma				
Overall rate	3/49 (6%)	1/48 (2%)	1/48 (2%)	2/49 (4%)
Adjusted rate	6.7%	2.4%	2.4%	4.4%
Terminal rate	3/36 (8%)	1/36 (3%)	1/33 (3%)	2/39 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.469N	P=0.325N	P=0.332N	P=0.498N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/49 (6%)	1/48 (2%)	1/48 (2%)	3/49 (6%)
Adjusted rate	6.7%	2.4%	2.4%	6.6%
Terminal rate	3/36 (8%)	1/36 (3%)	1/33 (3%)	2/39 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	631
Poly-3 test	P=0.510	P=0.325N	P=0.332N	P=0.657N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	3/49 (6%)	3/50 (6%)	1/49 (2%)	3/49 (6%)
Adjusted rate	6.8%	6.9%	2.3%	6.7%
Terminal rate	3/35 (9%)	3/36 (8%)	0/33 (0%)	3/39 (8%)
First incidence (days)	729 (T)	729 (T)	604	729 (T)
Poly-3 test	P=0.542N	P=0.653	P=0.312N	P=0.661N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Skin (Subcutaneous Tissue): Hemangiosarcoma				
Overall rate	4/50 (8%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	8.7%	0.0%	0.0%	0.0%
Terminal rate	3/36 (8%)	0/36 (0%)	0/33 (0%)	0/39 (0%)
First incidence (days)	604	—	—	—
Poly-3 test	P=0.017N	P=0.067N	P=0.067N	P=0.061N
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	8.5%	2.3%	6.8%	4.4%
Terminal rate	1/36 (3%)	1/36 (3%)	2/33 (6%)	1/39 (3%)
First incidence (days)	563	729 (T)	539	669
Poly-3 test	P=0.367N	P=0.202N	P=0.534N	P=0.350N
Skin (Subcutaneous Tissue): Fibrosarcoma, Sarcoma, or Fibrous Histiocytoma				
Overall rate	5/50 (10%)	1/50 (2%)	4/50 (8%)	4/50 (8%)
Adjusted rate	10.7%	2.3%	9.1%	8.6%
Terminal rate	2/36 (6%)	1/36 (3%)	3/33 (9%)	2/39 (5%)
First incidence (days)	563	729 (T)	539	472
Poly-3 test	P=0.553	P=0.120N	P=0.539N	P=0.507N
Spleen: Hemangiosarcoma				
Overall rate	4/50 (8%)	0/49 (0%)	0/48 (0%)	0/50 (0%)
Adjusted rate	8.8%	0.0%	0.0%	0.0%
Terminal rate	4/36 (11%)	0/36 (0%)	0/32 (0%)	0/39 (0%)
First incidence (days)	729 (T)	—	—	—
Poly-3 test	P=0.018N	P=0.067N	P=0.072N	P=0.060N
All Organs: Hemangiosarcoma				
Overall rate	8/50 (16%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	17.4%	4.6%	0.0%	8.8%
Terminal rate	5/36 (14%)	1/36 (3%)	0/33 (0%)	3/39 (8%)
First incidence (days)	604	604	—	686
Poly-3 test	P=0.143N	P=0.053N	P=0.005N	P=0.182N
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	8/50 (16%)	3/50 (6%)	1/50 (2%)	4/50 (8%)
Adjusted rate	17.4%	6.8%	2.3%	8.8%
Terminal rate	5/36 (14%)	2/36 (6%)	1/33 (3%)	3/39 (8%)
First incidence (days)	604	604	729 (T)	686
Poly-3 test	P=0.140N	P=0.113N	P=0.020N	P=0.182N
All Organs: Malignant Lymphoma				
Overall rate	29/50 (58%)	30/50 (60%)	16/50 (32%)	23/50 (46%)
Adjusted rate	62.3%	64.9%	35.9%	49.8%
Terminal rate	23/36 (64%)	22/36 (61%)	12/33 (36%)	21/39 (54%)
First incidence (days)	563	547	539	535
Poly-3 test	P=0.050N	P=0.479	P=0.008N	P=0.154N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
All Organs: Benign Neoplasms				
Overall rate	34/50 (68%)	25/50 (50%)	24/50 (48%)	28/50 (56%)
Adjusted rate	73.2%	55.7%	52.5%	59.7%
Terminal rate	28/36 (78%)	22/36 (61%)	16/33 (49%)	24/39 (62%)
First incidence (days)	604	521	539	424
Poly-3 test	P=0.160N	P=0.056N	P=0.028N	P=0.116N
All Organs: Malignant Neoplasms				
Overall rate	43/50 (86%)	38/50 (76%)	32/50 (64%)	35/50 (70%)
Adjusted rate	89.1%	80.0%	66.7%	72.3%
Terminal rate	32/36 (89%)	27/36 (75%)	19/33 (58%)	27/39 (69%)
First incidence (days)	563	521	323	472
Poly-3 test	P=0.024N	P=0.170N	P=0.006N	P=0.029N
All Organs: Benign or Malignant Neoplasms				
Overall rate	46/50 (92%)	44/50 (88%)	38/50 (76%)	43/50 (86%)
Adjusted rate	95.3%	92.7%	78.4%	87.4%
Terminal rate	35/36 (97%)	33/36 (92%)	23/33 (70%)	34/39 (87%)
First incidence (days)	563	521	323	424
Poly-3 test	P=0.087N	P=0.457N	P=0.012N	P=0.142N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for the liver, lung, ovary, pancreatic islets, 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 control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death		1		
Moribund sacrifice	7	6	5	4
Natural deaths	7	7	12	7
Survivors				
Terminal sacrifice	36	36	33	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(49)	(48)	(48)	(49)
Edema	10 (20%)	8 (17%)	8 (17%)	4 (8%)
Hemorrhage	1 (2%)			
Intestine small, duodenum	(48)	(48)	(46)	(46)
Cyst	1 (2%)			
Inflammation, chronic	1 (2%)		1 (2%)	
Epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	
Intestine small, jejunum	(46)	(49)	(48)	(48)
Hyperplasia, lymphoid				1 (2%)
Epithelium, hyperplasia			1 (2%)	
Intestine small, ileum	(45)	(46)	(49)	(49)
Diverticulum			1 (2%)	
Epithelium, hyperplasia		1 (2%)	2 (4%)	
Liver	(50)	(50)	(50)	(50)
Basophilic focus	3 (6%)		5 (10%)	2 (4%)
Clear cell focus	2 (4%)	1 (2%)	2 (4%)	3 (6%)
Cyst	2 (4%)			
Eosinophilic focus	7 (14%)	4 (8%)	8 (16%)	4 (8%)
Hematopoietic cell proliferation	4 (8%)	2 (4%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	5 (10%)	6 (12%)	1 (2%)
Infarct				2 (4%)
Infiltration cellular, mixed cell	6 (12%)	6 (12%)	7 (14%)	5 (10%)
Mixed cell focus	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Necrosis, focal	3 (6%)	5 (10%)	2 (4%)	4 (8%)
Tension lipidosis	2 (4%)			2 (4%)
Centrilobular, necrosis	6 (12%)	1 (2%)	2 (4%)	3 (6%)
Hepatocyte, depletion glycogen	3 (6%)	2 (4%)	1 (2%)	
Hepatocyte, karyomegaly	2 (4%)	4 (8%)		
Hepatocyte, vacuolization cytoplasmic	6 (12%)	3 (6%)	6 (12%)	3 (6%)
Kupffer cell, hyperplasia	2 (4%)	1 (2%)		
Kupffer cell, pigmentation	7 (14%)		2 (4%)	1 (2%)
Mesentery	(21)	(17)	(16)	(17)
Angiectasis	1 (5%)			
Hemorrhage		2 (12%)		
Necrosis	1 (5%)			
Fat, necrosis	12 (57%)	8 (47%)	11 (69%)	13 (76%)

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Alimentary System (continued)				
Pancreas	(50)	(48)	(49)	(49)
Atrophy	1 (2%)	2 (4%)	2 (4%)	
Cyst		1 (2%)	1 (2%)	
Acinus, cytoplasmic alteration		3 (6%)	2 (4%)	
Salivary glands	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia, lymphoid	25 (50%)	22 (44%)	26 (52%)	20 (40%)
Stomach, forestomach	(50)	(49)	(50)	(50)
Edema	2 (4%)	1 (2%)	2 (4%)	
Erosion	1 (2%)			1 (2%)
Hemorrhage				1 (2%)
Inflammation, chronic active		4 (8%)		1 (2%)
Ulcer			2 (4%)	
Epithelium, hyperplasia	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Stomach, glandular	(50)	(49)	(49)	(50)
Cyst	2 (4%)		2 (4%)	
Edema	2 (4%)		1 (2%)	1 (2%)
Erosion	1 (2%)	1 (2%)	1 (2%)	
Tooth			(1)	
Malformation			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	1 (2%)		1 (2%)
Mineralization	3 (6%)			
Thrombosis		1 (2%)		1 (2%)
Artery, inflammation, chronic		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(49)
Accessory adrenal cortical nodule	8 (16%)	5 (10%)	5 (10%)	10 (20%)
Hyperplasia, focal	1 (2%)	1 (2%)		
Hypertrophy, focal	1 (2%)	1 (2%)	2 (4%)	
Zona reticularis, vacuolization cytoplasmic		2 (4%)	1 (2%)	
Adrenal medulla	(50)	(48)	(49)	(49)
Hyperplasia	2 (4%)			2 (4%)
Islets, pancreatic	(49)	(48)	(48)	(49)
Hyperplasia	1 (2%)	4 (8%)	3 (6%)	1 (2%)
Parathyroid gland	(47)	(48)	(46)	(47)
Cyst	2 (4%)			1 (2%)
Pituitary gland	(49)	(50)	(49)	(49)
Pars distalis, angiectasis	1 (2%)		1 (2%)	1 (2%)
Pars distalis, cyst	1 (2%)	4 (8%)		3 (6%)
Pars distalis, hyperplasia, focal	6 (12%)	6 (12%)	1 (2%)	4 (8%)
Thyroid gland	(50)	(49)	(49)	(50)
Degeneration, cystic	26 (52%)	17 (35%)	12 (24%)	11 (22%)
Follicular cell, hyperplasia	6 (12%)	1 (2%)	5 (10%)	1 (2%)
General Body System				
None				

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Genital System				
Clitoral gland	(48)	(49)	(48)	(49)
Inflammation, chronic	1 (2%)	1 (2%)	4 (8%)	1 (2%)
Ovary	(50)	(49)	(50)	(49)
Angiectasis	5 (10%)	6 (12%)	5 (10%)	9 (18%)
Cyst	8 (16%)	13 (27%)	11 (22%)	14 (29%)
Thrombosis	1 (2%)			1 (2%)
Corpus luteum, hyperplasia	3 (6%)	1 (2%)	3 (6%)	
Granulosa cell, hyperplasia				2 (4%)
Interstitial cell, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis		3 (6%)	1 (2%)	
Hyperplasia, cystic	45 (90%)	45 (90%)	43 (86%)	46 (92%)
Inflammation, chronic	2 (4%)	2 (4%)		1 (2%)
Metaplasia, squamous		1 (2%)	2 (4%)	2 (4%)
Endometrium, hyperplasia, atypical				1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	22 (44%)	24 (48%)	18 (36%)	11 (22%)
Myelofibrosis	3 (6%)	1 (2%)	1 (2%)	
Lymph node	(16)	(9)	(7)	(4)
Iliac, hemorrhage	1 (6%)		1 (14%)	
Iliac, hyperplasia, lymphoid	3 (19%)			
Mediastinal, hemorrhage	1 (6%)	1 (11%)		
Renal, hyperplasia, lymphoid				1 (25%)
Lymph node, mandibular	(50)	(50)	(50)	(47)
Atrophy	2 (4%)		1 (2%)	1 (2%)
Hematopoietic cell proliferation	1 (2%)	2 (4%)		1 (2%)
Hemorrhage		3 (6%)	2 (4%)	3 (6%)
Hyperplasia, lymphoid	8 (16%)	9 (18%)	6 (12%)	9 (19%)
Pigmentation	22 (44%)	27 (54%)	25 (50%)	20 (43%)
Lymph node, mesenteric	(49)	(48)	(46)	(50)
Atrophy	1 (2%)			2 (4%)
Hematopoietic cell proliferation			2 (4%)	2 (4%)
Hemorrhage	3 (6%)	2 (4%)	3 (7%)	
Hyperplasia, lymphoid	6 (12%)	9 (19%)	4 (9%)	7 (14%)
Pigmentation	1 (2%)		1 (2%)	
Spleen	(50)	(49)	(48)	(50)
Hematopoietic cell proliferation	42 (84%)	30 (61%)	32 (67%)	29 (58%)
Hemorrhage		1 (2%)		1 (2%)
Hyperplasia, lymphoid	3 (6%)	1 (2%)	2 (4%)	3 (6%)
Metaplasia, osseous				1 (2%)
Pigmentation	29 (58%)	22 (45%)	30 (63%)	27 (54%)
Lymphoid follicle, atrophy			1 (2%)	1 (2%)
Lymphoid follicle, hyperplasia	5 (10%)	8 (16%)	13 (27%)	14 (28%)
Thymus	(49)	(49)	(50)	(47)
Atrophy	9 (18%)	3 (6%)	6 (12%)	4 (9%)
Hyperplasia, lymphoid	1 (2%)	3 (6%)	4 (8%)	2 (4%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	9 (18%)	4 (8%)	4 (8%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Edema	2 (4%)			1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Epidermis, hyperplasia	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis		3 (6%)	1 (2%)	1 (2%)
Skeletal muscle	(5)	(2)	(4)	(1)
Atrophy	1 (20%)	1 (50%)		
Inflammation, chronic	1 (20%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	2 (4%)			
Cyst epithelial inclusion	1 (2%)		1 (2%)	
Hemorrhage		2 (4%)	1 (2%)	
Necrosis		1 (2%)		
Peripheral nerve	(5)	(4)	(1)	(1)
Atrophy	3 (60%)	2 (50%)		
Inflammation, chronic		1 (25%)		
Spinal cord	(4)	(4)	(2)	(1)
Atrophy			1 (50%)	
Hemorrhage		1 (25%)	1 (50%)	
Necrosis		1 (25%)	1 (50%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema	1 (2%)	1 (2%)	1 (2%)	
Emphysema			1 (2%)	
Foreign body	1 (2%)			2 (4%)
Hemorrhage	6 (12%)	7 (14%)	6 (12%)	7 (14%)
Hyperplasia, lymphoid	1 (2%)		4 (8%)	1 (2%)
Infiltration cellular, polymorphonuclear		1 (2%)	1 (2%)	
Infiltration cellular, histiocyte	2 (4%)	1 (2%)	5 (10%)	
Inflammation, chronic	1 (2%)			1 (2%)
Thrombosis	1 (2%)	2 (4%)	1 (2%)	
Alveolar epithelium, hyperplasia		2 (4%)	1 (2%)	2 (4%)
Alveolar epithelium, metaplasia, bronchiolar		2 (4%)		

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Special Senses System				
Eye	(48)	(50)	(50)	(50)
Developmental malformation	1 (2%)			4 (8%)
Hemorrhage		2 (4%)		
Inflammation, chronic				2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Amyloid deposition				1 (2%)
Cyst				1 (2%)
Hyperplasia, lymphoid	8 (16%)	5 (10%)	5 (10%)	3 (6%)
Infarct	2 (4%)	2 (4%)	3 (6%)	5 (10%)
Inflammation, focal, mixed cell			1 (2%)	
Metaplasia, osseous	2 (4%)			1 (2%)
Nephropathy	16 (32%)	14 (29%)	7 (14%)	8 (16%)
Renal tubule, accumulation, hyaline droplet	2 (4%)	6 (12%)	4 (8%)	1 (2%)
Renal tubule, necrosis	2 (4%)		2 (4%)	1 (2%)
Renal tubule, pigmentation	2 (4%)			1 (2%)
Renal tubule, regeneration			1 (2%)	
Renal tubule, vacuolization cytoplasmic		1 (2%)	1 (2%)	
Urinary bladder	(50)	(49)	(48)	(50)
Edema		2 (4%)		1 (2%)
Hyperplasia, lymphoid	11 (22%)	7 (14%)	4 (8%)	10 (20%)
Inflammation, chronic		1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia		1 (2%)	1 (2%)	

APPENDIX C
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Mortelmans *et al.* (1986). Bromodichloromethane was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of bromodichloromethane. The high dose was limited by toxicity or in the absence of toxicity, 10,000 µg/plate was selected as the high dose. All trials were repeated (except TA98 with 30% hamster S9 in experiment 2)

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.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by McGregor *et al.* (1988) Bromodichloromethane was supplied as a coded aliquot by Radian Corporation. The high dose of bromodichloromethane was determined by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring cells resistant to trifluorothymidine (TFT), subcultures were exposed to medium containing thymidine, hypoxanthine, methotrexate, and glycine for 1 day; to medium containing thymidine, hypoxanthine, and glycine for 1 day; and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with bromodichloromethane continued for 4 hours, at which time the medium plus bromodichloromethane was removed, and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with TFT for selection of TFT-resistant cells, and cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. Because a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male F344 rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented by Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for bromodichloromethane to be considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a call of "questionable," and the absence of both a trend and peak response resulted in a "negative" call.

CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS

Testing was performed as reported by Anderson *et al.* (1990). Bromodichloromethane was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least four doses of bromodichloromethane; the high dose was limited by toxicity. In the absence of toxicity, 5 mg/mL was selected as the high dose. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

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

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.,* 1987). An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P<0.005$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with bromodichloromethane for 8.8 to 10 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with bromodichloromethane and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 11 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test

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

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was

positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.,* 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

Mouse Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by bromodichloromethane exposure. The standard three-exposure protocol is described in detail by Shelby *et al.* (1993). Male B6C3F_1 mice were injected intraperitoneally three times at 24-hour intervals with bromodichloromethane dissolved in corn oil. Vehicle control animals were injected with corn oil only. The positive control animals received injections of cyclophosphamide. The animals were killed 24 hours after the third injection, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs) were scored for the frequency of micronucleated cells in each of five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of 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 with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dose group is less than or equal to 0.025 divided by the number of 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, the 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-week toxicity study, peripheral blood samples were obtained from female B6C3F_1 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) in up to 10 mice per exposure group. In addition, the percentage of PCEs in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as described for PCEs in the bone marrow micronucleus test. Results of the 3-week study were accepted without repeat tests because additional test 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 judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

The results of *in vitro* mutagenicity tests with bromodichloromethane were mixed and it is possible that the volatility of this chemical was a factor in the outcome of some of the tests (Simmon *et al.,* 1977). Bromodichloromethane was tested for mutagenicity in *S. typhimurium* in two separate experiments at the same laboratory using a total of five tester strains in a standard preincubation assay (Table C1; Mortelmans *et al.,* 1986). No mutagenic activity was observed in any of the strains, with or without induced rat or hamster liver S9 activation enzymes. In contrast to the negative results in bacteria, tests for mutation induction in mouse lymphoma L5178Y/tk$^{+/-}$ cells were positive in the presence of induced rat liver S9; no mutagenic activity occurred in tests conducted without S9 (Table C2; McGregor *et al.,* 1988). In cytogenetic tests with cultured CHO cells, bromodichloromethane induced a small increase in SCEs in one of three trials conducted in the presence of induced rat liver S9 enzymes (Table C3; Anderson *et al.,* 1990); no significant increase in SCEs occurred without S9. Among the three SCE trials conducted with S9, there was variability in the responses and in the levels at which toxicity occurred, which may indicate fluctuating exposures to the volatile chemical. No induction of Abs occurred in CHO cells incubated with bromodichloromethane at concentrations up to 5,000 µg/mL, with or without S9 (Table C4; Anderson *et al.,* 1990).

Results of *in vivo* tests for chromosomal damage in mice were negative. No increases in the frequency of micronucleated PCEs were seen in bone marrow of male B6C3F$_1$ mice administered bromodichloromethane (200 to 500 mg/kg per day) by intraperitoneal injection for 3 days (Table C5). In the first of two trials in this assay, significant increases in micronucleated PCEs were seen at two of the four dose groups, but the responses were not correlated with dose. In a second trial, no significant increases in micronucleated cells were seen at any of the four dose groups, and the assay was judged to be negative overall. In a second micronucleus test, no induction of micronuclei was observed in peripheral blood NCEs of female B6C3F$_1$ mice administered up to 700 mg/L bromodichloromethane in drinking water for 3 weeks (Table C6). Although the 3-week exposure time was not long enough to reach equilibrium in the circulating erythrocyte population, which reaches steady state after approximately 35 days of exposure, there was no indication of a response in any of the five exposed groups tested, and the results were concluded to be negative.

TABLE C1
Mutagenicity of Bromodichloromethane in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Experiment 1							
TA100	0.0	118 ± 4.5	143 ± 7.1	131 ± 14.3	116 ± 3.8	116 ± 10.4	132 ± 7.8
	10.0	98 ± 8.1	140 ± 15.3		114 ± 1.8	117 ± 6.8	160 ± 7.2
	33.0	91 ± 1.2	126 ± 12.2	146 ± 8.6	106 ± 14.7	120 ± 3.2	135 ± 19.3
	100.0	100 ± 5.2	106 ± 3.6	136 ± 7.2	122 ± 4.4	117 ± 14.8	125 ± 8.1
	333.0	91 ± 8.9	117 ± 4.7	127 ± 13.9	112 ± 18.5	108 ± 11.3	123 ± 8.4
	1,000.0	99 ± 5.8	114 ± 4.1	29 ± 29.3[c]	108 ± 4.8	102 ± 2.8	26 ± 26.3
	3,333.0			Toxic			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		417 ± 10.7	377 ± 8.7	683 ± 19.1	966 ± 42.3	439 ± 14.4	456 ± 24.8
TA1535	0.0	36 ± 3.2	18 ± 0.9	35 ± 5.0	17 ± 0.0	36 ± 8.6	27 ± 5.8
	10.0	23 ± 3.6	30 ± 4.0	32 ± 4.0	24 ± 4.6	22 ± 3.8	
	33.0	15 ± 2.4	24 ± 3.8	20 ± 4.6	36 ± 2.5	22 ± 1.7	26 ± 7.8
	100.0	18 ± 0.6	28 ± 6.5	23 ± 4.3	32 ± 4.0	23 ± 3.3	22 ± 4.4
	333.0	16 ± 2.4	17 ± 1.5	17 ± 10.7	37 ± 3.7	20 ± 2.6	24 ± 5.8
	1,000.0	16 ± 1.7	5 ± 3.7[c]	4 ± 4.3[c]	29 ± 1.9	22 ± 4.0	9 ± 6.2
	3,333.0			Toxic			
Trial summary		Negative	Negative	Negative	Weakly Positive	Negative	Negative
Positive control		476 ± 28.6	342 ± 42.1	314 ± 9.5	245 ± 10.7	324 ± 11.1	178 ± 24.1
TA1537	0.0	8 ± 0.7	5 ± 1.9	7 ± 3.1	5 ± 0.9	8 ± 2.6	6 ± 0.9
	10.0	9 ± 2.3	4 ± 0.9		7 ± 0.9	9 ± 0.3	6 ± 1.0
	33.0	6 ± 1.3	7 ± 1.5	8 ± 0.3	7 ± 2.9	6 ± 1.2	7 ± 2.8
	100.0	8 ± 0.7	4 ± 0.3	7 ± 0.7	4 ± 0.0	6 ± 0.9	5 ± 1.5
	333.0	7 ± 0.7	5 ± 0.0	9 ± 2.0	7 ± 1.3	8 ± 2.6	8 ± 2.6
	1,000.0	4 ± 1.0	5 ± 2.2	0 ± 0.0[c]	0 ± 0.0[c]	9 ± 3.6	9 ± 0.9
	3,333.0			Toxic			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		151 ± 11.7	350 ± 41.7	319 ± 28.5	406 ± 11.5	292 ± 1.9	135 ± 8.7
TA98	0.0	26 ± 0.6	27 ± 1.0	36 ± 0.0	20 ± 3.6	44 ± 4.6	28 ± 2.3
	10.0	19 ± 5.3	23 ± 5.2		34 ± 1.2	27 ± 1.7	36 ± 4.0
	33.0	22 ± 3.2	22 ± 4.0	37 ± 4.6	28 ± 4.4	28 ± 2.1	28 ± 0.3
	100.0	21 ± 5.5	18 ± 1.5	34 ± 7.1	35 ± 2.7	29 ± 4.8	23 ± 2.5
	333.0	14 ± 2.4	18 ± 1.3	34 ± 5.4	29 ± 2.2	29 ± 5.2	26 ± 3.0
	1,000.0	16 ± 1.9	20 ± 2.8	0 ± 0.0[c]	14 ± 14.0	27 ± 2.8	21 ± 4.3
	3,333.0			Toxic			
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		749 ± 43.9	575 ± 33.3	691 ± 134.3	764 ± 21.3	313 ± 4.1	326 ± 10.7

TABLE C1
Mutagenicity of Bromodichloromethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	-S9 Trial 4	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
Experiment 2							
TA100	0.0	123 ± 5.5	116 ± 6.2	125 ± 5.5	137 ± 9.8	125 ± 6.4	139 ± 6.7
	0.025			153 ± 22.5	143 ± 8.6		140 ± 3.3
	0.050			168 ±10.3	147 ± 9.2		138 ± 9.5
	0.100			147 ± 14.6	134 ± 11.4		132 ± 7.1
	0.250			191 ± 7.8	132 ± 4.3		151 ± 10.2
	0.500			55 ± 18.7[c]	84 ± 4.0[c]		70 ± 24.7[c]
	100.0	115 ± 5.0	125 ± 3.0			127 ± 12.4	
	333.0	131 ± 5.8	125 ± 5.5			124 ± 3.5	
	1,000.0	122 ± 3.0	131 ± 2.7			119 ± 0.9	
	3,333.0	134 ± 5.7	115 ± 3.3			114 ± 2.3	
	10,000.0	89 ± 9.3[c]	89 ± 5.7[c]			79 ± 5.8[c]	
Trial summary		Negative	Negative	Equivocal	Negative	Negative	Negative
Positive control		900 ± 12.3	844 ± 5.1	772 ± 23.8	819 ± 32.9	695 ± 18.5	674 ± 20.3

Strain	Dose (µg/plate)	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA100	0.0	117 ± 7.1	141 ± 2.5	128 ± 4.9	136 ± 7.5	121 ± 9.3	141 ± 9.3
	0.025		111 ± 6.1		122 ± 7.8		130 ± 11.4
	0.050		140 ± 4.4		117 ± 2.2		175 ± 10.5
	0.100		115 ± 5.9		120 ± 7.8		135 ± 0.6
	0.250		111 ± 2.1		122 ± 5.9		122 ± 15.0
	0.500		32 ± 22.8[c]		65 ± 12.7[c]		15 ± 2.1[c]
	100.0	128 ± 5.8		130 ± 4.6		125 ± 3.5	
	333.0	118 ± 4.3		123 ± 3.3		117 ± 5.8	
	1,000.0	120 ± 12.2		146 ± 16.3		126 ± 4.9	
	3,333.0	123 ± 8.5		143 ± 7.9		132 ± 10.8	
	10,000.0	84 ± 9.8[c]		47 ± 8.7[c]		68 ± 8.8[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		664 ± 21.1	571 ± 28.3	635 ± 24.8	653 ± 24.7	624 ± 15.4	487 ± 16.7

TABLE C1
Mutagenicity of Bromodichloromethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	-S9 Trial 4	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
Experiment 2 (continued)							
TA1535	0.0	11 ± 1.5	9 ± 1.5	11 ± 1.8	14 ± 3.2	10 ± 1.2	14 ± 3.5
	0.025			15 ± 0.6	11 ± 0.9		20 ± 0.7
	0.050			20 ± 4.5	13 ± 1.0		14 ± 0.3
	0.100			25 ± 1.5	13 ± 1.5		12 ± 0.7
	0.250			22 ± 0.7	14 ± 2.8		10 ± 1.3
	0.500			5 ± 0.9[c]	7 ± 1.0[c]		5 ± 0.6[c]
	100.0	11 ± 0.3	10 ± 0.9			8 ± 1.3	
	333.0	10 ± 2.0	11 ± 0.7			11 ± 3.2	
	1,000.0	8 ± 0.6	13 ± 1.2			9 ± 0.7	
	3,333.0	13 ± 1.7	12 ± 3.3			7 ± 0.0	
	10,000.0	6 ± 0.9[c]	7 ± 0.7[c]			4 ± 0.9[c]	
Trial summary		Negative	Negative	Weakly Positive	Negative	Negative	Negative
Positive control		938 ± 15.5	940 ± 16.2	837 ± 28.7	883 ± 24.8	126 ± 14.0	695 ± 18.5

Strain	Dose (µg/plate)	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA1535	0.0	10 ± 1.5	16 ± 2.1	10 ± 1.2	19 ± 5.6	12 ± 2.3	14 ± 1.7
	0.025		17 ± 2.8		20 ± 0.7		18 ± 3.5
	0.050		18 ± 0.9		17 ± 3.3		23 ± 2.9
	0.100		22 ± 4.1		15 ± 2.0		21 ± 2.0
	0.250		13 ± 2.7		16 ± 2.9		18 ± 2.2
	0.500		6 ± 1.8[c]		7 ± 1.0[c]		6 ± 0.0[c]
	100.0	10 ± 2.0		10 ± 1.1		12 ± 2.1	
	333.0	9 ± 2.7		9 ± 1.5		9 ± 1.8	
	1,000.0	12 ± 2.1		8 ± 0.6		14 ± 0.7	
	3,333.0	10 ± 1.5		9 ± 2.3		17 ± 3.8	
	10,000.0	7 ± 0.9[c]		5 ± 0.7[c]		9 ± 1.2[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		141 ± 5.0	95 ± 4.5	130 ± 15.1	79 ± 7.3	115 ± 6.9	76 ± 6.4

TABLE C1
Mutagenicity of Bromodichloromethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	-S9 Trial 4	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
Experiment 2 (continued)							
TA97	0.0	142 ± 7.4	122 ± 7.9	144 ± 9.2	157 ± 5.8	170 ± 7.9	179 ± 17.9
	0.025			156 ± 7.2	152 ± 3.3		179 ± 6.0
	0.050			195 ± 14.5	166 ± 14.3		193 ± 3.3
	0.100			162 ± 1.7	160 ± 7.9		174 ± 25.3
	0.250			125 ± 19.0	169 ± 13.4		188 ± 1.9
	0.500			73 ± 14.6[c]	82 ± 15.0[c]		65 ± 11.3[c]
	100.0	132 ± 3.0	123 ± 7.9			172 ± 12.2	
	333.0	151 ± 7.3	131 ± 11.2			156 ± 5.2	
	1,000.0	135 ± 2.3	119 ± 5.7			142 ± 3.0	
	3,333.0	137 ± 6.6	127 ± 6.3			163 ± 2.6	
	10,000.0	65 ± 6.2[c]	95 ± 8.6[c]			83 ± 10.7[c]	
Trial summary		Negative	Negative	Equivocal	Negative	Negative	Negative
Positive control		572 ± 16.9	640 ± 21.1	541 ± 36.9	571 ± 13.0	710 ± 8.9	688 ± 20.3

Strain	Dose (µg/plate)	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA97	0.0	175 ± 9.0	153 ± 4.7	178 ± 5.4	185 ± 5.5	201 ± 10.7	203 ± 11.9
	0.025		149 ± 5.2		172 ± 18.6		167 ± 30.7
	0.050		188 ± 9.7		163 ± 8.6		192 ± 15.6
	0.100		177 ± 13.1		167 ± 8.8		209 ± 4.4
	0.250		163 ± 9.7		195 ± 7.7		183 ± 3.4
	0.500		55 ± 9.5[c]		71 ± 29.4[c]		71 ± 3.5[c]
	100.0	149 ± 5.8		191 ± 17.8		158 ± 14.5	
	333.0	174 ± 13.3		190 ± 4.8		184 ± 17.6	
	1,000.0	167 ± 11.3		156 ± 35.2		186 ± 15.6	
	3,333.0	161 ± 14.2		146 ± 24.1		191 ± 3.1	
	10,000.0	99 ± 14.7[c]		77 ± 11.0[c]		59 ± 5.7[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		660 ± 6.7	681 ± 22.0	653 ± 15.0	640 ± 17.9	650 ± 23.7	639 ± 18.3

TABLE C1
Mutagenicity of Bromodichloromethane in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate -S9 Trial 1	-S9 Trial 2	-S9 Trial 3	-S9 Trial 4	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2
Experiment 2 (continued)							
TA98	0.0	11 ± 1.8	20 ± 1.7	16 ± 3.2	20 ± 2.2	22 ± 1.8	22 ± 4.7
	0.025			25 ± 2.2	16 ± 2.9		22 ± 3.2
	0.050			35 ± 2.4	16 ± 1.2		22 ± 1.2
	0.100			26 ± 5.5	19 ± 3.2		33 ± 11.0
	0.250			22 ± 0.9	15 ± 2.8		19 ± 3.7
	0.500			10 ± 5.5[c]	9 ± 0.0[c]		7 ± 2.0[c]
	100.0	9 ± 1.9	27 ± 3.1			21 ± 2.0	
	333.0	9 ± 0.6	26 ± 1.2			23 ± 4.2	
	1,000.0	13 ± 1.5	22 ± 1.0			28 ± 11.0	
	3,333.0	13 ± 2.4	26 ± 3.3			26 ± 2.4	
	10,000.0	5 ± 0.6[c]	11 ± 1.9[c]			9 ± 0.3[c]	
Trial summary		Negative	Negative	Equivocal	Negative	Negative	Negative
Positive control		380 ± 8.3	443 ± 16.9	453 ± 24.7	424 ± 10.5	534 ± 11.4	639 ± 16.8

Strain	Dose (µg/plate)	+30% hamster S9	+10% rat S9 Trial 1	+10% rat S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
TA98	0.0	19 ± 2.7	19 ± 4.0	22 ± 3.5	12 ± 2.9	24 ± 2.0
	0.025	28 ± 2.1		20 ± 0.7		31 ± 5.7
	0.050	37 ± 1.2		16 ± 1.7		45 ± 2.6
	0.100	19 ± 3.5		20 ± 3.5		27 ± 5.7
	0.250	20 ± 4.5		17 ± 3.5		40 ± 3.6
	0.500	7 ± 1.2[c]		6 ± 1.5[c]		16 ± 2.8[c]
	100.0		19 ± 0.3		9 ± 0.7	
	333.0		18 ± 4.2		13 ± 2.3	
	1,000.0		25 ± 5.5		14 ± 2.9	
	3,333.0		25 ± 3.5		15 ± 1.5	
	10,000.0		10 ± 0.9[c]		5 ± 0.3[c]	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		384 ± 16.1	466 ± 15.8	565 ± 21.8	431 ± 7.8	372 ± 15.0

[a] Studies were performed at SRI International. The detailed protocol and these data are presented by Mortelmans *et al.* (1986).
[b] 0 µg/plate was the solvent control.
Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Bromodichloromethane[a]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction
-S9						
Trial 1						
Trial call: Negative						
Dimethylsulfoxide[c]		65	107	54	28	
		61	79	81	44	
		94	112	106	38	
		88	103	59	22	33
Bromodichloromethane	15.625	64	124	61	32	
		69	120	104	51	41
	31.25	83	119	107	43	
		72	108	103	48	45
	62.5	78	106	115	49	
		91	90	59	22	35
	125	91	71	117	43	
		81	91	110	45	44
	250	84	33	129	51	
		69	35	86	42	47
	500	Lethal				
		Lethal				
Methyl methanesulfonate[d]	15	24	7	409	560	
		36	18	358	328	444*
Trial 2						
Trial call: Negative						
Dimethylsulfoxide		109	103	101	31	
		99	98	74	25	
		111	100	76	23	
		110	98	85	26	26
Bromodichloromethane	200	93	79	75	27	
		105	83	67	21	24
	250	96	59	67	23	
		72	61	65	30	27
	300	148[e]	91	68	15	
		105[f]	51	86	27	
	350	52	33	43	27	
		91[f]	28	87	32	30
	400	82	20	106	43	
		110	20	112	34	38
Methyl methanesulfonate	15	41	31	252	206	
		40	22	115	139	172*

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Bromodichloromethane

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Trial call: Positive						
Dimethylsulfoxide		70	98	148	71	
		68	94	186	91	
		53	98	146	92	
		74	110	226	102	89
Bromodichloromethane	180	59	67	242	136	
		63	69	299	159	148*
	240	60	34	255	141	
		41	23	233	189	165*
	300	46	15	391	283	
		60	29	247	138	210*
	360	56	9	402	241	
		51	10	459	300	271*
	420	39	4	755	651	
		31	4	865	945	798*
	480	Lethal				
		Lethal				
Methylcholanthrene[d]	2.5	26	19	750	968	
		28	22	791	953	960*

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Bromodichloromethane

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Trial call: Positive						
Dimethylsulfoxide		107	110	81	25	
		111	89	91	27	
		110	101	101	31	
		129[e]	119	113	29	28
Bromodichloromethane	180	101	56	107	35	
		101	40	133	44	40
	240	79	39	100	42	
		121[e]	32	185	51	
	300	95	36	135	47	
		112	26	156	46	47*
	360	88	20	111	42	
		105	21	177	56	49*
	420	92	9	219	80	
		99	10	216	73	76*
	480	Lethal				
		Lethal				
Methylcholanthrene	2.5	81	49	680	282	
		89	50	666	251	266*

* Significantly different ($P \leq 0.05$) from the solvent control
[a] Study was performed at Inveresk Research International. The detailed protocol and these data are presented by McGregor *et al.* (1988).
[b] Mutant fraction = mutant cells/10^6 clonable cells.
[c] Solvent control
[d] Positive control
[e] Rejected, cloning efficiency greater than 170%
[f] Loss of sample set due to contamination

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Bromodichloromethane[a]

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Negative								
Untreated control		50	1,038	421	0.41	8.42	26.5	
		50	1,035	467	0.45	9.34	26.5	
Bromodichloromethane	50	50	1,024	452	0.44	9.04	26.5	-2.17
	160	50	1,038	471	0.45	9.42	26.5	0.57
	500	50	1,038	466	0.45	9.32	26.5	-0.50
	1,600	0[c]					0.0	
	5,000	0[d]					0.0	
					P=0.4761[e]			
Mitomycin-C[f]	0.001	50	1,032	707	0.69	14.14	26.5	51.83
	0.010	50	1,028	2,520	2.45	50.40	26.5	443.29
Trial 2								
Summary: Equivocal								
Untreated control		50	1,043	436	0.42	8.72	26.0	
		50	1,046	439	0.42	8.78	26.0	
Bromodichloromethane	500	50	1,045	427	0.41	8.54	26.0	-2.64
	1,000	50	1,047	516	0.49	10.32	26.0	17.43
	1,500	50	1,042	458	0.44	9.16	26.0	4.73
	2,000	50	1,040	518	0.50	10.36	26.0	18.68
	3,000	0[c]					31.0	
	4,000	0[d]						
					P=0.0016			
Mitomycin-C	0.001	50	1,046	1,402	1.34	28.04	26.0	219.36
	0.010	10	211	693	3.28	69.30	26.0	682.56

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Bromodichloromethane

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide[g]		50	1,047	480	0.46	9.60	26.0	
		50	1,047	470	0.45	9.40	26.0	
Bromodichloromethane	50	50	1,045	487	0.47	9.74	26.0	3.82
	160	50	1,038	496	0.48	9.92	26.0	6.45
	500	50	1,050	440	0.42	8.80	26.0	-6.65
	1,600	50	1,047	499	0.48	9.98	26.0	6.17
	5,000	0[c]					0.0	
					P=0.4476			
Cyclophosphamide[f]	0.3	50	1,037	595	0.57	11.90	26.0	27.82
	2.0	50	1,049	1,192	1.14	23.84	26.0	153.13
Trial 2								
Summary: Positive								
Untreated control		50	1,047	344	0.33	6.88	26.0	
		50	1,042	426	0.41	8.52	26.0	
Bromodichloromethane	2,000	50	1,042	449	0.43	8.98	26.0	5.40
	3,000	50	1,043	445	0.43	8.90	26.0	4.36
	4,000	50	1,045	533	0.51	10.66	26.0	24.76*
	5,000	50	1,038	511	0.49	10.22	26.0	20.42*
					P=0.0001			
Cyclophosphamide	0.3	50	1,043	667	0.64	13.34	26.0	56.42
	2.0	10	212	369	1.74	36.90	26.0	325.74

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Bromodichloromethane

Compound	Concentration (µg/mL)	Total Cells Scored	No. of Chromo-somes	No. of SCEs	SCEs/ Chromo-some	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome (%)
+S9 (continued)								
Trial 3								
Summary: Negative								
Dimethylsulfoxide		50	1,046	412	0.39	8.24	26.0	
Bromodichloromethane	160	50	1,040	470	0.45	9.40	26.0	14.74
	500	50	1,041	473	0.45	9.46	26.0	15.36
	1,000	50	1,037	422	0.41	8.44	26.0	3.32
	2,000	50	1,043	454	0.44	9.08	26.0	10.51
					P=0.2531			
Cyclophosphamide	0.1	50	1,049	547	0.52	10.94	26.0	32.39
	0.6	10	208	238	1.14	23.80	26.0	190.50

* Positive response (≥20% increase over untreated or solvent control)
[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol and these data are presented by Anderson *et al.* (1990). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Cytostatic
[d] Toxic
[e] Significance of SCEs/chromosome tested by the linear regression trend test versus log of the dose
[f] Positive control
[g] Solvent control

TABLE C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Bromodichloromethane[a]

Compound	Concentration (µL/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
-S9					
Trial 1					
Harvest time: 12 hours					
Summary: Negative					
Dimethylsulfoxide[b]		100	1	0.01	1.0
Bromodichloromethane	160	100	3	0.03	3.0
	500	100	3	0.03	2.0
	1,600	100	4	0.04	3.0
	5,000	100	1	0.01	1.0
					P=0.4994[c]
Mytomycin-C[d]	0.25	100	45	0.45	32.0
	1.00	100	60	0.60	45.0
-S9					
Trial 2					
Harvest time: 10.8 hours					
Summary: Negative					
Dimethylsulfoxide		100	3	0.03	3.0
Bromodichloromethane	250	100	4	0.04	4.0
	500	100	2	0.02	2.0
	1,000	100	3	0.03	3.0
	2,000	100	1	0.01	1.0
					P=0.8398
Mytomycin-C	0.25	100	24	0.24	22.0
	1.00	50	39	0.78	42.0

TABLE C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Bromodichloromethane

Compound	Concentration (µL/mL)	Total Cells Scored	Number of Aberrations	Aberrations/ Cell	Cells with Aberrations (%)
+S9					
Trial 1					
Harvest Time: 13 hours					
Summary: Negative					
Dimethylsulfoxide		100	0	0.00	0.0
Bromodichloromethane	160	100	0	0.00	0.0
	500	100	3	0.03	2.0
	1,600	100	1	0.01	1.0
	5,000	100	0	0.00	0.0
					P=0.3422
Cyclophosphamide[d]	15	100	11	0.11	9.0
	50	100	37	0.37	31.0
Trial 2					
Harvest Time: 13 hours					
Summary: Negative					
Dimethylsulfoxide		100	1	0.01	1.0
Bromodichloromethane	250	100	0	0.00	0.0
	500	100	3	0.03	3.0
	1,000	100	1	0.01	1.0
	2,000	100	1	0.01	1.0
					P=0.3857
Cyclophosphamide	15	100	32	0.32	28.0
	50	50	42	0.84	46.0

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol and these data are presented by Anderson *et al.* (1990).
[b] Solvent control
[c] Significance of percent cells with aberrations tested by the linear regression trend test versus log of the dose
[d] Positive control

TABLE C5
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Mice Treated with Bromodichloromethane by Intraperitoneal Injection[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated PCEs/ 1,000[b] PCEs	Pairwise P-value[c]	PCEs[b] (%)
Trial 1					
Corn oil[d]	0	5	0.90 ± 0.33		45.550 ± 1.15
Bromodichloromethane	200	5	2.20 ± 0.51	0.0097	47.650 ± 1.95
	300	5	1.50 ± 0.35	0.1102	39.280 ± 4.00
	400	3	3.17 ± 0.33	0.0004	33.900 ± 4.93
	500	4	1.63 ± 0.24	0.0833	34.725 ± 3.76
			P=0.033[e]		
Cyclophosphamide[f]	25	4	16.25 ± 2.20	0.0000	41.400 ± 2.88
Trial 2					
Corn oil	0	5	1.60 ± 0.53		47.500 ± 0.00
Bromodichloromethane	200	5	1.80 ± 0.46	0.3657	45.250 ± 1.85
	300	5	3.10 ± 0.46	0.0142	45.750 ± 2.14
	400	5	1.70 ± 0.56	0.4308	43.875 ± 1.60
	500	5	1.80 ± 0.12	0.3657	45.267 ± 1.17
			P=0.342		
Cyclophosphamide	25	5	16.50 ± 1.16	0.0000	47.540 ± 2.53

[a] Study was performed at Environmental Health Research and Testing, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
[b] PCE=polychromatic erythrocyte.
[c] Mean ± standard error
[d] Pairwise comparison with the vehicle control; dosed group values are significant at P≤0.008; positive control values are significant at P≤0.05 (ILS, 1990).
[e] Vehicle control
[f] Significance of micronucleated PCEs/1,000 PCEs by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)
Positive control

TABLE C6
Frequency of Micronuclei in Peripheral Blood Normochromatic Erythrocytes of Female Mice Following Administration of Bromodichloromethane in Drinking Water for 3 Weeks[a]

Compound	Dose (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCE[b]	Pairwise P-value[c]	PCEs[b] (%)
Water[d]	0	10	1.00 ± 0.15		1.870 ± 0.10
Bromodichloromethane	43.7	10	0.65 ± 0.17	0.8886	1.850 ± 0.11
	87.5	10	0.70 ± 0.13	0.8484	1.790 ± 0.10
	175	10	0.95 ± 0.14	0.5636	1.770 ± 0.08
	350	10	0.95 ± 0.19	0.5636	1.860 ± 0.11
	700	9	0.83 ± 0.17	0.7036	1.922 ± 0.07
			P=0.421[e]		

[a] Study was performed at SITEK Research Laboratories. The detailed protocol is presented by MacGregor *et al.* (1990)
[b] Mean ± standard error. PCE=polychromatic erythrocyte, NCE=normochromatic erythrocyte
[c] Pairwise comparison with the vehicle control; significant at P≤0.005 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX D
CLINICAL PATHOLOGY RESULTS

TABLE D1
Hematology and Clinical Chemistry Data for Male Rats in the 3-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	43.7 mg/L	87.5 mg/L	175 mg/L	350 mg/L	700 mg/L
n	10	10	10	10	10	10
Hematology						
Automated hematocrit (%)	44.5 ± 0.7	45.2 ± 0.4	45.3 ± 0.7	45.3 ± 0.4	45.5 ± 0.5	44.9 ± 0.6
Hemoglobin (g/dL)	14.8 ± 0.2	14.9 ± 0.2	14.9 ± 0.3	14.9 ± 0.1	14.9 ± 0.2	14.9 ± 0.2
Erythrocytes (10^6/µL)	7.75 ± 0.11	7.92 ± 0.10	7.88 ± 0.14	7.92 ± 0.07	7.91 ± 0.09	7.78 ± 0.12
Reticulocytes (10^5/µL)	0.47 ± 0.01	0.49 ± 0.01	0.47 ± 0.02	0.44 ± 0.01	0.46 ± 0.01	0.45 ± 0.02
Mean cell volume (fL)	57.4 ± 0.2	57.1 ± 0.4	57.5 ± 0.3	57.2 ± 0.2	57.6 ± 0.2	57.8 ± 0.2
Mean cell hemoglobin (pg)	19.1 ± 0.0	18.9 ± 0.1	18.9 ± 0.1	18.9 ± 0.2	18.9 ± 0.2	19.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.0 ± 0.1	32.9 ± 0.1	33.0 ± 0.1	32.8 ± 0.1*	33.2 ± 0.2
Platelets (10^3/µL)	863.9 ± 13.3	881.1 ± 16.9	832.6 ± 13.8	839.9 ± 13.1	831.6 ± 14.6	804.3 ± 14.9[b]**
Leukocytes (10^3/µL)	9.18 ± 0.46	10.10 ± 0.42	9.83 ± 0.42	10.99 ± 0.44*	9.93 ± 0.39	10.26 ± 0.63
Segmented neutrophils (10^3/µL)	0.86 ± 0.05	1.02 ± 0.04	0.90 ± 0.05	0.94 ± 0.04	0.97 ± 0.04	0.98 ± 0.08
Lymphocytes (10^3/µL)	8.07 ± 0.41	8.77 ± 0.38	8.67 ± 0.39	9.75 ± 0.44*	8.69 ± 0.35	8.97 ± .57
Activated lymphocytes (10^3/µL)	0.10 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.14 ± 0.02
Monocytes (10^3/µL)	0.10 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.0	0.10 ± 0.01
Basophils (10^3/µL)	0.010 ± 0.001	0.008 ± 0.002	0.011 ± 0.002	0.011 ± 0.001	0.007 ± 0.002	0.010 ± 0.001
Eosinophils (10^3/µL)	0.04 ± 0.00	0.05 ± 0.0	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00	0.06 ± 0.01
Clinical Chemistry						
Urea nitrogen (mg/dL)	14.1 ± 0.6	14.1 ± 0.5	14.1 ± 0.7	15.6 ± 0.5	14.2 ± 0.5	15.5 ± 0.4
Creatinine (mg/dL)	0.53 ± 0.02	0.58 ± 0.05	0.53 ± 0.02	0.53 ± 0.02	0.56 ± 0.02	0.53 ± 0.02
Total protein (g/dL)	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1	6.2 ± 0.1[b]	6.2 ± 0.0
Albumin (g/dL)	4.4 ± 0.1	4.5 ± 0.1	4.3 ± 0.2	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.0
Alanine aminotransferase (IU/L)	48 ± 2	49 ± 1	52 ± 2	47 ± 2	46 ± 1	47 ± 2
Alkaline phosphate (IU/L)	515 ± 8	515 ± 9	502 ± 17	480 ± 11	482 ± 10*	481 ± 9*
Creatine kinase (IU/L)	475 ± 57	435 ± 43	418 ± 34	460 ± 35	403 ± 36	395 ± 43
Sorbitol dehydrogenase (IU/L)	10 ± 1	11 ± 1	10 ± 1	11 ± 1	10 ± 1	9 ± 1
Bile acids (µmol/L)	26.1 ± 1.7	28.8 ± 2.2	28.8 ± 3.2	26.3 ± 1.8	23.8 ± 2.8	32.6 ± 1.8

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9

TABLE D2
Hematology Data for Female Mice in the 3-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	43.7 mg/L	87.5 mg/L	175 mg/L	350 mg/L	700 mg/L
n	10	10	10	10	10	9
Automated hematocrit (%)	47.7 ± 0.7	47.2 ± 0.5	48.8 ± 0.8	49.5 ± 1.1	46.8 ± 1.3	46.9 ± 0.4
Hemoglobin (g/dL)	16.0 ± 0.2	15.9 ± 0.2	16.4 ± 0.3	16.6 ± 0.4	15.7 ± 0.5	15.7 ± 0.1
Erythrocytes (10^6/µL)	9.99 ± 0.15	9.86 ± 0.11	10.20 ± 0.16	10.37 ± 0.25	9.72 ± 0.29	9.69 ± 0.06
Reticulocytes (10^5/µL)	3.32 ± 0.14	3.67 ± 0.17	3.17 ± 0.18	3.48 ± 0.17	3.46 ± 0.17	3.96 ± 0.29
Mean cell volume (fL)	47.8 ± 0.2	47.9 ± 0.3	47.8 ± 0.2	47.7 ± 0.2	48.2 ± 0.3	48.4 ± 0.3
Mean cell hemoglobin(pg)	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.1 ± 0.1	16.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.6 ± 0.1	33.6 ± 0.2	33.6 ± 0.2	33.7 ± 0.2	33.4 ± 0.2	33.5 ± 0.1
Platelets (10^3/µL)	866.7 ± 52.5	929.8 ± 26.0	864.5 ± 33.5	845.1 ± 31.4	991.9 ± 26.4	1,029.9 ± 35.4
Leukocytes (10^3/µL)	3.38 ± 0.30	3.78 ± 0.15	4.05 ± 0.24	3.33 ± 0.36	5.03 ± 0.26**	4.35 ± 0.38**
Segmented neutrophils (10^3/µL)	0.35 ± 0.04	0.43 ± 0.04	0.49 ± 0.07	0.38 ± 0.06	0.54 ± 0.05*	0.54 ± 0.06*
Lymphocytes (10^3/µL)	2.92 ± 0.27	3.23 ± 0.12	3.42 ± 0.18	2.86 ± 0.30	4.34 ± 0.23**	3.64 ± 0.33**
Activated lymphocytes (10^3/µL)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00
Monocytes (10^3/µL)	0.04 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.01*	0.07 ± 0.01
Basophils (10^3/µL)	0.003 ± 0.002	0.002 ± 0.001	0.006 ± 0.002	0.003 ± 0.002	0.010 ± 0.001*	0.004 ± 0.002
Eosinophils (10^3/µL)	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.01	0.06 ± 0.00	0.07 ± 0.01

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.

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

TABLE E1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 3-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	43.7 mg/L	87.5 mg/L	175 mg/L	350 mg/L	700 mg/L
n	10	10	10	10	10	10
Necropsy body wt.	199 ± 6	195 ± 6	193 ± 4	187 ± 6	186 ± 6	179 ± 5*
Heart						
Absolute	0.685 ± 0.022	0.656 ± 0.018	0.663 ± 0.011	0.629 ± 0.016	0.632 ± 0.014	0.630 ± 0.019
Relative	3.441 ± 0.058	3.375 ± 0.061	3.448 ± 0.050	3.366 ± 0.024	3.409 ± 0.069	3.532 ± 0.093
R. Kidney						
Absolute	0.752 ± 0.024	0.754 ± 0.027	0.753 ± 0.022	0.739 ± 0.023	0.741 ± 0.024	0.736 ± 0.022
Relative	3.774 ± 0.044	3.868 ± 0.060	3.905 ± 0.048	3.950 ± 0.026*	3.984 ± 0.049**	4.119 ± 0.076**
Liver						
Absolute	8.122 ± 0.259	7.987 ± 0.307	7.938 ± 0.244	7.652 ± 0.277	7.757 ± 0.349	7.597 ± 0.298
Relative	40.786 ± 0.556	40.931 ± 0.639	41.194 ± 0.835	40.863 ± 0.669	41.547 ± 0.697	42.414 ± 0.925
Lung						
Absolute	1.091 ± 0.036	1.100 ± 0.051	1.124 ± 0.033	1.071 ± 0.030	1.083 ± 0.038	1.051 ± 0.046
Relative	5.497 ± 0.186	5.656 ± 0.223	5.839 ± 0.143	5.758 ± 0.200	5.847 ± 0.211	5.894 ± 0.249
R. Testis						
Absolute	1.151 ± 0.041	1.099 ± 0.053	1.110 ± 0.037	1.011 ± 0.077	1.088 ± 0.029	0.988 ± 0.073
Relative	5.783 ± 0.137	5.617 ± 0.170	5.761 ± 0.159	5.347 ± 0.303	5.869 ± 0.140	5.486 ± 0.347
Thymus						
Absolute	0.411 ± 0.013	0.371 ± 0.015	0.413 ± 0.017	0.385 ± 0.008	0.370 ± 0.017	0.371 ± 0.015
Relative	2.070 ± 0.070	1.918 ± 0.090	2.156 ± 0.103	2.079 ± 0.088	2.000 ± 0.093	2.083 ± 0.092

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given as grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE E2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Mice in the 3-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	43.7 mg/L	87.5 mg/L	175 mg/L	350 mg/L	700 mg/L
n	10	10	10	10	10	10
Necropsy body wt.	22.6 ± 0.2	22.6 ± 0.4	21.3 ± 0.6*	21.4 ± 0.3*	20.3 ± 0.5**	21.3 ± 0.4**
Heart						
Absolute	0.105 ± 0.003	0.112 ± 0.002	0.099 ± 0.004	0.105 ± 0.005	0.100 ± 0.001	0.104 ± 0.002
Relative	4.655 ± 0.124	4.961 ± 0.139	4.632 ± 0.098	4.903 ± 0.219	4.939 ± 0.123	4.892 ± 0.125
R. Kidney						
Absolute	0.153 ± 0.003	0.153 ± 0.005	0.154 ± 0.007	0.157 ± 0.006	0.156 ± 0.005	0.157 ± 0.005
Relative	6.786 ± 0.144	6.768 ± 0.209	7.207 ± 0.264	7.328 ± 0.238	7.675 ± 0.153*	7.366 ± 0.169*
Liver						
Absolute	0.942 ± 0.020	0.966 ± 0.030	0.907 ± 0.032	0.969 ± 0.025	0.970 ± 0.034	0.971 ± 0.034
Relative	41.759 ± 0.763	42.668 ± 1.033	42.491 ± 0.842	45.241 ± 0.996	47.931 ± 2.011**	45.489 ± 1.027**
Lung						
Absolute	0.162 ± 0.006	0.158 ± 0.005	0.156 ± 0.007	0.154 ± 0.009	0.144 ± 0.002*	0.135 ± 0.004**
Relative	7.176 ± 0.259	6.993 ± 0.214	7.302 ± 0.223	7.173 ± 0.351	7.114 ± 0.184	6.359 ± 0.231
Thymus						
Absolute	0.060 ± 0.002	0.058 ± 0.002	0.054 ± 0.002	0.058 ± 0.003	0.063 ± 0.004	0.067 ± 0.003
Relative	2.670 ± 0.107	2.549 ± 0.084	2.568 ± 0.116	2.702 ± 0.131	3.092 ± 0.185*	3.146 ± 0.123*

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given as grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX F
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Bromodichloromethane

A single lot of bromodichloromethane (02107TG) was obtained from Aldrich Chemical Company, Inc. (Milwaukee, WI), by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH), and provided to the study laboratory for use in the 3-week and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory and the study laboratory (Southern Research Institute, Birmingham, AL). Reports on analyses performed in support of the bromodichloromethane studies are on file at the National Institute of Environmental Health Sciences.

Lot 02107TG of the chemical, a clear colorless liquid, was received in 100 g ampules. Since this material is sensitive to air, one of the ampules was divided into smaller aliquots and reampuled for individual purity and identity analyses. Some of the samples turned yellow during the reampuling process. The colored samples were not used for frozen reference, and the purity results reported are for uncolored samples. The material was identified as bromodichloromethane by the analytical chemistry laboratory using infared (IR), ultraviolet/visible (UV/Vis), and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy and by the study laboratory using IR and proton NMR. All the IR and NMR spectra were consistent with the literature spectra (*Aldrich,* 1981, 1992) of bromodichloromethane; however, the proton NMR spectrum observed by the analytical chemistry laboratory contained a singlet at 2.17 ppm that was not seen in the reference spectrum. The UV/Vis spectrum indicated no substantial absorption over the range of 200 to 800 nm relative to the blank spectrum. The IR, proton NMR, and carbon-13 NMR spectra are presented in Figures F1, F2, and F3, respectively.

The moisture content of lot 02107TG was determined by Galbraith Laboratories (Knoxville, TN) using Karl Fischer titration. The purity of this lot was determined by Galbraith Laboratories using elemental analysis, by the analytical laboratory using gas chromatography (GC) system A (Table F1), and by the study laboratory using GC system B. Karl Fischer titration indicated a moisture content of less than 0.24%. Elemental analyses for carbon, bromine, and chlorine were in agreement with the theoretical values for bromodichloromethane; the hydrogen content was approximately 10% below theoretical. Purity profiles were obtained by the analytical laboratory using GC system A. Two volatile impurities were found with areas 0.48% and 0.82% of the total peak area.

The purity profile of the test chemical dissolved in ethyl acetate obtained by the study laboratory using GC system B indicated a relative purity of 98.2% compared to a frozen reference sample supplied by the analytical chemistry laboratory, and a calculated chromatography purity of 99.8%. The overall purity of lot 02107TG was determined to be 98% or greater.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using GC system C. These studies indicated that bromodichloromethane was stable as a bulk chemical for 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored refrigerated, protected from light, in heat-sealed glass ampules. Stability was monitored by the study laboratory during the 3-week and 2-year studies using GC system B. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared once prior to the 3-week studies and approximately every 4 weeks during the 2-year studies by mixing bromodichloromethane with tap water (Table F2). Formulations were stored refrigerated in amber glass bottles, protected from air for up to 35 days.

Homogeneity studies of the 43.7 and 700 mg/L dose formulations were performed by the study laboratory using GC system D. Stability studies of the 1 and 20 µg/mL formulations of bromodichloromethane in tap water were performed by the analytical chemistry laboratory using GC system E (Table F1). Homogeneity was confirmed, and stability was confirmed for at least 35 days for the 20 µg/mL formulation stored in amber glass bottles at 5° C, and for at least 7 days in amber drinking water bottles under simulated animal room conditions if a loss of approximately 5% of the test chemical was acceptable.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC system D. During the 3-week studies, the dose formulations were analyzed once; five of seven dose formulations for rats and mice were within 10% of the target concentrations and all were within 12% of the target concentrations (Table F3). Animal room samples of these dose formulations were also analyzed; one of the ten animal room samples was within 10%, and all were within 30% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks (Table F4). Of the dose formulations analyzed and used in the 2-year studies, 71 of 73 were within 10% of the target concentrations; all were within 12% of the target concentration. The two formulations that were within 12% of the target concentration were used for dosing with permission of the NTP. None of the 24 animal room samples were within 10% of the target concentrations; all rat samples were within 25% of target, and mouse samples ranged from 14% to 62% of target. Water bottles were changed twice weekly.

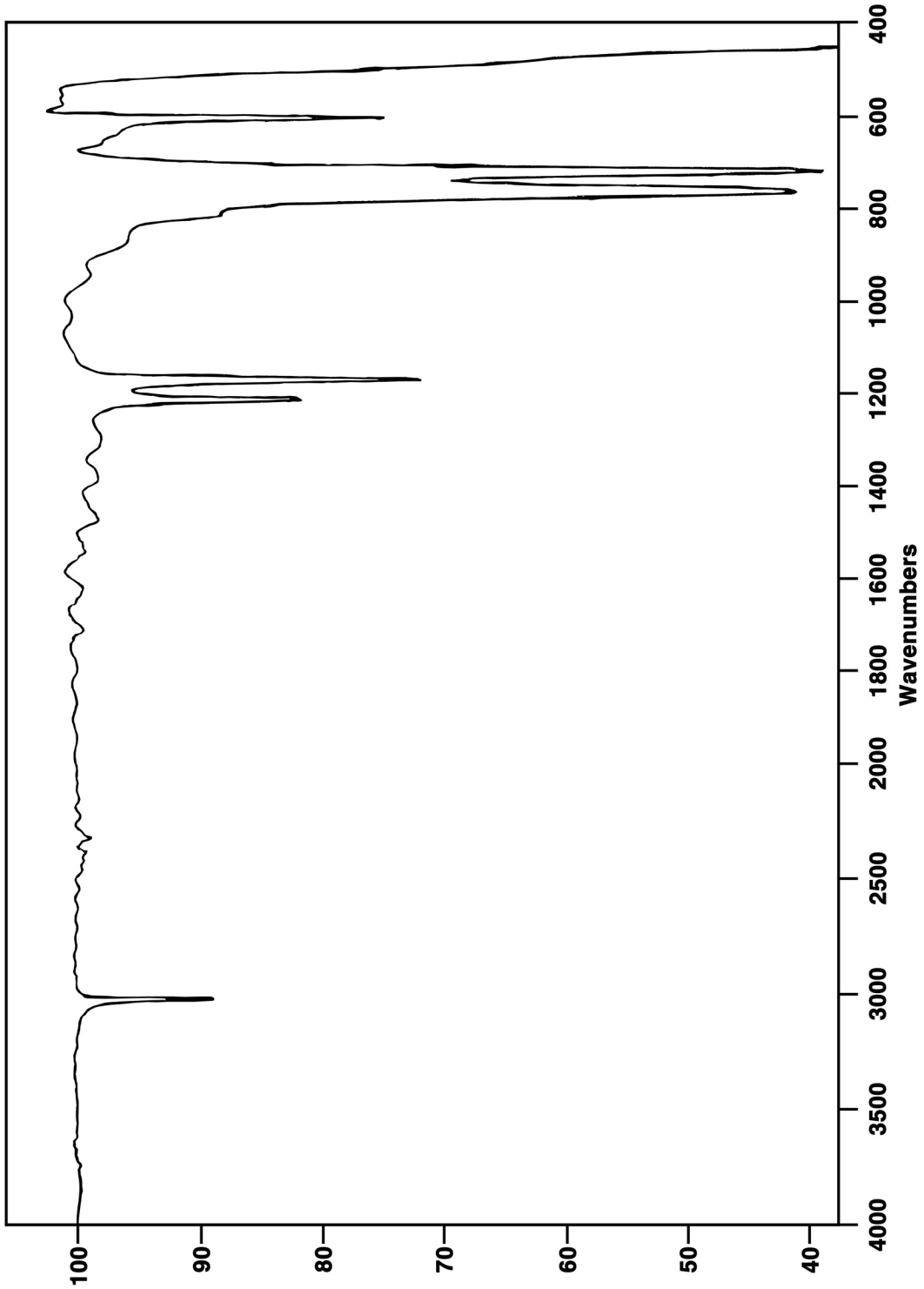


FIGURE F1
Infrared Absorption Spectrum of Bromodichloromethane

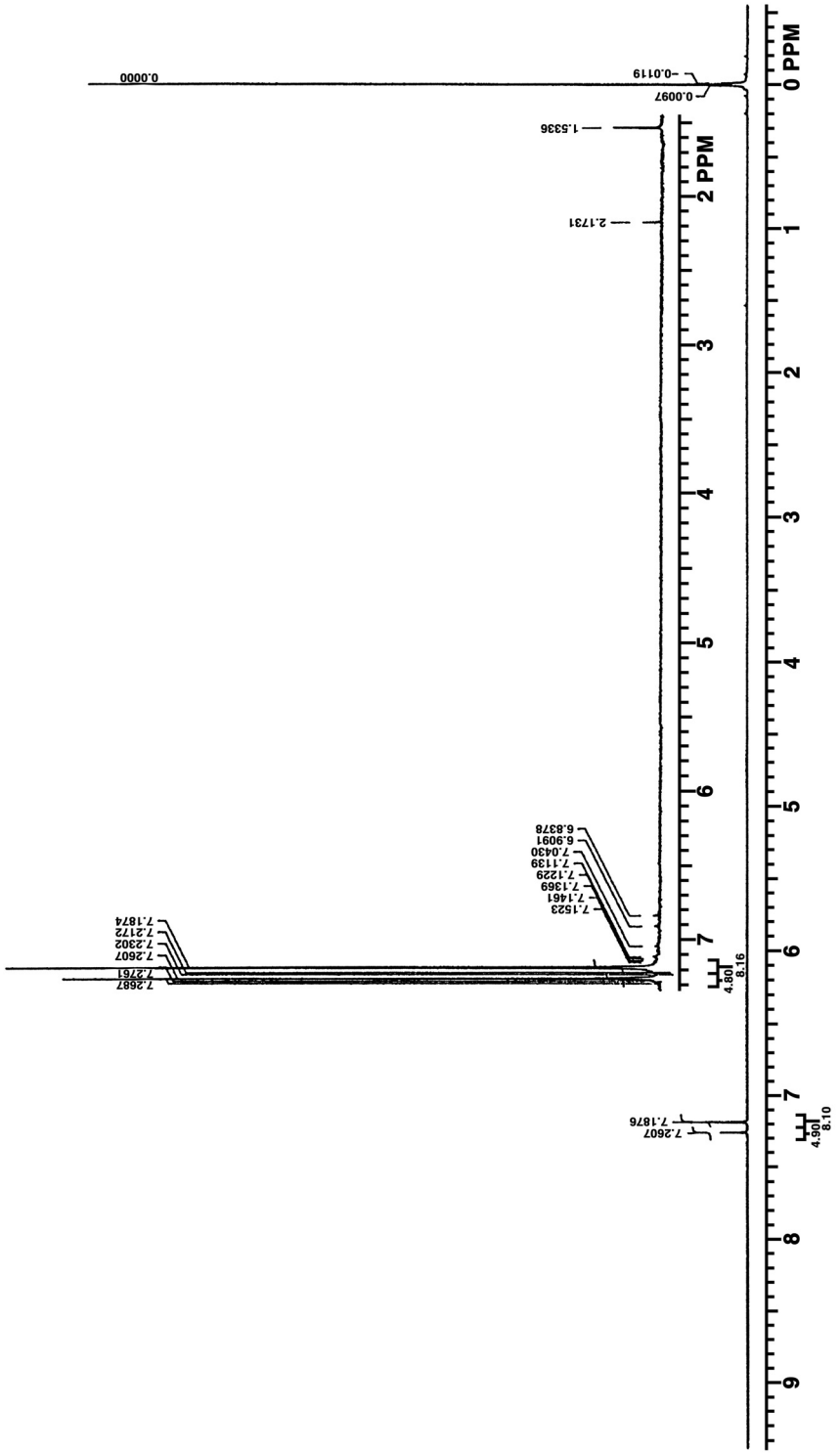


FIGURE F2
Proton Nuclear Magnetic Resonance Spectrum of Bromodichloromethane

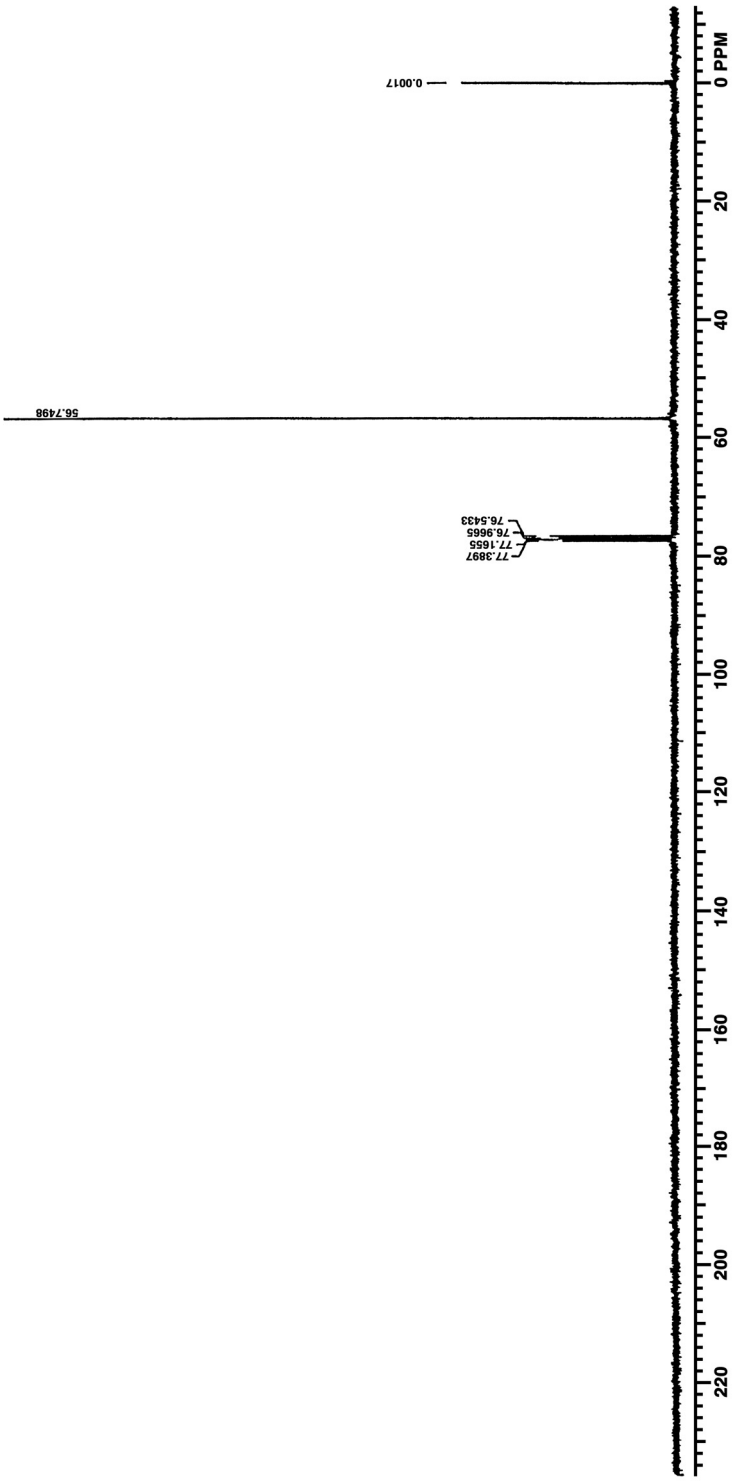


FIGURE F3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Bromodichloromethane

TABLE F1
Gas Chromatography Systems Used in the Drinking Water Studies of Bromodichloromethane[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry	Supelco Vocol 30 m × 0.25 mm, 1.5-µm film thickness (Supelco, Inc., Bellefonte, PA)	Helium at 4 mL/minute	40° C for 4 minutes, then 6° C/minute to 210° C, held for 2 minutes
System B			
Electron capture	Supelco Vocol 30 m × 0.53 mm, 3.0-µm film thickness (Supelco, Inc)	Nitrogen at approximately 8 mL/minute	40° C for 5 minute, then 10° C/minute to 170° C, held for 1 minute
System C			
Electron capture	Supelco Vocol 30 m × 0.53 mm, 3.0-µm film thickness (Supelco, Inc)	Helium at 8.2 mL/minute	40° C to 120° C at 10° C/minute, then 49° C/minute to 169° C, held for 1 minute
System D	Supelco Vocol 30 m × 0.53 mm, 3.0-µm film thickness (Supelco, Inc)	Nitrogen at approximately 8 mL/minute	40° C for 1 minute, then 5° C/minute to 150° C, held for 2 minutes
Electron capture			
System E	1% SP 1000 60/80 Carbopack B, 2.4 m × 2 mm (Supelco, Inc)	Helium at 10 mL/minute	150° C, held for approximately 16 minutes
Flame ionization			

[a] Gas chromatographs and the mass spectrometer were manufactured by Hewlett-Packard (Palo Alto, CA).

TABLE F2
Preparation and Storage of Dose Formulations in the Drinking Water Studies of Bromodichloromethane

3-Week Studies	2-Year Studies
Preparation	
Bromodichloromethane was pipetted into tap water contained in a glass mixing container and the solution was stirred for approximately 4 hours under a nitrogen headspace. The dose formulations were prepared once during the studies.	Initially, preparation of the dose formulations was the same as that for the 3-week studies. However, beginning July 20, 1999, the solution was stirred overnight, usually for a minimum of 17 hours. Throughout these studies, the dose formulations were prepared approximately every 4 weeks.
Chemical Lot Number	
02107TG	02107TG
Maximum Storage Time	
35 days	35 days
Storage Conditions	
Stored in amber glass bottles at 5° C	Same as 3-week studies
Study Laboratory	
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE F3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Week Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
July 29-30, 1998	July 30-31, 1998	43.7	38.5	–12
		43.7	38.4	–12
		43.7	39.4	–10
		87.5	84.4	–4
		175	173	–1
		350	332	–5
		700	667	–5
Animal Room Samples Rats				
July 29-30, 1998	August 21 and 24, 1998	43.7	32.9	–25
		87.5	74.3	–15
		175	157	–10
		350	303	–13
		700	616	–12
Mice				
July 29-30, 1998	August 21 and 24, 1998	43.7	30.8	–30
		87.5	70.3	–20
		175	147	–16
		350	276	–21
		700	568	–19

[a] Results of duplicate analyses

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target[b] (%)
Rats and Mice				
December 8, 1998	December 9-11, 1998	175	165	–6
		175	159	–9
		350	270[c]	–23
		350	307[c]	–12
		350	348	–1
		350	337	–4
		700	670	–4
		700	661	–6
		700	671	–4
		700	672	–4
December 14, 1998	December 14, 1998	350	336[d]	–4
March 2, 1999	March 3-4, 1999	175	167	–5
		175	170	–3
		175	171	–2
		175	168	–4
		350	326	–7
		350	185[c]	–47
		350	321	–8
		700	659	–6
		700	658	–6
March 12, 1999	March 23, 1999	350	300[c,d]	–14
March 24, 1999	March 25, 1999	350	338[d]	–4
April 27, 1999	April 28-30, 1999	175	169	–3
		175	167	–5
		175	173	–1
		175	96[c]	–45
		350	303[c]	–13
		350	323	–8
		350	307[e]	–12
		700	549[c]	–22
		700	655	–7
May 4, 1999	May 5-6, 1999	175	149[c,d]	–15
		350	293[c,d]	–16
		350	296[c,d]	–16
		700	623[c,d]	–11
May 6, 1999	May 7-9, 1999	350	340[d]	–3
		350	344[d]	–2
May 7, 1999	May 7-9, 1999	175	174[d]	–1
		700	658[d]	–6

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Rats and Mice (continued)				
July 20, 1999	July 21-23, 1999	175	164	–6
		175	163	–7
		350	327	–7
		350	323	–8
		700	624[c]	–11
		700	655	–6
July 27, 1999	July 28, 1999	700	638[d]	–9
September 14, 1999	September 15-16, 1999	175	165	–6
		175	166	–5
		350	332	–5
		350	327	–7
		700	651	–7
		700	641	–8
December 7, 1999	December 8-9, 1999	175	172	–2
		175	168	–4
		350	326	–7
		350	333	–5
		700	654	–7
		700	671	–4
February 1, 2000	February 2-3, 2000	175	165	–6
		175	163	–7
		350	331	–6
		350	333	–5
		700	662	–6
		700	599[c]	–14
February 3, 2000	February 4, 2000	700	618[c,d]	–12
February 9, 2000	February 11, 2000	700	675[d]	–4
April 25, 2000	April 26-27, 2000	175	161	–8
		175	150[c]	–14
		350	322	–8
		350	310[c]	–11
		700	638	–9
		700	623[c]	–11
May 2, 2000	May 3-4, 2000	175	168[d]	–4
		350	342[d]	–2
		700	654[d]	–7

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Rats and Mice (continued)				
July 18, 2000	July 19-20, 2000	175	148[c]	–16
		175	160	–8
		350	326	–7
		350	319	–9
		700	610[c]	–13
		700	640	–9
July 24, 2000	July 26-28, 2000	175	160[d]	–8
		700	588[c,d]	–16
July 27, 2000	July 27-28, 2000	700	602[c,d]	–14
July 31, 2000	August 1, 2000	700	651[d]	–7
September 12, 2000	September 14-15, 2000	175	167	–4
		175	161	–8
		350	330	–6
		350	336	–4
		700	667	–5
		700	654	–7
November 27, 2000	November 29-30, 2000	175	151[c]	–14
		350	293[c]	–16
		700	610[c]	–13
November 30, 2000	December 4, 2000	175	162[d]	–8
		350	316[d]	–10
		700	613[f]	–12
Animal Room Samples				
Rats				
December 8, 1998	January 4, 1999	175	149	–15
		700	605	–14
December 14, 1998	January 4, 1999	350	299	–15
July 20, 1999	August 24-25, 1999	175	146	–17
		350	294	–16
July 27, 1999	August 24-25, 1999	700	587	–16
February 1, 2000	March 8-9, 2000	175	140	–20
		350	298	–15
February 9, 2000	March 8-9, 2000	700	522	–25
September 12, 2000	October 16-17, 2000	175	147	–16
		350	288	–18
		700	525	–25

TABLE F4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Animal Room Samples (continued)				
Mice				
December 8, 1998	January 4-5, 1999	175	145	–17
		700	555	–21
December 14, 1998	January 4-5, 1999	350	300	–14
July 20, 1999	August 24-25, 1999	175	107	–39
		350	202	–42
July 27, 1999	August 24-25, 1999	700	584	–17
February 1, 2000	March 8-9, 2000	175	89	–49
		350	286	–18
February 9, 2000	March 8-9, 2000	700	436	–38
September 12, 2000	October 16-17, 2000	175	107	–39
		350	193	–45
		700	268	–62

[a] Results of duplicate analyses
[b] Reported percentages are based on unrounded raw data; therefore, some percentages may not be reproducible when calculated from the rounded concentration values presented here.
[c] Remixed, not used in studies
[d] Results of remix
[e] Remixed, but sample was used on five rat cages for 1 day with permission from the NTP.
[f] Not remixed and sample was used for the final week of the studies with permission from the NTP.

APPENDIX G
WATER AND COMPOUND CONSUMPTION IN THE 2-YEAR DRINKING WATER STUDIES OF BROMODICHLOROMETHANE

TABLE G1
Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 ppm			700 ppm		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ (mg/kg)
4	16.7	201	15.4	195	14	15.2	194	28	14.7	193	53
8	17.7	278	16.1	269	11	15.9	272	21	15.8	269	41
12	16.9	327	15.3	318	8	14.9	319	16	14.4	314	32
16	15.8	360	14.2	348	7	13.8	347	14	13.5	345	27
20	16.0	387	15.1	376	7	14.3	375	13	14.4	371	27
24	16.1	405	15.6	393	7	15.3	392	14	14.4	391	26
28	15.3	429	14.7	415	6	13.9	414	12	13.9	410	24
32	14.7	444	14.3	430	6	14.0	428	12	13.8	425	23
36	14.7	458	13.9	444	6	13.7	444	11	13.8	438	22
40	14.6	468	13.8	455	5	13.8	454	11	13.4	449	21
44	14.7	481	14.1	468	5	13.9	468	10	13.5	462	21
48	16.1	481	15.3	470	6	15.1	470	11	14.8	465	22
52	15.8	492	14.7	479	5	15.0	479	11	14.8	472	22
56	14.9	498	14.1	484	5	14.0	486	10	14.0	476	21
60	15.4	505	14.8	493	5	14.0	492	10	14.1	486	20
64	16.3	506	15.2	495	5	15.0	495	11	14.9	487	21
68	15.5	511	14.3	497	5	14.1	497	10	13.7	488	20
72	15.3	512	15.1	500	5	14.4	497	10	14.3	487	21
76	15.1	510	14.2	503	5	13.8	495	10	13.6	490	19
80	14.8	509	14.4	503	5	13.8	497	10	13.3	491	19
81	14.0	511	13.2	504	5	14.0	494	10	13.6	489	19
84	16.2	508	14.8	497	5	14.8	495	11	14.2	482	21
88	14.8	506	13.6	500	5	13.0	496	9	13.6	483	20
92	15.8	511	14.4	498	5	13.4	492	10	13.9	478	20
96	17.1	511	15.4	483	6	15.2	489	11	15.2	483	22
100	16.5	506	14.8	474	6	16.0	488	12	17.6	477	26
104	16.9	491	15.6	482	6	15.1	481	11	16.6	473	25
Mean for weeks											
1-13	17.1	268	15.6	261	11	15.4	262	21	15.0	259	42
14-52	15.4	441	14.6	428	6	14.3	427	12	14.0	423	24
53-104	15.6	507	14.6	494	5	14.3	493	10	14.5	484	21

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of bromodichloromethane consumed per kilogram body weight per day

TABLE G2
Water and Compound Consumption by Female Mice in the 2-Year Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 ppm			700 ppm		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ (mg/kg)
4	3.0	21.7	2.2	20.8	19	1.7	20.1	29	1.9	20.1	66
8	3.4	26.4	2.3	24.2	17	2.5	24.6	36	2.1	24.4	61
12	2.9	32.1	2.5	27.2	16	2.6	28.2	32	2.3	27.3	59
16	2.2	37.9	2.0	31.8	11	1.6	33.3	17	2.2	31.7	48
20	2.6	42.6	2.5	34.8	12	2.2	37.2	21	2.5	35.5	50
24	2.7	45.9	2.4	37.8	11	2.3	38.5	21	2.8	37.3	53
28	1.9	50.0	2.2	40.1	10	2.2	41.4	18	1.9	40.4	33
32	2.4	51.8	2.2	43.8	9	2.2	45.4	17	2.3	43.7	37
36	2.4	54.8	2.2	46.6	8	2.3	48.2	17	2.2	47.4	32
40	2.5	58.1	2.5	50.6	9	2.3	52.8	15	2.4	51.3	33
44	2.6	59.4	2.4	54.6	8	2.3	55.7	14	2.4	54.3	31
48	2.7	60.2	2.5	57.0	8	2.2	58.4	13	2.2	56.4	27
52	2.7	61.2	2.5	58.1	7	2.4	58.1	14	2.5	55.6	31
56	2.6	62.3	2.1	57.3	6	2.1	58.8	12	2.1	56.3	27
60	2.5	64.4	2.3	58.3	7	2.0	61.2	12	2.2	58.3	27
64	2.7	64.7	2.2	60.0	6	2.1	60.5	12	2.2	59.9	26
68	2.7	65.4	2.1	60.8	6	2.2	61.4	12	2.2	60.3	25
72	2.9	66.6	2.1	62.2	6	2.2	62.7	12	2.2	61.4	25
76	2.6	66.6	2.0	61.3	6	2.0	61.7	11	2.3	60.1	26
80	2.9	65.1	2.3	59.2	7	2.1	60.1	12	2.2	59.1	27
84	2.9	65.4	2.2	59.9	6	2.2	60.7	13	2.4	59.3	28
88	3.3	66.1	2.2	61.2	6	2.4	60.9	14	2.3	60.9	26
92	3.7	64.0	2.4	59.8	7	2.3	59.4	13	2.4	59.6	28
96	3.6	62.2	3.2	56.5	10	2.5	55.8	16	2.4	57.3	29
100	3.7	60.4	2.8	54.7	9	2.3	52.4	16	2.7	56.5	33
104	3.8	56.9	3.0	53.8	10	3.4	51.8	23	2.8	55.3	35
Mean for weeks											
1-13	3.1	26.7	2.4	24.1	17	2.3	24.3	33	2.1	23.9	62
14-52	2.5	52.2	2.3	45.5	9	2.2	46.9	17	2.3	45.4	38
53-104	3.1	63.9	2.4	58.8	7	2.3	59.0	14	2.3	58.8	28

[a] Grams of drinking water consumed per animal per day
[b] Milligrams of bromodichloromethane consumed per kilogram body weight per day

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

TABLE H1
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 H2
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	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-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 H3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	13.6 ± 0.46	12.8 – 14.5	25
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	25
Crude fiber (% by weight)	9.1 ± 0.63	7.9 – 10.5	25
Ash (% by weight)	5.0 ± 0.20	4.7 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.748 ± 0.053	0.670 – 0.850	12
Cystine	0.223 ± 0.027	0.150 – 0.250	12
Glycine	0.702 ± 0.043	0.620 – 0.750	12
Histidine	0.343 ± 0.023	0.310 – 0.390	12
Isoleucine	0.534 ± 0.041	0.430 – 0.590	12
Leucine	1.078 ± 0.059	0.960 – 1.140	12
Lysine	0.729 ± 0.065	0.620 – 0.830	12
Methionine	0.396 ± 0.053	0.260 – 0.460	12
Phenylalanine	0.611 ± 0.038	0.540 – 0.660	12
Threonine	0.492 ± 0.045	0.430 – 0.590	12
Tryptophane	0.129 ± 0.016	0.110 – 0.160	12
Tyrosine	0.378 ± 0.054	0.280 – 0.460	12
Valine	0.658 ± 0.049	0.550 – 0.710	12
Essential Fatty Acids (% of total diet)			
Linoleic	3.89 ± 0.278	3.49 – 4.54	12
Linolenic	0.30 ± 0.038	0.21 – 0.35	12
Vitamins			
Vitamin A (IU/kg)	5,458 ± 1,055	3,460 – 7,790	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.3 ± 17.06	52.0 – 110.0	12
Thiamine (ppm)[b]	7.8 ± 0.87	6.3 – 9.3	25
Riboflavin (ppm)	6.4 ± 2.11	4.20 – 11.20	12
Niacin (ppm)	78.6 ± 10.86	66.4 – 98.2	12
Pantothenic Acid (ppm)	23.1 ± 3.61	17.4 – 29.1	12
Pyridoxine (ppm)[b]	8.88 ± 2.05	6.4 – 12.4	12
Folic Acid (ppm)	1.84 ± 0.56	1.26 – 3.27	12
Biotin (ppm)	0.337 ± 0.13	0.225 – 0.704	12
Vitamin B_{12} (ppb)	64.8 ± 50.9	18.3 – 174.0	12
Choline (ppm)[b]	3,094 ± 292	2,700 – 3,790	12
Minerals			
Calcium (%)	1.003 ± 0.047	0.903 – 1.090	25
Phosphorus (%)	0.569 ± 0.027	0.517 – 0.618	25
Potassium (%)	0.668 ± 0.023	0.627 – 0.694	12
Chloride (%)	0.368 ± 0.033	0.300 – 0.423	12
Sodium (%)	0.189 ± 0.016	0.160 – 0.212	12
Magnesium (%)	0.200 ± 0.009	0.185 – 0.217	12
Sulfur (%)	0.176 ± 0.026	0.116 – 0.209	12
Iron (ppm)	177 ± 46.2	135 – 311	12
Manganese (ppm)	53.4 ± 6.42	42.1 – 63.1	12
Zinc (ppm)	52.5 ± 6.95	43.3 – 66.0	12
Copper (ppm)	6.64 ± 1.283	5.08 – 9.92	12
Iodine (ppm)	0.535 ± 0.242	0.233 – 0.972	12
Chromium (ppm)	0.545 ± 0.125	0.330 – 0.751	12
Cobalt (ppm)	0.23 ± 0.041	0.20 – 0.30	12

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE H4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.16 ± 0.061	0.10 – 0.30	25
Cadmium (ppm)	0.04 ± 0.007	0.04 – 0.07	25
Lead (ppm)	0.11 ± 0.104	0.05 – 0.54	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.19 ± 0.033	0.14 – 0.28	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	10.8 ± 2.94	9.04 – 21.1	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)	10 ± 2	10 – 20	25
Coliform (MPN/g)	0.7 ± 1.5	0.0 – 3.6	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.6 ± 1.48	2.1 – 7.7	25
N-Nitrosodimethylamine (ppb)[e]	1.7 ± 0.53	1.0 – 3.0	25
N-Nitrosopyrrolidine (ppb)[e]	2.9 ± 1.18	1.0 – 5.6	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	0.151 ± 0.121	0.023 – 0.499	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.217 ± 0.185	0.020 – 0.826	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 I
SENTINEL ANIMAL PROGRAM

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 randomly selected rats and male sentinel mice during the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
ELISA	
Mycoplasma arthriditis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	6, 12, and 18 months, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
MICE	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM (epizootic diarrhea of infant mice)	6, 12, and 18 months, study termination
GDVII (mouse encephalomyelitis virus)	6, 12, and 18 months, study termination
LCM (lymphocytic choriomeningitis virus)	6, 12, and 18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV (mouse hepatitis virus)	6, 12, and 18 months, study termination
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
MCMV (mouse cytomegalovirus)	Study termination
M. arthritidis	Study termination
Parvovirus	6, 12, and 18 months, study termination

RESULTS

All serology tests were negative.

APPENDIX J
SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

SINGLE-DOSE TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F$_1$ MICE

INTRODUCTION

Single dose toxicokinetic studies of bromodichloromethane were conducted in F344/N rats and B6C3F$_1$ mice of both sexes by intravenous injection (10 mg bromodichloromethane/kg body weight) and oral gavage administration (25, 50, or 100 mg/kg) in both corn oil and aqueous formulations. Plasma samples collected at early time points (up to 28 hours following administration) were analyzed to establish basic toxicokinetic parameters.

MATERIALS AND METHODS

Bromodichloromethane was procured in one lot (05122ES) from Sigma Aldrich Chemical Company, Inc. (Milwaukee, WI). The identity of the material was confirmed using infrared spectrometry and its purity was estimated to be 99% by gas chromatography (GC) with flame ionization detection as described in Appendix F. Formulations for intravenous injection and aqueous oral gavage administration were prepared by mixing bromodichloromethane with the vehicle (deionized water mixed 9:1 with Cremophor®, Sigma Aldrich lot 16H0043). Formulations for corn oil gavage administration were prepared by mixing bromodichloromethane with corn oil that had been previously analyzed to ensure that peroxide levels were below 3 mEq/kg.

Male and female rats (14 to 17 weeks of age at the start of the study) and mice (12 to 17 weeks of age at the start of the study) were procured from Taconic Laboratory Animals and Services (Germantown, NY) and quarantined for at least 11 days before the study. Animals were housed in polycarbonate cages with hardwood bedding and were given food and water *ad libitum*.

Groups of three rats and mice per sex were bled by retroorbital (rats) or cardiac (mice) puncture at specified time points after intravenous or oral gavage administration of bromodichloromethane (Table J1). Whole blood was collected in 2 mL glass tubes containing EDTA as the anticoagulant and centrifuged for 10 minutes at 5,000 rpm within 60 minutes of collection. Plasma was transferred into glass tubes and stored refrigerated (approximately 5° C) until analyzed.

For analysis, 50 to 200 µL of plasma were combined with 300 µL of a 160 mg/mL sodium sulfate solution in deionized water and 1 mL of internal standard solution (1,190 µg/mL dichloropropane in deionized water) into a headspace GC vial. The headspace autosampler (CTC Analytics, LEAP Technologies, Inc., Carrboro, NC) was programed to incubate the samples at 90° C for 15 minutes then inject 500 µL onto the GC column using a syringe heated to 95° C. The GC system (Agilent Technologies, Palo Alto, CA) used a VOCOL column, 30 m × 0.53 mm, 3.0 µm film thickness; Supelco, Bellefonte, PA), with an oven program (50° C for 1 minute, to 100° C at 10° C/minute, held for 5 minutes, to 150° C at 70° C/minute, held for 1 minute) and helium as the carrier gas. Component signals were collected on an electron capture detector with argon/methane makeup gas. The method was linear (coefficient of determination was 0.99 or greater) and demonstrated to be free of carryover or coeluting peaks. Acceptance criteria for precision [% relative standard deviation of quality control (QC) samples must be ≤15 %] and accuracy (% relative error for each QC level was ≤20% and the individual relative errors for at least 66% of the QC samples overall were ≤15%) were met for runs used in reporting data. The limit of quantitation (LOQ), defined as the lowest standard that met acceptance criteria for precision and accuracy, was 1.980 ng/mL.

Noncompartmental modeling with PROC NLIN in SAS Version 8.2 (SAS Institute, Inc., Cary, NC) was used to derive toxicokinetic parameters from those plasma bromodichloromethane measurements that were above the limit of quantitation for the method.

RESULTS

Rats

Mean plasma concentrations of bromodichloromethane versus time curves for male and female rats by each route and each vehicle were similar (Figures J1, J2, and J3). Noncompartmental toxicokinetic parameter estimates for these data are provided in Table J2. Area under the plasma concentration versus time curve (AUC) appears to increase with dose in both sexes. For males, bioavailability ranged from 0.193 to 0.599 in water:Cremophor® and from 0.119 to 0.510 in corn oil. Estimates of elimination rate constants and half-lives (k_{elim} and $t_{1/2}$, respectively) were different between intravenous and gavage routes with much lower rates of elimination and longer half-lives at high gavage doses with both vehicles. For females, bioavailability ranged from 0.240 to 0.495 in water:Cremophor® and 0.162 to 0.713 in corn oil. Estimates of elimination rate constants and half-lives followed the same trends as seen with male rats in both vehicles.

Mice

Mean plasma bromodichloromethane concentration versus time data for male and female mice in the intravenous and gavage studies are plotted in Figures J4, J5, and J6.

Noncompartmental toxicokinetic parameter estimates for these data are provided in Table J3. AUC estimates increase with dose for both male and female mice. Large interindividual variations observed at some time points gave large percent relative standard deviations in the mean plasma concentrations at these time points. For males, bioavailability ranged from 0.284 to 1.17 in water:Cremophor® and from 0.211 to 0.600 in corn oil. Estimates of elimination rate constants and half-lives did not change in a dose-linear fashion with either vehicle. For females, bioavailability ranged from 0.191 to 0.973 in the aqueous vehicle and from 0.033 to 0.583 in corn oil; similar to the data from male mice, half-lives and elimination rate constants did not vary in a linear fashion with dose. The large variability in the plasma data makes interpretation of these data unreliable.

DISCUSSION

The present studies were designed to evaluate the toxicokinetics and estimate the internal dose of bromodichloromethane when administered by intravenous injection or oral gavage to male and female rats and mice. Oral bioavailability of a bolus dose of bromodichloromethane in water:Cremophor® (9:1) and corn oil was also determined.

Following a single intravenous injection of 10 mg bromodichloromethane per kg body weight to male and female rats, bromodichloromethane initially cleared rapidly from the plasma, followed by a period of slower elimination and then a second, fast elimination period. Bromodichloromethane concentrations were below the LOQ for the method after approximately 480 minutes.

A single gavage administration of bromodichloromethane in water:Cremophor® to male and female rats gave similar elimination rate constants and half-lives for nominal doses of 25 and 50 mg/kg, but 100 mg/kg doses gave sharply lower elimination rate constants and approximately doubled the half-life of bromodichloromethane in plasma. Bioavailability increased with increasing dose in both sexes. A single gavage administration of bromodichloromethane in corn oil gave similar results as those shown in the aqueous vehicle; however, the 50 mg/kg dose in female rats appears to have given an increase in the elimination rate constant and subsequent decrease in half-life over the 25 and 100 mg/kg doses. This did not change the increase in bioavailability with increasing dose as also seen with the water:Cremophor® vehicle.

Following a single intravenous injection of 10 mg/kg to male and female mice, bromodichloromethane initially cleared rapidly from the plasma; plasma concentrations then leveled off for a short period that was followed by a another period of rapid elimination. Plasma bromodichloromethane concentrations were above the LOQ for the

method for approximately 60 minutes for male mice and approximately 45 minutes for female mice. Uncertainty in the data at the last time point above the LOQ for female mice makes interpretation of the terminal part of the elimination curve difficult.

A single gavage administration of bromodichloromethane in water:Cremophor® to male and female mice at nominal doses of 25, 50, or 100 mg/kg resulted in plasma concentrations with sufficient variability that trends in the data were difficult to distinguish. Bioavailability increased with increasing dose, but the elimination rate constants and half-lives showed no trends. With corn oil as the vehicle, similar results were observed. Concentrations of bromodichloromethane in plasma remained above the LOQ for approximately 500 minutes in male mice when administered in either vehicle, but stayed above the LOQ for 720 (water:Cremophor®) or 960 (corn oil) minutes in females.

Overall, these studies showed no sex-related differences in the kinetics of bromodichloromethane, nor was there much difference when bromodichloromethane was administered in an aqueous vehicle or a nonaqueous vehicle.

TABLE J1
Blood Collection Time Points in F344/N Rats and B6C3F$_1$ Mice after a Single Intravenous Injection or Gavage Dose of Bromodichloromethane

Route of Administration	Dose (mg/kg)	Blood Collection Time Points
Rats		
Intravenous injection	10	2, 5, 10, 15, and 30 minutes and 1, 2, 4, 6, 8, and 12 hours
Water:Cremophor® (9:1) gavage	25	2, 5, 7, 10, 15, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, and 8 hours
	50	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, 12, and 16 hours
	100	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 8, 12, 16, 24, and 28 hours
Corn oil gavage	25	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, 8, and 10 hours
	50	2, 5, 7, 15, 30, and 45 minutes and 1, 1.25, 1.5, 2, 4, 8, 12, 16, and 24 hours
	100	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 8, 12, 16, 24, and 28 hours
Mice		
Intravenous injection	10	2, 5, 10, 15, 20, 30, and 45 minutes and 1 and 1.25 hours
Water:Cremophor® (9:1) gavage	25	2, 5, 7, 10, 15, 20, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 8, and 16 hours
	50	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 6, 8, and 12 hours
	100	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 4, 8, and 12 hours
Corn oil gavage	25	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 6, and 8 hours
	50	2, 5, 7, 15, 30, and 45 minutes and 1, 1.25, 1.5, 2, 4, 8, and 12 hours
	100	2, 5, 7, 15, 30, and 45 minutes and 1, 1.5, 2, 3, 4, 8, 12, 16, 24, and 28 hours

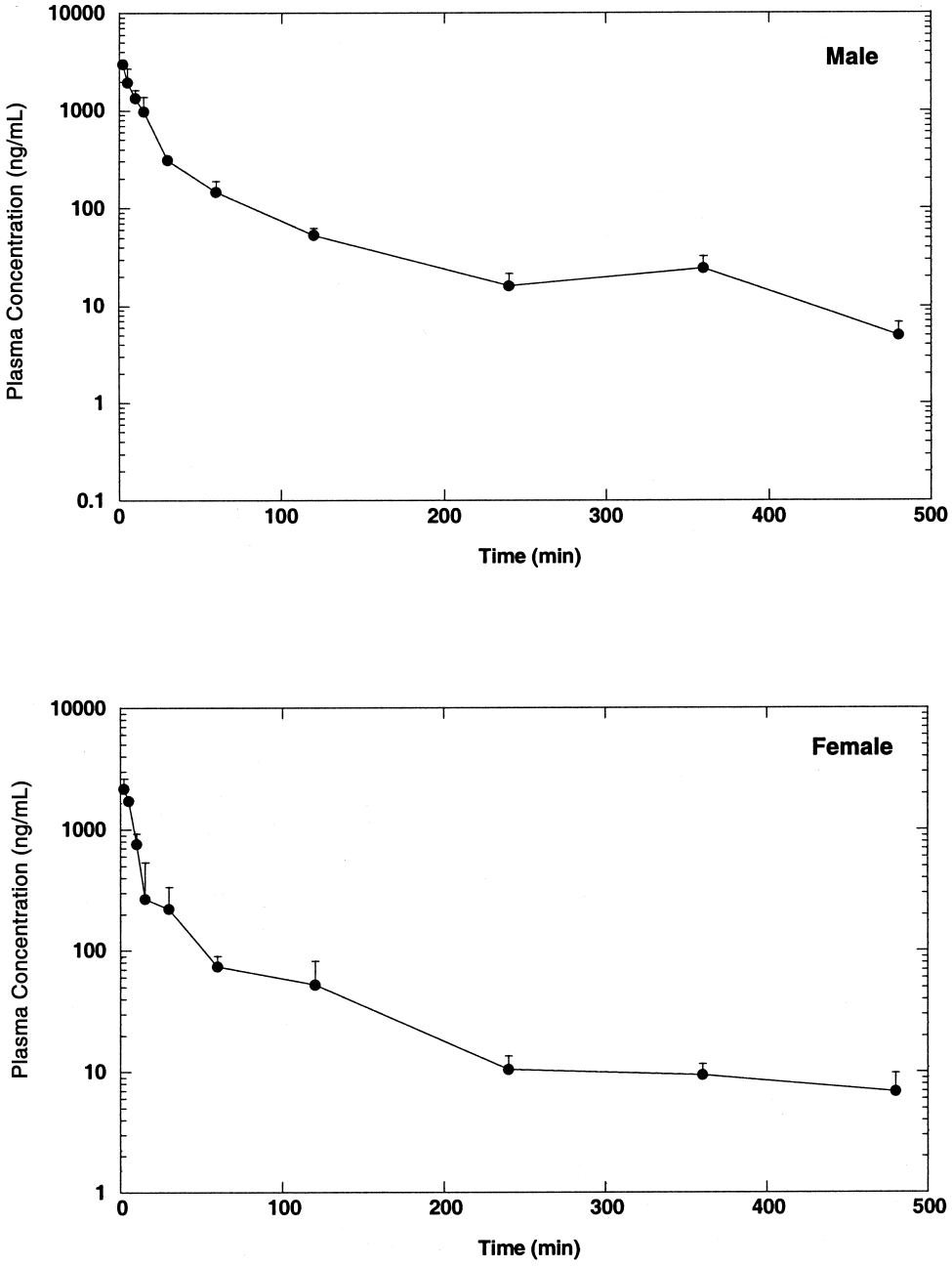
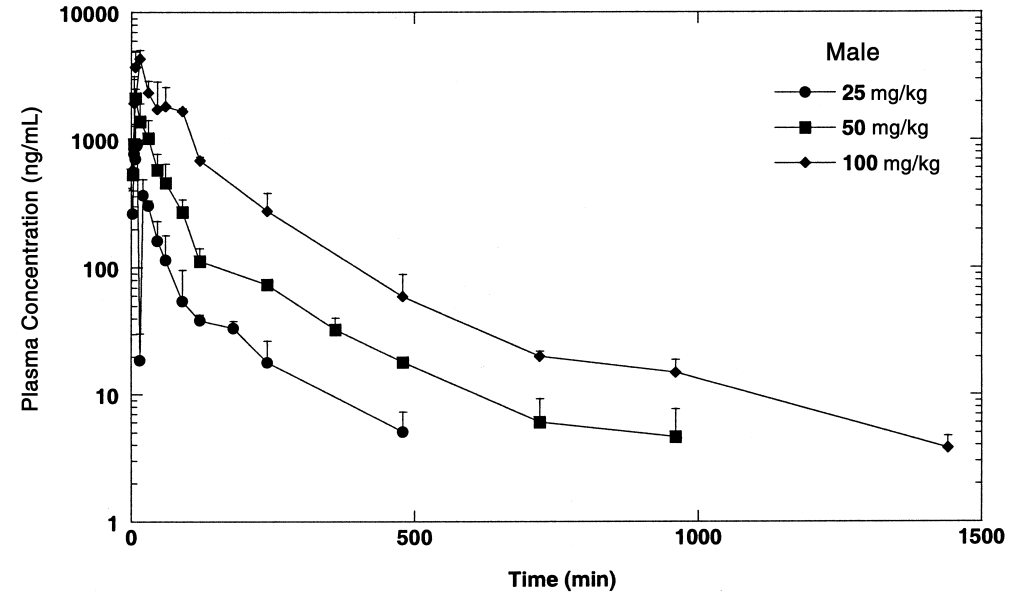


FIGURE J1
Plasma Concentrations of Bromodichloromethane in F344/N Rats after a Single Intravenous Injection of 10 mg/kg Bromodichloromethane



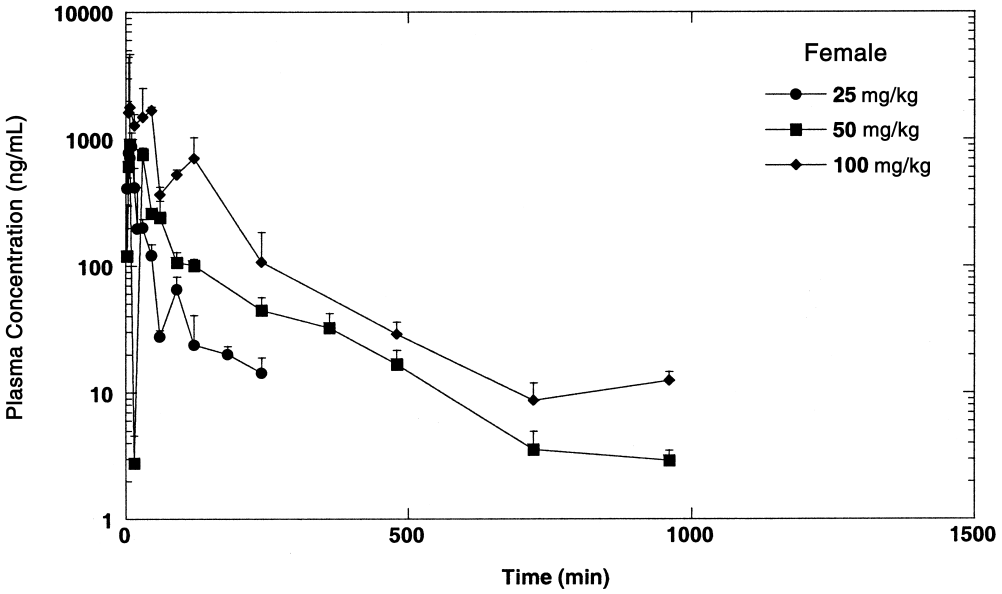


FIGURE J2
Plasma Concentrations of Bromodichloromethane in F344/N Rats after a Single Gavage Dose of Bromodichloromethane in Water:Cremophor® (9:1)

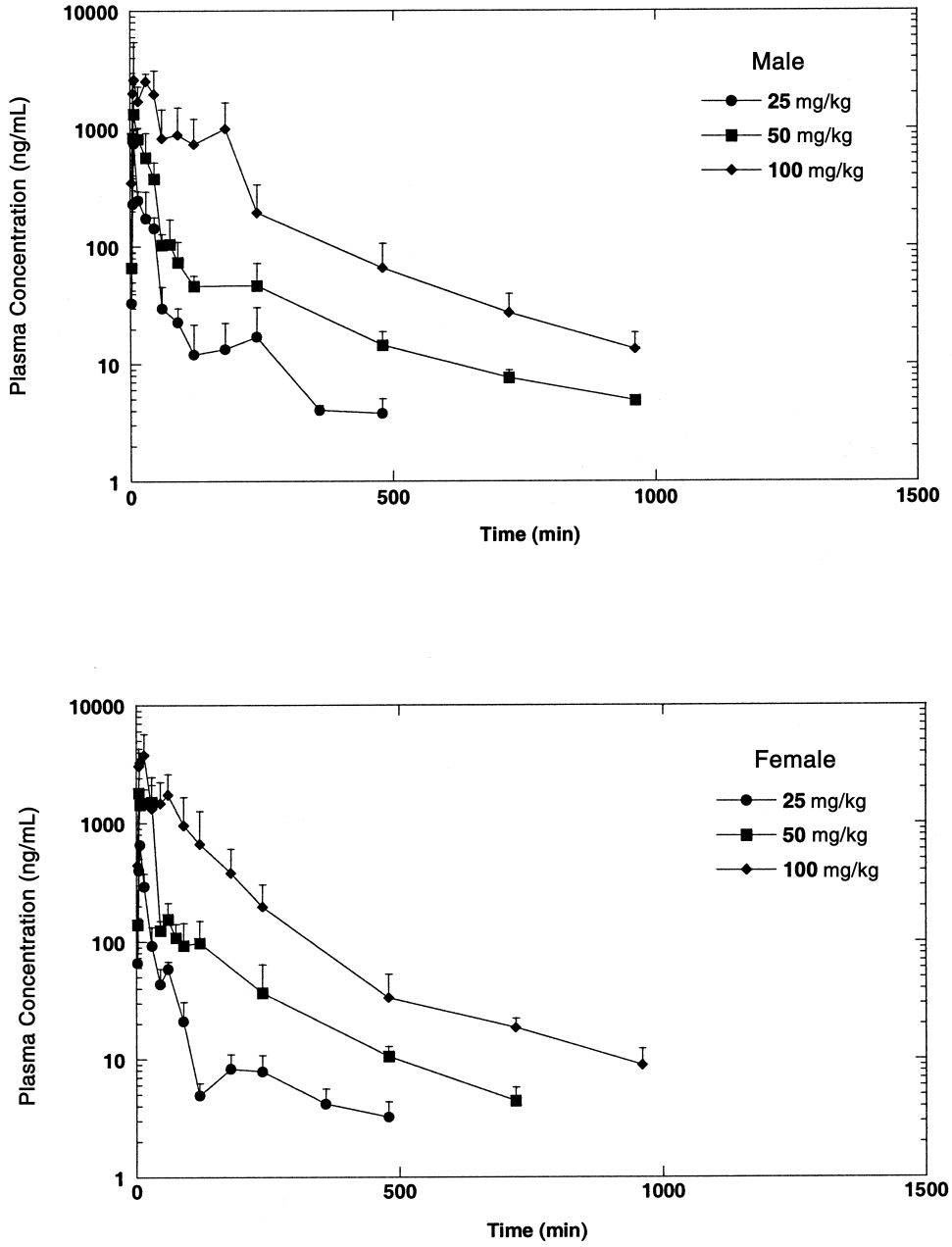


FIGURE J3
Plasma Concentrations of Bromodichloromethane in F344/N Rats after a Single Gavage Dose of Bromodichloromethane in Corn Oil

TABLE J2
Toxicokinetic Parameter Estimates in F344/N Rats after a Single Intravenous Injection or Gavage Dose of Bromodichloromethane[a]

	Nominal Dose (mg/kg)	Actual Dose[b] (mg/kg)	k_{elim} ($minutes^{-1}$)	$t_{1/2\ elim}$ (minutes)	AUC (µg • min/mL)	AUC/ Dose	Bioavailability[c]
Male							
Intravenous injection	10	9.34	0.00622	111	59,400	5.94	—[d]
Water:Cremophor® (9:1) gavage							
	25	23.47	0.00506	137	28,700	1.15	0.193
	50	47.30	0.00498	139	99,700	2.00	0.337
	100	87.85	0.00278	250	356,000	3.56	0.599
Corn oil gavage	25	24.03	0.00478	145	17,600	0.706	0.119
	50	47.10	0.00371	187	56,500	1.13	0.190
	100	95.70	0.00256	271	303,000	3.03	0.510
Female							
Intravenous injection	10	9.34	0.00835	83.0	39,000	3.90	—
Water:Cremophor® (9:1) gavage							
	25	23.47	0.00493	141	23,400	0.935	0.240
	50	47.30	0.00509	136	61,900	1.24	0.318
	100	87.85	0.00306	227	183,000	1.83	0.495
Corn oil gavage	25	24.03	0.00163	425	15,800	0.631	0.162
	50	47.10	0.00689	101	77,400	1.55	0.397
	100	95.70	0.00203	341	278,000	2.78	0.713

[a] Toxicokinetic parameters were calculated from the plasma concentration versus time curves, where each data point represented the mean of up to three samples. k_{elim} = Elimination rate constant; $t_{1/2}$ = elimination half-life, AUC = area under the plasma concentration versus time curve calculated using the trapezoidal rule

[b] Based on formulation analysis

[c] Calculated as as $AUC_{gavage}/AUC_{intravenous}$ H Nominal $Dose_{intravenous}$/Nominal $Dose_{gavage}$

[d] Not applicable to intravenous dosing

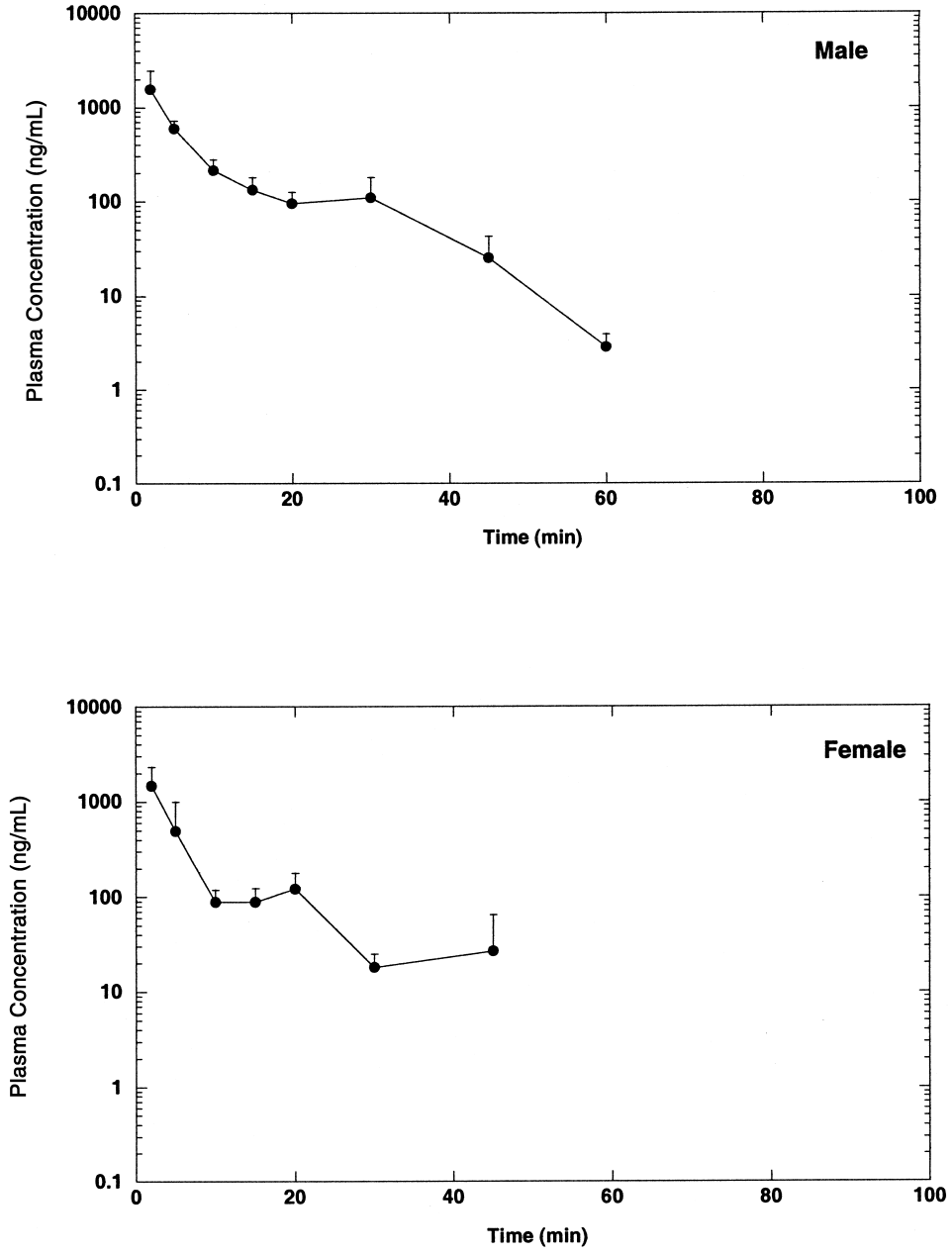


FIGURE J4
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice after a Single Intravenous Injection of 10 mg/kg Bromodichloromethane

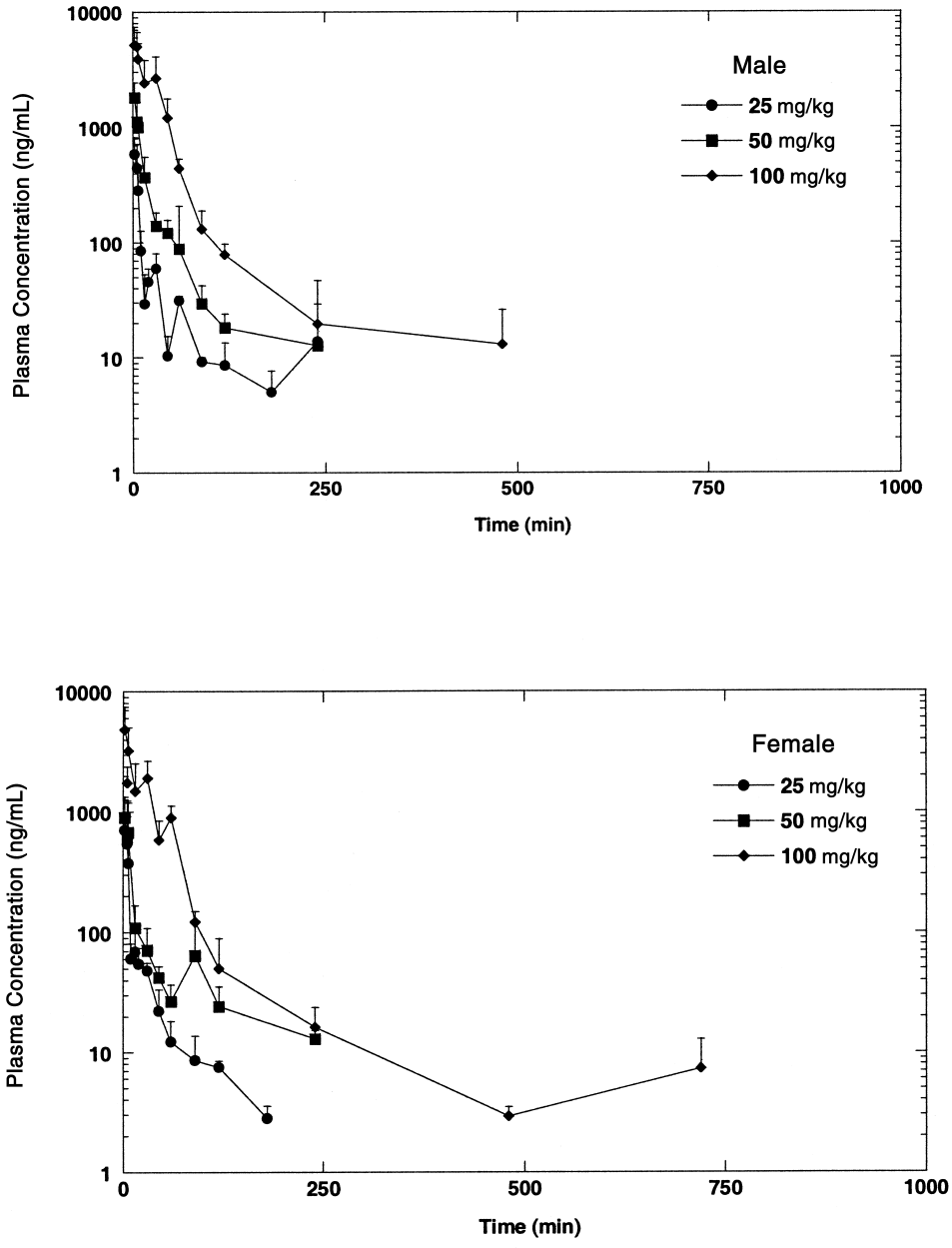
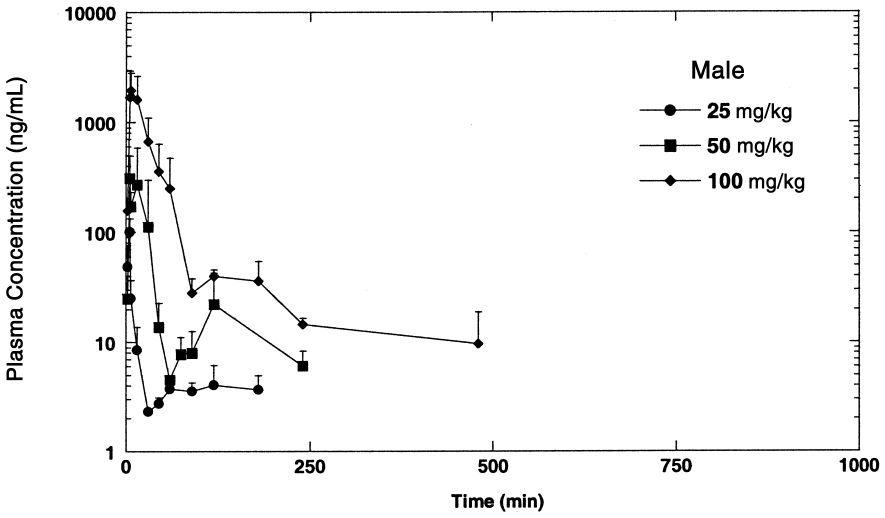


FIGURE J5
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice after a Single Gavage Dose of Bromodichloromethane in Water:Cremophor® (9:1)



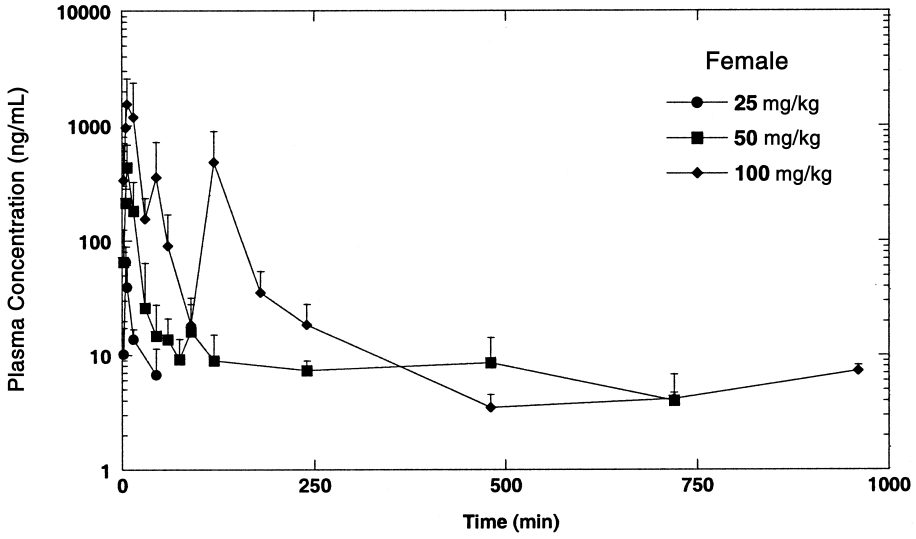


FIGURE J6
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice after a Single Gavage Dose of Bromodichloromethane in Corn Oil

TABLE J3
Toxicokinetic Parameter Estimates in B6C3F$_1$ Mice after a Single Intravenous Injection or Gavage Dose of Bromodichloromethane[a]

	Nominal Dose (mg/kg)	Actual Dose[b] (mg/kg)	k_{elim} ($minutes^{-1}$)	$t_{1/2\ elim}$ (minutes)	AUC[e] (µg • min/mL)	AUC/ Dose	Bioavailability[c]
Male							
Intravenous injection	10	8.54	0.070	9.91	12,600	1.26	—[d]
Water:Cremophor® (9:1) gavage							
	25	21.35	0.000643	107	7,820	0.313	0.284
	50	46.02	0.0714	9.71	21,000	0.427	0.339
	100	90.37	0.0195	35.5	148,000	1.48	1.17
Corn oil gavage	25	24.02	0.000066	1,050	6,650	0.266	0.211
	50	47.52	0.000520	1,320	20,700	0.415	0.329
	100	92.97	0.00452	153	64,200	0.642	0.600
Female							
Intravenous injection	10	8.54	0.0728	9.53	11,200	1.11	—
Water:Cremophor® (9:1) gavage							
	25	21.35	0.0276	25.1	5,300	0.212	0.191
	50	46.02	0.0107	64.7	14,300	0.290	0.261
	100	90.37	0.0472	14.7	108,000	1.08	0.973
Corn oil gavage	25	24.02	0.0330	21.0	926	0.0370	0.033
	50	47.52	0.00145	477	13,400	0.269	0.240
	100	92.97	0.00518	134	64,700	0.647	0.583

[a] Toxicokinetic parameters were calculated from the plasma concentration versus time curves, where each data point represented the mean of up to three samples. k_{elim} = Elimination rate constant; $t_{1/2}$ = elimination half-life, AUC = area under the plasma concentration versus time curve calculated using the trapezoidal rule

[b] Based on formulation analysis

[c] Calculated as as $AUC_{gavage}/AUC_{intravenous}$ H Nominal $Dose_{intravenous}$/Nominal $Dose_{gavage}$

[d] Not applicable to intravenous dosing

APPENDIX K
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL AND DOSE-RESPONSE ANALYSES

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL AND DOSE-RESPONSE ANALYSES

INTRODUCTION

In the single-dose toxicokinetic studies of bromodichloromethane (Appendix J) plasma time-course concentrations of bromodichloromethane were measured in male and female F344/N rats and B6C3F$_1$ mice after intravenous injection or gavage administration at several doses (Table K1).

MODEL DEVELOPMENT

Model Structure

The model used for bromodichloromethane represents blood, liver, muscle, kidney, skin, adipose tissue, gastrointestinal tract, and other rapidly perfused tissues as diffusion-limited compartments. Equations represent the concentrations of bromodichloromethane in each compartment. The gastrointestinal tract is divided into four subcompartments: stomach, duodenum, jejuno-ileum, and cecum/colon. The luminal spaces of the latter two are divided into three equal spaces each. Physiological parameters for the model compartments, tissue:blood partition coefficients for bromodichloromethane, and estimates derived from the model for fitted parameters are shown in Tables K2, K3, and K4, respectively.

Absorption

Absorption from the gastrointestinal tract is represented as a nonlinear process governed by Michaelis-Menten kinetics. Absorbed bromodichloromethane goes from the gastrointestinal tract lumen into the capillary space of the gastrointestinal tract tissue. The K_m constant in the Michaelis-Menten kinetics is the same for the other compartments of the gastrointestinal tract. The V_{max} constant is allowed to be larger for the stomach as compared to the rest of the gastrointestinal tract. Both K_m and V_{max} include an additional multiplicative constant that is used for simulating the experiments involving water:Cremophor® (versus corn oil) gavage; the same multiplier is used for the gastrointestinal tract in the intravenous injection study.

Rate constants for transit through the gastrointestinal tract were taken from the literature. The model includes two adjustable parameters to change the rate constants; a multiplier for the stomach emptying rate, and a multiplier for the rate of transit through the small intestine.

Distribution

The tissues in the model are diffusion-limited. That is, each tissue is divided into two subcompartments, one representing the capillary space and the other representing the rest of the tissue. The rate of diffusion between the capillary space and the rest of the tissue is represented by a permeability parameter, which is the ratio of the capillary-to-tissue diffusion rate to the tissue blood flow. Two permeabilities are used in the model: one for kidney and liver, which have discontinuous capillary walls, and one for the other tissues.

Partition coefficients for tissues were derived by dividing each tissue:air partition coefficient from Lilly *et al.* (1998) by the blood:air partition coefficient from the same source. The value for slowly perfused tissue was used for skin. The values for all other tissues (except adipose tissue) were approximately equal to one, so a value of one was used for those tissues.

The concentration of bromodichloromethane in plasma is assumed to equal its concentration in whole blood.

Metabolism

Metabolism is assumed to occur in the liver, kidney, and colon via two pathways: a saturable cytochrome P450 (CYP450) pathway (modeled using Michaelis-Menten kinetics) and a glutathione-*S*-transferase (GST) pathway (modeled using first-order kinetics). *In vitro* rate constants for the pathways were available from the literature (Ross and Pegram, 2004) in units of metabolism/amount of microsomal or cytosolic protein. Amounts of these proteins/tissues were also available from the literature (Kohn and Melnick, 2000). Amounts of microsomal protein/tissue for the lung and kidney as described in Kohn and Melnick (2000) were used for the large intestine in this model. The same amount of cytosolic protein/g tissue was used for all tissues in the model. Using the rates as given, it was not possible to produce a satisfactory fit of model results to the data. Therefore, an additional multiplicative constant was inserted into the metabolism terms in the equations. This constant was the same for both metabolic pathways in all tissues.

Excretion

Excretion occurs via three routes:

1) Urinary excretion is abstracted as removing bromodichloromethane from the blood as a first-order process.

2) Fecal elimination occurs when bromodichloromethane in the gastrointestinal tract is not absorbed completely before passing all the way through the gastrointestinal tract. Biliary excretion of bromodichloromethane is included in the model as a nonlinear process governed by Michaelis-Menten kinetics.

3) Respiratory elimination occurs via exhalation. Exhalation is modeled via a term in the differential equation for the concentration in the blood:

$$\frac{dA_B}{dt} = -alv C_B \Big/ P_{blood:air} + other\ terms$$

A_B = amount in blood,
C_B = concentration in blood
alv = alveolar ventilation rate (80% cardiac output)
$P_{blood:air}$ = the blood:air partition coefficient (from the literature).

Model Equations

The equations presented here are those used for modeling the absorption, distribution, metabolism, and excretion of bromodichloromethane in rats and mice.

Subscripts for tissues

Adipose	A
Blood	B
Gastrointestinal tract	G
Kidney	K
Liver	L
Muscle	M
Rapidly Perfused Tissues	R
Skin	S

Parameters

V_{XC}	Volume of capillary space of tissue X
V_{XT}	Volume of tissue space of tissue X
V_{lumI}	Volume of Ith gastrointestinal tract compartment lumen
Q_X	Rate of blood flow to tissue X (Q_G refers to the flow via the portal vein, Q_L to the rest of the flow to the liver)
Perm	Capillary permeability parameter for tissues other than liver and kidney
$Perm_{LK}$	Capillary permeability parameter for liver and kidney
T_X	Transit rate out of gastrointestinal tract segment X
$V_{maxCYPX}$	Maximum metabolic rate by CYP450 in tissue X
K_{mCYPX}	K_m parameter for Michaelis-Menten metabolism by CYP450 in tissue X
V_{rel}	Metabolic rate relative to amount determined *in vitro*
V_{maxA}, K_{mA}	Constants for absorption from gastrointestinal tract
AV_{rel}	Relative V_{max} for absorption rate for water:Cremophor® gavage as compared to corn oil gavage
AK_{rel}	Relative K_m for absorption rate for water:Cremophor® gavage as compared to corn oil gavage
A_{stom}	Relative V_{max} for absorption rate for stomach as compared to small intestine
V_{maxB}, K_{mB}	Constants for biliary excretion
K_K	Urinary excretion rate constant for bromodichloromethane
P_X	Tissue:blood partition coefficient in tissue X
K_{GSTX}	Rate constant for GST in tissue X

Variables

1) Amounts in tissues:
   - A_{XC} amount in tissue X's capillary space
   - A_{XT} amount in tissue X's tissue space
   - A_B amount in blood

   For the gastrointestinal tract subcompartments, a subscript I (value from 1 to 8) is added, i.e., A_{GCI} or A_{GTI}.

2) Amounts in gut lumen:
   - A_{GI} amount in gastrointestinal tract segment I

3) Concentrations in tissues and in gut lumen:
   - C_{XC} concentration in tissue X's capillary space
   - C_{XT} concentration in tissue X's tissue space
   - C_X concentration in tissue X

   The concentration in a compartment is equal to the amount in that compartment divided by the volume of the compartment. For the gastrointestinal tract subcompartments, a subscript I (value from 1 to 8) is added, i.e., CP_{GCI} or CP_{GTI}.

Equations

1) Distribution in tissues other than liver and blood:

For the kidney, $Perm_{LK}$ is used instead of Perm.

$$A'_{XT} = Perm \cdot Q_X \left(C_{XC} - {C_{XT}}/{P_X} \right)$$

$$A'_{XC} = -Perm \cdot Q_X \left(C_{XC} - {C_{XT}}/{P_X} \right) + Q_X \left(C_B - C_{XC} \right)$$

The equations for kidney and large intestine add the following term to the equation for A'_{XT}:

$$\frac{-V_{rel} V_{\max CYPX} C_{XT}}{K_{mCYPX} + C_{XT}} - V_{rel} K_{mGSTX} A_{XT}$$

The first term above is CYP450 metabolism and the 2nd term is GST metabolism.

The equations for the gastrointestinal tract tissues add the following term to the equation for A'_{XC}:

$$+ \frac{AV_{rel} V_{\max A} V_{lumI} C_{GI}}{AK_{rel} K_{mA} + C_{GI}}$$ (see the equations for gut lumen below).

2) Distribution in blood:

$$A'_B = -Q_A \left(C_B - C_{AC} \right) - Q_K \left(C_B - C_{KC} \right) - \left(Q_L + Q_G \right) \left(C_B - C_{LC} \right)$$
$$- Q_M \left(C_B - C_{MC} \right) - Q_R \left(C_B - C_{RC} \right) - Q_S \left(C_B - C_{SC} \right)$$

3) Distribution in liver:

$$A'_{LC} = -Perm_{LK} \cdot (Q_L + Q_G)\left(C_{LC} - {C_{LT}}/{PL}\right) + Q_L(C_B - C_{LC})$$
$$+ \sum_{i=1}^{4} Q_{Gi} C_{GCi} - Q_G C_{LC}$$

$$A'_{LT} = Perm_{LK} \cdot (Q_L + Q_G)\left(C_{LC} - {C_{LT}}/{PL}\right) - \frac{V_{rel} V_{\max CYPL} A_{LT}}{K_{mCYPL} + C_{LT}}$$
$$- V_{rel} K_{mGSTL} A_{LT} - \frac{V_{\max B} A_{LT}}{K_{mB} + C_{LT}}$$

4) Gut transport:

Gut lumen equations:
For stomach (subcompartment 1):

$$A'_{G1} = drink - T_1 A_{G1} - \frac{AV_{rel} A_{stom} V_{\max A} V_{lum1} C_{G1}}{AK_{rel} K_{mA} + C_{G1}} + \frac{V_{\max B} A_{LT}}{K_{mB} + C_{LT}}$$

where drink = (amount of water drunk in one day) × (concentration in drinking water) × (a piecewise constant function which integrates to 1 over 24 hours and has 90% of the drinking occurring in the first 12 hours).

For I = 2 to 8 (duodenum, jejuno-ileum, and colon):

$$A'_{GI} = T_{I-1} A_{GI-1} - T_I A_{GI} - \frac{AV_{rel} V_{\max A} V_{lumI} C_{GI}}{AK_{rel} K_{mA} + C_{GI}}$$

5) Initial conditions:

$A_B(0)$ = IV dose
$A_{G1}(0)$ = gavage dose

All other variables = 0 at time 0.

Statistical Method Used to Compare the Tumor Incidences from the Drinking Water Study with the Gavage Study Results

For intestine and kidney tumors, the incidence rates in the drinking water study did not reach high enough levels to reliably fit a dose-response curve. Therefore, a Weibull dose-response curve was fit to the tumor data from the gavage study (for each dose metric and for each of the two tissues) and the drinking water data were compared to the predicted incidence rates from the gavage study dose-response curve (using the same dose metric).

Because these are rare tumors and the data in both studies support a background rate of essentially zero, we fixed the background rate in the Weibull model (response at dose=0) at zero. Thus, the Weibull equation that was fit was:

$$\textit{Incidence rate} = 1 - \exp(-am^b),$$

where m_i is the dose metric value and a and b are parameters to be estimated as the model is fit. Because there are two Weibull parameters to estimate and two nonzero doses, the fit is virtually perfect (within the context of a nonlinear iterative search algorithm). Therefore, the quality of model fit cannot be evaluated.

To test for lack of agreement between the drinking water and gavage data, a simple binomial test was used of the hypothesis that the rate predicted by the gavage dose-response curve was the population proportion of responses at each of the dose metrics from the drinking water study.

Results and Discussion

Plots of model results versus data are shown in Figures K1 through K6c.

Model based estimates of 24-hour blood AUCs for bromodichloromethane and maximal rates and 24-hour cumulative flux of bromodichloromethane metabolism in the male F344/N rat liver, kidney, and large intestine via GST-mediated conjugation with glutathione or CYP450-mediated oxidation after administration by gavage in corn oil or in drinking water are presented in Tables 11 to 13 of the Discussion section of this Technical Report.

Observed and model-predicted neoplasm rates are presented in Figures K7 through K16. Statistical tests for significant difference between observed and model-predicted neoplasm incidence rates in the 2-year drinking water study are presented in Tables K5 through K7.

TABLE K1
Routes of Administration and Dose Concentrations in the Single-Dose Toxicokinetic Studies of Bromodichloromethane in F344/N Rats and B6C3F$_1$ Mice[a]

	Route	Doses
	Intravenous injection	10 mg/kg
	Gavage (corn oil vehicle)	25, 50, or 100 mg/kg
	Gavage (Water:Cremophor® vehicle)	25, 50, or 100 mg/kg

[a] These studies are fully described in Appendix J.

TABLE K2
Physiological Parameters for F344/N Rats and B6C3F$_1$ Mice in the Physiologically Based Pharmacokinetic Model of Bromodichloromethane

Parameters	Rats	Mice
Tissue Volumes[a]		
Adipose	0.111 (male) 0.075 (female)	0.1
Blood[b]	0.054	0.085
Gastrointestinal tract segments:		
Stomach	0.0046	0.0046
Duodenum	0.00112	0.00112
Jejuno-ileum	0.01288	0.01288
Cecum/colon	0.0084	0.0084
Kidneys	0.00848	0.0167
Liver	0.045	0.0549
Muscle	0.45	0.384
Rapidly perfused tissues	0.143	0.143
Skin	0.17	0.1653
Tissue Capillary Space[c]		
Adipose	2	2
Gastrointestinal tract	3	3
Kidneys	16	24
Liver	21	31
Muscle	4	4
Rapidly perfused tissues	7.1	7.1
Skin	2	3
Cardiac Output (L/hr)[d]	15 $BW^{0.74}$	15 $BW^{0.74}$
Tissue Blood Flow[e]		
Adipose	7	7
Gastrointestinal tract	15.5	15.5
Kidneys	14.1	9.1
Liver (not including portal vein)	2	2
Muscle	27.8	15.9
Rapidly perfused tissues	24.8	24.8
Skin	5.8	5.8

TABLE K2
Physiological Parameters for F344/N Rats and B6C3F$_1$ Mice in the Physiologically Based Pharmacokinetic Model of Bromodichloromethane

Parameters	Rats	Mice
Gastrointestinal Tract Segments		
Stomach	0.025726	0.025726
Duodenum	0.006264	0.006264
Jejuno-ileum	0.072033	0.072033
Cecum/colon	0.046978	0.046978
Gastrointestinal Tract Lumen Volumes[d]	0.056 BW	0.056 BW
Stomach	0.009541	0.009541
Duodenum	0.002323	0.002323
Jejuno-ileum	0.026714	0.026714
Cecum/colon	0.001742	0.001742
Gastrointestinal Tract Transit Rates[e]		
Stomach (hr^{-1})[f]	0.36 × R_{stom}	0.36 × R_{stom}
Duodenum (hr^{-1})[g]	3.57 × R_{SI}	3.57 × R_{SI}
Jejuno-ileum (hr^{-1})[g,h]	0.31 × R_{SI}	0.31 × R_{SI}
Cecum/colon (hr^{-1})	0.067	0.067

[a] V_{XC} + V_{XT}, as a fraction of body weight
[b] Total volume; the V_B parameter in the model is this amount minus the amount in tissue capillary space
[c] V_{XC}, as percentage of total tissue volume
[d] BW = body weight in kg
[e] Q_x, as percentage of cardiac output
[f] for R_{stom}, see Table K4
[g] for R_{SI}, see Table K4
[h] Rates for three subsegments of the jejuno-ileum and cecum/colon are this amount multiplied by 3.0

TABLE K3
Partition Coefficients for Bromodichloromethane for the Physiologically Based Pharmacokinetic Model of Bromodichloromethane

Partition Coefficient	Value
Adipose:blood	17
Skin:blood	0.4
All other tissues:blood	1
Blood:air	31.4

TABLE K4
Derived Parameter Estimates for F344/N Rats and B6C3F$_1$ Mice from the Physiologically Based Pharmacokinetic Model of Bromodichloromethane[a]

Parameter (units)	Rats		Mice	
	Male	Female	Male	Female
Permeabilities				
Perm	1.638594	0.629458	0.290633	0.432807
$Perm_{LK}$	42.68347	26.98896	40.89798	36.53595
Metabolic Parameter				
V_{rel}	23.29967	35.24916	46.97358	29.84173
Biliary Excretion				
V_{maxB} (L/min)	1.229998	0.764273	0.385424	1.759659
K_{mB} (mg/L)	1.058669	6.513537	8.990431	32.99509
Absorption Constants				
V_{maxA} (mg/L/min)	70,665.28	91,328.49	143,867.6	136,326.7
K_{mA} (mg/L)	64,906.14	27,529.45	30,049.25	29,695.75
AV_{rel}	1.340279	0.462036	4.49193	2.205106
AK_{rel}	0.991894	1.301624	0.332596	0.436053
A_{stom}	6.878511	7.341864	12.2023	15.82483
R_{stom}	1.067202	0.804296	7.333084	6.292762
R_{SI}	12.13361	7.962234	1.170236	0.844749
Urinary Excretion				
K_k (min^{-1})	5.352958	5.208892	5.783327	9.422341

[a] Derived by fitting the physiologically based pharmacokinetic model to the animal data.

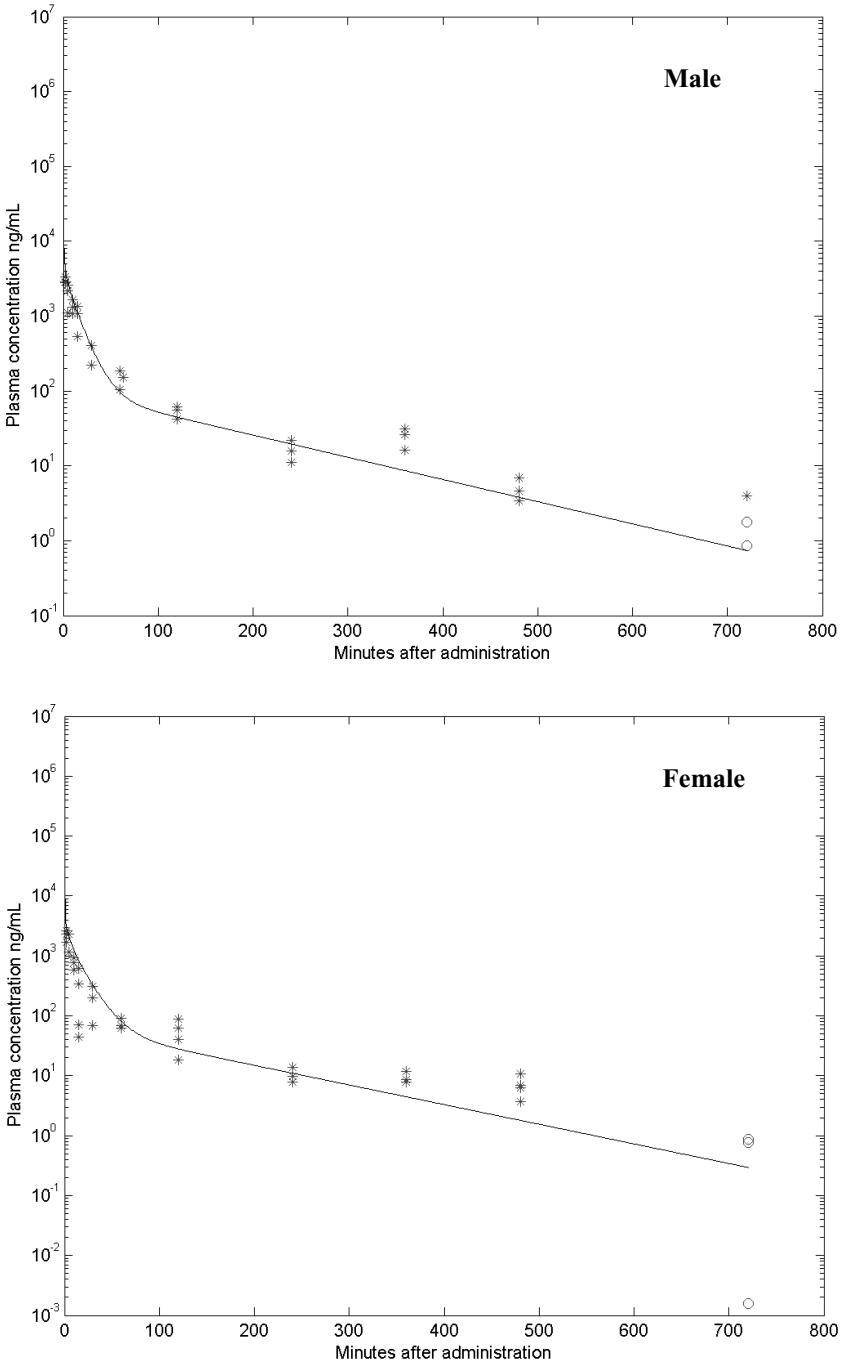


FIGURE K1
Plasma Concentrations of Bromodichloromethane in F344/N Rats
Following a Single Intravenous Injection of 10 mg/kg Bromodichloromethane
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

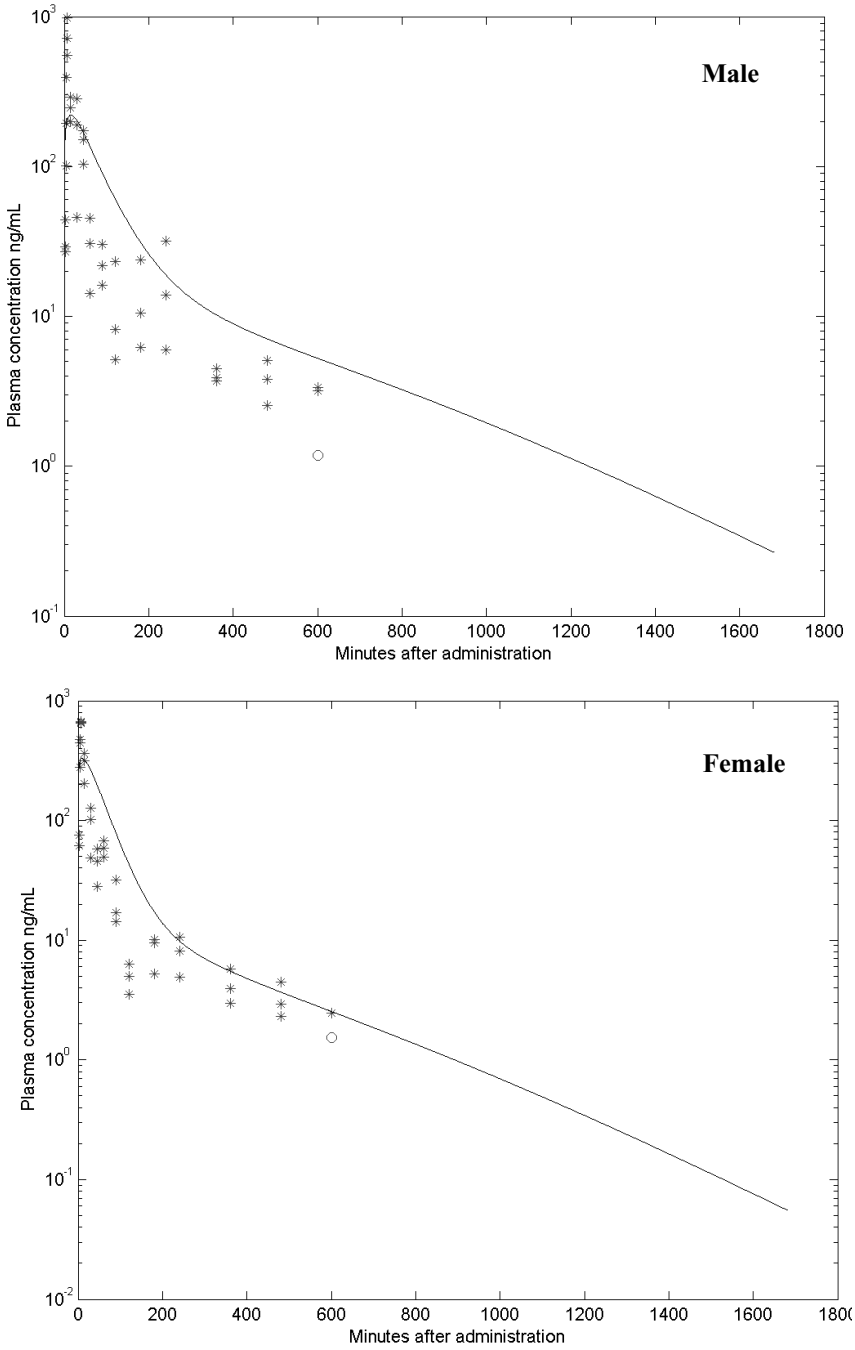


FIGURE K2a
Plasma Concentrations of Bromodichloromethane in F344/N Rats
Following a Single Gavage Administration of 25 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

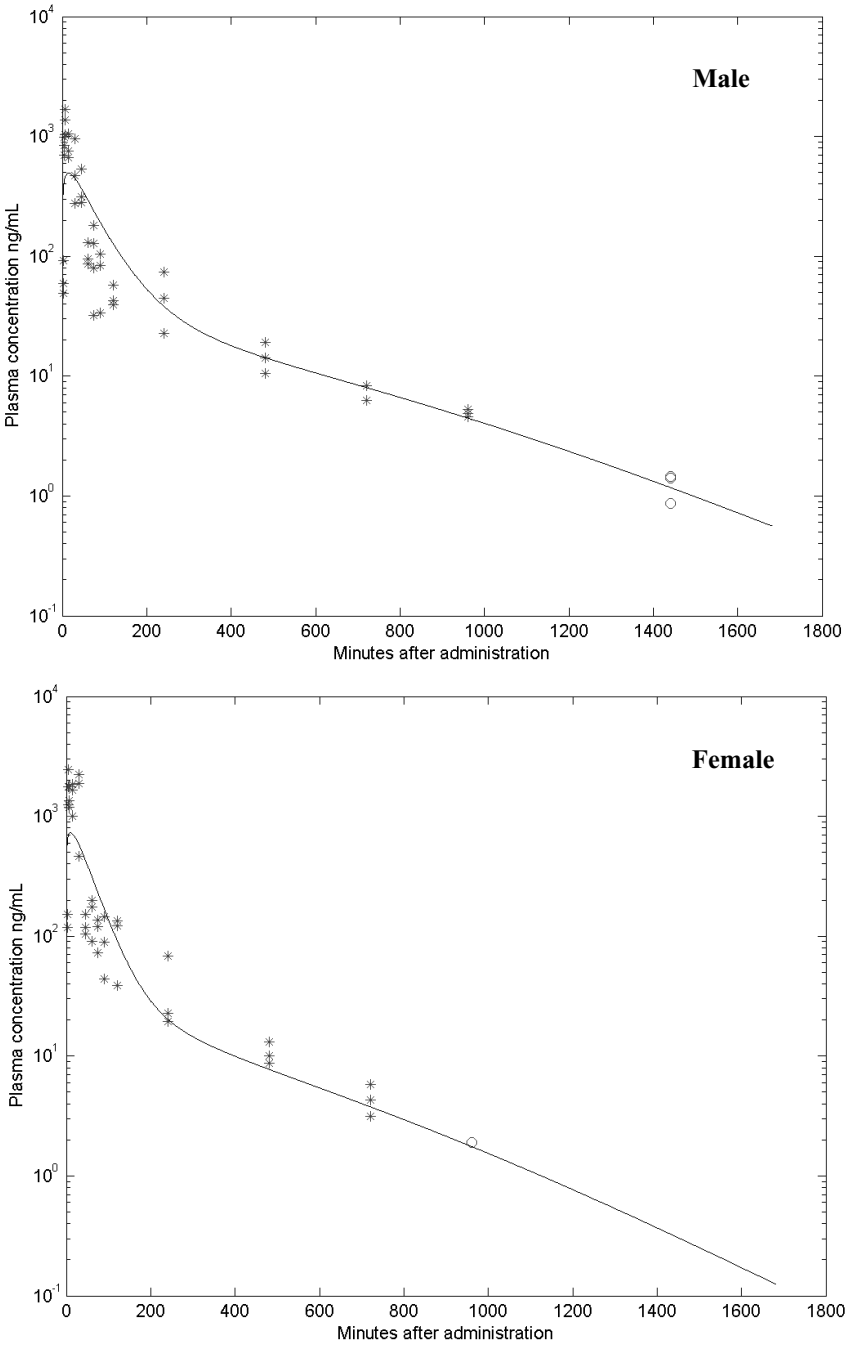


FIGURE K2b
Plasma Concentrations of Bromodichloromethane in F344/N Rats
Following a Single Gavage Administration of 50 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

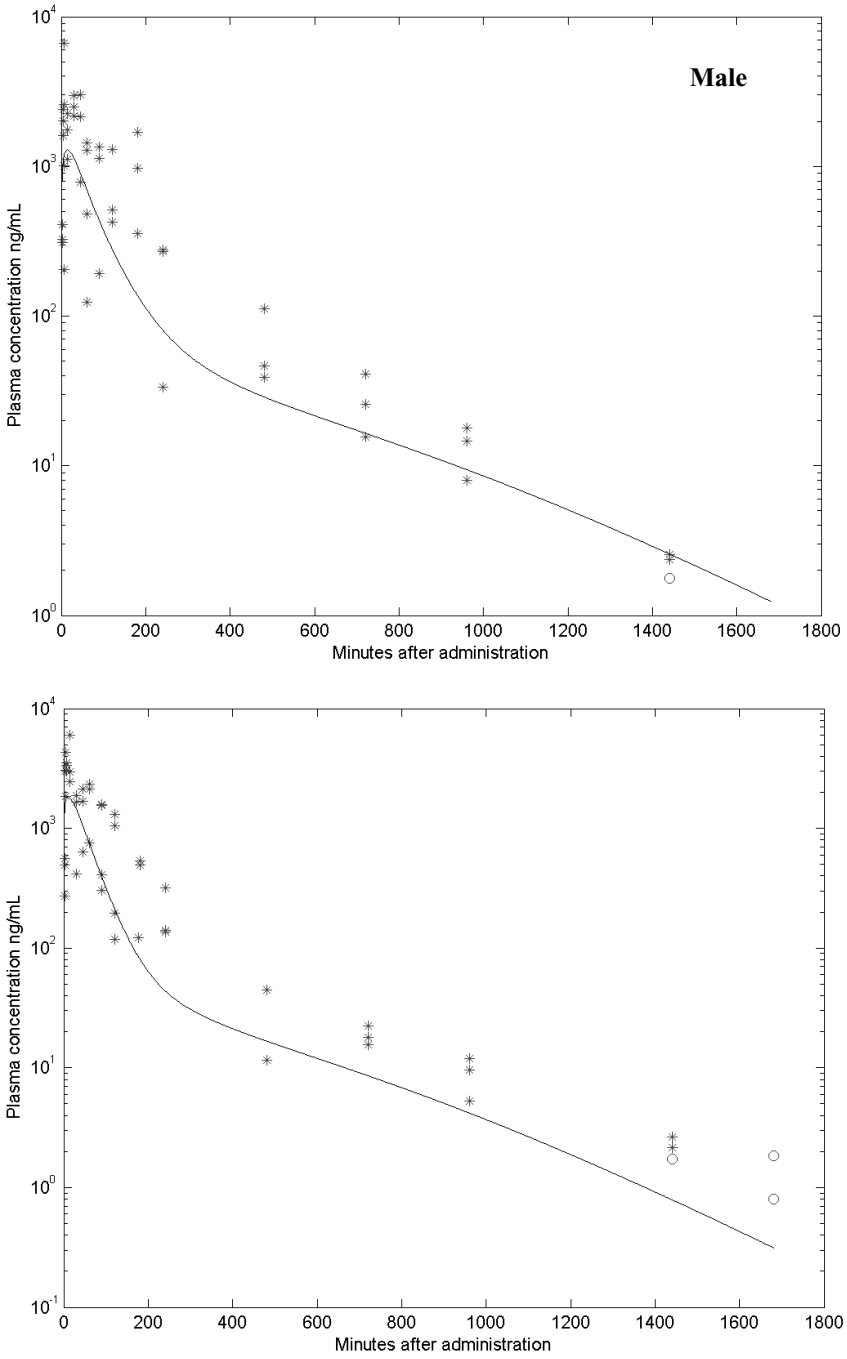


FIGURE K2c
Plasma Concentrations of Bromodichloromethane in F344/N Rats
Following a Single Gavage Administration of 100 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

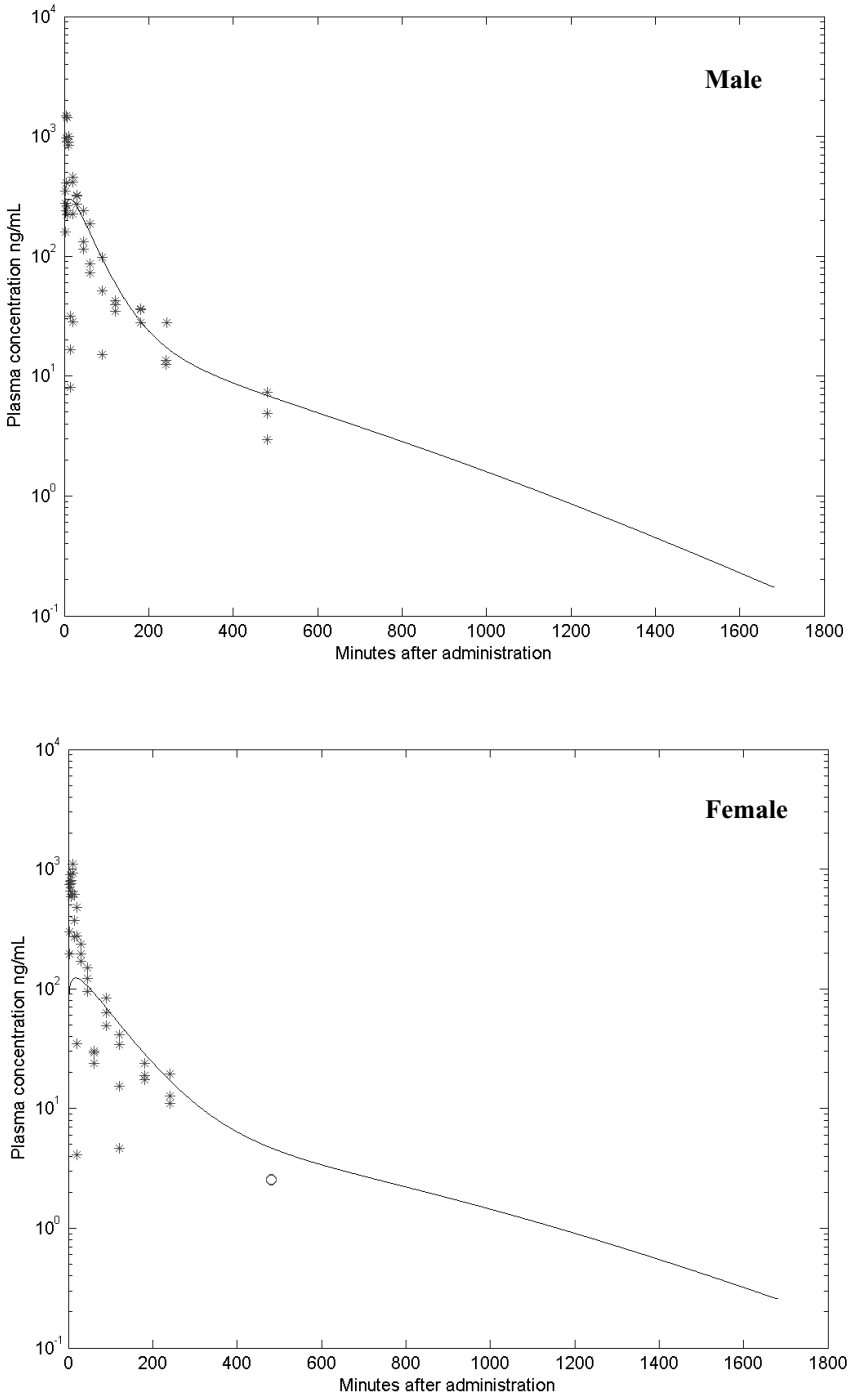


FIGURE K3a
Plasma Concentrations of Bromodichloromethane in F344/N Rats Following a Single Gavage Administration of 25 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

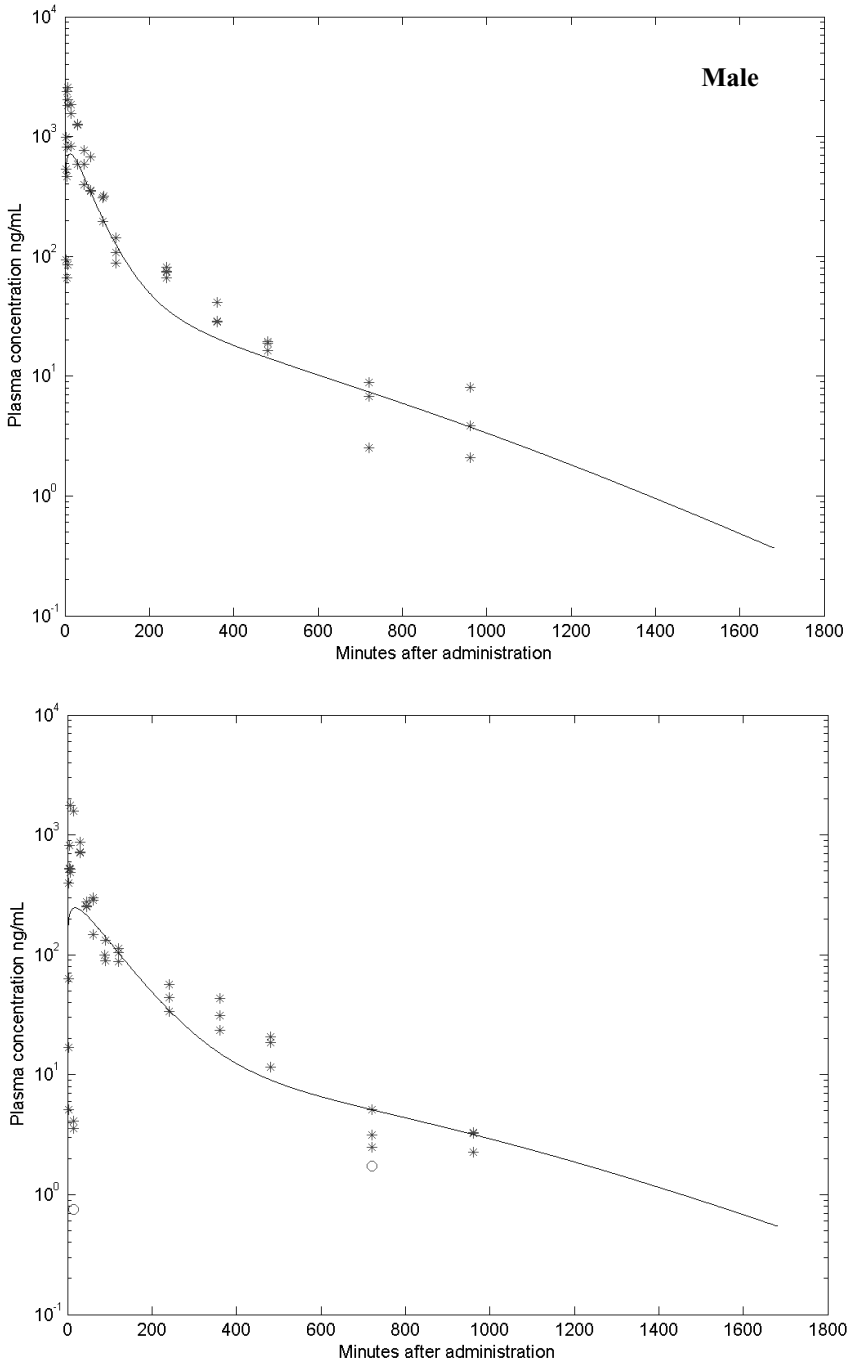


FIGURE K3b
Plasma Concentrations of Bromodichloromethane in F344/N Rats Following a Single Gavage Administration of 50 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

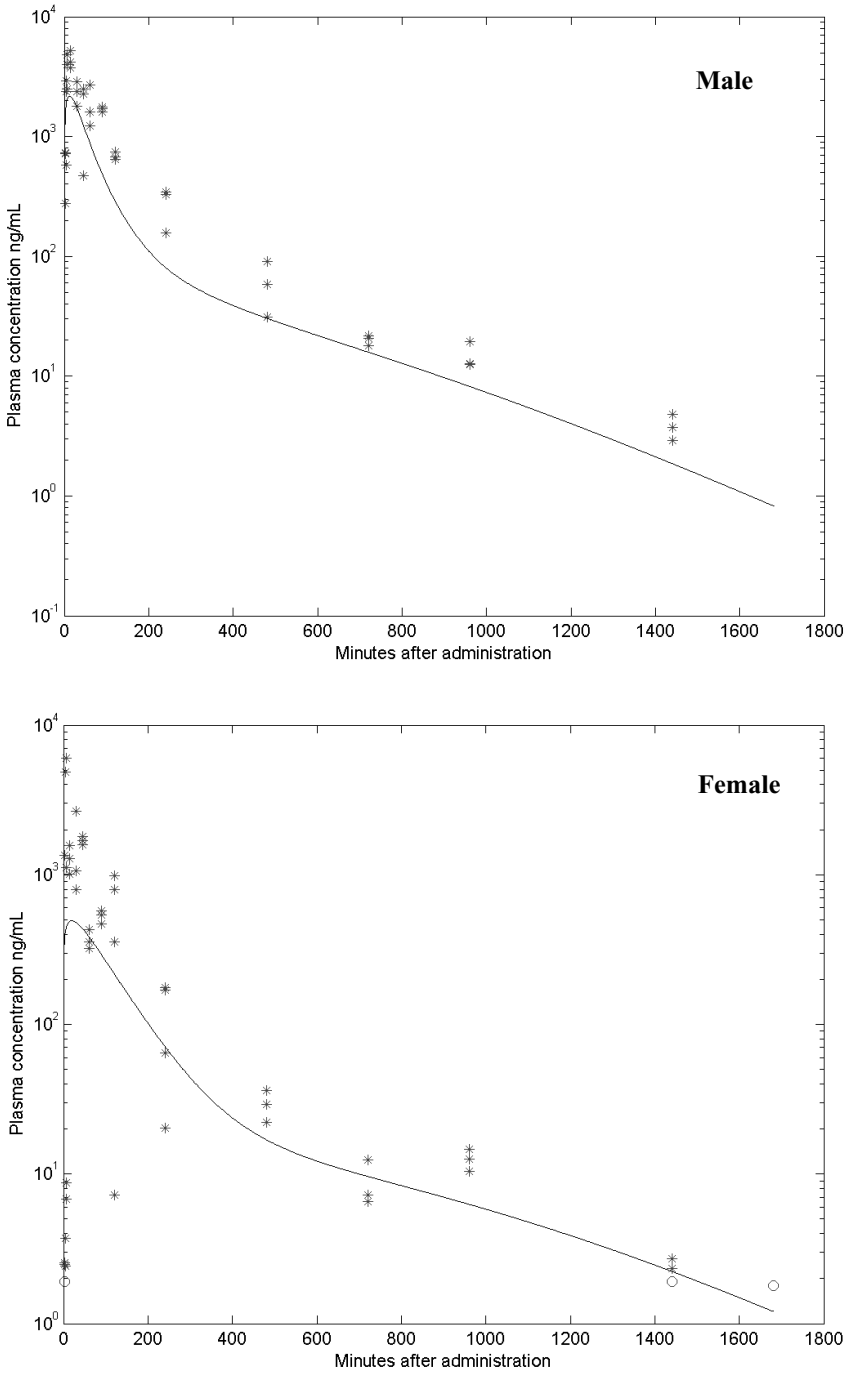


FIGURE K3c
Plasma Concentrations of Bromodichloromethane in F344/N Rats Following a Single Gavage Administration of 100 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

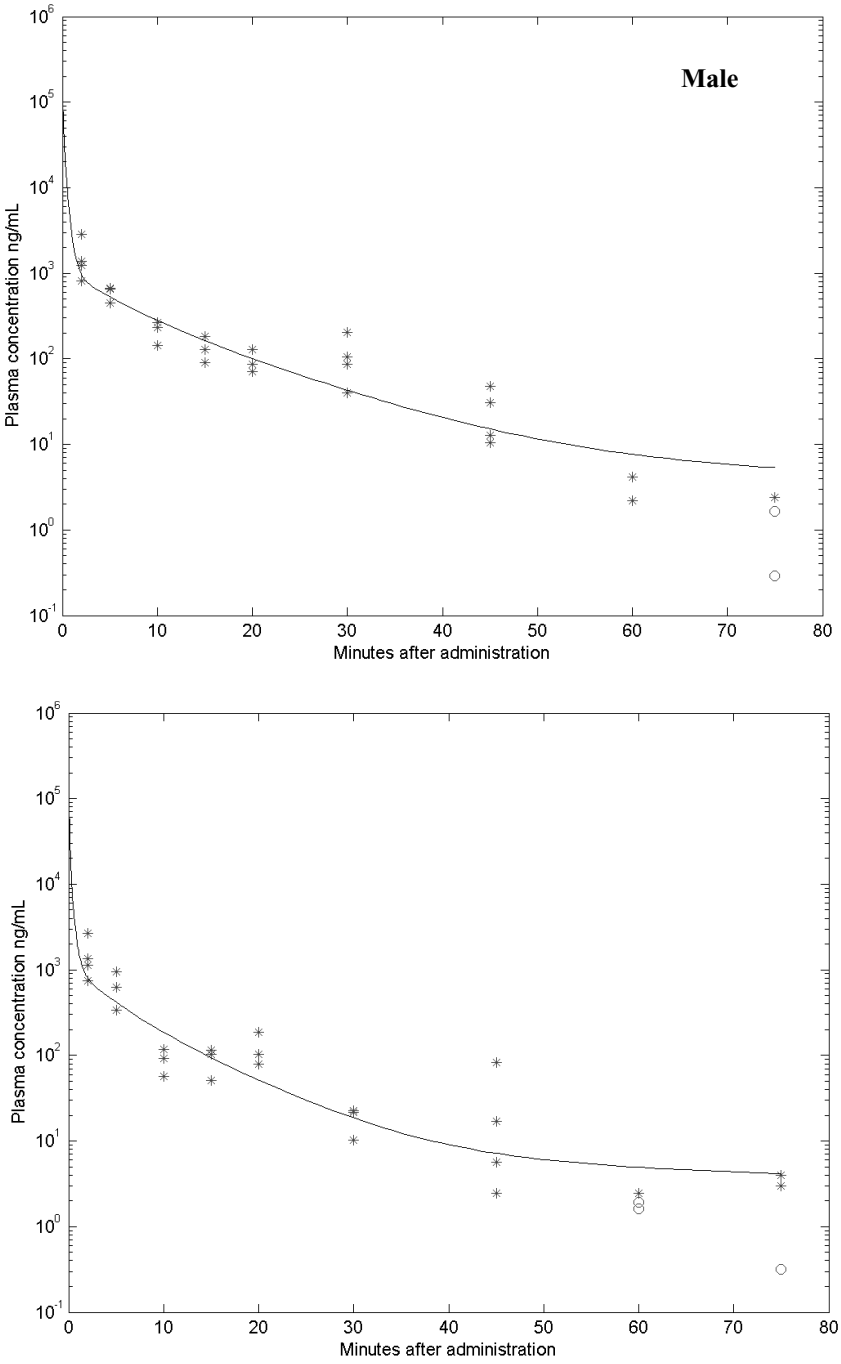


FIGURE K4
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice Following a Single Intravenous Injection of 10 mg/kg Bromodichloromethane
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

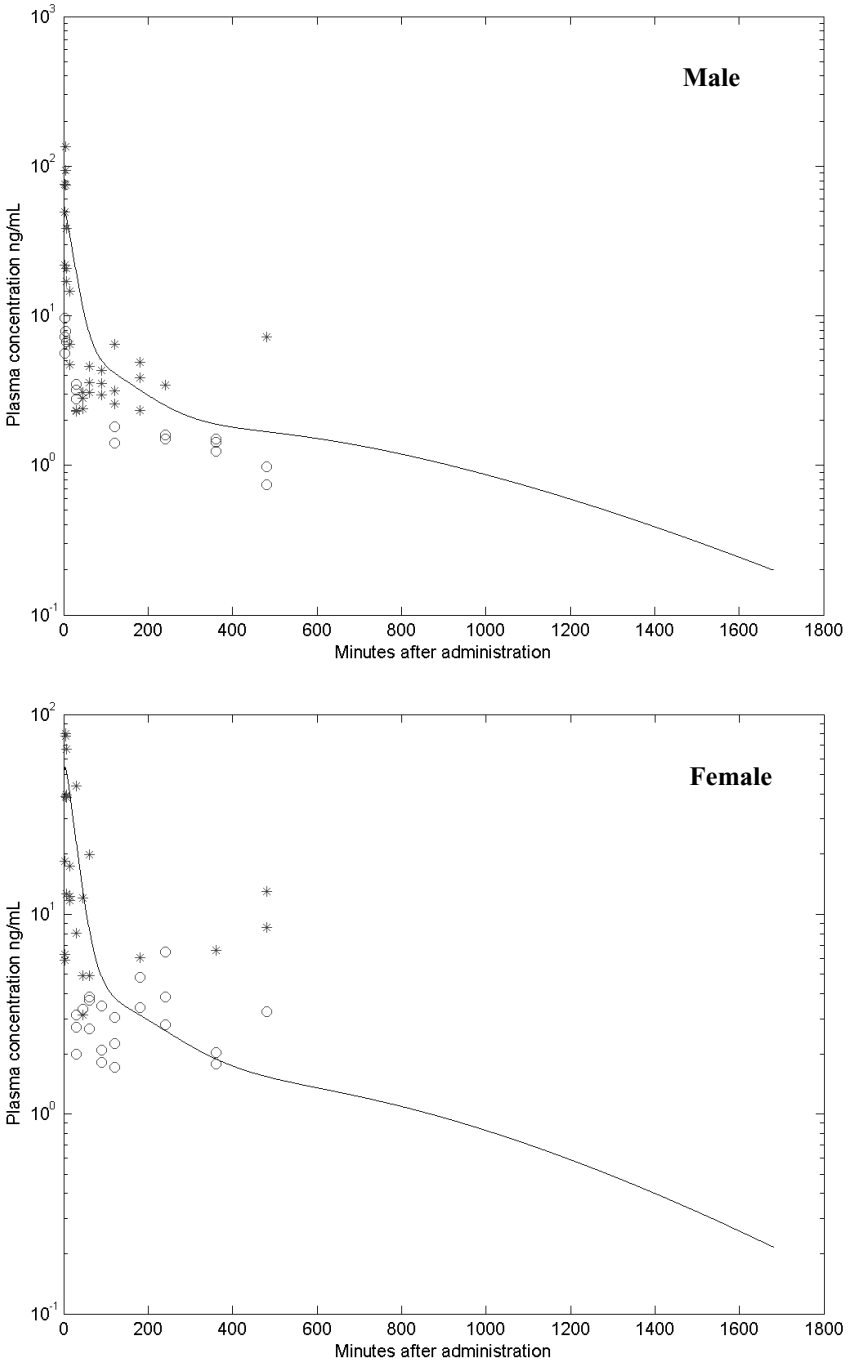


FIGURE K5a
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice
Following a Single Gavage Administration of 25 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

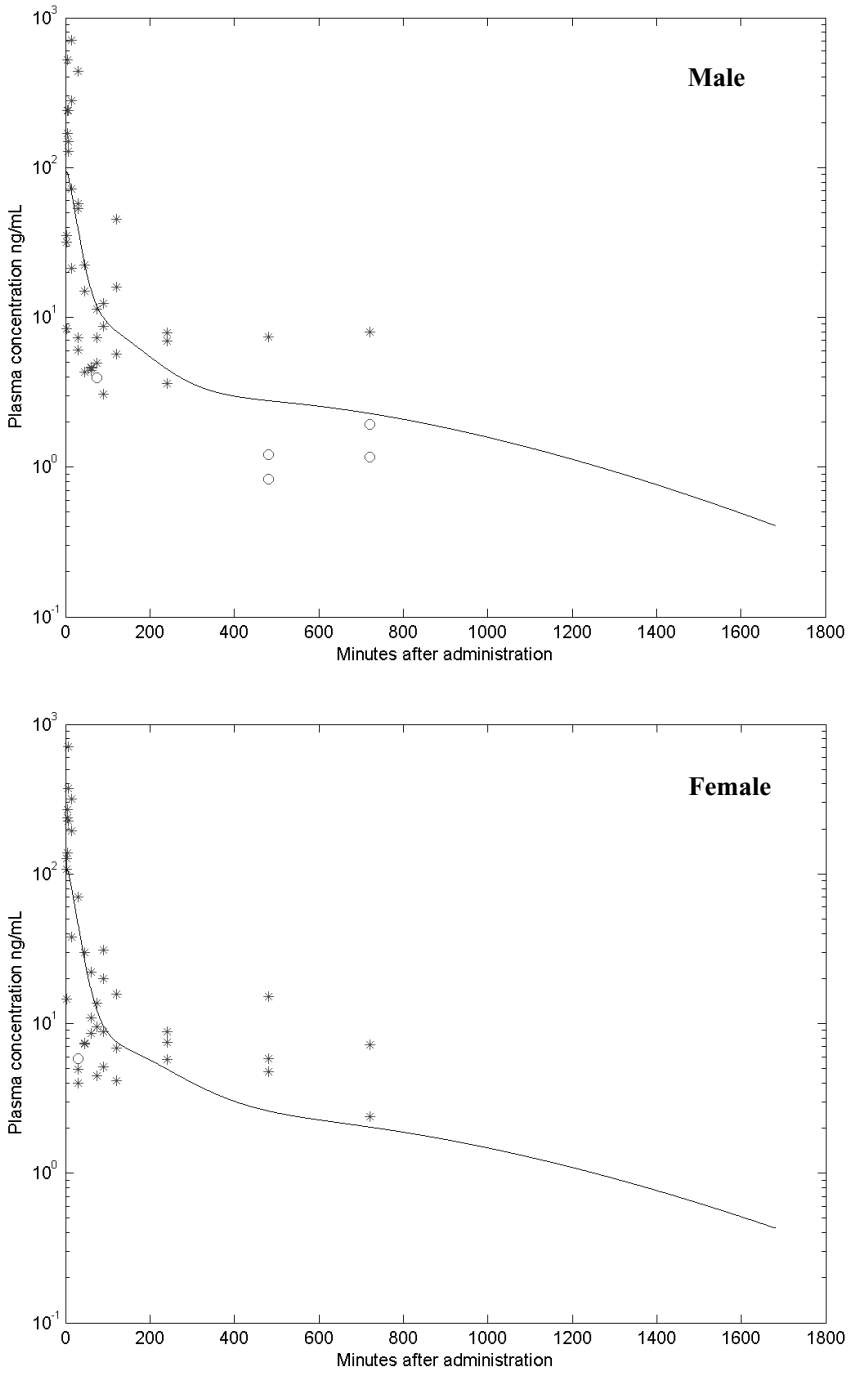


FIGURE K5b
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice
Following a Single Gavage Administration of 50 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

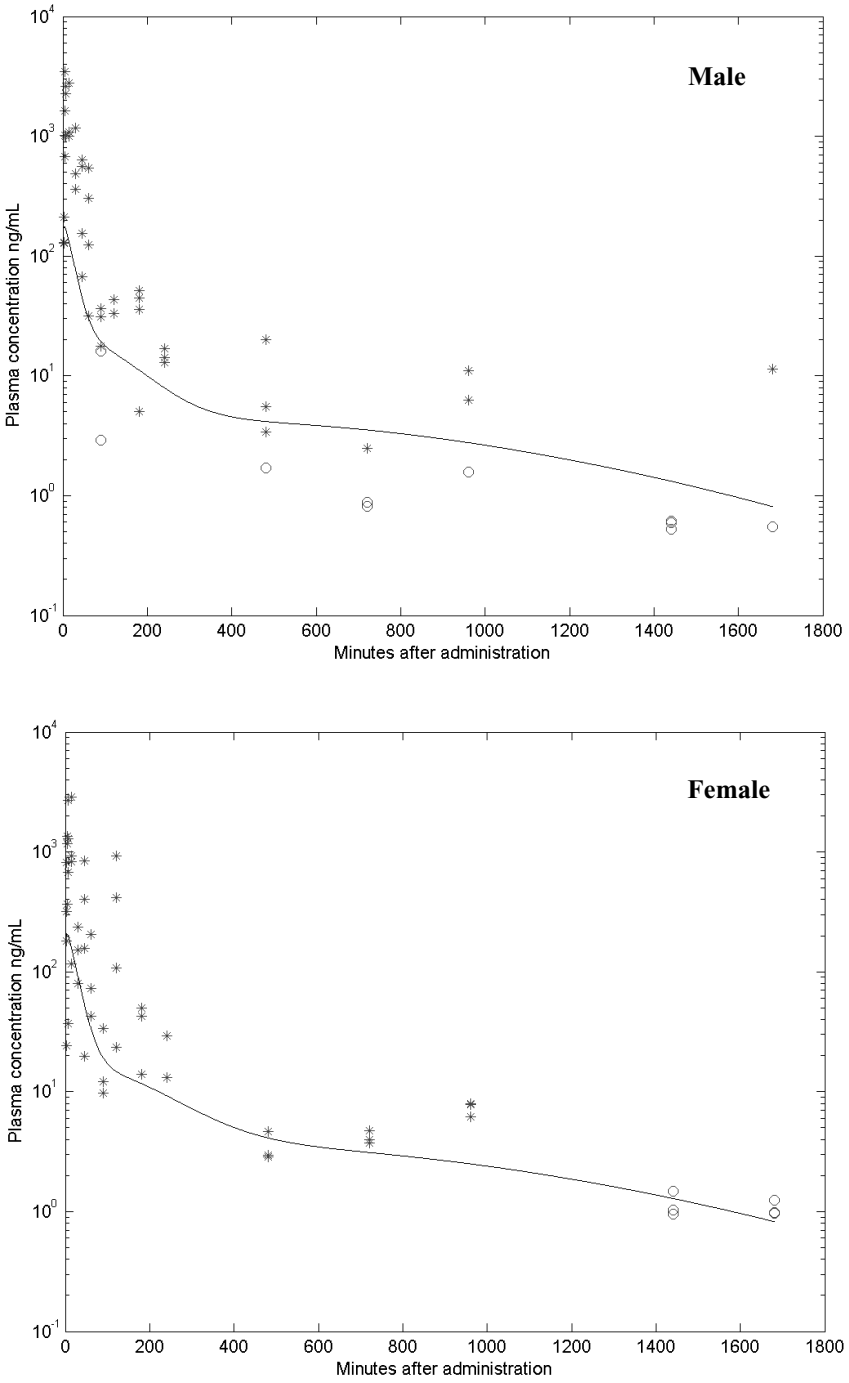


FIGURE K5c
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice Following a Single Gavage Administration of 100 mg/kg Bromodichloromethane in Corn Oil
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

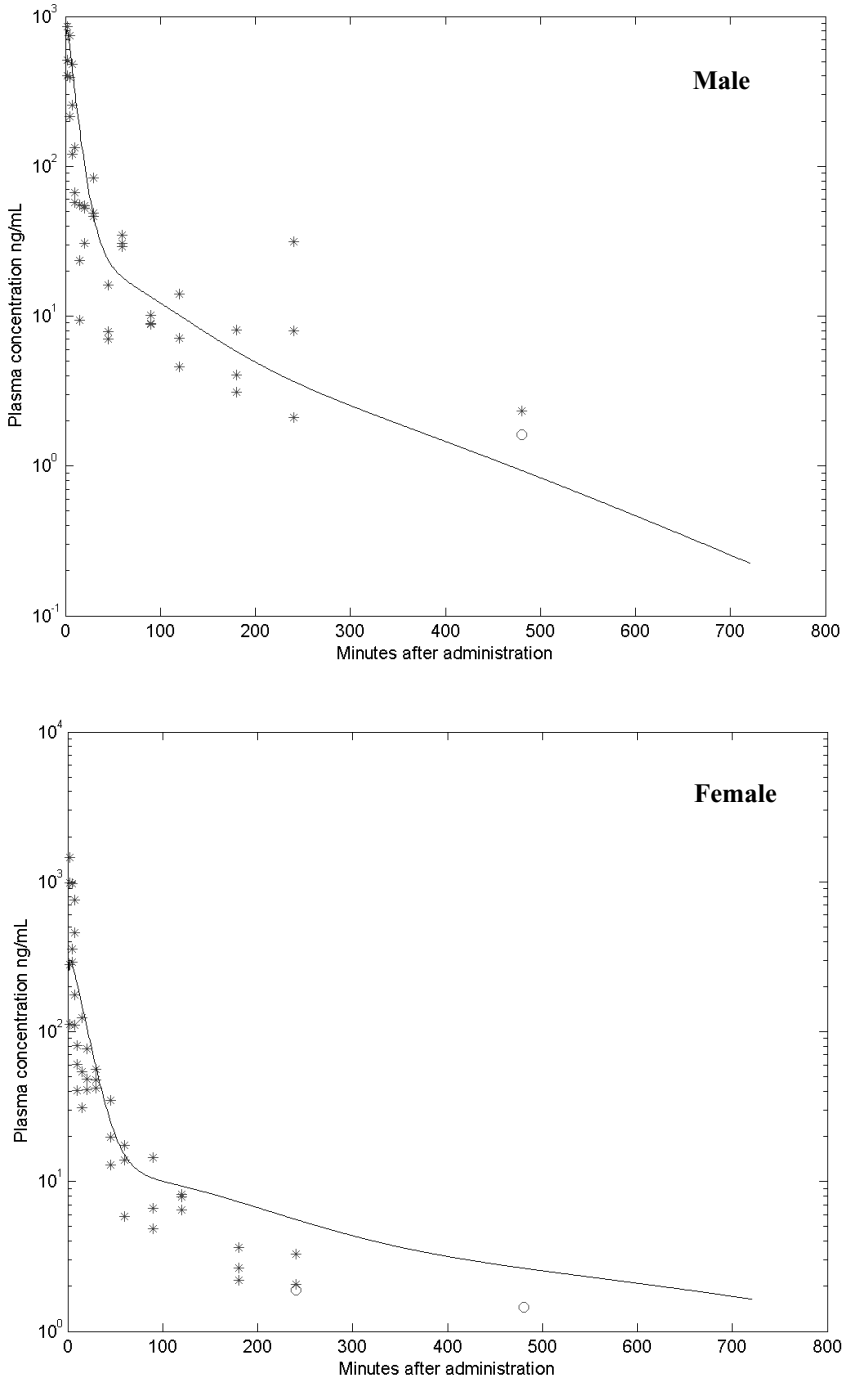


FIGURE K6a
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice Following a Single Gavage Administration of 25 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

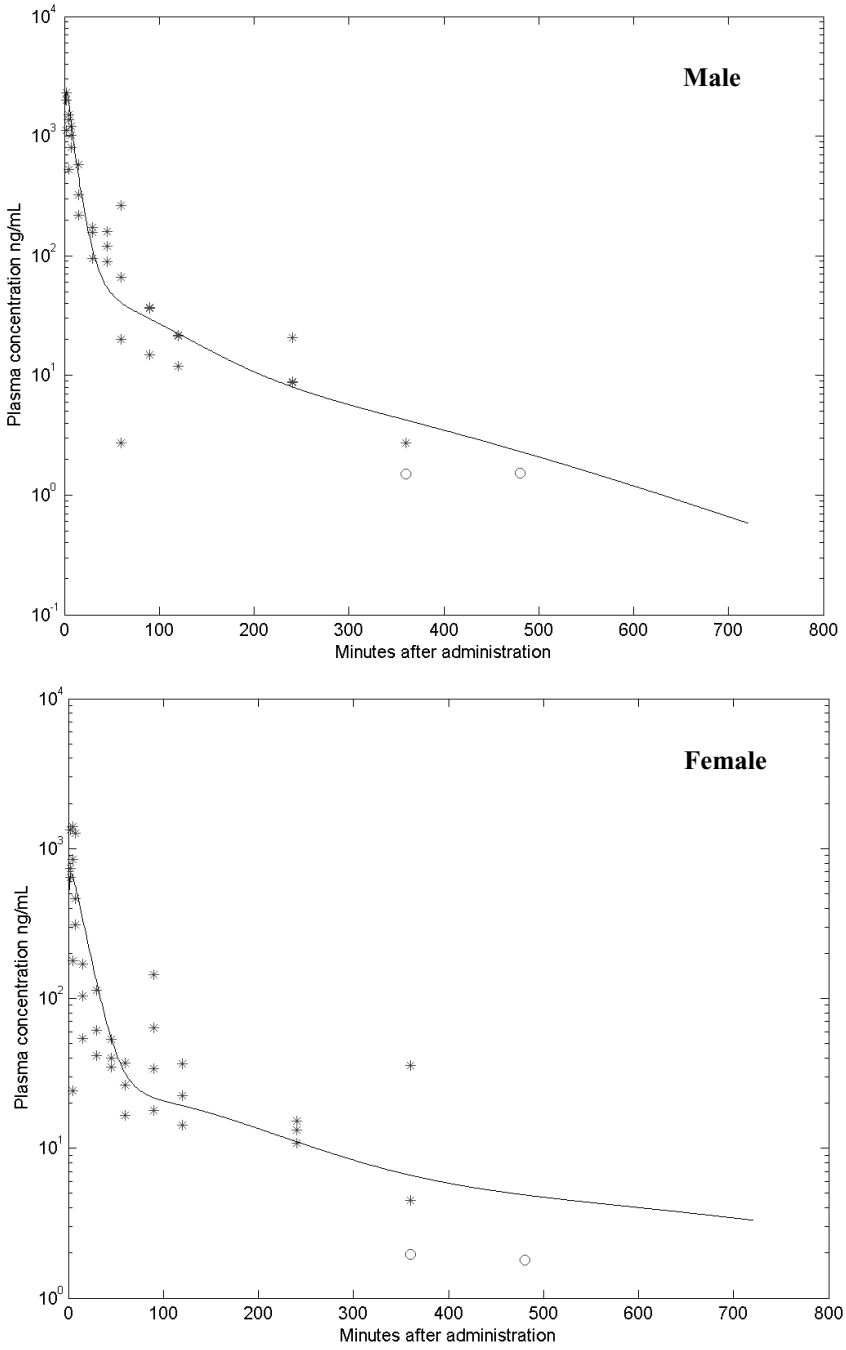


FIGURE K6b
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice Following a Single Gavage Administration of 50 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

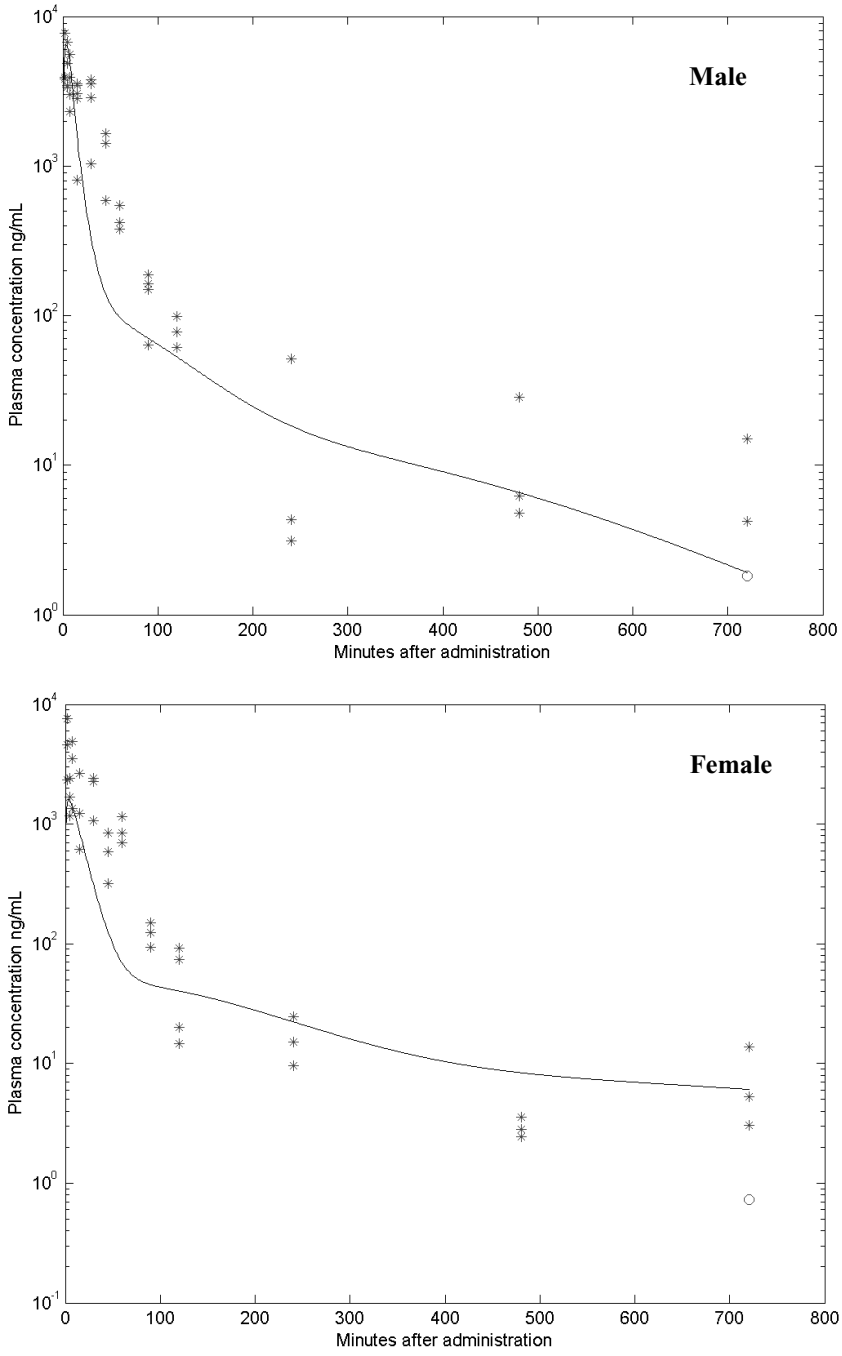


FIGURE K6c
Plasma Concentrations of Bromodichloromethane in B6C3F$_1$ Mice Following a Single Gavage Administration of 100 mg/kg Bromodichloromethane in Water:Cremophor® (9:1)
Model results (solid line) versus data (asterisks); circles are data points below the limit of quantitation.

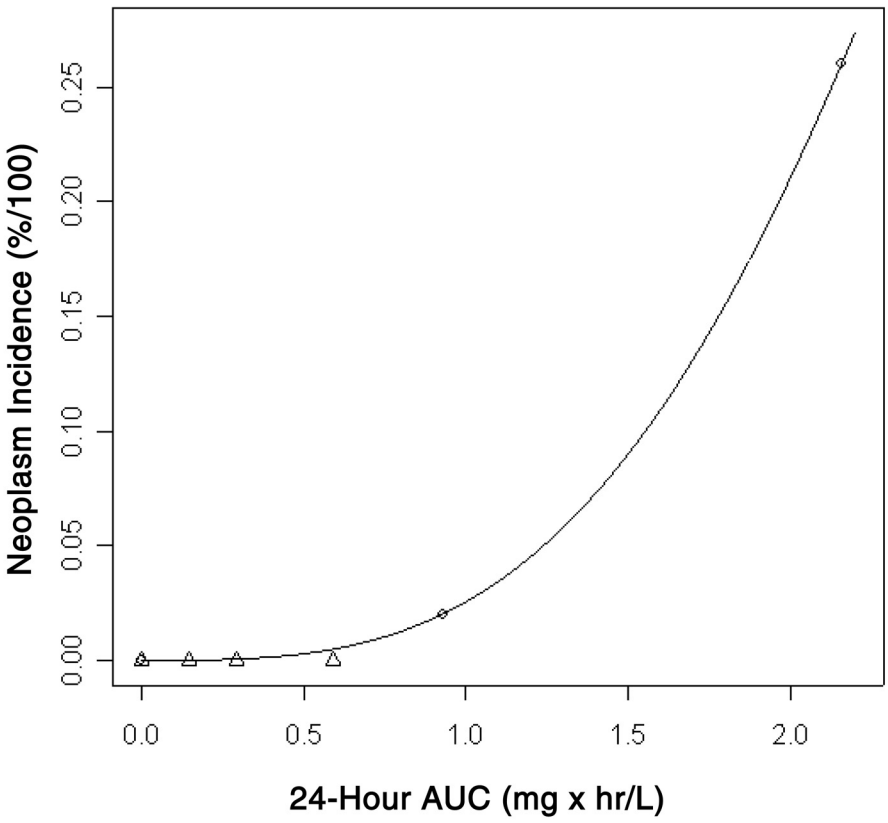


FIGURE K7
Observed and Predicted Incidences of Neoplasms in the Kidney using 24-Hour AUC as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=0.02566207, b=3.19753159

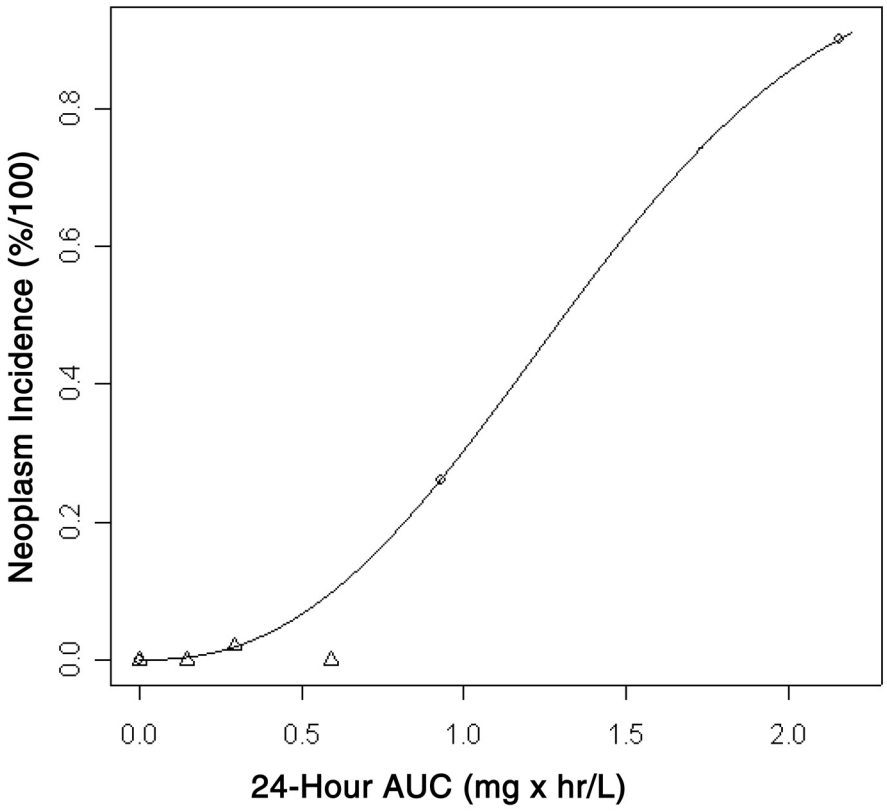


FIGURE K8
Observed and Predicted Incidences of Neoplasms in the Large Intestine using 24-Hour AUC as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=0.3604632, b=2.4079693

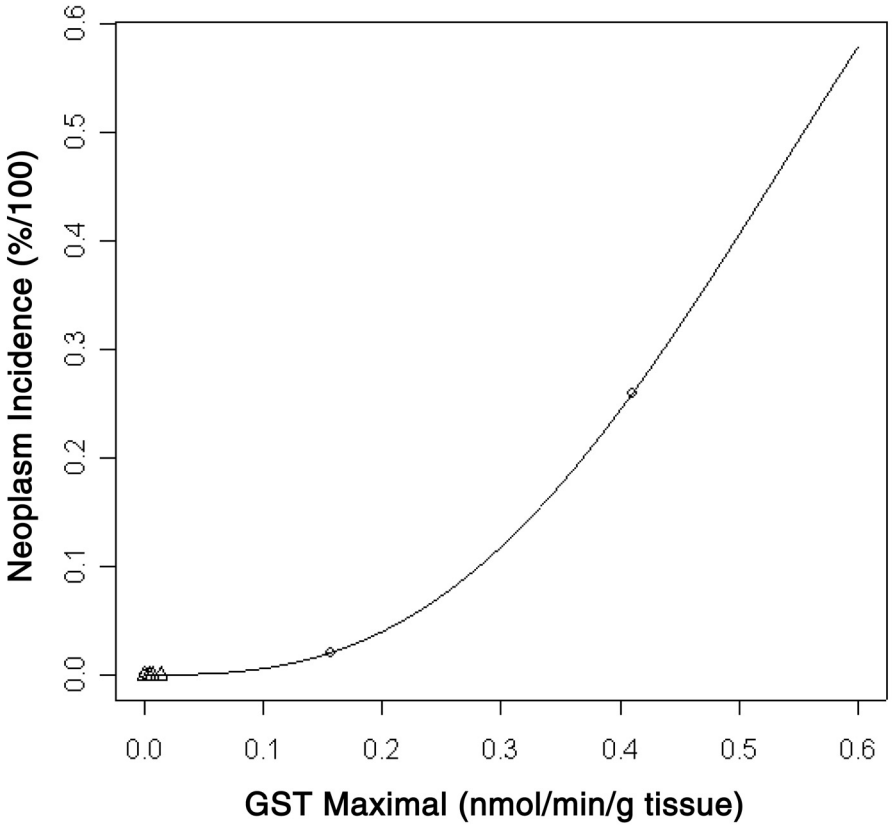


FIGURE K9
Observed and Predicted Incidences of Neoplasms in the Kidney using GST Maximal Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=3.587949, b=2.78685

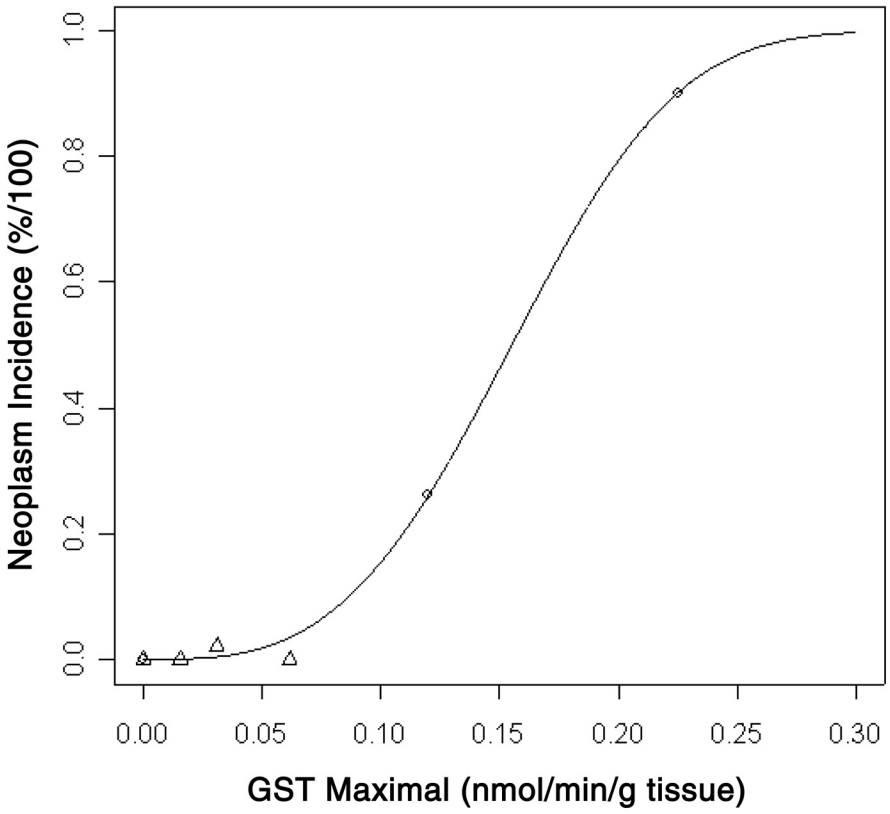


FIGURE K10
Observed and Predicted Incidences of Neoplasms in the Large Intestine using GST Maximal Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○); NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=287.549111, b=3.236354

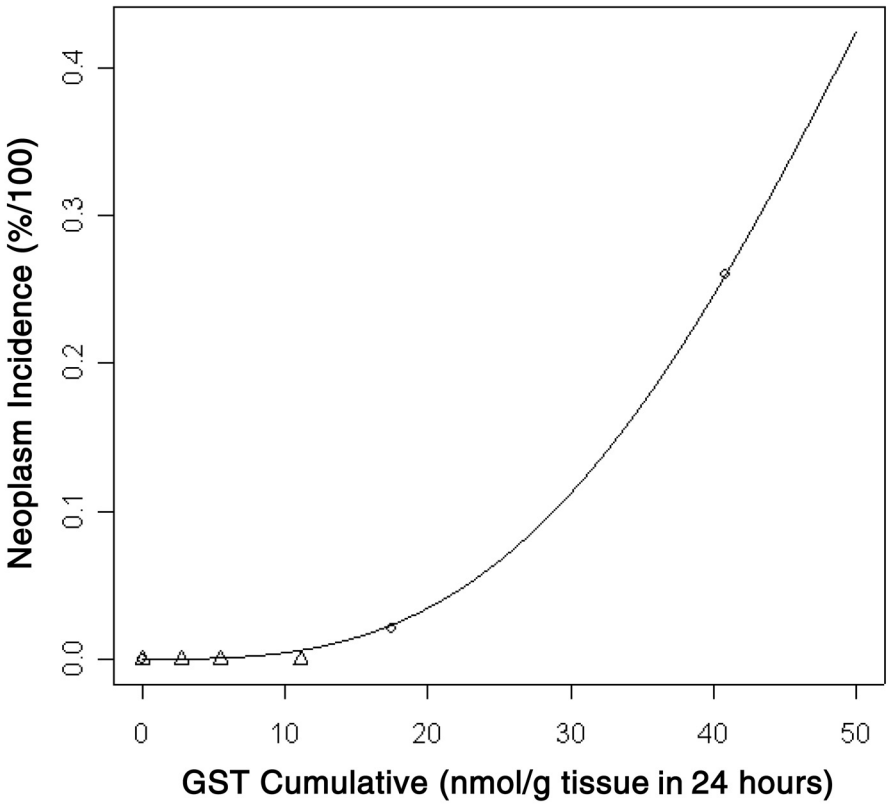


Figure K11
Observed and Predicted Incidences of Neoplasms in the Kidney using GST Cumulative Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=4.190033 × 10^{-6}, b=3.012981

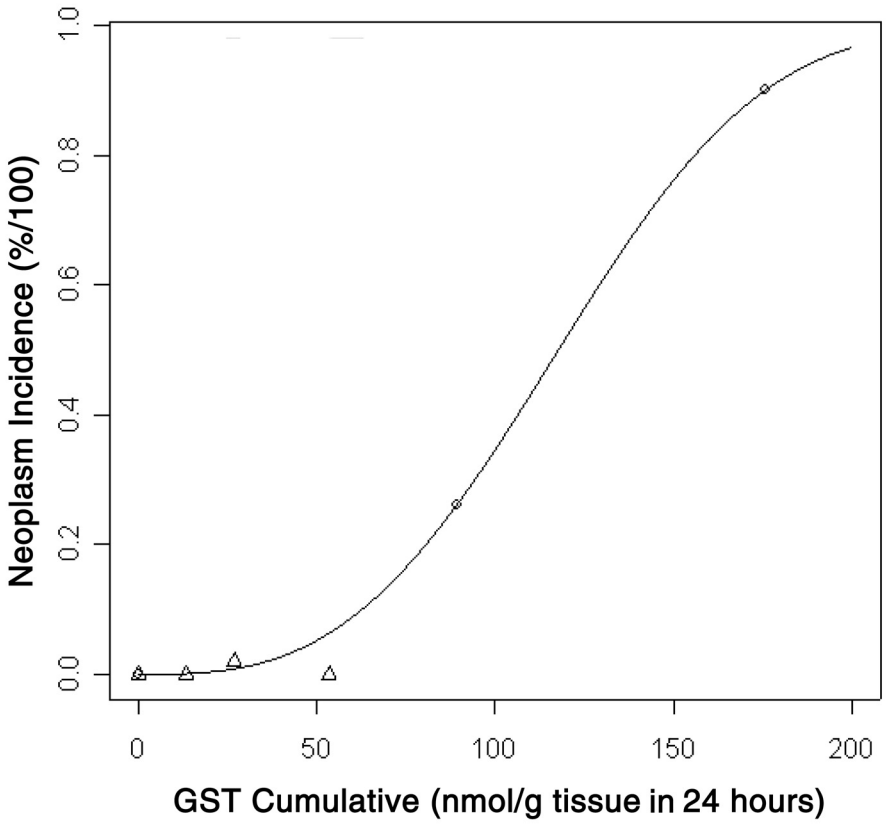


FIGURE K12
Observed and Predicted Incidences of Neoplasms in the Large Intestine using GST Cumulative Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: $a=4.353217 \times 10^{-7}$, b=2.994225

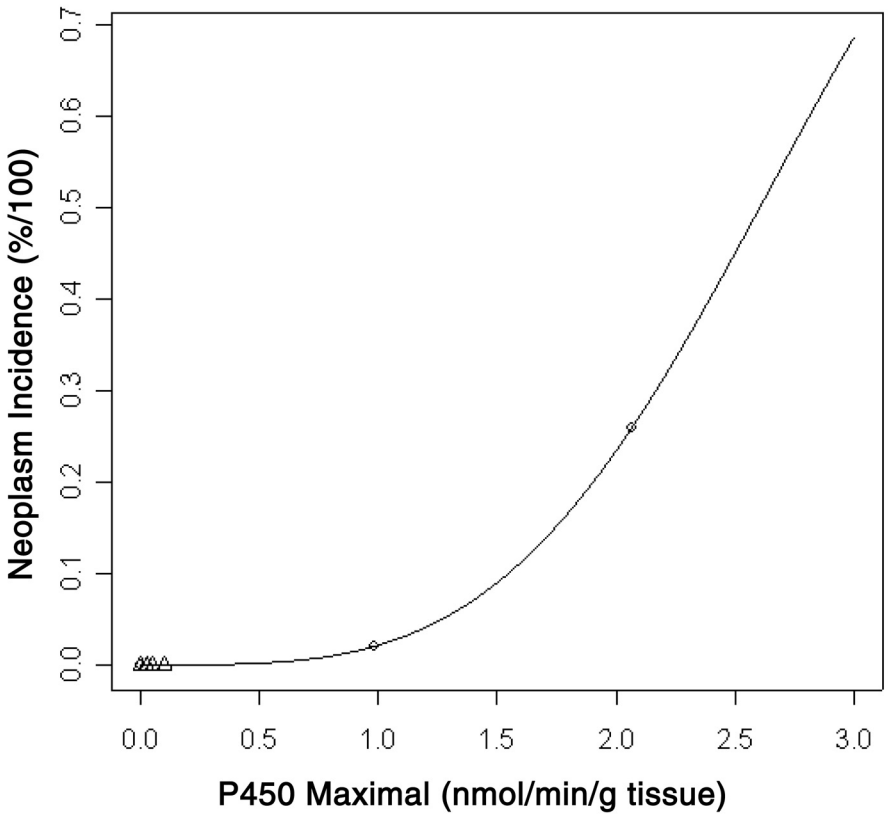


FIGURE K13
Observed and Predicted Incidences of Neoplasms in the Kidney using P450 Maximal Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=0.02156349, b=3.62345291

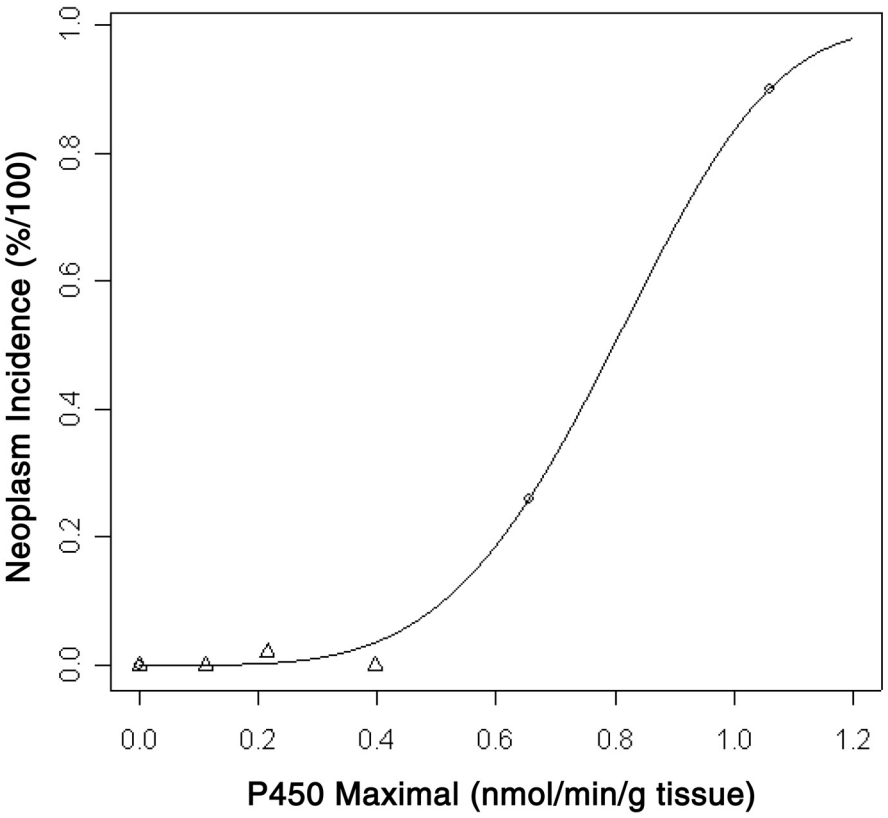


FIGURE K14
Observed and Predicted Incidences of Neoplasms in the Large Intestine using P450 Maximal Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=1.798367, b=4.239183

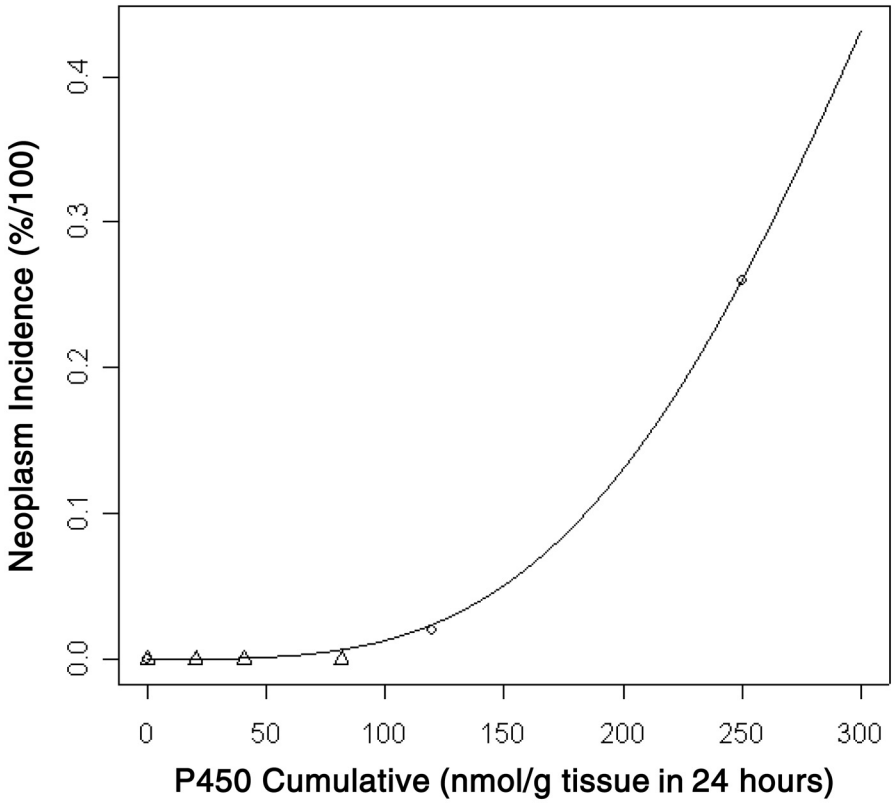


FIGURE K15
Observed and Predicted Incidences of Neoplasms in the Kidney using P450 Cumulative Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: a=1.627143 × 10^{-9}, b=3.447344

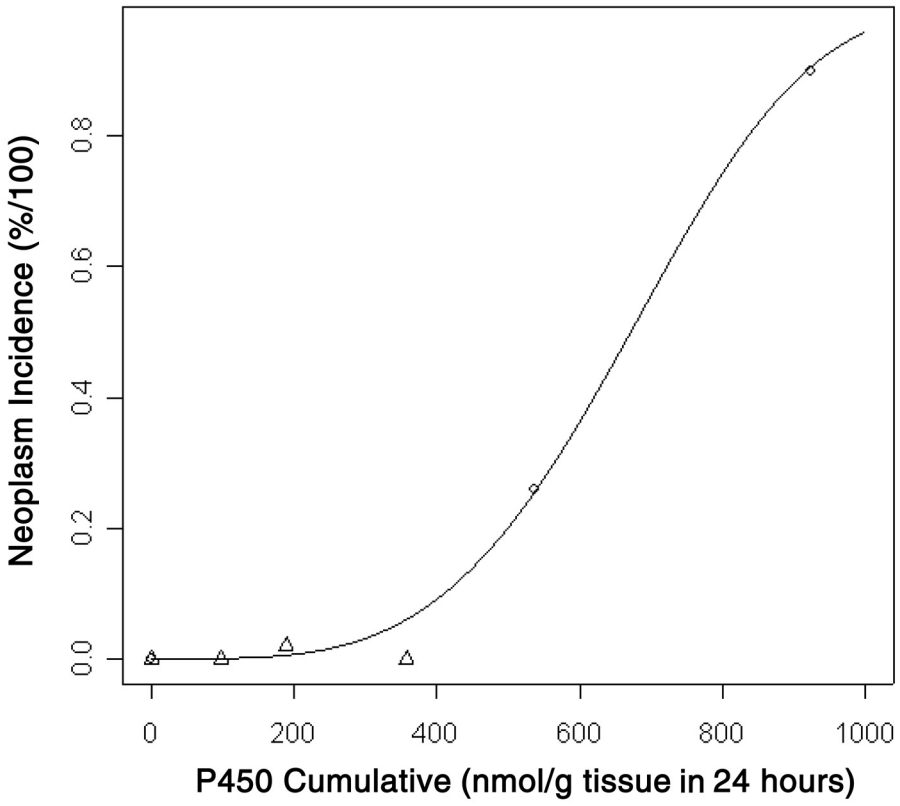


FIGURE K16
Observed and Predicted Incidences of Neoplasms in the Large Intestine using P450 Cumulative Metabolism as the Dose Metric
Observed neoplasm incidences are from the previous 2-year corn oil gavage (○; NTP, 1987) and current 2-year drinking water (△) studies. The solid line (—) shows predicted neoplasm incidences based on the Weibull model fit to the gavage data.
Model parameters: $a=1.000811 \times 10^{-11}$, b=3.833928

TABLE K5
Observed and Predicted Incidences of Neoplasms in the Kidney and Large Intestine of Male F344/N Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years: 24-Hour Blood AUC as the Dose Metric[a]

Exposure Concentration (mg/L)	Observed Neoplasm Rate (Number/50 animals)	24-Hour Blood AUC		
		AUC (mg × hr/L)	Predicted Neoplasm Rate (Number/50 animals)	P Value[b] H_0: Rate=Predicted
Large Intestine				
175	0 (0)	0.146	0.003498545 (0.175)	1
350	0.02 (1)	0.293	0.018579264 (0.929)	0.6085
700	0 (0)	0.591	0.096600054 (4.83)	0.0135
Kidney				
175	0 (0)	0.146	0.00005461 (0.003)	1
350	0 (0)	0.293	0.00050638 (0.025)	1
700	0 (0)	0.591	0.0047632 (0.238)	1

[a] Predicted neoplasm rates are based on the dose response from the 2-year corn oil gavage study (NTP, 1987) using this dose metric.
AUC=area under the curve
[b] Probability of significant difference between observed and model-predicted neoplasm incidences for the 2-year drinking water study

TABLE K6
Observed and Predicted Incidences of Neoplasms in the Kidney and Large Intestine of Male F344/N Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years: GST-Mediated Conjugation with Glutathione as the Dose Metric[a]

Exposure Concentration (mg/L)	Observed Neoplasm Rate (Number/50 animals)	GST Maximal Metabolism			GST Cumulative Metabolism		
		Activity (nmol/min/g tissue)	Predicted Neoplasm Rate (Number/50 animals)	P Value[b] H_0: Rate=Predicted	Activity[c] (nmol/g tissue)	Predicted Neoplasm Rate (Number/50 animals)	P Value[b] H_0: Rate=Predicted
Kidney							
175	0 (0)	0.0034	0.000000473 (0.00002)	1	2.73	0.000086367 (0.004)	1
350	0 (0)	0.0068	0.000003269 (0.0002)	1	5.49	0.000708562 (0.035)	1
700	0 (0)	0.0138	0.000023494 (0.001)	1	11.1	0.005898 (0.295)	1
Large Intestine							
175	0 (0)	0.0156	0.000408258 (0.02)	1	13.4	0.001031316 (0.052)	1
350	0.02 (1)	0.0311	0.003801232 (0.19)	0.1734	26.9	0.00827954 (0.414)	0.3401
700	0 (0)	0.0619	0.034717232 (1.7358)	0.4219	53.6	0.06341181 (3.171)	0.0752

[a] Predicted neoplasm rates are based on the dose response from the 2-year corn oil gavage study (NTP, 1987) using this dose metric. GST=glutathione-*S*-transferase
[b] Probability of significant difference between observed and model-predicted neoplasm incidences for the 2-year drinking water study
[c] Within a 24-hour period

TABLE K7
Observed and Predicted Incidences of Neoplasms in the Kidney and Large Intestine of Male F344/N Rats Exposed to Bromodichloromethane in Drinking Water for 2 Years: P450-Mediated Oxidation as the Dose Metric[a]

Exposure Concentration (mg/L)	Observed Neoplasm Rate (Number/50 animals)	P450 Maximal Metabolism Activity (nmol/min/g tissue)	Predicted Neoplasm Rate (Number/50 animals)	P Value[b] H_0: Rate=Predicted	P450 Cumulative Metabolism Activity[c] (nmol/g tissue)	Predicted Neoplasm Rate (Number/50 animals)	P Value[b] H_0: Rate=Predicted
Kidney							
175	0 (0)	0.025	0.000000034 (0.000002)	1	20.4	0.00005323 (0.003)	1
350	0 (0)	0.051	0.000000448 (0.00002)	1	40.8	0.000580496 (0.029)	1
700	0 (0)	0.101	0.00000532 (0.0003)	1	81.7	0.006340631 (0.317)	1
Large Intestine							
175	0 (0)	0.112	0.000167611 (0.008)	1	97.4	0.000420971 (0.021)	1
350	0.02 (1)	0.215	0.002656961 (0.133)	0.1246	189	0.005333129 (0.267)	0.2346
700	0 (0)	0.397	0.035182258 (1.760)	0.4241	358	0.060031838 (3.00)	0.0742

[a] Predicted neoplasm rates are based on the dose response from the 2-year corn oil gavage study (NTP, 1987) using this dose metric. P450=cytochrome P450
[b] Probability of significant difference between observed and model-predicted neoplasm incidences for the 2-year drinking water study
[c] Within a 24-hour period

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