

NTP TECHNICAL REPORT
ON THE
TOXICOLOGY AND CARCINOGENESIS
STUDIES OF DIBROMOACETIC ACID
(CAS NO. 631-64-1)
IN F344/N RATS AND B6C3F₁ MICE
(DRINKING WATER STUDIES)

Scheduled Peer Review Date: September 27-28, 2005

NOTICE

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National Toxicology Program

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

FOREWORD

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

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

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

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

Details about ongoing and completed NTP studies, abstracts of all NTP Technical Reports, and full versions of the completed reports are available at the NTP's World Wide Web site: <http://ntp.niehs.nih.gov>. In addition, printed copies of these reports are available from NTP as supplies last by contacting (919) 541-1371.

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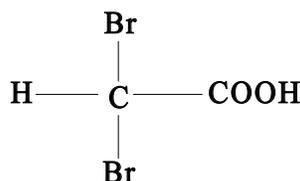
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ABSTRACT



DIBROMOACETIC ACID

CAS No. 631-64-1

Chemical Formula: $\text{C}_2\text{H}_2\text{Br}_2\text{O}_2$ Molecular Weight: 217.86

Synonyms: Acetic acid, dibromo (9CI); dibromoethanoic acid; dibromoacetate

Dibromoacetic acid is a water disinfection by-product. Dibromoacetic acid was nominated to the National Toxicology Program by the United States Environmental Protection Agency for toxicity and carcinogenicity studies in rats and mice because of widespread human exposure and because a related dihaloacetate, dichloroacetate, was found to be carcinogenic to the liver of rats and mice. Drinking water was selected as the route of exposure to mimic human exposure to this chemical. Male and female F344/N rats and B6C3F₁ mice were exposed to dibromoacetic acid (greater than 99% pure) in drinking water for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium* and peripheral blood erythrocytes of exposed mice.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg/L dibromoacetic acid in drinking water for 2 weeks, which is equivalent to average daily doses of approximately 17, 32, 67, 134, 270 (males), and 257 (females) mg dibromoacetic acid/kg body weight. All rats survived to the end of the study. Mean body weight gains of 1,000 mg/L males and of 500 mg/L females were significantly greater than those of the

controls. Water consumption by exposed and control groups was similar. Liver weights of exposed males and females were significantly increased. Right testis weights of males exposed to 500 mg/L or greater were significantly decreased. The incidences of hepatocytic cytoplasmic alteration were significantly increased in males exposed to 500 mg/L or greater and in 2,000 mg/L females, and testicular lesions, characterized by a delay in spermiation and retained spermatids, were noted in 500 and 1,000 mg/L males.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg/L dibromoacetic acid (equivalent to average daily doses of approximately 0, 24, 47, 95, 178, and 370 mg/kg to males and 0, 22, 53, 88, 166, and 309 mg/kg to females) in drinking water for 2 weeks. All mice survived to the end of the study. Mean body weight gains of 250 and 500 mg/L males were significantly greater than those of the controls. Water consumption by exposed and control groups was similar. Liver weights of males and females in the 1,000 and 2,000 mg/L groups were significantly increased. Thymus weights of males and females in the 1,000 and 2,000 mg/L groups were significantly less than those of controls. The incidences of thymus atrophy were significantly increased in 2,000 mg/L males and females. The incidences of morphological changes to the germinal epithelial of the testes were increased in males exposed to 1,000 or 2,000 mg/L.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female rats were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg/L dibromoacetic acid (equivalent to average daily doses of approximately 10, 20, 40, 90, and 166 mg/kg to males and 12, 23, 48, 93, and 181 mg/kg to females) in drinking water for 3 months. All rats survived to the end of the study. Mean body weights of male and female rats in the 2,000 mg/L group were significantly less than those of controls. Water consumption by the 2,000 mg/L males at weeks 1 and 13 and by females at week 13 was less than that by controls. Small decreases in the erythron and platelet counts occurred in rats exposed to 2,000 mg/L; minimally impaired erythropoiesis was also seen in 1,000 mg/L rats. Liver weights of all exposed groups of males and females were

significantly increased. Male rats in the 2,000 mg/L groups had significantly decreased testis weights. Testicular atrophy was noted in the 2,000 mg/L group, and retained spermatids were observed in the 500 and 1,000 mg/L groups. In the pituitary gland of male rats exposed to 2,000 mg/L, the incidence of cellular hypertrophy was significantly increased. The incidences of hepatocellular vacuolization were significantly increased in males exposed to 500 mg/L or greater and in females exposed to 2,000 mg/L. Hematopoietic cell proliferation was noted in females in the 2,000 mg/L group.

3-MONTH STUDY IN MICE

Groups of 10 male and 10 female mice were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg/L dibromoacetic acid (equivalent to average daily doses of approximately 16, 30, 56, 115, and 230 mg/kg to males and 17, 34, 67, 132, and 260 mg/kg to females) in drinking water for 3 months. All mice survived to the end of the study. Mean body weights and body weight gains of female mice in the 2,000 mg/L group and the mean body weight gain of 2,000 mg/L males were significantly less than those of controls. Water consumption by males in the 2,000 mg/L group was decreased at weeks 1 and 13 relative to controls. Small decreases in mean cell hemoglobin and platelet counts occurred in 2,000 mg/L male mice. Liver weights of males and females exposed to 500 mg/L or greater were significantly increased. Hepatocellular cytoplasmic vacuolization was present in most mice and the severity was increased in 1,000 and 2,000 mg/L males and females. The incidences of abnormal testicular morphology were significantly increased in 1,000 and 2,000 mg/L males.

2-YEAR STUDY IN RATS

Groups of 50 male and 50 female rats were exposed to drinking water containing 0, 50, 500, and 1,000 mg/L dibromoacetic acid for 2 years (equivalent to average daily doses of approximately 2, 20, and 40 mg/kg to males and 2, 25, and 45 mg/kg to females). Survival of exposed rats was similar to that of the control groups. Mean body weights of 1,000 mg/L males and females were less than those of the controls after week 29 and week 53, respectively, and those of 500 mg/L males and females were less after week 57 and week 85, respectively. Water

consumption by males and females exposed to 1,000 mg/L was less than that by controls during year 2 of the study.

The incidence of malignant mesothelioma was significantly increased in 1,000 mg/L male rats. A positive trend in the incidence of mononuclear cell leukemia occurred in female rats, and the incidence in 1,000 mg/L females was significantly increased. The incidences of cystic degeneration of the liver were significantly increased in all exposed groups of male rats. The incidences of alveolar epithelial hyperplasia were significantly increased in 500 and 1,000 mg/L females, and the incidences of nephropathy were significantly increased in all exposed groups of females.

2-YEAR STUDY IN MICE

Groups of 50 male and 50 female rats were exposed to drinking water containing 0, 50, 500, and 1,000 mg/L dibromoacetic acid for 2 years (equivalent to average daily doses of approximately 4, 45, and 87 mg/kg to males and 4, 35, and 65 mg/kg to females). Mean body weights of 50 and 500 mg/L male mice were greater than those of the controls after week 85. Water consumption by exposed mice was generally similar to that by controls throughout the study.

The incidences of liver neoplasms occurred with positive trends in male and female mice. The incidences of multiple hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in all exposed groups of males and in 500 and 1,000 mg/L females. The incidences of hepatoblastoma were significantly increased in 500 and 1,000 mg/L males, and the incidences of hepatocellular carcinoma were significantly increased in 1,000 mg/L males and 500 mg/L females. The incidences of alveolar/bronchiolar adenoma occurred with positive trends in males and females, and the incidence in 500 mg/L male mice was significantly greater than that in controls.

GENETIC TOXICOLOGY

Dibromoacetic acid was mutagenic in *Salmonella typhimurium* strain TA100 with and without rat or hamster liver metabolic activation enzymes (S9); no activity was detected in strain TA98, with or without S9. Increased frequencies of micronucleated normochromatic erythrocytes were observed in peripheral blood samples from male, but not female, mice administered dibromoacetic acid in drinking water for 3 months.

CONCLUSIONS

Under the conditions of these studies, there was *some evidence of carcinogenic activity** of dibromoacetic acid in male rats based on an increased incidence of malignant mesothelioma. There was *some evidence of carcinogenic activity* of dibromoacetic acid in female rats based on an increased incidence and positive trend of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of dibromoacetic acid in male and female mice based on increased incidences of hepatocellular neoplasms and hepatoblastoma (males only). Increased incidences of lung neoplasms in male mice were also considered to be exposure related. The slight increased incidence of lung neoplasms in female mice may have been related to dibromoacetic acid exposure.

Exposure to dibromoacetic acid for two years caused increased incidences of cystic degeneration of the liver in male rats, increased incidences of alveolar epithelial hyperplasia and nephropathy in female rats, and increased incidences of splenic hematopoiesis in male mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Dibromoacetic Acid

| | Male F344/N Rats | Female F344/N Rats | Male B6C3F ₁ Mice | Female B6C3F ₁ Mice |
|---|--|---|---|--|
| Concentrations in drinking water | 0, 50, 500, or 1,000 mg/L | 0, 50, 500, or 1,000 mg/L | 0, 50, 500, or 1,000 mg/L | 0, 50, 500, or 1,000 mg/L |
| Body weights | 500 and 1,000 mg/L groups less than the control group | 1,000 mg/L group less than the control group | 50 and 500 mg/L groups greater than the control group | Exposed groups similar to the control group |
| Survival rates | 34/50, 24/50, 30/50, 28/50 | 35/50, 39/50, 35/50, 32/50 | 31/50, 38/50, 34/50, 31/50 | 38/50, 35/50, 32/50, 32/50 |
| Nonneoplastic effects | <u>Liver</u> : degeneration, cystic (3/50, 9/50, 11/50, 15/50) | <u>Lung</u> : alveolar epithelium, hyperplasia (3/50, 7/50, 13/50, 14/50) <u>Kidney</u> : nephropathy (18/50, 32/50, 37/50, 40/50) | <u>Spleen</u> : hematopoiesis (18/49, 20/50, 28/50, 38/50) | None |
| Neoplastic effects | <u>All organs</u> : malignant mesothelioma (3/50, 1/50, 0/50, 10/50) | <u>All organs</u> : mononuclear cell leukemia (11/50, 13/50, 16/50, 22/50) | <u>Liver</u> : hepatocellular adenoma (18/49, 37/50, 37/50, 42/50); hepatocellular carcinoma (14/49, 9/50, 19/50, 26/50); hepatocellular adenoma or carcinoma (28/49, 41/50, 42/50, 47/50); hepatoblastoma (0/49, 4/50, 6/50, 18/50) <u>Lung</u> : alveolar/bronchiolar adenoma (7/49, 5/50, 17/50, 12/50); alveolar/bronchiolar adenoma or carcinoma (12/49, 12/50, 22/50, 17/50) | <u>Liver</u> : hepatocellular adenoma (19/49, 26/50, 32/50, 35/49); hepatocellular carcinoma (3/49, 3/50, 12/50, 8/49); hepatocellular adenoma or carcinoma (22/49, 28/50, 37/50, 37/49) |
| Equivocal findings | None | None | None | <u>Lung</u> : alveolar/bronchiolar adenoma (1/50, 3/50, 3/50, 6/50); alveolar/bronchiolar adenoma or carcinoma (2/50, 5/50, 5/50, 7/50) |
| Level of evidence of carcinogenic activity | Some evidence | Some evidence | Clear evidence | Clear evidence |
| Genetic toxicology | | | | |
| <i>Salmonella typhimurium</i> gene mutations: | | Positive in TA100 with and without S9; negative in TA98 with or without S9 | | |
| Micronucleated erythrocytes | | | | |
| Mouse peripheral blood <i>in vivo</i> : | | Positive in males; negative in females | | |

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
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The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on dibromoacetic acid on September 27-28, 2005, 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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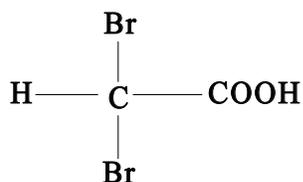
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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

NOTE: A summary of the Technical Reports Review Subcommittee's remarks will appear in a future draft of this report.

INTRODUCTION



DIBROMOACETIC ACID

CAS No. 631-64-1

Chemical Formula: $\text{C}_2\text{H}_2\text{Br}_2\text{O}_2$ Molecular Weight: 217.86

Synonyms: Acetic acid, dibromo (9CI); dibromoethanoic acid, dibromoacetate

CHEMICAL AND PHYSICAL PROPERTIES

Dibromoacetic acid is a deliquescent crystal (melting point, 48° C; boiling point, 195° C at 250 mm mercury) (Weast, 1983). It is a moderately strong acid, having a pK_a of 1.39 (Urbansky, 2000). In dilute solutions at pH greater than 6, more than 99.99% of the chemical exists as the dissociated carboxylate anion, dibromoacetate. Thus, under most conditions of exposure, and in biological tissues, this chemical exists as the carboxylate anion. Although dibromoacetic acid was the test article for this Technical Report, in the animal, it is described as dibromoacetate after it leaves the stomach.

PRODUCTION, USE, AND HUMAN EXPOSURE

Chloroacetates are formed when drinking water supplies containing natural organic matter (e.g., humic or fulvic acids) are disinfected with chlorine-containing oxidizing compounds such as chlorine gas, hypochlorous acid, and hypochlorite. If bromide is present in the source water, it may be oxidized to hybromous acid-hypobromite ion, which can react with organic matter to form brominated organic compounds. The reaction of brominated and/or chlorinated oxidizing agents with natural organic matter produces mixed brominated and chlorinated acetic acids,

including mono-, di-, and trichloroacetic acid; mono-, di-, and tribromoacetic acid; bromochloroacetic acid; bromodichloroacetic acid; and chlorodibromoacetic acid. The relative amount of brominated haloacetates produced in chlorinated drinking water is a function of the bromide concentration in the source water and the initial bromine/chlorine ratio.

Coagulation prior to chlorination removes much of the disinfection by-product precursors from source water and thereby reduces the amount of disinfection by-products formed during disinfection. Although possible reactions of haloacetates in water are decarboxylation and nucleophilic substitution (hydrolysis), because these processes are very slow in ambient water, most decreases in concentrations of haloacetates in drinking water distribution systems are likely due to biodegradation (Urbansky, 2001).

Haloacetates are second to trihalomethanes as the most commonly detected disinfection by-products in surface drinking water supplies in the United States (Liang and Singer, 2003). The relative amounts of these two families of chemicals as well as other disinfection by-products produced in drinking water supplies are affected by the nature and concentration of the organic precursor materials, water temperature, pH, the type of disinfectant, the disinfectant dose, and contact time (Liang and Singer, 2003; Huang *et al.*, 2004). For example, increasing the pH from 6 to 8 increases trihalomethane formation, decreases trihaloacetate formation, and has little effect on mono- and dihaloacetate levels. Bromoform and dibromoacetate were the predominant disinfection by-products measured in water from the Sea of Galilee (approximately 2 mg bromide/L) that was pretreated with chlorination for algae control and then disinfected with chlorine dioxide and chloramines (Richardson *et al.*, 2003). Controlled laboratory studies also showed that dibromoacetate is produced by chlorine dioxide treatment (Richardson *et al.*, 2003) or ozonation (Huang *et al.*, 2004) of water containing high ambient bromide concentrations.

Levels of haloacetic acids in drinking water are regulated by the United States Environmental Protection Agency (40 CFR, § 141.64) under the disinfection by-product rule, the sum of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid is limited to 60 µg/L (60 ppb). This level is

believed to reduce risks from cancer as well as reproductive and developmental toxicity. A nationwide study of disinfection by-product occurrence in diverse geographic regions of the United States was conducted between October 2000 and April 2002 (Weinberg *et al.*, 2002). In this study, 12 water treatment plants that had different source water quality and bromide levels and that employed the major disinfectants chlorine, chloramines, ozone, and chlorine dioxide were sampled quarterly. Concentrations of dibromoacetate in the finished water ranged up to 18 µg/L and estimates of dibromoacetate concentration in the distribution systems ranged up to 22 µg/L.

Much higher levels of human exposure to dichloroacetate have occurred from therapeutic use of this agent to treat lactic acidosis; dichloroacetate acts by stimulating the mitochondrial pyruvate dehydrogenase complex, resulting in increased oxidation of glucose, lactate, and pyruvate (Stacpoole *et al.*, 1998). Dichloroacetate is also a metabolite of trichloroethylene. Health effects of dichloroacetate may be relevant to dibromoacetate because of qualitative similarities in the metabolism of the chlorinated/brominated dihaloacetates.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Dihaloacetates are rapidly absorbed from the gastrointestinal tract after oral exposure. The maximum blood concentration of dibromoacetate in F344/N rats was reached 1 hour after administration by gavage (Schultz *et al.*, 1999).

Dihaloacetates exhibit low binding to rat plasma proteins (Schultz *et al.*, 1999). Forty-eight hours after oral administration of ¹⁴C-dichloroacetate in male F344/N rats, 5% to 8% of administered radiolabel was measured in the liver, 5% to 10% in muscle, 3.3% to 4.5% in skin, 1.4% to 2.6% in blood, and 1.0% to 1.7% in the intestines (Lin *et al.*, 1993). Dibromoacetate was measured in the testicular interstitial fluid of male Sprague-Dawley rats given five daily gavage doses of 250 mg dibromoacetate/kg body weight (Holmes *et al.*, 2001). The level of dibromoacetate in testicular fluid peaked at 79 µg/mL (approximately 370 µM) 30 minutes after the last dose, and the half-life was approximately 1.5 hours. Dibromoacetate was administered to Sprague-Dawley rats in drinking

water at concentrations ranging from 125 to 1,000 ppm (mg/L) with exposures beginning 14 days before cohabitation and continuing through gestation and lactation (Christian *et al.*, 2001). Quantifiable levels of dibromoacetate were measured in parental and fetal plasma, placental tissue, amniotic fluid, and milk. Thus, dibromoacetate crosses the placenta and is taken up by fetal tissue.

The oral bioavailability of dibromoacetate was reported to be 30% in male F344/N rats (Schultz *et al.*, 1999). The lower bioavailability of dibromoacetate compared to dichloroacetate is due to greater first-pass metabolism of dibromoacetate in the liver. Biotransformation of dihaloacetates to glyoxylate occurs primarily in liver cytosol of rats and humans by a glutathione-dependent process (James *et al.*, 1997) catalyzed by glutathione-S-transferase zeta (GST ζ) (Tong *et al.*, 1998a). This enzyme also catalyzes the penultimate step in the tyrosine degradation pathway. Glyoxylate is the only stable metabolite of dichloroacetate formed by purified GST ζ (Tong *et al.*, 1998b). The major metabolites identified in the urine or blood of F344/N rats or B6C3F₁ mice administered dichloroacetate are glyoxylate, glycolate, and oxalate (Lin *et al.*, 1993; Narayanan *et al.*, 1999). In addition to these metabolites, approximately 30% of radioactivity from orally administered ¹⁴C-dichloroacetic acid was exhaled as carbon dioxide (Lin *et al.*, 1993; Xu *et al.*, 1995). Mice metabolize dichloroacetate at approximately twice the rate of rats (Gonzalez-Leon *et al.*, 1999).

During GST ζ -mediated oxygenation of dichloroacetate or other dihaloacetates to glyoxylate, glutathione is required but not consumed. The reaction scheme for the GST ζ -mediated biotransformation of dihaloacetates (Figure 1) involves displacement of a halide by glutathione to form S-(α -halocarboxymethyl)glutathione, hydrolysis of this intermediate to form S-(α -hydroxycarboxymethyl)glutathione, and elimination of glutathione to produce glyoxylate (Tong *et al.*, 1998b). Among the brominated/chlorinated dihaloacetates, the relative rates of glyoxylate formation catalyzed by purified GST ζ are bromochloroacetate > dichloroacetate > dibromoacetate. In an interspecies comparison of the kinetics of dichloroacetate metabolism, the K_m with human liver cytosol was smaller than that with rat or mouse cytosol; however, the relative rates of metabolism to glyoxylate (V_{max}/K_m) were mouse > rat > human (Tong *et al.*, 1998b). Glyoxylate can undergo transamination to glycine, decarboxylation to form carbon dioxide, and oxidation to oxalate.

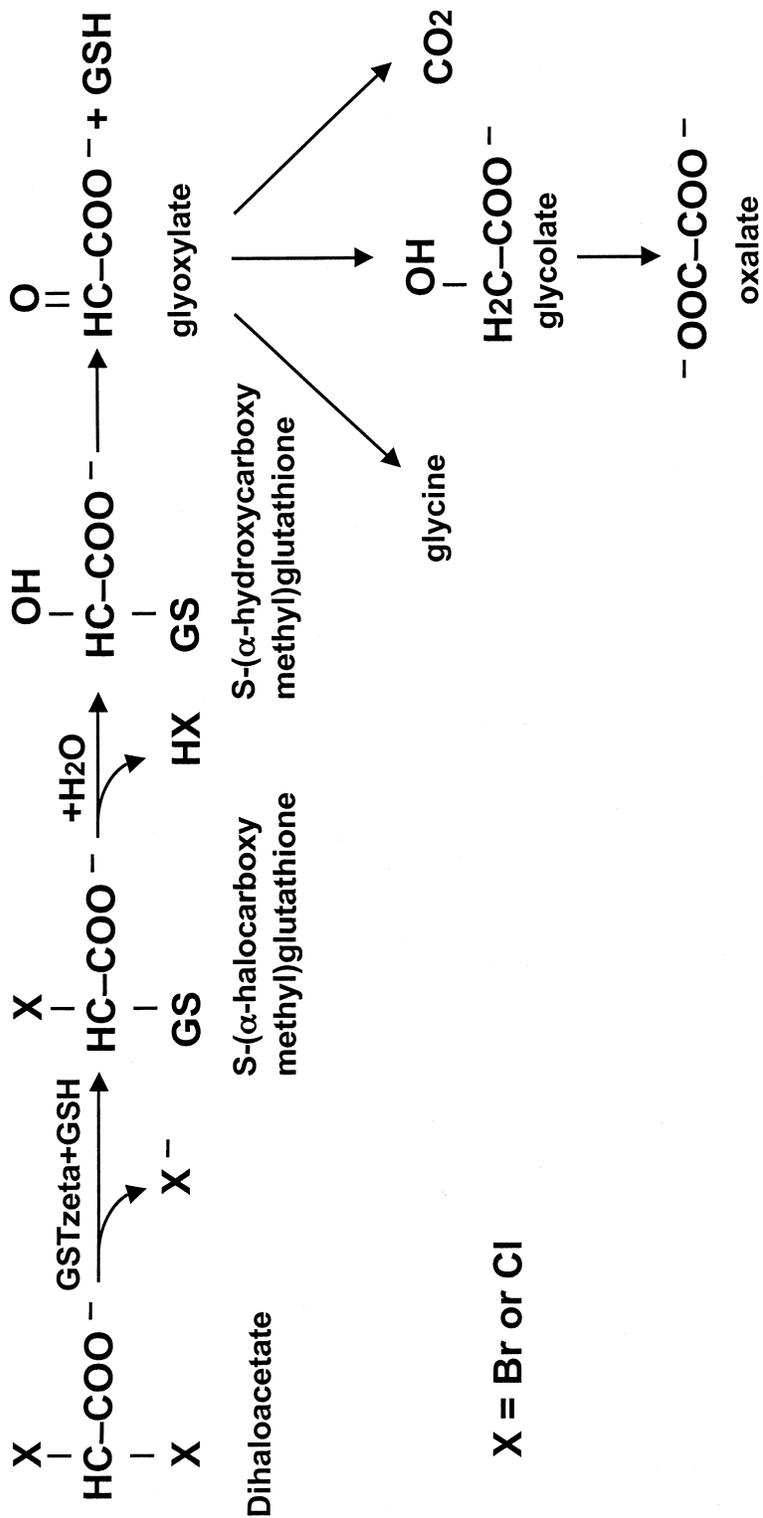


FIGURE 1
 Scheme for the Biotransformation of Dihaloacetates (Tong *et al.*, 1998b)

After gavage administration, elimination of dichloroacetate in rats occurs by exhalation as carbon dioxide and excretion of metabolites in the urine (Lin *et al.*, 1993). Elimination half-lives of dihaloacetates in blood are less than 4 hours; for dibromoacetate, the plasma half-life after intravenous injection is approximately 30 to 40 minutes (Schultz *et al.*, 1999). Elimination of dihaloacetates is primarily by metabolism; less than 3% of an intravenous dose (500 μmol dibromoacetate/kg body weight; 109 mg/kg) is excreted as the parent compound in urine and less than 0.1% is eliminated in feces. Bromine substitution of dihaloacetates increases the rate of metabolic clearance; dichloroacetate is cleared at half the rate of dibromoacetate. Pretreatment of male F344/N rats with 0.2 or 2.0 g dichloroacetate/L drinking water for 2 weeks reduced the rate of metabolic clearance of subsequent intravenous or gavage doses of dichloroacetate by sixfold (Gonzalez-Leon *et al.*, 1997). In addition, pretreatment caused increased blood concentration-time profiles and elimination half-lives for dichloroacetate, decreased formation of carbon dioxide, and increased renal excretion of dichloroacetate. Dichloroacetate pretreatment also inhibited the conversion of dichloroacetate to glyoxylate, oxalate, or glycolate in hepatic cytosol.

Metabolic clearance of dichloroacetate was also decreased in male B6C3F₁ mice pretreated with 2 g dichloroacetate/L drinking water for 14 days; however, the effect in mice was less marked than that in rats (Gonzalez-Leon *et al.*, 1999). Pretreatment of male B6C3F₁ mice with 1 g dichloroacetic acid/L drinking water for 2 weeks resulted in a threefold decrease in the rate of metabolism of dichloroacetate in liver cytosol, and similar reduction in the rate of formation of glyoxylate, oxalate, and glycolate (Austin and Bull, 1997). Elimination of dichloroacetate in children or adults is also reduced as a result of prior exposure to therapeutic doses of this agent (Curry *et al.*, 1991; Stacpoole *et al.*, 1998). The reduced elimination of dichloroacetate in pretreated animals is due to irreversible inactivation of GST ζ ; the degradation rate constant for GST ζ in the liver of male F344/N rats given five daily intraperitoneal injections of 0.30 mmol dichloroacetate/kg body weight (38 mg/kg) is 0.21/day (Anderson *et al.*, 1999). Treatment of male F344/N rats with 0.2 g dichloroacetate/L drinking water for 7 days reduces liver GST ζ activity by 90%; this reduction markedly decreases the metabolic elimination of dichloroacetate and increases its oral bioavailability (Saghir and Schultz, 2002).

Dibromoacetate and bromochloroacetate are also suicide substrates for GST ζ ; 12 hours after a single injection of these dihaloacetates, GST ζ activity in the rat liver is reduced to 17% and 19%, respectively, of that in controls. Hydrolysis of *S*-(α -halocarboxymethyl)glutathione forms a hemithioacetal that eliminates glutathione and yields glyoxylate; however, this intermediate may inactivate GST ζ by covalently binding to a nucleophilic site on the enzyme (Wempe *et al.*, 1999). Thus, hydrolysis of this intermediate and inactivation of GST ζ are competing reactions. Based on the differential effect of dichloroacetate-induced inactivation of GST ζ on the elimination of (+)-bromochloroacetate versus (-)-bromochloroacetate in male F344/N rats, Schultz and Sylvester (2001) suggested that an additional GST isoenzyme that is not inactivated by dihaloacetates might provide a minor contribution to the formation of glyoxylate in non-pretreated animals.

TOXICITY

Experimental Animals

The LD₅₀ for dibromoacetate in male Sprague Dawley rats was reported to be 1,737 mg/kg body weight (Linder *et al.*, 1994a). In male Sprague-Dawley rats gavaged daily with 250 mg dibromoacetate/kg, weight loss was evident by week 3, neurological signs including excitability, awkward gait, atypical limb movement, and abnormal posturing were evident by week 4, and labored breathing, light tremor, and difficulty in moving were observed during week 6 of treatment (Linder *et al.*, 1995).

Studies comparing the effects of dichloroacetate in mouse liver with those of trichloroacetate indicate that these agents likely operate by different mechanisms. Drinking water exposure of male B6C3F₁ mice to 2 g dichloroacetate/L for 37 weeks caused hepatomegaly, cytomegaly, focal necrosis, and accumulation of glycogen in hepatocytes; in contrast, exposure of male mice to 2 g trichloroacetate/L drinking water caused marked accumulation of lipofuscin (indicator of lipid peroxidation), modest accumulation of glycogen, and no evidence of focal necrosis (Bull *et al.*, 1990). After 52 weeks of exposure, the incidences of hyperplastic nodules and hepatocellular adenoma and carcinoma were increased in dichloroacetate- and trichloroacetate-exposed mice

compared to controls. Similar to dichloroacetate, dibromoacetate and bromochloroacetate caused liver glycogen accumulation in B6C3F₁ mice (Kato-Weinstein *et al.*, 2001). In addition, dibromoacetate produced a dose-dependent decrease in serum glucose concentration and transient increases (2 to 4 weeks) in liver cell replication rates and in the activity of the peroxisomal enzyme cyanide-insensitive acyl-CoA oxidase. Dibromoacetate and dichloroacetate induced peroxisomal palmitoyl-CoA oxidase activity in primary rat hepatocyte cultures (Walgren *et al.*, 2004).

In a two-generation drinking water study, absolute and relative liver weights and kidney weights were increased in pups exposed to 50 ppm or greater concentrations of dibromoacetate (Christian *et al.*, 2002). No microscopic changes were associated with these weight increases.

Exposure of adolescent male and female F344/N rats to 0.2 to 1.5 g dibromoacetate/L drinking water for 6 months produced concentration-related neuromuscular toxicity (Moser *et al.*, 2004). Effects of exposure to dibromoacetate included decreased grip strength, mild gait abnormalities, and decreased sensorimotor responsiveness; neuropathological findings included degeneration of spinal cord nerve fibers and spinal cord cellular vacuolization at the 0.6 and 1.5 g/L concentrations. Similar neuromuscular effects had been reported in F344/N rats exposed to dichloroacetate (Moser *et al.*, 1999).

In an evaluation of cytotoxic potency of drinking water disinfection by-products in Chinese hamster ovary cells, dibromoacetate was less potent than bromoacetate and 3-chloro-4-(dichloromethyl)-5-hydroxy-2[5H]-furanone, but more potent than chloroacetate, potassium bromate, tribromoacetate, dichloroacetate, and trichloroacetate (Plewa *et al.*, 2002).

Humans

No studies on the toxicity of dibromoacetate in humans were found in a review of the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Spermatotoxicity in male rats has been identified as one of the most sensitive toxic endpoints following exposure to dibromoacetate and dichloroacetate. In adult male Sprague-Dawley rats administered a single gavage dose of 1,250 mg dibromoacetate/kg body weight, a transient decline in serum testosterone, increases in abnormal sperm head shapes, fusion of spermatid flagella, decreases in epididymal sperm counts, reduction in sperm motility, and delayed spermiation described as abnormal retention of Step 19 spermatids near the lumen of Stage IX to Stage XII tubules were observed (Linder *et al.*, 1994a). In follow-up studies with oral doses of dibromoacetate ranging from 2 to 270 mg/kg for up to 79 days, reduction in epididymal sperm counts and altered spermiation were observed at 10 mg/kg or higher doses (Linder *et al.*, 1994b, 1997a). No abnormalities were detected in the testes or epididymides of rats exposed to 2 mg/kg. In addition, adverse effects on sperm morphology and sperm motility were detected at the 90 mg/kg dose level, and large atypical residual bodies were seen in the lumen and epithelium of seminiferous tubules and in the epididymides. The formation of atypical residual bodies was suggested to be a result of impairment of degradative processes of Sertoli cells. In spite of the changes in sperm quality caused by dibromoacetate, the germinal epithelium appeared intact and there were no obvious changes in sperm production in exposed rats. Seminiferous tubule atrophy was observed only in the testes of rats dosed with 250 mg dibromoacetate/kg for 42 days and then held without exposure for an additional 186 days (Linder *et al.*, 1997a). The primary spermatotoxic effects of dibromoacetate appear to be delayed spermiation (retention of mature sperm), formation of atypical residual bodies, abnormal sperm morphology, and decreased sperm motility.

Testicular effects caused by dibromoacetate in rats are similar to those of dichloroacetate; however, the potency of dibromoacetate-induced testicular toxicity is greater than that of dichloroacetate (Linder *et al.*, 1997b).

Administration of bromochloroacetate to male Sprague-Dawley rats also decreased epididymal sperm counts, decreased the number of motile sperm, increased the number of epididymal sperm with misshapen heads or tail defects, increased the number of atypical residual bodies in seminiferous tubules, and increased the number of Step 19 spermatids retained in Stages X and XI of the spermatogenic cycle (Klinefelter *et al.*, 2002).

Fertility of male Sprague-Dawley rats was not altered by daily gavage treatment with up to 50 mg dibromoacetate/kg body weight for up to 79 days; however, male fertility was compromised in rats treated for 15 days or more with 250 mg/kg (Linder *et al.*, 1995). The latter evaluation was made through natural breeding and after artificial insemination of female rats with sperm collected from exposed rats. The fertility of cauda epididymal sperm was also reduced in male rats exposed to 8 mg bromochloroacetate/kg or greater doses for 14 days (Klinefelter *et al.*, 2002). The latter evaluation was made by *in utero* insemination. In fertility assessments by intrauterine insemination, the ED₅₀ for decreased male fertility of cauda epididymal sperm collected from Sprague-Dawley rats exposed by gavage for 14 days to dibromoacetate (3.5 mg/kg, 16.1 µmol/kg) was similar to the ED₅₀ for bromochloroacetate (2.7 mg/kg, 15.6 µmol/kg) (Kaydos *et al.*, 2004).

Holtzman rats were administered dibromoacetate by gavage at doses ranging from 62.5 to 250 mg/kg per day during the first 8 days of pregnancy to determine whether exposure to dibromoacetate during early pregnancy affects female reproduction (Cummings and Hedge, 1998). No effects were detected on the number of implantation sites, number of pups per litter, number of resorptions, or pup weights on day 20; however, serum levels of estradiol were elevated in exposed dams. In a follow-up study, gavage dosing of 90-day old female Sprague-Dawley rats with 10 to 270 mg dibromoacetate/kg for 14 days caused a dose-related alteration in estrous cyclicity, with a tendency toward longer periods of persistent estrus (Balchak *et al.*, 2000). In addition, *in vitro* exposure of preovulatory follicles to dibromoacetate (50 µg/mL for 24 hours) caused an elevation in estradiol release and suppression of progesterone secretion stimulated with human chorionic gonadotropin; thus, the disruption of estrous cyclicity by dibromoacetate was attributed to alteration of ovarian steroid production. Elevations in circulating estradiol in female rats exposed to dibromoacetate were attributed to suppression of estradiol catabolism because serum estradiol levels were elevated in ovariectomized rats implanted with estradiol-containing capsules and then treated with dibromoacetate (Goldman and Murr, 2003). Daily exposure of female Dutch-belted rabbits to approximately 1 to 50 mg dibromoacetate/kg body weight per day in drinking water beginning on gestation day 15 and continuing through 24 weeks did not produce any gross abnormalities of the reproductive tract or viscera, but did reduce the number of primordial follicles in prepubertal and adult rabbits

(Bodensteiner *et al.*, 2004). This exposure covers the fetal and neonatal periods when the primordial follicle pool in rabbits is formed. Reduction in the population of primordial follicles could result in early reproductive senescence.

Exposure of Sprague-Dawley rats to 4 to 800 ppm dibromoacetate in drinking water from gestation day 15 through adulthood induced delays in pubertal development (delayed preputial separation in males and delayed vaginal opening in females at 400 ppm), and decreases in the fertility of cauda epididymal sperm at 600 and 800 ppm (Klinefelter *et al.*, 2004). The incidences of atypical residual bodies, delayed spermiation, misshapen sperm heads, and atrophic seminiferous tubules were increased at 400 ppm and greater concentrations; sperm motility and fertility were decreased at 600 and 800 ppm. Altered steroidogenesis was suggested to be a contributor to the pubertal delays.

Dibromoacetate was administered to Sprague-Dawley rats in drinking water at concentrations ranging from 125 to 1,000 ppm (mg/L) with exposures beginning 14 days before cohabitation and continuing through gestation and lactation (Christian *et al.*, 2001). The only reported reproductive and developmental effects were reductions in mating performance at 1,000 ppm and decreases in pup body weights at 250 ppm and greater levels. In a two-generation reproductive toxicity study, Sprague-Dawley rats were given dibromoacetate in drinking water at concentrations of 0, 250, 500, or 650 ppm (Christian *et al.*, 2002). No effects on estrous cyclicity, mating, fertility, implantation sites, litter sizes, pup viability, or pup sex ratios were observed in the parental and F₁ generation female rats. In parental or F₁ generation male rats, increased incidences of delayed sperm production (retention of Step 19 spermatids in Stage IX and Stage X seminiferous tubules), atypical residual bodies in the testis, abnormal sperm shape, and epididymal abnormalities (atrophy, residual bodies, and hypospermia) were observed in the 250 ppm or 650 ppm exposure groups. Delays in preputial separation and vaginal opening were observed in 650 ppm groups of F₁ generation rats. In contrast to the effect of dibromoacetate on follicular development in rabbits (Bodensteiner *et al.*, 2004), no effect on ovarian follicular histology was observed in rats exposed to dibromoacetate. For this effect, the rabbit may be a more sensitive species.

Twenty-four hour exposures of mouse embryo cultures to haloacetates produced adverse effects on neural tube closure, craniofacial development, and heart development (Hunter *et al.*, 1996). For induction of neural tube defects, dibromoacetate was more potent than dichloroacetate.

Humans

No human studies have been reported on reproductive or developmental effects of dibromoacetate *per se*; however, several studies have indicated an association between exposure to disinfection by-products and alterations in reproductive function or fetal development, including spontaneous abortions, stillbirths, low birth weight, and birth defects (Nieuwenhuijsen *et al.*, 2000).

CARCINOGENICITY

Experimental Animals

No studies have been reported on the carcinogenicity of dibromoacetate in animals. In contrast, several studies have shown that dichloroacetate administered in drinking water is carcinogenic to the liver of rats and mice. Hepatocellular adenomas and carcinomas were induced in male B6C3F₁ mice exposed to 5 g dichloroacetate/L drinking water for 61 weeks (Herren-Freund *et al.*, 1987). The same dose of trichloroacetate was also carcinogenic to male mice; however, the incidences of hepatocellular adenoma and carcinoma in mice exposed to trichloroacetate (8 of 22 and 7 of 22, respectively) were less than those induced with dichloroacetate (25 of 26 and 21 of 26, respectively). In a follow-up drinking water study, the incidences of liver tumors in male B6C3F₁ mice administered 0, 0.05, 0.5, 3.5, or 5 g dichloroacetate/L for 60 weeks were 7%, 24%, 11%, 100%, and 90%, respectively, and the mean daily doses of dichloroacetate in the exposed groups were estimated to be 7.6, 77, 410, and 486 mg dichloroacetate/kg body weight, respectively (DeAngelo *et al.*, 1991). Liver tumor incidences in the 0.05 and 0.5 g/L groups were not significantly different from controls. However, when male B6C3F₁ mice were exposed to 0.5 g dichloroacetate/L drinking water for 104 weeks (mean daily dose, 93 mg/kg), the incidences of hepatocellular carcinoma and adenoma were 63% and 42%, respectively, in animals that survived to the end of the

study; the combined incidences of liver tumors were 3/20 in controls and 18/24 in dichloroacetate-exposed mice (Daniel *et al.*, 1992).

Hepatocellular adenomas and carcinomas were induced in female B6C3F₁ mice exposed to 20 mmol dichloroacetate/L drinking water (2.6 g/L) for 576 days (82 weeks) and hepatocellular adenomas were induced in female mice exposed to 6.67 mmol dichloroacetate/L (0.83 g/L). Hepatocellular adenomas and carcinomas were also induced in female B6C3F₁ mice exposed to 20 mmol trichloroacetate/L drinking water (3.3 g/L) for 576 days, and hepatocellular carcinomas were induced in female mice exposed to 6.7 mmol trichloroacetate/L (1.1 g/L) (Pereira, 1996). Dichloroacetate induced a predominance of eosinophilic hepatic foci and tumors, which consistently stained for the presence of glutathione-S-transferase (GST)- π ; in contrast, tumors induced by trichloroacetate were predominantly basophilic and lacked GST- π . In addition, hepatocyte proliferation was increased after 5 days of exposure to dichloroacetate or trichloroacetate, but not after 12 or 33 days of exposure.

Based on an analyses of the time- and dose-dependent relationships for the effects of dichloroacetate on the induction of preneoplastic and neoplastic lesions in the livers of exposed mice (DeAngelo *et al.*, 1999), it was suggested that dichloroacetate-induced hepatocarcinogenesis is due to selective growth of a phenotypic cell-type (basophilic and/or clear cell focal lesions) that does not respond to normal homeostatic control mechanisms, such as cells that are resistant to mitoinhibition (Carter *et al.*, 2003). Hepatocellular necrosis and regenerative hyperplasia, as well as steatosis, were not associated with the development of tumors or preneoplastic lesions.

Dichloroacetate is also carcinogenic to the liver of rats. Male F344/N rats were exposed to 0, 0.05, 0.5, or 1.6 g dichloroacetate/L drinking water for 100 weeks (DeAngelo *et al.*, 1996). Mean daily doses of dichloroacetate were 3.6, 40, and 139 mg/kg body weight for the three exposed groups. The incidences of hepatocellular adenoma or carcinoma combined in rats that survived more than 78 weeks were 3% (1/33) in controls and 0% (0/26), 24.1% (7/29), and 28.6% (8/28) in the respective exposed groups. The liver cancer response was not associated with peroxisome proliferation, hepatocellular necrosis, or sustained hepatocyte proliferation.

Several studies have examined potential mechanisms of hepatocarcinogenesis of dihaloacetates. Single gavage administration of halogenated acetic acids induced lipid peroxidation and formation of 8-hydroxydeoxyguanosine adducts in nuclear DNA in the liver of male B6C3F₁ mice; the relative potencies for these effects were dibromoacetate \approx bromochloroacetate > dichloroacetate > trichloroacetate (Austin *et al.*, 1996). These results suggest that DNA damage from oxidative stress induced by these agents may contribute to the hepatocarcinogenic process. In male B6C3F₁ mice exposed to 2.0 g dichloroacetate/L drinking water hepatocyte division rates were increased after 14 days of exposure; after 28 and 280 days of exposure, hepatocyte division rates were reduced in livers of dichloroacetate-treated mice compared to controls (Stauber and Bull, 1997). Altered hepatic foci and liver tumors in dichloroacetate-treated mice showed higher immunoreactivity to the oncoproteins *c-Jun* and *c-Fos* and higher rates of cell division than the surrounding nonlesioned liver tissue. Increased cell replication rates in hepatic foci and tumors and decreased rates in normal hepatocytes of mice exposed to dichloroacetate provide a selective growth advantage to initiated cells. In a follow-up study, incubation of primary hepatocyte cultures from untreated male B6C3F₁ mice with 0.5 to 2.0 mM dichloroacetate enhanced the formation of anchorage-independent colonies in soft agar (Stauber *et al.*, 1998). A fourfold increase in colony formation was measured when hepatocytes were obtained from mice pretreated with 0.5 g dichloroacetate/L drinking water for 2 weeks. Although dichloroacetate did not induce *c-Jun* expression in hepatocyte monolayers, the colonies promoted by dichloroacetate were immunoreactive with *c-Jun* antibody. These results suggest that dichloroacetate was selective for *c-Jun*⁺ subpopulations.

Gavage administration of 500 mg dichloroacetate/kg body weight to female B6C3F₁ mice for 5 days caused decreased DNA methylation and increased mRNA expression of the *c-myc* proto-oncogene (Pereira *et al.*, 2001). Administration of 3.2 g dichloroacetate/L drinking water for 36 weeks increased the incidence and multiplicity of liver tumors but not kidney tumors in *N*-methyl-*N*-nitrosourea-initiated mice. Thus, hypomethylation of *c-myc* and increased expression of this gene may be involved in the promotion of liver tumors by dichloroacetate. Exposure of female B6C3F₁ mice and male F344/N rats to 1,000 or 2,000 mg dibromoacetate/L drinking water for 28 days caused a decrease in the 5-methylcytosine content of DNA and hypomethylation and increased mRNA expression

of the *c-myc* and insulin-like growth factor II genes (Tao *et al.*, 2004). Treatment with dibromoacetate also caused glycogen accumulation and peroxisome proliferation in the mouse and rat liver. Thus, dibromoacetate and dichloroacetate induce similar biochemical and molecular effects, some of which may be involved in hepatocarcinogenesis.

The mutational spectrum at codon 61 of the *H-ras* gene was different in liver tumors obtained from male B6C3F₁ mice exposed to 500 mg dichloroacetate/L drinking water for 76 weeks compared to liver tumors from control mice (Anna *et al.*, 1994). Although the frequency of liver tumors with *H-ras* codon 61 mutations was not significantly different in dichloroacetate-exposed (62%) and control (69%) mice, the dichloroacetate-treated mice had increased CAA-CTA and decreased CAA-AAA mutations. Hence, base-substitution mutations may be involved in the hepatocarcinogenicity of dichloroacetic acid.

Humans

No studies have been reported on the carcinogenicity of dibromoacetate in humans *per se*; however, several studies have examined cancer risks associated with exposure to disinfection by-products. 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 to 1.34) for bladder cancer and 1.38 (95% CI: 1.01 to 1.87) for rectal cancer (Morris *et al.*, 1992). A population-based case-control study in Colorado also found an association between prolonged exposures to chlorinated surface water and increased bladder cancer risk in men and women for both smokers and nonsmokers (McGeehin *et al.*, 1993). An elevation in brain cancer risk was also associated with exposure to chlorinated surface water (Cantor *et al.*, 1999).

Dichloroacetate is listed as likely to be carcinogenic in humans by the United States Environmental Protection Agency (USEPA, 2003). Based on sufficient evidence of carcinogenicity in experimental animals, dichloroacetate was listed by the International Agency for Research on Cancer (IARC, 2004) as possibly carcinogenic to humans (Group 2B).

GENETIC TOXICITY

Several studies have demonstrated genotoxicity of dihaloacetates. Both dibromoacetate and dichloroacetate induced DNA damage in the *Escherichia coli* SOS repair assay and both were mutagenic in *Salmonella typhimurium* strain TA100 in an Ames fluctuation test (Giller *et al.*, 1997). Dibromoacetate and dichloroacetate were also reported to be mutagenic in *S. typhimurium* strains TA98 and TA100 with and without metabolic activation (Kargalioglu *et al.*, 2002). In each of these strains, dibromoacetate was a more potent mutagen than dichloroacetate; in strain RSJ100, a derivative of TA1535 containing a rat GSTT1-1 gene, dichloroacetate was mutagenic, but dibromoacetate was not. Dibromoacetate also induced DNA strand breaks in Chinese hamster ovary cells as measured by the Comet assay (Plewa *et al.*, 2002); dichloroacetate was not genotoxic in this assay. Administration of dibromoacetate or bromochloroacetate in drinking water to male B6C3F₁ mice at concentrations of 0.1, 0.5, or 2.0 g/L for 3 weeks produced increases in the content of 8-hydroxydeoxyguanosine in liver nuclear DNA; this effect, indicative of oxidative DNA damage, was not seen after administration of dichloroacetate (Parrish *et al.*, 1996). A metabolite of dihaloacetate biotransformation, glyoxylate, is mutagenic in *S. typhimurium* strains TA97, TA100, and TA104 in the absence of S9; glyoxylate was mutagenic in strain TA102 in the presence of S9 (Sayato *et al.*, 1987).

Dichloroacetate induced prophage lambda in *E. coli* and mutations in *S. typhimurium* strain TA100 that were primarily GC→AT transitions (DeMarini *et al.*, 1994). An increase in mutant frequency was observed in the liver of transgenic male B6C3F₁ mice (harboring the bacterial *lacI* gene) that were exposed to 1.0 or 3.5 g dichloroacetate/L drinking water for 60 weeks (Leavitt *et al.*, 1997); mutations were primarily GC→AT transitions, as well as transitions and transversions at TA sites. In addition, dichloroacetate induced gene mutations and chromosome aberrations in L5178Y/TK^{+/-} mouse lymphoma cells (Harrington-Brock *et al.*, 1998). Male B6C3F₁ mice were exposed to dichloroacetate in drinking water at concentrations of 0.5, 1, 2, or 3.5 g/L for up to 31 weeks and evaluated for genotoxic effects in peripheral blood (Fusco *et al.*, 1996). Small but significant increases were observed in the frequencies of polychromatic and normochromatic erythrocytes; in addition, the authors suggested that dichloroacetate may also cause DNA crosslinking because DNA migration from leukocytes of exposed mice

was decreased in the Comet assay. In contrast to many of the above studies that indicate genotoxic activity of dichloroacetate, Fox *et al.* (1996) reported that sodium dichloroacetate did not induce mutations in *Salmonella*, *E. coli*, or L5178Y mouse lymphoma cells, did not induce chromosomal aberrations in Chinese hamster ovary cells, and did not induce micronuclei in rat bone marrow cells.

STUDY RATIONALE

Dibromoacetic acid was nominated to the NTP by the United States Environmental Protection Agency for toxicity and carcinogenicity studies in rats and mice because of widespread human exposure to this brominated water disinfection by-product and because a related dihaloacetate, dichloroacetate, was found to be carcinogenic to the liver of rats and mice. Drinking water was selected as the route of exposure to mimic human exposure to this chemical.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF DIBROMOACETIC ACID

Dibromoacetic acid was obtained from Fluka (Buchs, Switzerland) in one lot (46019/1 55196). Lot 46019/1 55196 was used in the 2-week, 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH) and by the study laboratory at Southern Research Institute (Birmingham, AL). Karl Fischer titration, elemental analysis, and melting point determination were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

Lot 46019/1 55196, a clumped white powder or moist white crystalline solid, was identified as dibromoacetic acid by the study laboratory using infrared spectroscopy and by the analytical chemistry laboratory using infrared, ultraviolet/visible, proton and carbon-13 nuclear magnetic resonance spectroscopy. The purity of lot 46019/1 55196 was determined by the analytical laboratory using functional group titration, ion chromatography, and high performance liquid chromatography (HPLC) and by the study laboratory using HPLC.

Karl Fischer titration indicated 0.27% water. Elemental analyses for carbon, hydrogen, and bromine were in agreement with the theoretical values for dibromoacetic acid. The melting point determination was slightly higher (38.6° C) than that given by the manufacturer's certificate of analysis and Materials Safety Data Sheet (32° to 38° C). Functional group titration indicated a purity of greater than 100% which was consistent with the manufacturer's certificate of analysis. Ion Chromatography (IC) indicated one major peak and two impurities with a combined area of 3.5% (3.2% and 0.27%). Using HPLC and standard addition, the 3.2% impurity seen by IC was found to have the same retention time as monobromoacetic acid (MBA) and was shown to be overestimated by IC. HPLC, with standard addition, indicated one major peak and one impurity with a relative peak area of 0.34%. The overall purity was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC. These studies indicated that dibromoacetic acid showed no degradation after 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed amber glass containers.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once during the 2-week studies, four times during the 3-month studies, and approximately every 2 weeks throughout the 2-year studies. The dose formulations were prepared by mixing dibromoacetic acid with tap water. Formulations were adjusted to pH 5 with 0.1 N sodium hydroxide and stored in sealed opaque glass or Nalgene[®] containers at 5° C for up to 42 days.

Homogeneity studies of 125 and 2,000 mg/mL formulations were conducted by the study laboratory and stability studies of a 10 µg/mL formulation (pH 5) were conducted by the analytical study laboratory using IC.

Homogeneity was confirmed, and stability was confirmed for up to 42 days when stored at 5° C in sealed opaque glass or Nalgene[®] containers, and at animal room conditions for up to 3 hours.

Periodic analyses of the dose formulations of dibromoacetic acid were conducted by the study laboratory using IC. During the 2-week studies, the dose formulations were analyzed once; all five of the dose formulations for rats and mice were within 10% of the target concentrations, with no value greater than 10% of the target concentration. Animal room samples of these dose formulations were also analyzed; all 23 samples analyzed were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 20 of the dose formulations used for rats and mice were within 10% of the target concentrations, with no value greater than 8% of the target concentration. Animal room samples of these dose formulations were also analyzed; 21 of 25 samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 7 to 8 weeks. All 34 of the dose

formulations analyzed were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; 23 of 24 samples were within 10% of the target concentrations.

2-WEEK STUDIES

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Animals were quarantined for 12 (mice) or 13 (rats) days and were 6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg dibromoacetic acid/L in the drinking water for 15 days. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded daily for rats and mice. Water consumption was recorded weekly for a 7-day period. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on the kidney, liver, lung, and testis of 0 and 2,000 mg/L rats and mice; these tissues were examined to a no-effect level in the remaining exposure groups.

3-MONTH STUDIES

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to dibromoacetic acid and to determine the appropriate exposure concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 weeks old. Rats were quarantined for 11 (males) or 12 (females) days and mice were quarantined for 13 (females) or 14 (males) days and were 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross

observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female rats and mice were exposed to 0, 125, 250, 500, 1,000, or 2,000 mg dibromoacetic acid/L in the drinking water for 3 months; groups of 10 male and 10 female special study rats and mice were exposed to the same concentrations for 4 weeks. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Water consumption was measured weekly for a 7-day period. 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.

Blood was collected from the retroorbital sinus of special study rats on days 4 and 21 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats) analyses. Animals were anesthetized with a CO₂/O₂ mixture. The parameters measured are listed in Table 1. Blood samples for hematology were placed in tubes containing EDTA. Erythrocyte, platelet, and leukocyte counts; hematocrit value; hemoglobin concentration; and mean cell volume, hemoglobin, and hemoglobin concentration were determined using a Technicon H-1™ (Bayer HealthCare LLC, Tarrytown, NY) with reagents from Bayer, Inc. (Tustin, CA), R&D Systems, Inc. (Minneapolis, MN), or Fisher Scientific (Norcross, GA). Reticulocytes were counted using a Coulter Model Elite XL Flow Cytometer (Coulter Corp., Miami, FL) with reagents supplied by the manufacturer or Molecular Probes (Eugene, OR). Samples for clinical chemistry analyses were placed in tubes with no anticoagulant. Samples were analyzed using a Hitachi 911 automated analyzer (Roche Diagnostics Corporation, Indianapolis, IN) with reagents from Boehringer Mannheim Biochemicals (Indianapolis, IN) or Sigma Chemical Co. (St. Louis, MO), except sorbitol dehydrogenase was measured using a Cobas Fara chemistry analyzer (Roche Diagnostics Corporation).

On day 22 and for 5 days thereafter, 0.4 mg/mL BrdU was added to the drinking water of groups of 10 special study rats and mice. These animals were weighed and then sacrificed by CO₂ asphyxiation on day 27 for cell proliferation studies. The liver was weighed and the brain, colon, duodenum, kidney, liver, and urinary bladder were collected and fixed in 10% neutral buffered formalin for 18 to 24 hours, embedded in paraffin, and shipped to NTP. The duodenum and liver from 0, 500, 1,000, and 2,000 mg/L rats and mice were shipped to Pathology Associates Division of Charles River Laboratories (Frederick, MD) for analyses of labeled and unlabeled hepatocytes to determine the BrdU labeling index. Tissue sections were incubated with a monoclonal antibody to BrdU (Becton Dickenson 1:100 for rats and Serotec 1:100 for mice) and reagents required for the avidin-biotin peroxidase (Vectasin ABC peroxidase kit, Vector Laboratories, Burlingame, CA) method for the detection of the antigen-antibody complex. BrdU incorporated into cells in S phase was localized by the chromagen 3,3'-diaminobenzidine tetrahydrochloride, and tissues were counterstained with hematoxylin. The percentage of nuclear-stained hepatocytes was scored in at least 3,000 hepatocytes in 10 fields per slide at 200×

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on 10 male and 10 female core study rats and mice exposed to 0, 500, 1,000, or 2,000 mg/L. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered

saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus of core study animals were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on 0 and 2,000 mg/L rats and mice. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats and mice were exposed to 0, 50, 500, or 1,000 mg dibromoacetic acid/L in the drinking water for 105 to 106 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F₁ mice were obtained from Taconic Farms, Inc. (Germantown, NY) for use in the 2-year studies. Rats and mice were quarantined for 14 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats 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 (Appendix L).

Animal Maintenance

Male rats were housed up to three per cage, female rats and mice were housed five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated twice weekly.

Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded at 4-week intervals beginning at week 5.

Water consumption was recorded weekly for the first 13 weeks, then for 7 days every 4 weeks. Body weights were recorded on day 1, weekly during the first 13 weeks, at 4-week intervals thereafter, and at terminal sacrifice.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 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 liver and lung of male and female rats and mice; the epididymis, kidney, mediastinal lymph node, mesentery, peritoneum, seminal vesicle, spleen, and testis of males rats; the kidney, pancreatic islets, spleen, uterus, and vagina of female rats; the bone marrow, eye, harderian gland,

kidney, mandibular lymph node, pancreatic islets, and parathyroid gland of male mice; and the mesenteric lymph node, pancreatic islets, spleen, and thymus of 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 Dibromoacetic Acid

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|--|--|--|
| Study Laboratory Southern Research Institute (Birmingham, AL) | 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 | F344/N rats B6C3F ₁ mice |
| Animal Source Taconic Farms, Inc. (Germantown, NY) | Taconic Farms, Inc. (Germantown, NY) | Taconic Farms, Inc. (Germantown, NY) |
| Time Held Before Studies Rats: 13 days Mice: 12 days | Rats: 11 (males) or 12 (females) days Mice: 14 (males) or 13 (females) days | 14 days |
| Average Age When Studies Began 6 weeks | 6 weeks | 6 weeks |
| Date of First Exposure Rats: November 25, 1998 Mice: November 24, 1998 | Rats: February 15 (males) or 16 (females), 1999 Mice: February 17 (females) or 18 (males), 1999 | Rats: March 8, 2000 Mice: February 23, 2000 |
| Duration of Exposure 15 days | 14 weeks | 105 to 106 weeks |
| Date of Last Exposure Rats: December 9, 1998 Mice: December 8, 1999 | Rats: May 17 (males) or 18 (females), 1999 Mice: May 19 (females) or 20 (males), 1999 | Rats: March 14, 2002 Mice: February 28, 2002 |
| Necropsy Dates December 9, 1998 | Rats: May 17 (males) or 18 (females), 1999 Mice: May 19 (females) or 20 (males), 1999 | Rats: March 6-14, 2002 Mice: February 20-28, 2002 |
| Average Age at Necropsy 8 weeks | 19 weeks | 110 to 111 weeks |
| Size of Study Groups 5 males and 5 females | Core: 10 males and 10 females Special Study: 10 males and 10 females | 50 males and 50 females |
| Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights. | Same as 2-week studies | Same as 2-week studies |
| Animals per Cage Rats: 5 Mice: 1 (males); 5 (females) | Rats: 5 Mice: 1 (males); 5 (females) | Rats: 3 (males); 5 (females) Mice: 1 (males); 5 (females) |
| Method of Animal Identification Tail tattoo | Tail tattoo | Tail tattoo |

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

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|--|--|--|
| Diet | | |
| Irradiated NTP-2000 wafer rodent feed (Zeigler Brothers, Inc., Gardners, PA), available <i>ad libitum</i> | Same as 2-week studies | Same as 2-week studies |
| Water | | |
| Tap water (Birmingham, AL, municipal water supply) provided in amber glass bottles (Wheaton, Millville, NJ) with Teflon®-lined caps and stainless steel sipper tubes (Allentown Caging, Allentown, NJ), available <i>ad libitum</i> , changed twice weekly | Same as 2-week studies | Same as 2-week studies |
| Cages | | |
| Solid bottom polycarbonate (Lab Products, Inc., Maywood, NJ) changed once (male mice) or twice weekly | Same as 2-week studies | Same as 2-week studies |
| Bedding | | |
| Heat-treated irradiated hardwood chips (P.J. Murphy Forest Products, Inc.; Montville, NJ); changed with cage once (male mice) or twice weekly | Same as 2-week studies | Same as 2-week studies |
| Cage Filters | | |
| Reemay® spun-bonded polyester (Andico, Birmingham, AL) changed every 2 weeks | Same as 2-week studies | Same as 2-week studies |
| Racks | | |
| Stainless steel (Lab Products, Inc., Maywood, NJ) changed every 2 weeks | Same as 2-week studies | Same as 2-week studies |
| Animal Room Environment | | |
| Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour | Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour | Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room/Chamber air changes: 10/hour |
| Exposure Concentrations | | |
| 0, 125, 250, 500, 1,000, or 2,000 mg/L in drinking water | 0, 125, 250, 500, 1,000, or 2,000 mg/L in drinking water | 0, 50, 500, or 1,000 mg/L in drinking water |
| Type and Frequency of Observation | | |
| Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily. Water consumption was recorded weekly. | Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings were recorded weekly. Water consumption was recorded weekly. | Observed twice daily; animals were weighed initially, weekly for the first 13 weeks, at 4-week intervals thereafter, and at the end of the studies; clinical findings were recorded at 4-week intervals beginning at week 5. Water consumption was recorded weekly for the first 13 weeks, and every 4 weeks thereafter. |

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

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|--|---|--|
| <p>Method of Sacrifice CO₂ asphyxiation</p> | <p>CO₂ asphyxiation</p> | <p>CO₂ asphyxiation</p> |
| <p>Necropsy Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.</p> | <p>Necropsies were performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.</p> | <p>Necropsies were performed on all animals.</p> |
| <p>Clinical Pathology None</p> | <p>Blood was collected from the retroorbital sinus of special study rats on days 4 and 21 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats). Hematology: hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials Clinical chemistry: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids</p> | <p>None</p> |
| <p>Histopathology Microscopic examinations were performed on 0 and 2,000 mg/L animals. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: kidney, liver, lung, testis, and thymus.</p> | <p>Complete histopathologic examinations were performed on core study animals exposed to 0 or 2,000 mg/L. In addition to gross lesions and tissue masses, the following tissues were examined to a no-effect level: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, gallbladder (mice), heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.</p> | <p>Complete histopathologic examinations were performed on all animals. 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 and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.</p> |

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

| 2-Week Studies | 3-Month Studies | 2-Year Studies |
|--|---|----------------|
| <p>Sperm Motility and Vaginal Cytology None</p> | <p>At the end of the studies, sperm samples were collected from core study male animals in the 0, 500, 1,000, and 2,000 mg/L groups for sperm count and motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from core study female animals in the 0, 500, 1,000, and 2,000 mg/L groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.</p> | None |
| <p>Cell Proliferation None</p> | <p>From days 22 to 27, BrdU was added to the drinking water of special study rats and mice. On day 27, the livers of these animals were weighed, and the brain, colon, duodenum, kidney, liver, and urinary bladder were collected and fixed. The duodenum and liver of 0, 500, 1,000, and 2,000 mg/L rats and mice were evaluated for BrdU-labeled and unlabeled cells.</p> | None |

STATISTICAL METHODS

Survival Analyses

The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes 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, A5, B1, B5, C1, C5, D1, and D5 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, B3, C3, and D3) 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, B3, C3, and D3 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 k th 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/N rats and B6C3F₁ mice (Portier *et al.*, 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each 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, clinical chemistry, spermatid, and epididymal spermatozoal data, which have

typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973). Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. The BrdU labeling index was analyzed for significance using the Student's *t*-test (two sided, unequal variance).

Historical Control Data

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

QUALITY ASSURANCE METHODS

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of dibromoacetic acid was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and increases in the frequency of micronucleated erythrocytes in peripheral blood of exposed mice. The protocols for these studies and the results are given in Appendix E.

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

DNA reactivity combined with *Salmonella* mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the *Salmonella* test was shown to be the most predictive *in vitro* indicator for rodent carcinogenicity (89% of the

Salmonella mutagens are rodent carcinogens) (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). Additionally, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.*, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (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

2-WEEK STUDY

All rats survived to the end of the study (Table 2). Final mean body weights of male and female rats were similar to those of the controls; mean body weight gains of 1,000 mg/L males and of 500 mg/L females were significantly greater than those of the controls. Water consumption by exposed and control groups was similar. Drinking water concentrations of 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 17, 31, 67, 134, and 270 mg dibromoacetic acid/kg body weight to males and 17, 33, 67, 135, and 257 mg/kg body weight to females. There were no clinical findings related to dibromoacetic acid exposure.

Liver weights of all exposed groups of males and females were significantly increased (Table G1). Absolute and relative heart weights of male rats in the 2,000 mg/L group and relative heart weights of all other groups of males and females exposed to 500 mg/L or greater were significantly less than those of the controls. Right testis weights of males exposed to 500 mg/L or greater were significantly decreased.

Histopathologically, testicular lesions were noted in the 500 and 1,000 mg/L groups. The lesions were characterized by delays in spermiation. Step 19 spermatids were retained and found in the outermost part of seminiferous epithelium adjacent to the lumen in stages IX and X of the spermatogenic cycle. These changes were occasionally accompanied by large residual bodies. The overall severity of the lesions was mild. In the 1,000 mg/L group, all five animals had delayed spermiation with retained spermatids. In the 500 mg/L group, four animals had delayed spermiation with retained spermatids, and a single animal was without abnormalities. No abnormalities were observed in the 250 mg/L group.

TABLE 2
Survival, Body Weights, and Water Consumption of Rats
in the 2-Week Drinking Water Study of Dibromoacetic Acid

| 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 |
| Male | | | | | | | |
| 0 | 5/5 | 102 ± 3 | 161 ± 4 | 58 ± 2 | | 14.5 | 14.8 |
| 125 | 5/5 | 101 ± 4 | 169 ± 6 | 68 ± 3 | 105 | 15.4 | 15.4 |
| 250 | 5/5 | 104 ± 4 | 167 ± 8 | 62 ± 4 | 104 | 14.3 | 14.5 |
| 500 | 5/5 | 100 ± 3 | 167 ± 5 | 67 ± 2 | 104 | 15.2 | 15.1 |
| 1,000 | 5/5 | 102 ± 4 | 171 ± 4 | 69 ± 3* | 106 | 15.6 | 15.3 |
| 2,000 | 5/5 | 101 ± 3 | 166 ± 5 | 65 ± 3 | 103 | 15.2 | 15.6 |
| Female | | | | | | | |
| 0 | 5/5 | 90 ± 3 | 120 ± 3 | 30 ± 2 | | 12.1 | 11.8 |
| 125 | 5/5 | 86 ± 1 | 121 ± 2 | 35 ± 3 | 101 | 12.9 | 12.2 |
| 250 | 5/5 | 93 ± 2 | 129 ± 2 | 36 ± 1 | 107 | 13.6 | 12.7 |
| 500 | 5/5 | 87 ± 3 | 125 ± 3 | 38 ± 1* | 104 | 13.5 | 12.3 |
| 1,000 | 5/5 | 84 ± 2 | 118 ± 4 | 34 ± 2 | 98 | 13.0 | 11.8 |
| 2,000 | 5/5 | 89 ± 3 | 124 ± 4 | 35 ± 1 | 103 | 12.8 | 12.0 |

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

^a Number of animals surviving at 2 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.

The incidences of minimal, diffuse hepatocytic cytoplasmic alteration were significantly increased in males exposed to 500 mg/L or greater and in 2,000 mg/L females (males: 0 mg/L, 0/5; 125 mg/L, 0/5; 250 mg/L, 2/5; 500 mg/L, 5/5; 1,000 mg/L, 5/5; 2,000 mg/L, 5/5; females: 0 mg/L, 0/5; 1,000 mg/L, 0/5; 2,000 mg/L, 5/5). Affected hepatocytes were considered minimally enlarged, granular or vacuolated (0% to 25% greater than normal). The hepatocellular change caused the appearance of tightly packed cells with resulting compression of sinusoids. The cytoplasm of affected cells appeared pale and stained lightly eosinophilic. The hepatocellular cytoplasm in control rats was characterized by a more homogenous and darker eosinophilic staining appearance.

Exposure Concentration Selection Rationale: Based on the lack of lethality, clinical signs of toxicity, water consumption changes, and body weight changes, dibromoacetic acid exposure concentrations selected for the 3-month drinking water study in rats were 125, 250, 500, 1,000, and 2,000 mg/L. The 2,000 mg/L concentration provides a dose of dibromoacetic acid that has been reported to cause decreases in body weight gain and neurologic effects in rats with exposures longer than 2 weeks.

3-MONTH STUDY

All rats survived to the end of the study (Table 3). Final mean body weights and body weight gains of male and female rats in the 2,000 mg/L groups were significantly less than those of the controls. Water consumption by 2,000 mg/L males and females was less than that by controls at weeks 1 and 13. Water consumption by all exposed groups of females at week 13 was generally less than that by controls. Drinking water concentrations of 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 10, 20, 40, 90, and 166 mg dibromoacetic acid/kg body weight to males and 12, 23, 48, 93, and 181 mg/kg to females. There were no clinical findings related to dibromoacetic acid exposure.

TABLE 3
Survival, Body Weights, and Water Consumption of Rats
in the 3-Month Drinking Water Study of Dibromoacetic Acid

| Concentration (mg/L) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Water Consumption ^c | |
|-------------------------|-----------------------|-----------------------------------|-----------|-----------|--|-----------------------------------|---------|
| | | Initial | Final | Change | | Week 1 | Week 13 |
| Male | | | | | | | |
| 0 | 10/10 | 82 ± 1 | 327 ± 5 | 245 ± 4 | | 14.1 | 16.4 |
| 125 | 10/10 | 80 ± 1 | 334 ± 6 | 253 ± 6 | 102 | 14.1 | 15.3 |
| 250 | 10/10 | 82 ± 2 | 355 ± 4 | 253 ± 4 | 103 | 14.7 | 16.1 |
| 500 | 10/10 | 82 ± 1 | 334 ± 4 | 253 ± 4 | 102 | 15.1 | 16.5 |
| 1,000 | 10/10 | 83 ± 1 | 334 ± 6 | 251 ± 6 | 102 | 16.5 | 16.5 |
| 2,000 | 10/10 | 81 ± 2 | 298 ± 3** | 217 ± 3** | 91 | 13.1 | 15.7 |
| Female | | | | | | | |
| 0 | 10/10 | 78 ± 1 | 192 ± 3 | 114 ± 2 | | 13.5 | 13.2 |
| 125 | 10/10 | 79 ± 2 | 190 ± 3 | 111 ± 2 | 99 | 13.3 | 12.7 |
| 250 | 10/10 | 77 ± 1 | 190 ± 2 | 113 ± 2 | 99 | 12.8 | 11.9 |
| 500 | 10/10 | 76 ± 2 | 190 ± 3 | 114 ± 4 | 99 | 14.3 | 12.5 |
| 1,000 | 10/10 | 76 ± 1 | 188 ± 3 | 111 ± 3 | 98 | 14.2 | 11.6 |
| 2,000 | 10/10 | 77 ± 1 | 169 ± 2** | 92 ± 2** | 88 | 12.6 | 11.4 |

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test

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

^b Weights and weight changes are given as mean ± standard error.

^c Water consumption is expressed as grams per animal per day.

Liver weights of all exposed groups of males and females were significantly increased (Table G2). Kidney weights of males and females exposed to 500 mg/L or greater were significantly increased. Heart weights of male and female rats in the 2,000 mg/L groups were decreased. Male rats in the 2,000 mg/L group had significantly decreased testis weights. Lung weights were decreased in the 2,000 mg/L females, and thymus weights were decreased in 2,000 mg/L males and in 1,000 and 2,000 mg/L females.

Hematology and clinical chemistry data are listed in Tables 4 and F1. There were changes in the erythron that would be consistent with a very minimal ineffective erythropoiesis response in male and female rats. The erythron alterations were generally characterized by decreases in hematocrit, hemoglobin, mean cell volume, and mean cell hemoglobin values. These changes were small (~8% or less decrease) and occurred in the 1,000 and 2,000 mg/L male and female groups at multiple time points. In males, these alterations were transient and most consistently occurred in the 2,000 mg/L group on day 21; by week 13, there were no differences in the erythron variables between exposed and control animals. In females, the decreases occurred on day 21 and, though ameliorated, were also present at week 14. There were no changes in red blood cell counts, which suggests that the treatment effect on erythropoiesis resulted in a minimal decrease in erythrocyte size but not the number of circulating red blood cells and was not deemed clinically significant.

In the 2,000 mg/L male and female rats, a small decrease in platelet counts (~15% or less decrease) occurred on day 21 (males) and at week 14 (males and females). The resulting platelet counts suggested either a small effect on platelet production, release, or peripheral distribution and were not considered clinically significant.

In the female rats, there were changes in the leukon that would generally be consistent with a physiological stress- or steroid-induced response; the changes were characterized by decreases in white blood cell and lymphocyte counts (Table F1). These changes were not dose-related, were mild (~20% decrease), and occurred in the 1,000 and 2,000 mg/L female groups at week 14.

TABLE 4
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study
of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|------------------------------------|--------------|--------------|--------------|--------------|--------------|----------------|
| Male | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 9 |
| Hematocrit (%) | | | | | | |
| Day 4 | 45.3 ± 0.8 | 42.5 ± 0.9 | 44.2 ± 0.7 | 43.7 ± 0.5 | 45.1 ± 0.9 | 45.7 ± 0.6 |
| Day 21 | 46.2 ± 0.7 | 45.5 ± 0.6 | 45.6 ± 0.6 | 45.3 ± 0.5 | 45.5 ± 0.6 | 42.8 ± 0.6** |
| Week 14 | 46.8 ± 0.3 | 45.7 ± 0.5 | 45.4 ± 0.6 | 45.3 ± 0.5* | 45.5 ± 0.3 | 45.3 ± 0.3 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 14.7 ± 0.3 | 13.9 ± 0.3 | 14.5 ± 0.2 | 14.3 ± 0.2 | 14.7 ± 0.3 | 15.1 ± 0.2 |
| Day 21 | 15.4 ± 0.2 | 15.0 ± 0.2 | 15.2 ± 0.2 | 15.0 ± 0.2 | 15.0 ± 0.2 | 14.2 ± 0.2** |
| Week 14 | 15.4 ± 0.1 | 15.1 ± 0.2 | 15.0 ± 0.1* | 15.1 ± 0.1 | 15.1 ± 0.1 | 15.0 ± 0.1 |
| Erythrocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 7.30 ± 0.12 | 6.90 ± 0.15 | 7.20 ± 0.11 | 7.11 ± 0.11 | 7.49 ± 0.16 | 7.66 ± 0.12 |
| Day 21 | 7.65 ± 0.13 | 7.49 ± 0.11 | 7.49 ± 0.10 | 7.42 ± 0.06 | 7.63 ± 0.10 | 7.26 ± 0.11 |
| Week 14 | 9.08 ± 0.08 | 8.86 ± 0.10 | 8.78 ± 0.11 | 8.80 ± 0.09 | 8.85 ± 0.07 | 8.89 ± 0.06 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 62.0 ± 0.3 | 61.6 ± 0.3 | 61.5 ± 0.4 | 61.4 ± 0.2 | 60.3 ± 0.5* | 59.7 ± 0.2** |
| Day 21 | 60.5 ± 0.3 | 60.8 ± 0.5 | 60.9 ± 0.4 | 61.1 ± 0.4 | 59.7 ± 0.3 | 59.0 ± 0.3* |
| Week 14 | 51.6 ± 0.2 | 51.5 ± 0.2 | 51.7 ± 0.1 | 51.5 ± 0.2 | 51.5 ± 0.2 | 50.9 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 20.1 ± 0.1 | 20.1 ± 0.1 | 20.1 ± 0.1 | 20.0 ± 0.1 | 19.7 ± 0.2 | 19.8 ± 0.1* |
| Day 21 | 20.1 ± 0.1 | 20.1 ± 0.2 | 20.4 ± 0.1 | 20.3 ± 0.1 | 19.7 ± 0.1* | 19.6 ± 0.1** |
| Week 14 | 17.0 ± 0.1 | 17.0 ± 0.1 | 17.1 ± 0.1 | 17.2 ± 0.1 | 17.0 ± 0.1 | 16.9 ± 0.1 |
| Platelets (10 ³ /μL) | | | | | | |
| Day 4 | 875.8 ± 15.7 | 920.8 ± 25.7 | 909.1 ± 13.9 | 939.0 ± 26.3 | 868.7 ± 18.5 | 828.5 ± 24.5 |
| Day 21 | 869.2 ± 18.6 | 883.7 ± 13.5 | 859.6 ± 17.0 | 894.3 ± 19.7 | 836.8 ± 9.9 | 742.2 ± 14.8** |
| Week 14 | 636.1 ± 9.9 | 629.0 ± 14.2 | 634.7 ± 12.6 | 646.5 ± 8.9 | 610.8 ± 8.5 | 572.2 ± 12.3** |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.5 ± 0.1 | 5.3 ± 0.1 | 5.3 ± 0.1 | 5.4 ± 0.1 | 5.2 ± 0.0* | 4.9 ± 0.1** |
| Day 21 | 6.3 ± 0.1 | 6.5 ± 0.1 | 6.6 ± 0.1* | 6.5 ± 0.1 | 6.5 ± 0.1 | 6.0 ± 0.1 |
| Week 14 | 6.7 ± 0.0 | 6.8 ± 0.1 | 6.9 ± 0.1* | 6.9 ± 0.1* | 7.0 ± 0.1** | 6.9 ± 0.1** |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.0 ± 0.1 | 3.9 ± 0.1 | 4.0 ± 0.1 | 4.0 ± 0.1 | 3.9 ± 0.0 | 3.8 ± 0.1* |
| Day 21 | 4.5 ± 0.0 | 4.7 ± 0.1 | 4.7 ± 0.1* | 4.7 ± 0.1 | 4.7 ± 0.1* | 4.5 ± 0.1 |
| Week 14 | 4.8 ± 0.0 | 4.9 ± 0.1* | 5.1 ± 0.1** | 5.0 ± 0.1** | 5.1 ± 0.0** | 5.2 ± 0.1** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 745 ± 17 | 754 ± 14 | 769 ± 25 | 805 ± 22* | 818 ± 17* | 788 ± 17* |
| Day 21 | 556 ± 8 | 509 ± 11** | 515 ± 16* | 516 ± 11* | 504 ± 19** | 479 ± 7** |
| Week 14 | 200 ± 10 | 182 ± 4 | 201 ± 10 | 174 ± 9 | 186 ± 6 | 192 ± 6 |
| Bile acids (μmol/L) | | | | | | |
| Day 4 | 34.9 ± 3.1 | 33.9 ± 2.2 | 35.9 ± 2.2 | 38.0 ± 2.7 | 52.8 ± 3.3** | 53.2 ± 2.9** |
| Day 21 | 24.9 ± 1.5 | 26.2 ± 1.2 | 31.7 ± 1.4** | 26.0 ± 0.9 | 29.0 ± 1.7 | 24.2 ± 1.1 |
| Week 14 | 20.4 ± 2.1 | 16.6 ± 1.7 | 13.6 ± 0.7* | 18.5 ± 1.7 | 17.8 ± 1.9 | 16.9 ± 1.5 |

TABLE 4
Selected Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|------------------------------------|--------------|--------------|--------------|--------------|--------------|----------------|
| Female | | | | | | |
| Hematology | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 9 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | | | | | | |
| Day 4 | 43.5 ± 0.6 | 44.5 ± 0.5 | 45.1 ± 0.7 | 45.8 ± 1.2 | 45.5 ± 0.7 | 46.6 ± 1.0 |
| Day 21 | 46.2 ± 0.3 | 45.4 ± 0.4 | 44.9 ± 0.5 | 46.8 ± 0.7 | 44.2 ± 0.4** | 43.4 ± 0.8** |
| Week 14 | 42.4 ± 0.3 | 42.9 ± 0.4 | 42.7 ± 0.3 | 42.4 ± 0.5 | 41.5 ± 0.5 | 40.9 ± 0.5 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 14.3 ± 0.2 | 14.6 ± 0.2 | 14.8 ± 0.3 | 15.2 ± 0.4 | 15.1 ± 0.3 | 15.4 ± 0.3 |
| Day 21 | 15.6 ± 0.1 | 15.3 ± 0.2 | 15.3 ± 0.2 | 15.7 ± 0.2 | 15.0 ± 0.2* | 14.7 ± 0.2** |
| Week 14 | 13.7 ± 0.1 | 13.9 ± 0.1 | 13.9 ± 0.1 | 13.8 ± 0.1 | 13.5 ± 0.2 | 13.2 ± 0.1* |
| Erythrocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 7.53 ± 0.11 | 7.71 ± 0.12 | 7.76 ± 0.15 | 7.96 ± 0.19 | 7.90 ± 0.14 | 8.23 ± 0.16** |
| Day 21 | 7.98 ± 0.07 | 7.77 ± 0.06 | 7.70 ± 0.10 | 8.08 ± 0.13 | 7.73 ± 0.13 | 7.79 ± 0.15 |
| Week 14 | 8.07 ± 0.05 | 8.11 ± 0.05 | 8.13 ± 0.05 | 8.13 ± 0.10 | 8.06 ± 0.09 | 7.97 ± 0.08 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 57.8 ± 0.3 | 57.8 ± 0.4 | 58.2 ± 0.3 | 57.5 ± 0.2 | 57.7 ± 0.3 | 56.7 ± 0.5 |
| Day 21 | 57.9 ± 0.3 | 58.4 ± 0.5 | 58.4 ± 0.2 | 58.0 ± 0.5 | 57.2 ± 0.6 | 55.7 ± 0.3** |
| Week 14 | 52.5 ± 0.2 | 53.0 ± 0.2 | 52.6 ± 0.2 | 52.1 ± 0.2 | 51.5 ± 0.2* | 51.3 ± 0.2** |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 18.7 ± 0.1 |
| Day 21 | 19.6 ± 0.1 | 19.7 ± 0.1 | 19.9 ± 0.1 | 19.5 ± 0.1 | 19.4 ± 0.1 | 18.9 ± 0.1** |
| Week 14 | 17.0 ± 0.1 | 17.1 ± 0.1 | 17.2 ± 0.1 | 17.0 ± 0.1 | 16.7 ± 0.1* | 16.6 ± 0.1* |
| Platelets (10 ³ /μL) | | | | | | |
| Day 4 | 766.0 ± 19.4 | 802.3 ± 23.6 | 800.1 ± 24.8 | 794.9 ± 25.8 | 820.2 ± 21.1 | 744.6 ± 12.5 |
| Day 21 | 722.9 ± 19.2 | 815.3 ± 24.9 | 803.8 ± 14.8 | 806.1 ± 25.1 | 754.4 ± 22.4 | 705.3 ± 19.8 |
| Week 14 | 690.2 ± 15.6 | 661.5 ± 10.5 | 689.0 ± 15.4 | 674.7 ± 10.7 | 660.1 ± 13.9 | 608.2 ± 10.9** |
| Clinical Chemistry | | | | | | |
| n | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.6 ± 0.1 | 5.6 ± 0.1 | 5.7 ± 0.1 | 5.6 ± 0.1 | 5.3 ± 0.1 | 5.2 ± 0.1* |
| Day 21 | 6.2 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.6 ± 0.1* | 6.7 ± 0.1** | 6.4 ± 0.1* |
| Week 14 | 6.7 ± 0.1 | 7.1 ± 0.1* | 7.4 ± 0.1** | 7.3 ± 0.1** | 7.5 ± 0.1** | 7.4 ± 0.1** |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.1 ± 0.1 | 4.3 ± 0.1 | 4.3 ± 0.1 | 4.3 ± 0.1 | 4.0 ± 0.1 | 4.0 ± 0.1 |
| Day 21 | 4.6 ± 0.1 | 4.8 ± 0.1* | 4.8 ± 0.1* | 4.9 ± 0.1** | 5.1 ± 0.1** | 5.0 ± 0.1** |
| Week 14 | 5.2 ± 0.2 | 5.6 ± 0.1* | 5.9 ± 0.1** | 5.8 ± 0.1** | 6.0 ± 0.1** | 5.9 ± 0.1** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 550 ± 13 | 596 ± 24 | 582 ± 15 | 642 ± 25** | 674 ± 31** | 613 ± 26** |
| Day 21 | 374 ± 10 | 355 ± 8 | 341 ± 12* | 356 ± 10 | 317 ± 9** | 302 ± 10** |
| Week 14 | 165 ± 8 | 162 ± 7 | 162 ± 6 | 152 ± 5 | 149 ± 5 | 142 ± 6* |
| Bile acids (μmol/L) | | | | | | |
| Day 4 | 21.5 ± 2.0 | 22.9 ± 2.2 | 25.1 ± 2.1 | 25.9 ± 1.9 | 32.9 ± 3.3** | 44.8 ± 4.8** |
| Day 21 | 24.0 ± 2.4 | 17.5 ± 0.9* | 20.3 ± 1.2 | 20.4 ± 1.6 | 18.2 ± 0.9 | 18.1 ± 0.7 |
| Week 14 | 22.9 ± 2.1 | 25.6 ± 2.9 | 20.7 ± 2.7 | 22.5 ± 2.8 | 23.0 ± 3.1 | 24.9 ± 2.8 |

* 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.

On day 4, there were increases in serum alkaline phosphatase activities and bile acid concentrations in male and female rats. Alkaline phosphatase activity was increased in a nondose-related manner in the 500 mg/L or greater males and females; bile acid concentrations were increased in the 1,000 and 2,000 mg/L groups. The increases in these variables were transient, and on day 21, they had either disappeared or been replaced by decreases. For example, alkaline phosphatase activities were decreased by less than 20% in exposed male and female animals on day 21. The increases observed for alkaline phosphatase activity and bile acid concentration on day 4 were consistent with an acute cholestatic event that was transient and abrogated on day 21; the mechanism for this effect is unknown.

On day 4, there were small decreases (~10% or less) in serum albumin and/or total protein concentration in 2,000 mg/L male and female groups. This was a transient finding, which was replaced by increased albumin and/or total protein concentration values on day 21. By week 14, significantly increased concentrations (at ~15% or less) occurred in all exposed groups of male and female rats. The increased total protein concentration was probably related to the increased albumin concentration and, since, there are no known conditions in which increased albumin production occurs, it would be consistent with a physiological dehydration-type response.

On day 21 and at week 14, decreases in alanine aminotransferase activity occurred in exposed male and female animals, which could suggest an altered enzyme metabolism or inhibition. Other scattered changes in the hematological and clinical chemistry variables occurred but were not considered toxicologically relevant.

Cell proliferation in the liver of 1,000 and 2,000 mg/L male rats, but not in female rats, was significantly greater than in the controls (Table 5). Eight of 10 males in the 1,000 mg/L group had labeling indices greater than the highest individual control value (data not shown). The biological significance of this difference is not clear since the increases were not dose-related and increases in liver weights were the same for male and female rats.

TABLE 5
BrdU Labeling Index in the Liver of Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--------------------|-------------|--------------------------|--------------------------|--------------|
| Male | | | | |
| Labeling Index (%) | 0.20 ± 0.10 | 1.07 ± 1.29 | 0.48 ± 0.26** | 0.77 ± 0.48* |
| Female | | | | |
| Labeling Index (%) | 0.93 ± 0.37 | 0.83 ± 0.40 ^b | 1.16 ± 0.71 ^b | 0.88 ± 0.66 |

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test

** $P \leq 0.01$

^a Data are presented as mean ± standard deviation (n=10).

^b n=9

Treatment-related histopathological changes were noted in the liver, pituitary gland, epididymis, testes, and spleen. Although treatment-related organ weight changes were noted in the kidney, heart, lung, and thymus, no corresponding microscopic changes were noted.

Testicular atrophy was noted in the 2,000 mg/L group, and retained spermatids were observed in the 500 and 1,000 mg/L groups; 200 mg/L was the no-observed-effect level (NOEL) for testicular lesions (Table 6; Plates 1 and 2). Testicular atrophy was characterized by marked degeneration/loss of germinal epithelium, giant/syncytial cell formation, vacuolation of Sertoli cells, mild nonsuppurative inflammation around some venules, and more prominent interstitial cells. The testicular atrophy correlated with the decreased right testis weight noted in this group. Gradation of the testicular lesions was as follows: minimal (grade 1), depletion of approximately 5% or less of germinal epithelium; mild (grade 2), depletion of approximately 6% to 20% of germinal epithelium; moderate (grade 3), depletion of approximately 21% to 50% of germinal epithelium; marked (grade 4), depletion of approximately 51% or greater of germinal epithelium. Hypospermia was significantly increased in the epididymis, and sperm motility and spermatid heads were significantly reduced in 2,000 mg/L males (Tables 6 and H1).

Testicular changes seen in groups treated with 500 and 1,000 mg/L were similar to those seen in the 14-day study. The lesions were characterized by delays in spermiation. Step 19 spermatids were retained and found in the

TABLE 6
Incidences of Selected Nonneoplastic Lesions in Rats in the 3-Month Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--|---------|----------|----------|------------------------|------------|------------|
| Male | | | | | | |
| Testes ^a | 10 | 10 | 10 | 10 | 10 | 10 |
| Delayed Spermiation, Retained Spermatids ^b | 0 | 0 | 0 | 8** (1.6) ^c | 8** (2.0) | 0 |
| Germinal Epithelium, Atrophy | 0 | 0 | 0 | 0 | 0 | 10** (3.4) |
| Epididymis | 10 | 10 | 10 | 10 | 10 | 9 |
| Hypospermia | 0 | 0 | 0 | 0 | 0 | 9** (3.1) |
| Pituitary Gland | 10 | 10 | 10 | 10 | 10 | 10 |
| Pars Distalis, Hypertrophy | 1 (1.0) | 0 | 0 | 0 | 0 | 6* (1.3) |
| Liver | 10 | 10 | 10 | 10 | 10 | 10 |
| Hepatocyte, Vacuolization, Cytoplasmic | 3 (1.0) | 5 (1.0) | 7 (1.0) | 9* (1.3) | 9* (1.3) | 10** (2.0) |
| Female | | | | | | |
| Liver | 10 | 10 | 10 | 10 | 10 | 10 |
| Hepatocyte, Vacuolization, Cytoplasmic | 0 | 0 | 1 (1.0) | 0 | 3 (1.0) | 10** (1.5) |
| Spleen | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematopoietic Cell Proliferation | 0 | 0 | 0 | 0 | 0 | 4* (1.3) |

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

outermost part of the seminiferous epithelium adjacent to the lumen in stages IX and X of the spermatogenic cycle.

These changes were occasionally accompanied by large residual bodies. The overall severity of the lesions was mild.

In the pituitary gland of male rats exposed to 2,000 mg/L, cellular hypertrophy was noted (Table 6). This alteration was characterized by increased cytoplasmic pallor, enlargement, and vacuolation of the cells of the pituitary gland pars distalis. This change is considered to be secondary to the testicular atrophy. The hypertrophic cells are commonly called “castration cells” and were seen following orchietomy, considered to be due to hypertrophy of the FSH LH secreting cells (MacKenzie and Boorman, 1990).

In the liver, an exposure concentration-related increase in the centrilobular to midzonal glycogen-like hepatocellular vacuolization was noted in males in all exposed groups and in females in the 1,000 and 2,000 mg/L groups (Table 6). Involvement was characterized as minimal to mild.

In the spleen, minimal to mild hematopoietic cell proliferation was noted in females in the 2,000 mg/L group (Table 6). This change was characterized by minimal increases in the myeloid and erythroid precursors in the red pulp.

Exposure Concentration Selection Rationale: Based on decreased body weight gain, organ weight effects, and severity of testicular lesions observed at 2,000 mg/L, dibromoacetic acid exposure concentrations selected for the 2-year drinking water study in rats were 50, 500, and 1,000 mg/L.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 7 and in the Kaplan-Meier survival curves (Figure 2). Treatment with dibromoacetic acid had no effect on survival of male or female rats.

TABLE 7
Survival of Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|---------|-----------------|-----------------|------------|
| Male | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Accidental death ^a | 0 | 0 | 0 | 1 |
| Moribund | 4 | 15 | 12 | 12 |
| Natural deaths | 12 | 11 | 8 | 9 |
| Animals surviving to study termination | 34 | 24 | 30 ^c | 28 |
| Percent probability of survival at end of study ^b | 68 | 48 | 60 | 57 |
| Mean survival (days) ^c | 683 | 676 | 652 | 666 |
| Survival analysis ^d | P=0.784 | P=0.092 | P=0.406 | P=0.332 |
| Female | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 4 | 5 | 9 | 14 |
| Natural deaths | 11 | 6 ^f | 6 ^f | 4 |
| Animals surviving to study termination | 35 | 39 ^f | 35 ^f | 32 |
| Percent probability of survival at end of study | 70 | 78 | 70 | 64 |
| Mean survival (days) | 690 | 705 | 693 | 683 |
| Survival analysis | P=0.232 | P=0.427N | P=1.000N | P=0.674 |

^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 three animals that died during the last week of the study.

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

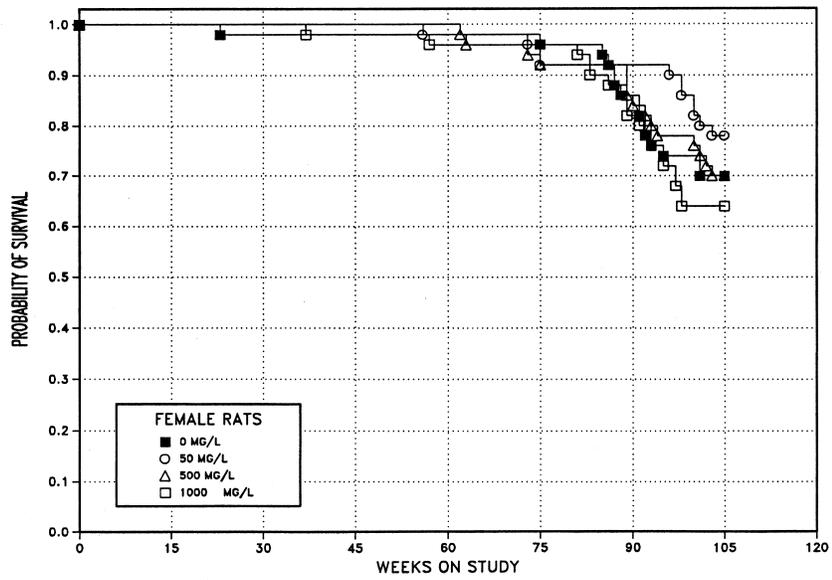
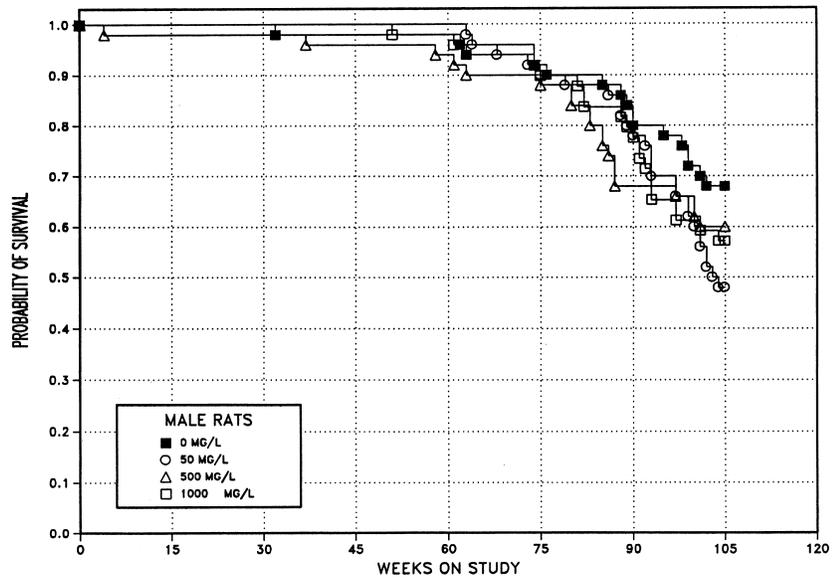


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 1,000 mg/L males and females were less than those of the controls after week 29 and week 49, respectively, and those of 500 mg/L males were less after week 57 (Figure 3; Tables 8 and 9). Water consumption by males and females exposed to 1,000 mg/L was less than that by controls during year 2 of the study (Tables J1 and J2). Drinking water concentrations of 50, 500, and 1,000 mg/L resulted in average daily doses of approximately 2, 20, and 40 mg/kg to males and 2, 25, and 45 mg/kg to females.

Toxicokinetics

After a single gavage dose of dibromoacetic acid (25, 50, or 125 mg/kg) to male and female F344/N rats, the plasma concentration versus time data can be described by a one-compartment model with no lag phase and first-order absorption and elimination for both males and females (Appendix N). A comparison of the absorption and elimination rate constants for all three gavage dose groups to the absorption rate constant following a single intravenous administration of 25 mg/kg indicated that the gavage profiles were characteristic of a flip-flop model, where the initial upward phase of the profile is a measure of elimination and the terminal linear phase actually reflects absorption. The elimination half-life was approximately 6 to 12 minutes. Calculated values for clearance, volume of distribution, and area under the plasma concentration versus time curves (AUC) extrapolated to infinity were not accurate due to the flip-flop kinetics; however, the $AUC_{0-1 \text{ hour}}$ values were not affected by extrapolation in the terminal linear phase. This parameter increased with increasing dose, and the increase was approximately dose-proportional. Bioavailability increased with dose and ranged from approximately 43% to 61% for males and 64% to 91% for females.

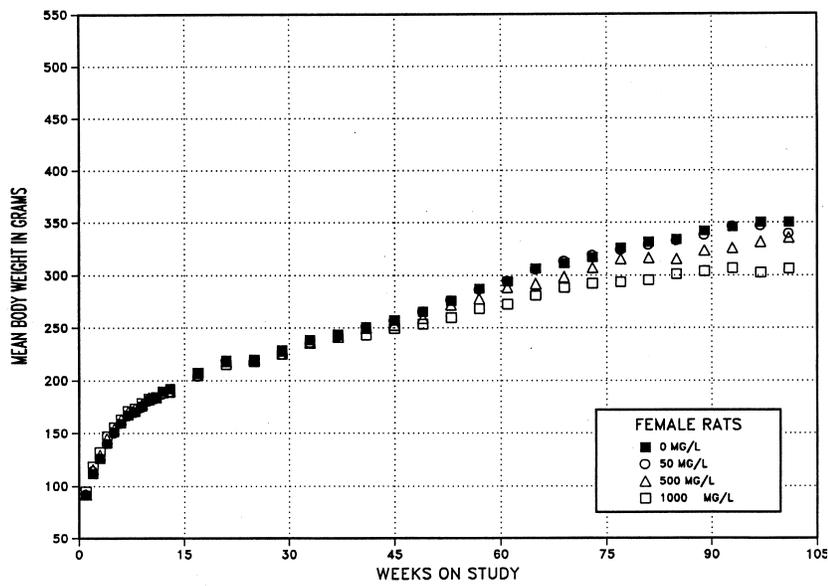
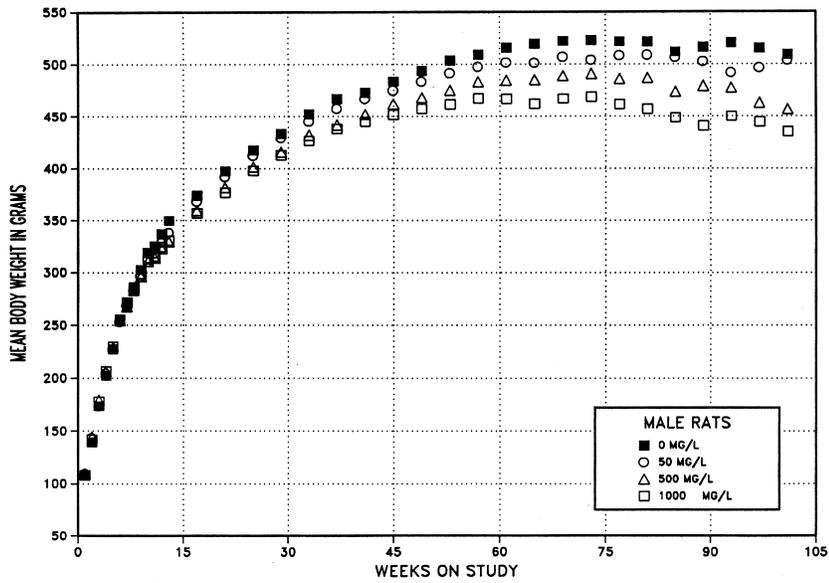


FIGURE 3
Growth Curves for Male and Female Rats Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

TABLE 8
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| Weeks on Study | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 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 | 108 | 50 | 110 | 102 | 50 | 110 | 102 | 50 | 108 | 101 | 50 |
| 2 | 140 | 50 | 143 | 103 | 50 | 145 | 104 | 50 | 142 | 102 | 50 |
| 3 | 174 | 50 | 173 | 100 | 50 | 179 | 103 | 50 | 178 | 102 | 50 |
| 4 | 203 | 50 | 205 | 101 | 50 | 206 | 102 | 49 | 206 | 102 | 50 |
| 5 | 227 | 50 | 227 | 100 | 50 | 229 | 101 | 49 | 230 | 101 | 50 |
| 6 | 254 | 50 | 253 | 99 | 50 | 255 | 100 | 49 | 255 | 100 | 50 |
| 7 | 271 | 50 | 268 | 99 | 50 | 268 | 99 | 49 | 272 | 100 | 50 |
| 8 | 286 | 50 | 283 | 99 | 50 | 283 | 99 | 49 | 285 | 100 | 50 |
| 9 | 303 | 50 | 296 | 98 | 50 | 296 | 98 | 49 | 299 | 99 | 50 |
| 10 | 319 | 50 | 314 | 98 | 50 | 311 | 97 | 49 | 313 | 98 | 50 |
| 11 | 325 | 50 | 319 | 98 | 50 | 314 | 97 | 49 | 316 | 97 | 50 |
| 12 | 337 | 50 | 329 | 98 | 50 | 323 | 96 | 49 | 325 | 96 | 50 |
| 13 | 350 | 50 | 338 | 97 | 50 | 330 | 94 | 49 | 331 | 95 | 50 |
| 17 | 374 | 50 | 368 | 99 | 50 | 360 | 96 | 49 | 357 | 96 | 50 |
| 21 | 398 | 50 | 392 | 99 | 50 | 382 | 96 | 49 | 377 | 95 | 50 |
| 25 | 418 | 50 | 413 | 99 | 50 | 401 | 96 | 49 | 398 | 95 | 50 |
| 29 | 434 | 50 | 430 | 99 | 50 | 416 | 96 | 49 | 413 | 95 | 50 |
| 33 | 452 | 49 | 445 | 99 | 50 | 432 | 96 | 49 | 427 | 94 | 50 |
| 37 | 467 | 49 | 458 | 98 | 50 | 442 | 95 | 49 | 438 | 94 | 50 |
| 41 | 473 | 49 | 467 | 99 | 50 | 452 | 96 | 48 | 445 | 94 | 50 |
| 45 | 484 | 49 | 475 | 98 | 50 | 462 | 96 | 48 | 452 | 94 | 50 |
| 49 | 494 | 49 | 484 | 98 | 50 | 468 | 95 | 48 | 457 | 93 | 50 |
| 53 | 503 | 49 | 491 | 98 | 50 | 475 | 94 | 48 | 462 | 92 | 49 |
| 57 | 509 | 49 | 498 | 98 | 50 | 483 | 95 | 48 | 467 | 92 | 48 |
| 61 | 516 | 49 | 501 | 97 | 50 | 485 | 94 | 47 | 467 | 91 | 48 |
| 65 | 519 | 47 | 501 | 97 | 48 | 485 | 93 | 45 | 462 | 89 | 47 |
| 69 | 522 | 47 | 507 | 97 | 47 | 489 | 94 | 45 | 467 | 90 | 47 |
| 73 | 523 | 47 | 504 | 96 | 47 | 491 | 94 | 45 | 469 | 90 | 47 |
| 77 | 522 | 45 | 508 | 98 | 45 | 486 | 93 | 44 | 462 | 89 | 44 |
| 81 | 522 | 45 | 509 | 98 | 44 | 487 | 93 | 42 | 457 | 88 | 43 |
| 85 | 512 | 45 | 506 | 99 | 44 | 474 | 93 | 40 | 449 | 88 | 41 |
| 89 | 516 | 43 | 503 | 97 | 41 | 479 | 93 | 34 | 441 | 85 | 40 |
| 93 | 521 | 40 | 492 | 95 | 38 | 477 | 92 | 34 | 450 | 87 | 35 |
| 97 | 515 | 39 | 496 | 96 | 35 | 463 | 90 | 34 | 445 | 86 | 32 |
| 101 | 509 | 36 | 504 | 99 | 30 | 457 | 90 | 31 | 435 | 86 | 30 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 254 | | 251 | 99 | | 250 | 99 | | 251 | 99 | |
| 14-52 | 444 | | 437 | 99 | | 424 | 96 | | 418 | 94 | |
| 53-101 | 516 | | 502 | 97 | | 479 | 93 | | 456 | 89 | |

TABLE 9
Mean Body Weights and Survival of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| Weeks on Study | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 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 | 92 | 50 | 92 | 101 | 50 | 92 | 101 | 50 | 95 | 104 | 50 |
| 2 | 112 | 50 | 114 | 102 | 50 | 116 | 104 | 50 | 119 | 106 | 50 |
| 3 | 126 | 50 | 126 | 100 | 50 | 130 | 103 | 50 | 132 | 105 | 50 |
| 4 | 140 | 50 | 140 | 100 | 50 | 145 | 103 | 50 | 147 | 105 | 50 |
| 5 | 152 | 50 | 150 | 99 | 50 | 154 | 102 | 50 | 156 | 103 | 50 |
| 6 | 160 | 50 | 159 | 100 | 50 | 163 | 102 | 50 | 163 | 102 | 50 |
| 7 | 167 | 50 | 166 | 99 | 50 | 170 | 102 | 50 | 171 | 102 | 50 |
| 8 | 171 | 50 | 170 | 99 | 50 | 174 | 102 | 50 | 173 | 102 | 50 |
| 9 | 176 | 50 | 174 | 99 | 50 | 178 | 101 | 50 | 179 | 102 | 50 |
| 10 | 181 | 50 | 181 | 100 | 50 | 184 | 102 | 50 | 183 | 101 | 50 |
| 11 | 185 | 50 | 183 | 99 | 50 | 184 | 100 | 50 | 184 | 98 | 50 |
| 12 | 190 | 50 | 187 | 98 | 50 | 189 | 99 | 50 | 189 | 99 | 50 |
| 13 | 192 | 50 | 189 | 98 | 50 | 190 | 99 | 50 | 189 | 98 | 50 |
| 17 | 207 | 50 | 204 | 99 | 50 | 205 | 99 | 50 | 207 | 100 | 50 |
| 21 | 219 | 50 | 219 | 100 | 50 | 219 | 100 | 50 | 215 | 98 | 50 |
| 25 | 220 | 49 | 218 | 99 | 50 | 220 | 100 | 50 | 218 | 99 | 50 |
| 29 | 229 | 49 | 225 | 98 | 50 | 228 | 100 | 50 | 225 | 98 | 50 |
| 33 | 239 | 49 | 235 | 99 | 50 | 236 | 99 | 50 | 236 | 99 | 50 |
| 37 | 244 | 49 | 242 | 99 | 50 | 242 | 99 | 50 | 241 | 99 | 49 |
| 41 | 251 | 49 | 251 | 100 | 50 | 250 | 100 | 50 | 243 | 97 | 49 |
| 45 | 258 | 49 | 257 | 100 | 50 | 252 | 98 | 50 | 249 | 97 | 49 |
| 49 | 265 | 49 | 265 | 100 | 50 | 259 | 98 | 50 | 254 | 96 | 49 |
| 53 | 276 | 49 | 276 | 100 | 50 | 272 | 99 | 50 | 260 | 94 | 49 |
| 57 | 287 | 49 | 286 | 100 | 49 | 278 | 97 | 50 | 268 | 93 | 49 |
| 61 | 294 | 49 | 295 | 100 | 49 | 288 | 98 | 50 | 272 | 93 | 48 |
| 65 | 306 | 49 | 305 | 100 | 49 | 292 | 95 | 48 | 281 | 92 | 48 |
| 69 | 312 | 49 | 313 | 101 | 49 | 298 | 96 | 48 | 288 | 93 | 48 |
| 73 | 317 | 49 | 319 | 101 | 49 | 307 | 97 | 47 | 292 | 92 | 48 |
| 77 | 326 | 48 | 324 | 99 | 46 | 316 | 97 | 46 | 294 | 90 | 48 |
| 81 | 332 | 48 | 329 | 99 | 46 | 317 | 95 | 46 | 296 | 89 | 48 |
| 85 | 334 | 48 | 333 | 100 | 46 | 316 | 95 | 46 | 301 | 90 | 45 |
| 89 | 342 | 43 | 338 | 99 | 46 | 324 | 95 | 45 | 304 | 89 | 44 |
| 93 | 346 | 39 | 347 | 100 | 46 | 326 | 94 | 41 | 307 | 89 | 40 |
| 97 | 350 | 37 | 347 | 99 | 45 | 332 | 95 | 39 | 303 | 86 | 36 |
| 101 | 351 | 35 | 340 | 97 | 40 | 336 | 96 | 37 | 306 | 87 | 32 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 157 | | 156 | 100 | | 159 | 102 | | 160 | 102 | |
| 14-52 | 237 | | 235 | 99 | | 235 | 99 | | 232 | 98 | |
| 53-101 | 321 | | 319 | 100 | | 308 | 96 | | 290 | 91 | |

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant mesothelioma and mononuclear cell leukemia and neoplasms or nonneoplastic lesions of the liver, lung, kidney, and pituitary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Malignant Mesothelioma: The incidence of malignant mesothelioma in male rats was significantly increased in the 1,000 mg/L group (Tables 10 and A3). The incidence in the 1,000 mg/L group exceeded the historical range in controls from drinking water studies (Tables 10 and A4a). Mesothelioma was seen on the abdominal wall and serosal surface of several abdominal organs (e.g., mesentery, peritoneum, testes, epididymis, prostate and seminal vesicles, pancreas, adrenal gland, spleen, mesenteric lymph nodes, kidney, urinary bladder, stomach, and skeletal muscle). The distinction between benign and malignant mesothelioma is not clear (Hall, 1990). Because neoplastic cells were found throughout the peritoneum, all mesotheliomas were classified as malignant.

Microscopically, mesothelioma consisted of exophytic, papillary projections of one or more layers of plump, cuboidal mesothelial cells with prominent, basophilic, round nuclei and abundant eosinophilic to amphophilic cytoplasm, on a supporting pedunculated, fibrovascular stromal stalk (Plates 3 and 4). The amount of the stromal component varied considerably, and at times the pleomorphic mesothelial cells took on a tubular appearance or occurred in packets that were densely packed. In NTP studies, chemically induced mesotheliomas have been seen almost exclusively in male rats.

TABLE 10
Incidences of Malignant Mesothelioma in Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|-------------------------------------|-----------|-----------|----------------|-------------|
| Malignant Mesothelioma ^a | | | | |
| Overall rate ^b | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) | 10/50 (20%) |
| Adjusted rate ^c | 6.9% | 2.4% | 0.0% | 22.6% |
| Terminal rate ^d | 2/34 (6%) | 1/24 (4%) | 0/30 (0%) | 2/28 (7%) |
| First incidence (days) | 591 | 729 (T) | — ^f | 512 |
| Poly-3 test ^e | P<0.001 | P=0.325N | P=0.137N | P=0.035 |

(T) Terminal sacrifice

^a Historical incidence for 2-year drinking water studies with controls given NTP-2000 diet (mean ± standard deviation): 15/250 (6.0% ± 4.2%), range 0%-12%

^b Number of neoplasm-bearing animals/number of animals necropsied

^c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^d Observed incidence at terminal kill

^e 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 lower incidence in an exposed group is indicated by N.

^f Not applicable; no neoplasms in animal group

Mononuclear Cell Leukemia: A positive trend in the incidence of mononuclear cell leukemia occurred in female rats (Tables 11 and B3), and the incidence in 1,000 mg/L females was significantly increased. A significant increase in mononuclear cell leukemia also occurred in 50 mg/L males (Tables 11 and A3). The incidences of mononuclear cell leukemia in 50 and 500 mg/L males and in 500 and 1,000 mg/L females exceeded the historical control ranges from drinking water studies (Tables 11, A4b, and B4). Mononuclear cell leukemia is one of the most common spontaneously occurring hematopoietic system neoplasms seen in F344/N rats. Mononuclear cell leukemia is a rapidly progressive, lethal neoplastic disease that first develops in the spleen, with infiltrates of neoplastic cells also occurring in the liver, lung, lymph nodes, bone marrow, and most other organs (Elwell *et al.*, 1996). There was no evidence of splenic toxicity in males; hence, the decrease in mononuclear cell leukemia incidence in the 1,000 mg/L group compared to that in the 50 mg/L group was not due to toxicity in the spleen at the higher exposure concentration.

TABLE 11
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|-------------|
| Male | | | | |
| Mononuclear Cell Leukemia ^a | | | | |
| Overall rate ^b | 17/50 (34%) | 31/50 (62%) | 24/50 (48%) | 13/50 (26%) |
| Adjusted rate ^c | 37.0% | 66.2% | 56.2% | 30.2% |
| Terminal rate ^d | 11/34 (32%) | 15/24 (63%) | 16/30 (53%) | 5/28 (18%) |
| First incidence (days) | 437 | 446 | 423 | 386 |
| Poly-3 test ^e | P=0.026N | P=0.003 | P=0.051 | P=0.325N |
| Female | | | | |
| Mononuclear Cell Leukemia ^f | | | | |
| Overall rate | 11/50 (22%) | 13/50 (26%) | 16/50 (32%) | 22/50 (44%) |
| Adjusted rate | 24.3% | 27.1% | 34.7% | 47.4% |
| Terminal rate | 8/35 (23%) | 8/39 (21%) | 11/35 (31%) | 12/32 (38%) |
| First incidence (days) | 605 | 509 | 519 | 395 |
| Poly-3 test | P=0.006 | P=0.474 | P=0.195 | P=0.016 |

^a Historical incidence for 2-year drinking water studies with controls given NTP-2000 diet (mean ± standard deviation): 79/250 (31.6% ± 3.3%), range 26%-34%

^b Number of neoplasm-bearing animals/number of animals necropsied

^c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^d Observed incidence at terminal kill

^e 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.

^f Historical incidence: 47/200 (23.5% ± 4.4%), range 20%-30%

Liver: The incidences of cystic degeneration in male rats were significantly increased in all exposed groups (Tables 12 and A5). However, the overall change was minimal to mild. Microscopically, this change consisted of focal areas of severe hepatocytic vacuolization in which a number of adjacent hepatocytes had ruptured and coalesced, forming large cystic spaces containing a faintly eosinophilic, flocculent material.

Lung: The incidences of alveolar epithelial hyperplasia were significantly increased in 500 and 1,000 mg/L females (Tables 12 and B4). Microscopically, hyperplasia of alveolar epithelium consisted of focal thickening of the alveolar septa caused by increased numbers of prominent, cuboidal type II pneumocytes, with maintenance of normal alveolar septal architecture. There was a marginal increase in the incidence of alveolar/bronchiolar

TABLE 12
Incidences of Selected Nonneoplastic Lesions in Rats
in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------------------------|------------------|------------------|------------------|
| Male | | | | |
| Liver ^a Degeneration, Cystic ^b | 50 3 (1.0) ^c | 50 9* (1.4) | 50 11* (1.5) | 50 15** (1.3) |
| Female | | | | |
| Lung Alveolar Epithelium, Hyperplasia | 50 3 (1.3) | 50 7 (1.9) | 50 13** (1.7) | 50 14** (1.9) |
| Kidney Nephropathy | 50 18 (1.1) | 50 32** (1.3) | 50 37** (1.4) | 50 40** (1.3) |

* 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

adenoma or carcinoma in 1,000 mg/L females (2/50, 3/50, 2/50, 5/50; Table B3), and the incidence exceeded the historical control range for 2-year drinking water studies [8/200 (4.0% \pm 1.6%), range 2%-6%].

Kidney: The incidences of nephropathy were significantly increased in all exposed groups of females (Tables 12 and B4). Nephropathy consisted of focal to multifocal regenerative renal tubules surrounded by a thickened basement membrane, glomerular thickening, tubular protein casts, and chronic inflammatory infiltrates with fibrosis. In a number of cases graded minimal, the lesions consisted almost exclusively of a few tubular protein casts.

Pituitary Gland: The incidence of pars distalis adenoma was significantly decreased in 1,000 mg/L males (23/50, 21/49, 17/49, 8/50; Table A3). The incidence of adenoma in 1,000 mg/L males was less than the range in historical controls [89/244 (36.4% \pm 8.7%), range 26%-46%] while that in the control group was at the upper end of the historical control range.

MICE

2-WEEK STUDY

All mice survived to the end of the study (Table 13). Final mean body weights of male and female mice were similar to those of the controls; mean body weight gains of 250 and 500 mg/L males were significantly greater than that of the controls. Water consumption by exposed and control groups was similar. Drinking water concentrations of 0, 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 0, 24, 47, 95, 178, and 370 mg dibromoacetic acid/kg body weight to males and 0, 22, 53, 88, 166, and 309 mg/kg to females. There were no clinical findings related to dibromoacetic acid exposure.

TABLE 13
Survival, Body Weights, and Water Consumption of Mice
in the 2-Week Drinking Water Study of Dibromoacetic Acid

| 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 |
| Male | | | | | | | |
| 0 | 5/5 | 22.4 ± 0.4 | 25.2 ± 0.4 | 2.8 ± 0.3 | | 4.3 | 4.6 |
| 125 | 5/5 | 22.0 ± 0.5 | 24.4 ± 0.6 | 2.5 ± 0.3 | 97 | 4.2 | 4.4 |
| 250 | 5/5 | 21.6 ± 0.4 | 25.5 ± 0.4 | 3.8 ± 0.2* | 101 | 4.4 | 4.2 |
| 500 | 5/5 | 22.6 ± 0.4 | 26.4 ± 0.4 | 3.8 ± 0.2* | 105 | 4.4 | 4.6 |
| 1,000 | 5/5 | 22.5 ± 0.3 | 25.7 ± 0.3 | 3.2 ± 0.2 | 102 | 4.2 | 4.1 |
| 2,000 | 5/5 | 21.5 ± 0.4 | 24.5 ± 0.4 | 3.0 ± 0.2 | 97 | 4.1 | 4.1 |
| Female | | | | | | | |
| 0 | 5/5 | 18.7 ± 0.2 | 20.5 ± 0.4 | 1.9 ± 0.4 | | 3.2 | 3.5 |
| 125 | 5/5 | 18.5 ± 0.5 | 20.5 ± 0.3 | 1.9 ± 0.4 | 100 | 3.4 | 3.1 |
| 250 | 5/5 | 18.6 ± 0.3 | 20.6 ± 0.3 | 1.9 ± 0.2 | 100 | 3.5 | 4.7 |
| 500 | 5/5 | 18.7 ± 0.4 | 21.0 ± 0.4 | 2.3 ± 0.4 | 102 | 3.1 | 3.7 |
| 1,000 | 5/5 | 18.7 ± 0.3 | 21.4 ± 0.3 | 2.7 ± 0.3 | 104 | 3.0 | 3.4 |
| 2,000 | 5/5 | 18.5 ± 0.3 | 20.9 ± 0.4 | 2.4 ± 0.2 | 102 | 2.8 | 3.1 |

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

^a Number of animals surviving at 2 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.

Liver weights of males in the 1,000 and 2,000 mg/L groups and females in the 500, 1,000, and 2,000 mg/L groups were significantly increased (Table G3). Thymus weights of males and females in the 1,000 and 2,000 mg/L groups were significantly decreased compared to controls. Gross lesions observed in the thymus and liver of male and female mice were considered to be related to exposure to dibromoacetic acid.

The incidences of thymus atrophy were increased in males exposed to 1,000 or 2,000 mg/L and in 2,000 mg/L females (males: 0 mg/L, 0/5; 125 mg/L, 0/5; 250 mg/L, 0/5; 500 mg/L, 0/5; 1,000 mg/L, 4/5; 2,000 mg/L, 5/5; females: 0/5, 0/5, 0/5, 0/5, 0/5, 5/5). This lesion consisted of a minimal to mild decrease in the thickness of the thymic cortex.

The incidences of morphological changes to the germinal epithelium in the testis were significantly increased in males exposed to 1,000 or 2,000 mg/L (0/5, 0/5, 0/5, 1/5, 5/5, 5/5). This change was characterized by the presence of large (15 to 35 microns) round to oval amphophilic to eosinophilic bodies (interpreted as atypical residual bodies) in seminiferous tubules, and occasional spermatid retention. The atypical residual bodies were present free in the lumen and also near the basement membrane of the seminiferous tubules.

Exposure Concentration Selection Rationale: Based on lack of lethality, clinical signs of toxicity, and body weight changes, dibromoacetic acid exposure concentrations selected for the 3-month drinking water study in mice were 125, 250, 500, 1,000, and 2,000 mg/L.

3-MONTH STUDY

All mice survived to the end of the study (Table 14). Final mean body weights of 2,000 mg/L females and body weight gains of 2,000 mg/L males and females were significantly less than those of the controls. Water consumption by males in the 2,000 mg/L group was decreased at weeks 1 and 13 relative to control mice. Drinking water concentrations of 125, 250, 500, 1,000, and 2,000 mg/L resulted in average daily doses of approximately 16, 30, 56, 115, and 230 mg dibromoacetic acid/kg body weight to males and 17, 34, 67, 132, and 260 mg/kg to females. There were no clinical findings related to dibromoacetic acid exposure.

TABLE 14
Survival, Body Weights, and Water Consumption of Mice
in the 3-Month Drinking Water Study of Dibromoacetic Acid

| Concentration (mg/L) | Survival ^a | Mean Body Weight ^b (g) | | | Final Weight Relative to Controls (%) | Water Consumption ^c | |
|-------------------------|-----------------------|-----------------------------------|--------------|-------------|--|-----------------------------------|---------|
| | | Initial | Final | Change | | Week 1 | Week 13 |
| Male | | | | | | | |
| 0 | 10/10 | 23.0 ± 0.3 | 38.2 ± 1.1 | 15.2 ± 0.9 | | 4.4 | 3.7 |
| 125 | 10/10 | 22.9 ± 0.2 | 39.1 ± 0.6 | 16.3 ± 0.6 | 102 | 4.0 | 3.5 |
| 250 | 10/10 | 23.6 ± 0.2 | 40.1 ± 0.4 | 16.5 ± 0.4 | 105 | 4.2 | 3.6 |
| 500 | 10/10 | 22.7 ± 0.5 | 39.6 ± 1.0 | 17.0 ± 0.9 | 104 | 4.0 | 3.1 |
| 1,000 | 10/10 | 23.1 ± 0.2 | 37.6 ± 0.9 | 14.4 ± 1.0 | 98 | 4.1 | 3.1 |
| 2,000 | 10/10 | 23.1 ± 0.2 | 35.1 ± 1.1 | 12.0 ± 1.1* | 92 | 3.8 | 2.9 |
| Female | | | | | | | |
| 0 | 10/10 | 18.4 ± 0.2 | 30.0 ± 1.2 | 11.6 ± 1.0 | | 3.2 | 3.0 |
| 125 | 10/10 | 18.4 ± 0.3 | 30.4 ± 1.0 | 12.0 ± 0.9 | 101 | 3.0 | 3.0 |
| 250 | 10/10 | 18.6 ± 0.2 | 30.5 ± 0.8 | 11.9 ± 0.8 | 102 | 3.0 | 3.1 |
| 500 | 10/10 | 18.7 ± 0.2 | 29.7 ± 0.8 | 10.9 ± 0.9 | 99 | 3.0 | 2.9 |
| 1,000 | 10/10 | 18.2 ± 0.4 | 29.5 ± 0.6 | 11.3 ± 0.7 | 98 | 3.1 | 3.1 |
| 2,000 | 10/10 | 18.7 ± 0.3 | 26.5 ± 0.6** | 7.8 ± 0.6** | 88 | 2.6 | 3.0 |

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

** $P \leq 0.01$

^a Number of animals surviving at 3 months/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.

The hematology data for mice in the 3-month toxicity study of dibromoacetic acid are listed in Table F2. In general, mice were affected much less than rats when administered the same exposure concentrations of dibromoacetic acid. Of the erythron effects observed in the rats, only the mean cell hemoglobin values demonstrated a similar decrease. This change was small (~3%) and only occurred in the 2,000 mg/L male mice. Small decreases in platelet counts (~16%) and white blood cell counts (~23%) in the 2,000 mg/L male mice were also observed in rats. Other changes in the hematological variables in male mice were not considered toxicologically relevant; no changes occurred in female mice.

Lung weights were increased in 1,000 and 2,000 mg/L females. Liver weights of males and females exposed to 500 mg/L or greater were significantly increased (Table G4).

There were no significant increases between exposed and control mice in BrdU labeling index in the liver (Table 15); the decrease in 2,000 mg/L males was not considered biologically significant.

TABLE 15
BrdU Labeling Index in the Liver of Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--------------------|-------------|-------------|-------------|--------------|
| Male | | | | |
| Labeling Index (%) | 0.71 ± 0.24 | 0.40 ± 0.20 | 0.53 ± 0.33 | 0.28 ± 0.15* |
| Female | | | | |
| Labeling Index (%) | 2.58 ± 1.09 | 2.12 ± 1.41 | 1.96 ± 0.70 | 1.82 ± 1.96 |

* Significantly different ($P \leq 0.05$) from the control group by Student's *t*-test

^a Data are presented as mean ± standard deviation (n=10).

Hepatocellular cytoplasmic vacuolization was present in most mice and the severity was increased in 1,000 and 2,000 mg/L males and females [males: 0 mg/L, 10/10 (1.6); 125 mg/L, 9/10 (1.6); 250 mg/L, 10/10 (1.5); 500 mg/L, 10/10 (1.5); 1,000 mg/L, 10/10 (2.1); 2,000 mg/L, 10/10 (2.9); females: 10/10 (1.5), 10/10 (1.5), 8/8 (1.5), 10/10 (1.7), 10/10 (2.3), 10/10 (2.7)]. The increased severity of vacuolization correlated in general with the increased liver weight. No corresponding liver morphological change to explain the statistically significant increased organ weight was noted in the 500 mg/L group. The cytoplasmic vacuoles were more accentuated in the periportal regions and had morphological characteristics suggestive of being glycogen like, such as ragged outline and no displacement of the nuclei.

The incidences of abnormal testicular morphology were significantly increased in 1,000 and 2,000 mg/L males [0/10, 0/10, 0/10, 0/10, 9/10 (1.0), 10/10 (2.0)]. This change was characterized by the presence of large (15 to 35 micron) round to oval amphophilic to eosinophilic bodies (interpreted as atypical residual bodies) in seminiferous tubules, occasional spermatid retention, and rare vacuolization of Sertoli cells (Plates 5 and 6). The atypical residual bodies were present free in the lumen and also near the basement membrane of the seminiferous tubules. They were also observed in the head and tail of the epididymis. The severity grades for this change were based upon the extent of atypical residual bodies within seminiferous tubules, with minimal severity representing only a few atypical residual bodies within scattered tubules and marked severity representing many atypical residual bodies within a majority of tubules. The change in the epididymis is considered to represent downstream collection of cells from the testes. There was no effect on epididymal sperm concentration, sperm motility, or spermatid heads (Table H3).

Exposure Concentration Selection Rationale: Based on decreased body weight gain and the severity of microscopic lesions at 2,000 mg/L, dibromoacetic acid exposure concentrations selected for the 2-year drinking water study in mice were 50, 500, and 1,000 mg/L.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 16 and in the Kaplan-Meier survival curves (Figure 4). Treatment with dibromoacetic acid had no effect on survival of male or female mice.

TABLE 16
Survival of Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|---------|----------|-----------------|------------|
| Male | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Other ^a | 1 | 0 | 0 | 0 |
| Moribund | 4 | 6 | 3 | 6 |
| Natural deaths | 14 | 6 | 13 | 13 |
| Animals surviving to study termination | 31 | 38 | 34 | 31 |
| Percent probability of survival at end of study ^b | 63 | 76 | 68 | 62 |
| Mean survival (days) ^c | 685 | 703 | 689 | 690 |
| Survival analysis ^d | P=0.428 | P=0.221N | P=0.813N | P=1.000 |
| Female | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Moribund | 2 | 2 | 5 | 7 |
| Natural deaths | 10 | 13 | 13 | 11 |
| Animals surviving to study termination | 38 | 35 | 32 ^e | 32 |
| Percent probability of survival at end of study | 76 | 70 | 64 | 64 |
| Mean survival (days) | 704 | 700 | 695 | 697 |
| Survival analysis | P=0.276 | P=0.658 | P=0.273 | P=0.309 |

^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 of the study

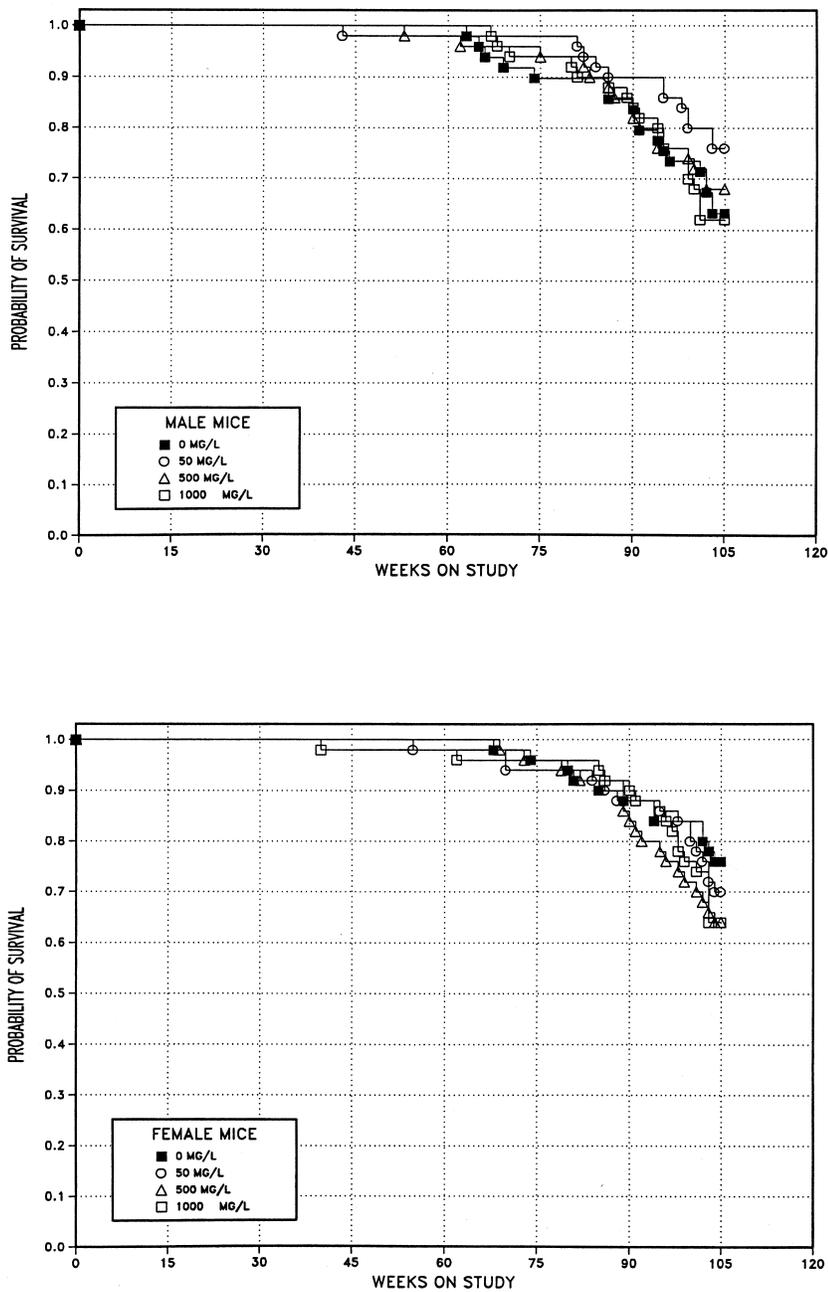


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 50 and 500 mg/L male mice were greater than those of the controls after week 85 (Tables 17 and 18; Figure 5). Mean body weights of exposed groups of female mice were generally similar to those of the controls throughout the study. Water consumption by exposed mice was generally similar to that by controls throughout the study (Tables J3 and J4). Drinking water concentrations of 50, 500, and 1,000 mg/L resulted in average daily doses of approximately 4, 45, and 87 mg/kg to males and 4, 35, and 65 mg/kg to females.

Toxicokinetics

In toxicokinetic studies similar to those conducted in rats, male and female B6C3F₁ mice were given a single gavage dose (70, 175, or 350 mg/kg) or a single intravenous injection (70 mg/kg) of dibromoacetic acid (Appendix N). The single gavage doses gave results that were described by the same model as in the rat study, with an elimination half-life range of approximately 2 to 7 minutes for males and females. The AUC_{0-20 minute} values increased with increasing dose, and the increase was greater than dose-proportional; this effect was attributed to a dose-dependent change in the terminal linear phase. Bioavailability increased with dose for both sexes of mice and ranged from approximately 11% to 44%.

TABLE 17
Mean Body Weights and Survival of Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| Weeks on Study | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 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 | 21.9 | 50 | 22.1 | 101 | 50 | 22.4 | 102 | 50 | 22.5 | 103 | 50 |
| 2 | 24.2 | 50 | 24.4 | 101 | 50 | 24.9 | 103 | 50 | 24.7 | 102 | 50 |
| 3 | 25.7 | 50 | 25.9 | 101 | 50 | 26.3 | 102 | 50 | 26.2 | 102 | 50 |
| 4 | 27.1 | 50 | 27.4 | 101 | 50 | 27.8 | 103 | 50 | 27.5 | 102 | 50 |
| 5 | 28.9 | 50 | 29.2 | 101 | 50 | 29.3 | 101 | 50 | 29.1 | 101 | 50 |
| 6 | 30.2 | 50 | 30.6 | 101 | 50 | 30.6 | 101 | 50 | 30.1 | 100 | 50 |
| 7 | 31.9 | 50 | 32.4 | 102 | 50 | 32.2 | 101 | 50 | 31.9 | 100 | 50 |
| 8 | 33.4 | 50 | 33.8 | 101 | 50 | 33.4 | 100 | 50 | 33.1 | 99 | 50 |
| 9 | 34.8 | 50 | 35.1 | 101 | 50 | 34.4 | 99 | 50 | 33.9 | 97 | 50 |
| 10 | 35.9 | 50 | 36.3 | 101 | 50 | 35.6 | 99 | 50 | 35.2 | 98 | 50 |
| 11 | 37.4 | 50 | 37.9 | 101 | 50 | 36.9 | 99 | 50 | 36.6 | 98 | 50 |
| 12 | 38.3 | 50 | 39.1 | 102 | 50 | 38.3 | 100 | 50 | 37.8 | 99 | 50 |
| 13 | 39.7 | 50 ^a | 40.2 | 101 | 50 | 39.3 | 99 | 50 | 38.9 | 98 | 50 |
| 17 | 43.7 | 50 | 44.8 | 103 | 50 | 43.0 | 98 | 50 | 42.7 | 98 | 50 |
| 21 | 47.0 | 50 | 47.8 | 102 | 50 | 46.8 | 100 | 50 | 46.5 | 99 | 50 |
| 25 | 49.4 | 50 | 49.6 | 100 | 50 | 48.9 | 99 | 50 | 49.3 | 100 | 50 |
| 29 | 50.0 | 50 | 50.5 | 101 | 50 | 59.8 | 100 | 50 | 50.0 | 100 | 50 |
| 33 | 50.9 | 49 | 51.3 | 101 | 50 | 50.5 | 99 | 50 | 50.8 | 100 | 50 |
| 37 | 52.2 | 49 | 52.6 | 101 | 50 | 51.7 | 99 | 50 | 51.8 | 99 | 50 |
| 41 | 52.1 | 49 | 52.9 | 102 | 50 | 52.2 | 100 | 50 | 52.2 | 100 | 50 |
| 45 | 52.2 | 49 | 52.5 | 101 | 49 | 52.3 | 100 | 50 | 52.3 | 100 | 50 |
| 49 | 52.1 | 49 | 53.0 | 102 | 49 | 52.4 | 101 | 50 | 52.8 | 101 | 50 |
| 53 | 52.2 | 49 | 53.3 | 102 | 49 | 52.6 | 101 | 50 | 53.1 | 102 | 50 |
| 57 | 51.1 | 49 | 52.7 | 103 | 49 | 52.6 | 103 | 49 | 52.8 | 103 | 50 |
| 61 | 52.0 | 49 | 53.3 | 103 | 49 | 53.1 | 102 | 49 | 53.1 | 102 | 50 |
| 65 | 52.5 | 48 | 54.0 | 103 | 49 | 53.4 | 102 | 48 | 53.8 | 103 | 50 |
| 69 | 51.7 | 46 | 54.0 | 104 | 49 | 53.3 | 103 | 48 | 52.6 | 102 | 48 |
| 73 | 52.1 | 45 | 54.1 | 104 | 49 | 53.0 | 102 | 48 | 52.6 | 101 | 47 |
| 77 | 51.6 | 44 | 54.8 | 104 | 49 | 52.9 | 103 | 47 | 52.0 | 101 | 47 |
| 81 | 51.8 | 44 | 53.8 | 104 | 48 | 52.1 | 101 | 47 | 52.0 | 100 | 46 |
| 85 | 50.7 | 44 | 52.8 | 104 | 46 | 51.9 | 102 | 45 | 50.5 | 100 | 45 |
| 89 | 48.7 | 42 | 51.7 | 106 | 45 | 51.5 | 106 | 43 | 50.3 | 103 | 44 |
| 93 | 48.1 | 39 | 51.0 | 106 | 45 | 51.6 | 107 | 40 | 49.3 | 103 | 41 |
| 97 | 47.3 | 36 | 49.9 | 106 | 43 | 50.5 | 107 | 38 | 48.3 | 102 | 38 |
| 101 | 46.7 | 36 | 50.0 | 107 | 40 | 51.1 | 109 | 36 | 46.6 | 100 | 34 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 31.5 | | 31.9 | 101 | | 31.6 | 101 | | 31.3 | 100 | |
| 14-52 | 50.0 | | 50.6 | 101 | | 49.7 | 100 | | 49.8 | 100 | |
| 53-101 | 51.5 | | 52.6 | 104 | | 52.3 | 104 | | 51.4 | 102 | |

^a Number of animals weighed for this week is less than the number surviving

TABLE 18
Mean Body Weights and Survival of Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| Weeks on Study | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 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 | 18.4 | 50 | 18.6 | 101 | 50 | 18.8 | 102 | 50 | 18.5 | 101 | 50 |
| 2 | 19.4 | 50 | 19.3 | 100 | 50 | 19.7 | 102 | 50 | 29.7 | 102 | 50 |
| 3 | 20.5 | 50 | 20.4 | 100 | 50 | 20.8 | 102 | 50 | 20.7 | 101 | 50 |
| 4 | 21.2 | 50 | 21.1 | 100 | 50 | 21.5 | 101 | 50 | 21.3 | 101 | 50 |
| 5 | 22.2 | 50 | 22.2 | 100 | 50 | 22.7 | 102 | 50 | 22.5 | 101 | 50 |
| 6 | 23.4 | 50 | 23.3 | 100 | 50 | 23.9 | 102 | 50 | 23.5 | 100 | 50 |
| 7 | 24.3 | 50 | 24.2 | 100 | 50 | 24.7 | 102 | 50 | 24.4 | 100 | 50 |
| 8 | 24.6 | 50 | 24.9 | 101 | 50 | 25.4 | 103 | 50 | 24.9 | 101 | 50 |
| 9 | 25.7 | 50 | 25.7 | 100 | 50 | 26.3 | 102 | 50 | 25.8 | 100 | 50 |
| 10 | 26.7 | 50 | 26.1 | 98 | 50 | 27.4 | 103 | 50 | 26.7 | 100 | 50 |
| 11 | 28.0 | 50 | 27.8 | 99 | 50 | 28.3 | 101 | 50 | 27.8 | 99 | 50 |
| 12 | 28.4 | 50 | 28.3 | 100 | 50 | 29.4 | 104 | 50 | 27.9 | 98 | 50 |
| 13 | 29.8 | 50 | 29.0 | 97 | 50 | 30.0 | 101 | 50 | 29.2 | 98 | 50 |
| 17 | 34.8 | 50 | 34.0 | 98 | 50 | 34.7 | 100 | 50 | 33.0 | 95 | 50 |
| 21 | 38.9 | 50 | 38.2 | 98 | 50 | 38.4 | 99 | 50 | 36.3 | 93 | 50 |
| 25 | 44.0 | 50 | 44.0 | 100 | 50 | 43.5 | 99 | 50 | 42.1 | 96 | 50 |
| 29 | 47.6 | 50 | 47.9 | 101 | 50 | 46.7 | 98 | 50 | 45.0 | 95 | 50 |
| 33 | 49.7 | 50 | 50.4 | 101 | 50 | 49.2 | 99 | 50 | 47.1 | 95 | 50 |
| 37 | 53.5 | 50 | 54.6 | 102 | 50 | 52.4 | 98 | 50 | 50.4 | 94 | 50 |
| 41 | 56.2 | 50 | 56.2 | 100 | 50 | 54.3 | 97 | 50 | 52.4 | 93 | 49 |
| 45 | 57.0 | 50 | 57.9 | 102 | 50 | 55.5 | 97 | 50 | 53.9 | 95 | 49 |
| 49 | 58.0 | 50 | 59.1 | 103 | 50 | 57.3 | 99 | 50 | 55.6 | 96 | 49 |
| 53 | 60.1 | 50 | 60.4 | 101 | 50 | 59.2 | 99 | 50 | 57.3 | 95 | 49 |
| 57 | 60.9 | 50 | 60.6 | 100 | 49 | 59.8 | 98 | 50 | 58.2 | 96 | 49 |
| 61 | 63.4 | 50 | 62.1 | 98 | 49 | 61.2 | 97 | 50 | 60.0 | 95 | 49 |
| 65 | 64.5 | 50 | 62.3 | 98 | 49 | 62.0 | 98 | 50 | 61.0 | 96 | 48 |
| 69 | 64.1 | 49 | 62.7 | 98 | 49 | 62.5 | 98 | 50 | 61.2 | 96 | 48 |
| 73 | 63.8 | 49 | 63.1 | 99 | 47 | 62.9 | 99 | 49 | 61.1 | 96 | 48 |
| 77 | 64.3 | 48 | 63.6 | 99 | 47 | 63.3 | 98 | 48 | 62.0 | 96 | 48 |
| 81 | 63.2 | 47 | 63.1 | 100 | 47 | 61.3 | 97 | 47 | 60.7 | 96 | 48 |
| 85 | 63.1 | 46 | 61.8 | 98 | 46 | 61.8 | 98 | 46 | 59.2 | 94 | 48 |
| 89 | 63.2 | 45 | 62.5 | 99 | 44 | 61.4 | 97 | 45 | 60.4 | 96 | 46 |
| 93 | 63.6 | 44 | 63.9 | 101 | 44 | 61.9 | 97 | 40 | 60.9 | 96 | 44 |
| 97 | 60.8 | 42 | 62.1 | 102 | 43 | 59.2 | 97 | 38 | 58.3 | 96 | 42 |
| 101 | 59.0 | 42 | 59.5 | 101 | 40 | 57.9 | 98 | 35 | 56.6 | 96 | 38 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 24.0 | | 23.9 | 100 | | 24.5 | 102 | | 24.1 | 100 | |
| 14-52 | 48.9 | | 49.1 | 100 | | 48.0 | 98 | | 46.2 | 95 | |
| 53-101 | 62.5 | | 62.1 | 99 | | 61.1 | 98 | | 59.8 | 96 | |

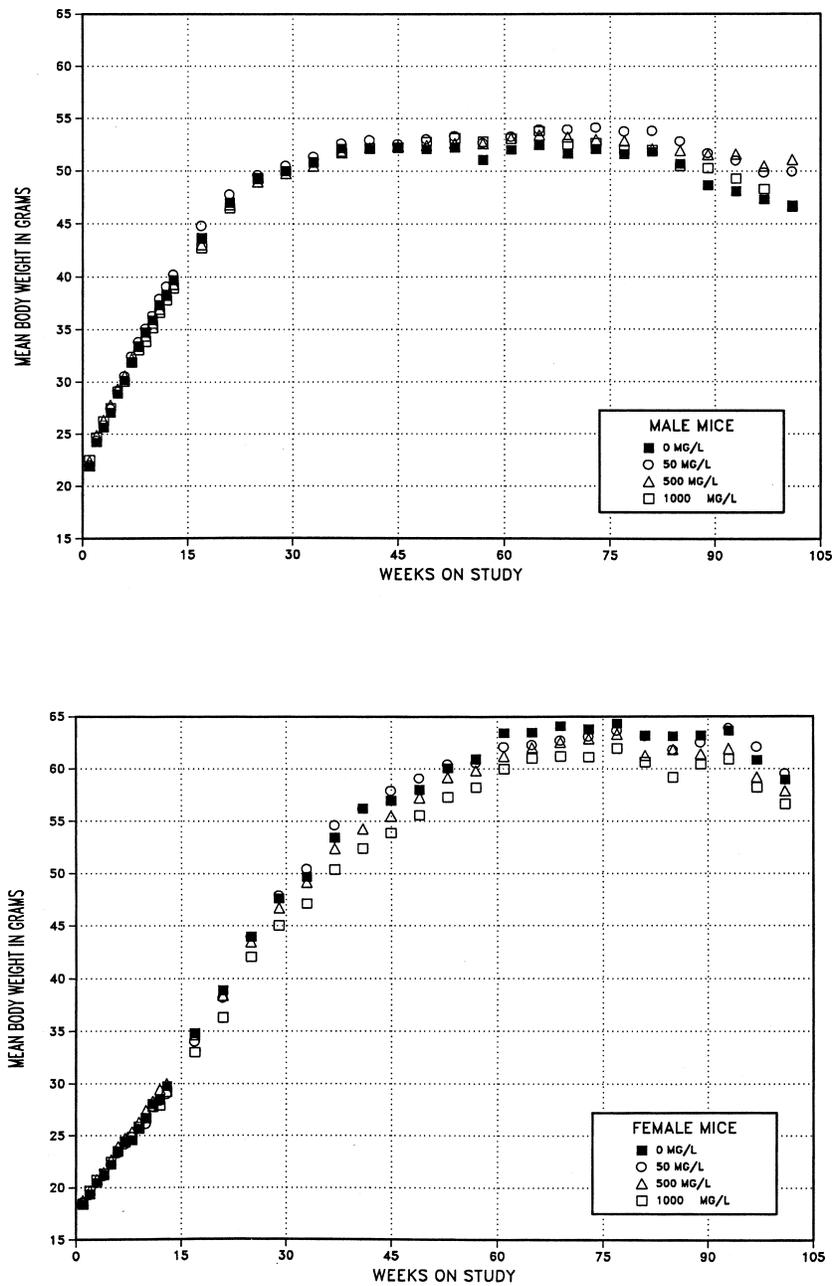


FIGURE 5
Growth Curves for Male and Female Mice Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms or nonneoplastic lesions of the liver, lung, eye, and spleen. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Liver: The incidences of liver neoplasms occurred with positive trends in male and female mice (Tables 19, C3, and D3). The incidences of multiple hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in all exposed groups of males. The incidences of hepatoblastoma were significantly increased in 500 and 1,000 mg/L males, and the incidence of hepatocellular carcinoma was significantly increased in 1,000 mg/L males. The incidences of hepatocellular adenoma, carcinoma, and adenoma or carcinoma (combined) in all groups of males (except carcinoma at 50 mg/L) exceeded, or were at the high end of, the historical ranges in drinking water controls (Tables 19 and C4a). The incidence of hepatoblastoma in 1,000 mg/L males also exceeded the historical control range. The incidences of multiple hepatocellular adenoma and hepatocellular adenoma or carcinoma (combined) were significantly increased in 500 and 1,000 mg/L females. The incidence of hepatocellular carcinoma was significantly increased in 500 mg/L females. The incidences of hepatocellular adenoma and adenoma or carcinoma (combined) in 500 and 1,000 mg/L females exceeded the historical ranges in controls (Tables 19 and D4a).

Hepatocellular adenoma consisted of one or more discrete masses with distinct borders that caused definite compression of the surrounding normal parenchyma. Adenomas usually were composed of hepatocytes that appeared similar to the surrounding parenchyma except that the normal lobular architecture was not apparent and plates of neoplastic hepatocytes intersected the surrounding normal hepatocytes at sharp angles, rather than merging with them (Plates 7 and 8). Hepatocellular carcinoma consisted of one or more discrete masses that generally had irregular borders due to localized areas of growth of neoplastic hepatocytes into the surrounding

TABLE 19
Incidences of Neoplasms of the Liver in Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------------|-------------|-------------|-------------|
| Male | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 50 |
| Hepatocellular Adenoma, Multiple ^a | 6 | 17* | 24* | 27** |
| Hepatocellular Adenoma (includes multiple) ^b | | | | |
| Overall rate ^c | 18/49 (37%) | 37/50 (74%) | 37/50 (74%) | 42/50 (84%) |
| Adjusted rate ^d | 41.6% | 78.0% | 80.1% | 88.7% |
| Terminal rate ^e | 14/31 (45%) | 31/38 (82%) | 29/34 (85%) | 29/31 (94%) |
| First incidence (days) | 596 | 573 | 573 | 469 |
| Poly-3 test ^f | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Hepatocellular Carcinoma, Multiple | 3 | 2 | 5 | 15** |
| Hepatocellular Carcinoma (includes multiple) ^g | | | | |
| Overall rate | 14/49 (29%) | 9/50 (18%) | 19/50 (38%) | 26/50 (52%) |
| Adjusted rate | 31.3% | 19.4% | 40.7% | 54.9% |
| Terminal rate | 6/31 (19%) | 6/38 (16%) | 10/34 (29%) | 14/31 (45%) |
| First incidence (days) | 451 | 659 | 573 | 476 |
| Poly-3 test | P<0.001 | P=0.141N | P=0.236 | P=0.016 |
| Hepatocellular Adenoma or Carcinoma ^h | | | | |
| Overall rate | 28/49 (57%) | 41/50 (82%) | 42/50 (84%) | 47/50 (94%) |
| Adjusted rate | 61.2% | 85.8% | 88.3% | 96.0% |
| Terminal rate | 17/31 (55%) | 33/38 (87%) | 30/34 (88%) | 30/31 (97%) |
| First incidence (days) | 451 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.004 | P<0.001 | P<0.001 |
| Hepatoblastoma, Multiple | 0 | 0 | 3 | 2 |
| Hepatoblastoma (includes multiple) ⁱ | | | | |
| Overall rate | 0/49 (0%) | 4/50 (8%) | 6/50 (12%) | 18/50 (36%) |
| Adjusted rate | 0.0% | 8.7% | 13.3% | 39.1% |
| Terminal rate | 0/31 (0%) | 3/38 (8%) | 3/34 (9%) | 9/31 (29%) |
| First incidence (days) | — ^j | 717 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.072 | P=0.019 | P<0.001 |
| Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma | | | | |
| Overall rate | 28/49 (57%) | 41/50 (82%) | 43/50 (86%) | 48/50 (96%) |
| Adjusted rate | 61.2% | 85.8% | 90.4% | 97.4% |
| Terminal rate | 17/31 (55%) | 33/38 (87%) | 31/34 (91%) | 30/31 (97%) |
| First incidence (days) | 451 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.004 | P<0.001 | P<0.001 |

TABLE 19
Incidences of Neoplasms of the Liver in Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|-------------|-------------|-------------|-------------|
| Female | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 49 |
| Hepatocellular Adenoma, Multiple | 11 | 14 | 23** | 26** |
| Hepatocellular Adenoma (includes multiple) ^k | | | | |
| Overall rate | 19/49 (39%) | 26/50 (52%) | 32/50 (64%) | 35/49 (71%) |
| Adjusted rate | 41.4% | 56.8% | 69.7% | 75.9% |
| Terminal rate | 18/38 (47%) | 22/35 (63%) | 26/32 (81%) | 28/32 (88%) |
| First incidence (days) | 653 | 694 | 573 | 636 |
| Poly-3 test | P<0.001 | P=0.098 | P=0.004 | P<0.001 |
| Hepatocellular Carcinoma, Multiple | 1 | 1 | 5 | 3 |
| Hepatocellular Carcinoma (includes multiple) ^l | | | | |
| Overall rate | 3/49 (6%) | 3/50 (6%) | 12/50 (24%) | 8/49 (16%) |
| Adjusted rate | 6.5% | 6.6% | 26.7% | 17.7% |
| Terminal rate | 1/38 (3%) | 2/35 (6%) | 9/32 (28%) | 7/32 (22%) |
| First incidence (days) | 658 | 694 | 617 | 671 |
| Poly-3 test | P=0.019 | P=0.659 | P=0.009 | P=0.094 |
| Hepatocellular Adenoma or Carcinoma ^m | | | | |
| Overall rate | 22/49 (45%) | 28/50 (56%) | 37/50 (74%) | 37/49 (76%) |
| Adjusted rate | 47.6% | 61.2% | 79.5% | 79.8% |
| Terminal rate | 19/38 (50%) | 24/35 (69%) | 28/32 (88%) | 29/32 (91%) |
| First incidence (days) | 653 | 694 | 573 | 636 |
| Poly-3 test | P<0.001 | P=0.131 | P<0.001 | P<0.001 |
| Hepatoblastoma | 1 | 0 | 1 | 1 |

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

** $P \leq 0.01$

^a Number of animals with lesion

^b Historical incidence for 2-year drinking water studies with controls given the NTP-2000 diet (mean \pm standard deviation): 84/197 (34.3% \pm 22.3%), range 34%-63%

^c Number of animals with neoplasm/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 the differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposed group is indicated by N.

^g Historical incidence: 57/197 (23.3% \pm 15.5%), range 18%-42%

^h Historical incidence: 122/197 (49.7% \pm 31.1%), range 48%-85%

ⁱ Historical incidence: 11/197 (4.5% \pm 6.2%), range 0%-13%

^j Not applicable; no neoplasms in animal group

^k Historical incidence: 93/248 (37.6% \pm 18.0%), range 18%-61%

^l Historical incidence: 28/248 (11.3% \pm 9.1%), range 4%-26%

^m Historical incidence: 110/248 (44.4% \pm 18.1%), range 20%-63%

normal parenchyma. The neoplastic hepatocytes often were somewhat atypical, but the major distinguishing feature of carcinoma was the presence of abnormal patterns of growth. The most common abnormal growth pattern was formation of trabeculae of neoplastic hepatocytes that were three or more cell layers thick, while less commonly the neoplastic cells formed glandular structures or solid masses (Plate 9).

Hepatoblastoma consisted of well-demarcated hypercellular areas, often with fluid or blood filled spaces and multifocal necrosis. The tumors were composed of pleomorphic collections of polygonal to elongate cells with ovoid to pyriform, hyperchromatic nuclei and variable amounts of eosinophilic to amphophilic cytoplasm. These cells were often arranged in palisading rows or glandular, “rosette” formations. Mitotic figures were numerous (Plate 10). Hepatoblastomas were frequently diagnosed in mice that also had hepatocellular adenoma or carcinoma. There was no consistent difference in the microscopic appearance of the liver neoplasm between the control and exposed groups. Hepatoblastomas are rare neoplasms with a low spontaneous incidence in mice (Harada *et al.*, 1999). They are considered part of the continuum in the development of hepatocellular neoplasms, are often observed within or adjacent to an existing hepatocellular adenoma or carcinoma, and are considered an undifferentiated variant of hepatocellular neoplasms. With chemicals that are hepatocarcinogens, there appears to be a positive association between increased incidences of hepatoblastoma and increased incidences of hepatocellular carcinoma in mice; the malignant potential of hepatoblastoma appears to be similar to that of hepatocellular carcinoma. In NTP 2-year studies, as many as 6 (mean incidence 1.5%) hepatoblastomas have occurred in individual male control groups (all routes) fed the NTP-2000 diet; the highest control incidence was observed in a drinking water control group.

Lung: The incidences of alveolar/bronchiolar adenoma occurred with a positive trend in males and females (Tables 20, C3, and D3). The incidence of adenoma in 500 mg/L male mice was significantly greater than that in controls. Alveolar/bronchiolar carcinoma occurred in control and exposed mice. The incidences of alveolar/bronchiolar adenoma in 500 and 1,000 mg/L males exceeded the historical range in drinking water controls, and that of 1,000 mg/L females was at the upper end of the historical control range (Tables 20, C4b, and

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------------------|-------------|-------------|-------------|
| Male | | | | |
| Number Examined Microscopically | 49 | 50 | 50 | 50 |
| Alveolar Epithelium, Hyperplasia ^a | 2 (1.5) ^b | 6 (1.7) | 6 (2.3) | 7 (1.9) |
| Alveolar/bronchiolar Adenoma, Multiple | 0 | 1 | 2 | 4 |
| Alveolar/bronchiolar Adenoma (includes multiple) ^c | | | | |
| Overall rate ^d | 7/49 (14%) | 5/50 (10%) | 17/50 (34%) | 12/50 (24%) |
| Adjusted rate ^e | 16.3% | 10.8% | 38.4% | 26.6% |
| Terminal rate ^f | 5/31 (16%) | 4/38 (11%) | 15/34 (44%) | 8/31 (26%) |
| First incidence (days) | 482 | 596 | 604 | 489 |
| Poly-3 test ^g | P=0.019 | P=0.326N | P=0.016 | P=0.178 |
| Alveolar/bronchiolar Carcinoma, Multiple | 2 | 1 | 2 | 3 |
| Alveolar/bronchiolar Carcinoma (includes multiple) ^h | 5 | 8 | 8 | 7 |
| Alveolar/bronchiolar Adenoma or Carcinoma ⁱ | | | | |
| Overall rate | 12/49 (24%) | 12/50 (24%) | 22/50 (44%) | 17/50 (34%) |
| Adjusted rate | 27.7% | 25.7% | 49.4% | 37.3% |
| Terminal rate | 8/31 (26%) | 9/38 (24%) | 18/34 (53%) | 11/31 (36%) |
| First incidence (days) | 482 | 596 | 604 | 489 |
| Poly-3 test | P=0.066 | P=0.513N | P=0.027 | P=0.226 |

TABLE 20
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-----------|------------|------------|------------|
| Female | | | | |
| Number Examined Microscopically | 50 | 50 | 50 | 50 |
| Alveolar/bronchiolar Adenoma ^j | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) | 6/50 (12%) |
| Adjusted rate | 2.2% | 6.5% | 6.7% | 13.2% |
| Terminal rate | 1/38 (3%) | 2/35 (6%) | 1/32 (3%) | 4/32 (13%) |
| First incidence (days) | 729 (T) | 490 | 553 | 636 |
| Poly-3 test | P=0.044 | P=0.307 | P=0.297 | P=0.054 |
| Alveolar/bronchiolar Carcinoma ^k | 1 | 2 | 2 | 2 |
| Alveolar/bronchiolar Adenoma or Carcinoma ^l | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 4.4% | 10.8% | 11.0% | 15.4% |
| Terminal rate | 2/38 (5%) | 3/35 (9%) | 2/32 (6%) | 5/32 (16%) |
| First incidence (days) | 729 (T) | 490 | 553 | 636 |
| Poly-3 test | P=0.095 | P=0.219 | P=0.210 | P=0.076 |

(T) Terminal sacrifice

^a Number of animals with lesion

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

^c Historical incidence for 2-year drinking water studies with controls given NTP-2000 diet (mean ± standard deviation: 26/199 (10.5% ± 7.7%), range 6%-20%)

^d Number of animals with neoplasm/number of animals with lung examined microscopically

^e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^f Observed incidence at terminal kill

^g 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 lower incidence in an exposed group is indicated by N.

^h Historical incidence: 16/199 (6.4% ± 3.9%), range 6%-10%

ⁱ Historical incidence: 41/199 (16.5% ± 10.7%), range 12%-26%

^j Historical incidence: 13/250 (5.2% ± 4.2%), range 2%-12%

^k Historical incidence: 5/250 (2.0% ± 1.4%), range 0%-4%

^l Historical incidence: 16/250 (6.4% ± 3.9%), range 2%-12%

D4b). A non statistically significant increase in the incidence of alveolar hyperplasia was noted in all treated groups of males. Alveolar/bronchiolar adenoma consisted of well demarcated hypercellular masses distorting the normal septal architecture characterized by well-differentiated cuboidal to round cells forming papillary projections into the alveolar or bronchiolar lumens and slight compression of the surrounding parenchyma.

Alveolar/bronchiolar carcinoma consisted of more irregular hypercellular masses distorting the normal septal architecture characterized by fairly pleomorphic, polygonal to columnar cells forming papillary and solid

projections into alveolar or bronchiolar lumens, with variable peripheral compression and invasion. There was no consistent difference in the microscopic appearance of the lung neoplasms between the control and treated groups.

Malignant lymphoma: A significant increase in the incidence of malignant lymphoma occurred in 50 mg/L male mice compared to controls (0 mg/L, 0/50; 50 mg/L, 5/50; 500 mg/L, 3/50; 1,000 mg/L, 2/50; Table C3), and the incidence exceeded the historical control range [5/199 (2.0% ± 2.5%), range 0%- 6%]. However there was a negative trend in the incidences of malignant lymphoma in the female mice, and the incidence in the 1,000 mg/L group was significantly decreased (27/50, 24/50, 17/50, 14/50; Table D3). In females, the incidences in the control and 50 mg/L groups were near the upper end of the historical control range while the incidence in the 1,000 mg/L group was near the middle of the historical control range [91/250 (36.4 ± 22.7), range 10%-58%].

Eye: The incidence of cataract was significantly increased in 1,000 mg/L male mice (0/49, 1/50, 1/50, 6/50; Table C5). Cataract, unilateral or bilateral, consisted of one or more areas within the lens composed of swelling, degeneration and fragmentation of the lens fibers. These were most often located just under the lens capsule. Because the eye was not routinely checked in NTP studies until recently, the real historical data for this cataract is unknown. However, according to Geiss and Yoshitomi (1999), severe mature cataracts are uncommon in old B6C3F₁ mice; focal posterior subcapsular degeneration is more frequent (up to 15%). It is therefore suggested that the apparent increased incidence of cataracts in the present study is not treatment-related.

Spleen: The incidence of hematopoietic cell proliferation was significantly increased male mice exposed to 1,000 mg/L (0 mg/L, 18/49; 50 mg/L, 20/50; 500 mg/L, 28/50; 1,000 mg/L, 38/50; Table C5). Hematopoietic cell proliferation consisted of an increase in the normal active hematopoietic tissue, expanding the red pulp. Both myeloid and erythroid cell series were affected; megakaryocytes were often increased as well.

GENETIC TOXICOLOGY

Dibromoacetic acid, tested over a concentration range of 33 to 10,000 $\mu\text{g}/\text{plate}$, was mutagenic in *Salmonella typhimurium* strain TA100, with and without 30% rat or hamster liver metabolic activation enzymes (S9); no activity was detected in strain TA98, with or without rat or hamster liver S9 enzymes (Table E1). Increased frequencies of micronucleated normochromatic erythrocytes (NCEs) were observed in peripheral blood samples from male B6C3F₁ mice administered 125 to 2,000 mg dibromoacetic acid/L in drinking water for 3 months; no increases in NCEs were seen in female mice similarly exposed (Table E2). No evidence of bone marrow toxicity, as measured by the percentage of immature (polychromatic) erythrocytes among total erythrocytes, was observed in either male or female mice.

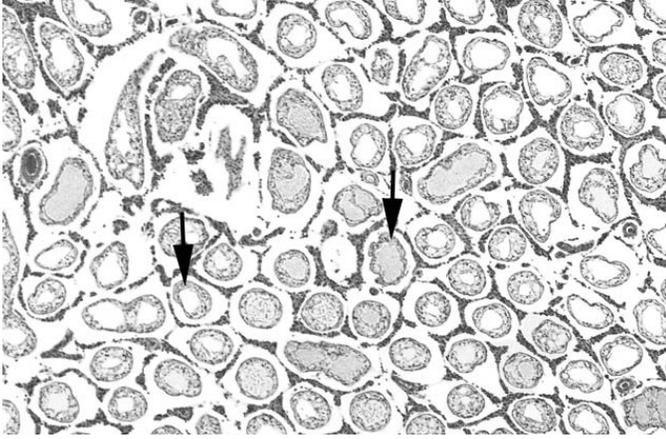


PLATE 1

Marked (grade 4) testicular atrophy (end stage lesion) in a male rat exposed to 2,000 mg/L dibromoacetic acid in drinking water for 3 months. Note that all seminiferous tubules (arrows) in the field are atrophic. H&E; 10 \times

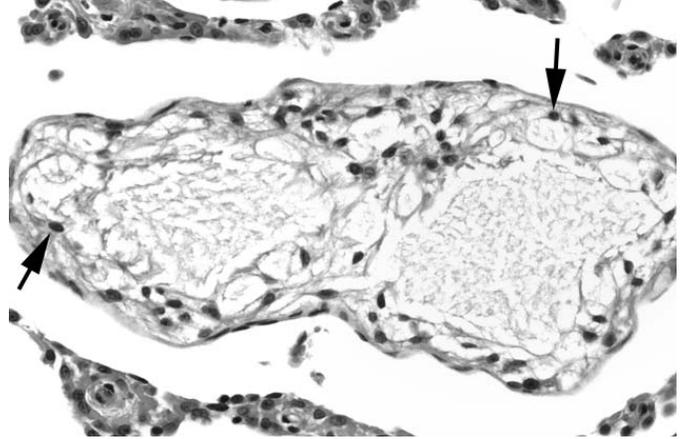


PLATE 2

Testicular atrophy (end stage lesion) in a male rat exposed to 2,000 mg/L dibromoacetic acid in drinking water for 3 months. The seminiferous tubules are lined only by Sertoli cells (arrows). H&E; 80 \times

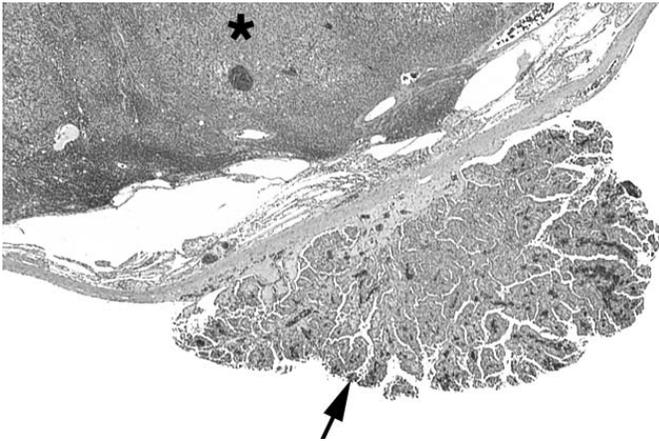


PLATE 3

Malignant mesothelioma (arrow) growing on the serosal surface of the testes in a male rat exposed to 1,000 mg/L dibromoacetic acid in drinking water for 2 years. There is also a spontaneous interstitial cell neoplasm (asterisk). H&E; 8 \times

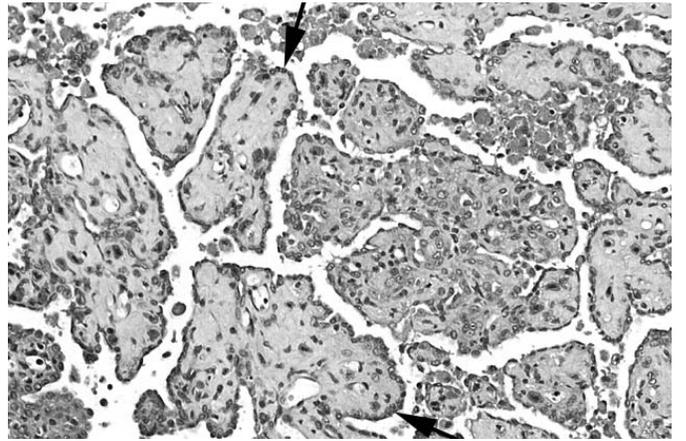


PLATE 4

Higher magnification of Plate 3 showing the structure of papillary exophytic projection (arrows) in the malignant mesothelioma. H&E; 50 \times

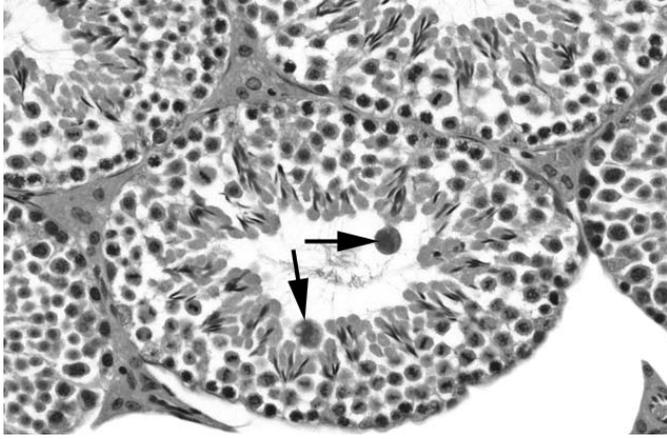


PLATE 5
Two abnormal residual bodies formation (arrows) in the testis of a male mouse exposed to 2,000 mg/L dibromoacetic acid in drinking water for 3 months. H&E; 80 \times

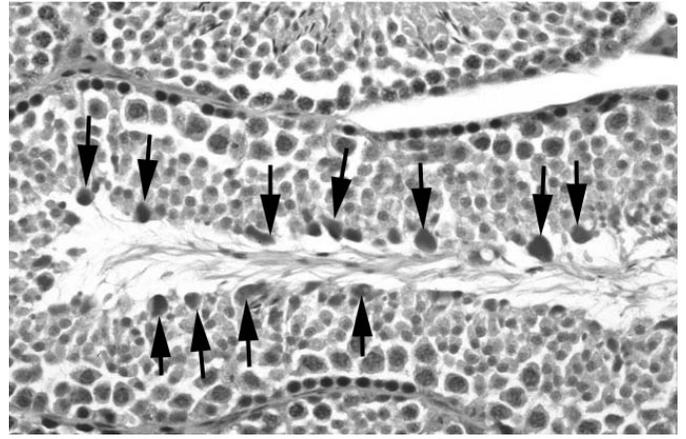


PLATE 6
Numerous abnormal residual bodies (arrows) in the testis of the same mouse presented in Plate 5. H&E; 80 \times

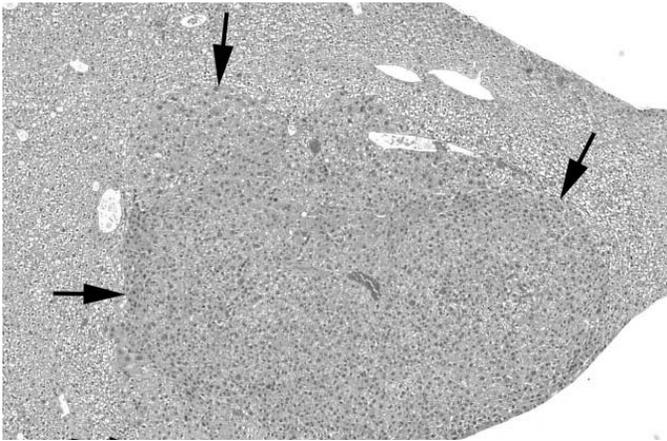


PLATE 7
Hepatocellular adenoma in a male mouse exposed to 1,000 mg/L dibromoacetic acid in drinking water for 2 years. The neoplasm had distinct borders, causing definite compression of the surrounding normal parenchyma (arrows). H&E; 10 \times

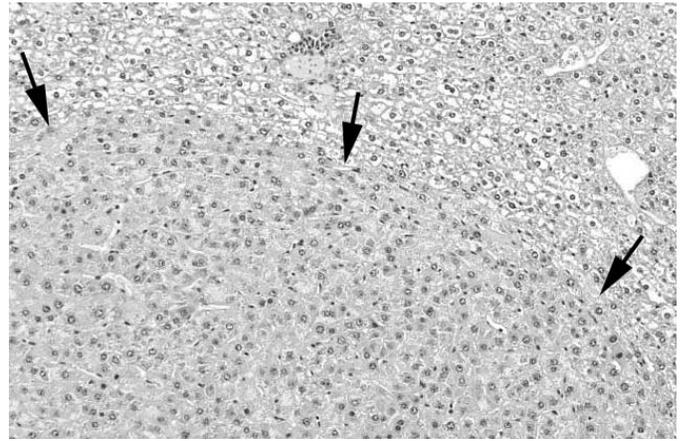


PLATE 8
Hepatocellular adenoma in a male mouse exposed to 1,000 mg/L dibromoacetic acid in drinking water for 2 years. Note the hepatocytes have a relatively normal appearance, the lack of normal lobular architecture, and the plates of neoplastic hepatocytes intersect the surrounding normal hepatocytes at sharp angles rather than merging with them (arrows). H&E; 20 \times

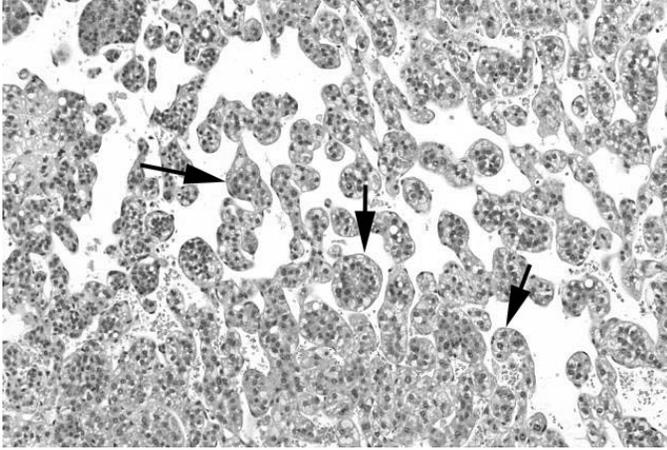


PLATE 9

Hepatocellular carcinoma in a male mouse exposed to 1,000 mg/L dibromoacetic acid in drinking water for 2 years. The neoplastic hepatocytes are somewhat atypical, with formation of trabeculae that are three or more cell layers thick (arrows). H&E; 10 \times

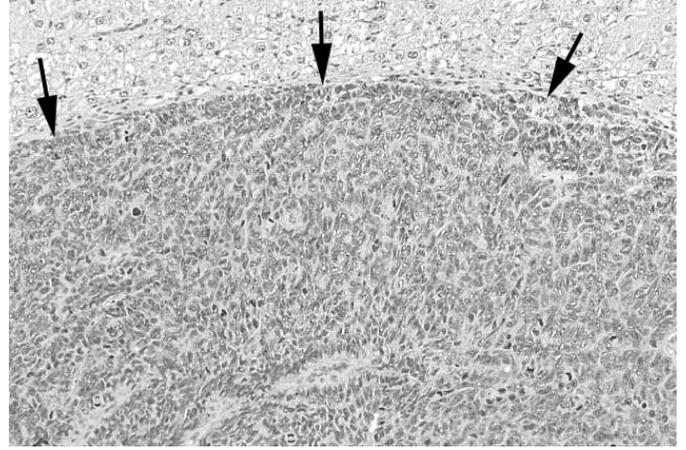


PLATE 10

Hepatoblastoma in a male mouse exposed to 1,000 mg/L dibromoacetic acid in drinking water for 2 years. The hypercellular neoplasm is well-demarcated (arrows) and composed of a pleomorphic collection of polygonal to elongate cells with ovoid to pyriform, hyperchromatic nuclei. These cells are arranged in palisading rows or glandular, "rosette" formations. H&E; 10 \times

DISCUSSION AND CONCLUSIONS

Dibromoacetic acid is a drinking water disinfection by-product formed by the reaction of chlorine oxidizing agents with natural organic matter in source water containing bromide. The United States Environmental Protection Agency regulates drinking water levels of dibromoacetic acid, along with monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, and monobromoacetic acid at a limit of 60 µg/L for the sum of these haloacetic acids (40 CFR, § 141.64). Toxicity and carcinogenicity studies of dibromoacetic acid in rats and mice were nominated to the NTP by the USEPA because of widespread human exposure to this brominated water disinfection by-product and because a related dihaloacetic acid, dichloroacetic acid, was found to be carcinogenic to the liver of mice (Herren-Freund *et al.*, 1987; DeAngelo *et al.*, 1991; Daniel *et al.*, 1992) and rats (DeAngelo *et al.*, 1996). Dibromoacetic acid was administered in drinking water to mimic the major route of human exposure to this chemical.

In 2-week and 3-month drinking water studies in rats, exposure to dibromoacetic acid (adjusted to pH 5.0) caused reductions in body weight gain in the 2,000 mg/L groups of males and females, increases in liver weights at all exposure concentrations (125 mg/L and higher), and minimal to mild hepatocellular cytoplasmic vacuolization at 500 mg/L or greater in males and in 2,000 mg/L females. The lowest exposure concentration in the 3-month study, 125 mg/L, resulted in an average daily dose of 10 mg dibromoacetic acid/kg body weight to males and 12 mg/kg to females. Hepatocyte cytoplasmic vacuolization in males exposed to 1,000 or 2,000 mg/L was associated with marginal increases in DNA replication, indicative of slight increases in hepatocyte proliferation. In a two-generation study, absolute and relative liver weights were increased in parental and F1 generation male and female Sprague-Dawley rats exposed to 50, 250, or 650 ppm (mg/L) dibromoacetic acid in drinking water; however, no corresponding microscopic changes in the liver were reported in that study (Christian *et al.*, 2002).

Testicular lesions induced in rats in 2-week and 3-month studies were similar to those described previously (Linder *et al.*, 1994b; 1997a) and were characterized by a delay in spermiation with retention of step 19 spermatids in the seminiferous epithelium adjacent to the lumen of stage IX and stage X tubules of the spermatogenic cycle. These changes were accompanied by the presence of atypical residual bodies. The lowest concentration of dibromoacetic acid showing this effect in the 3-month study was 500 mg/L, equivalent to a mean daily dose of 40 mg/kg.

Decreased testicular weight and incidence of testicular atrophy were observed in the 2,000 mg/L group (166 mg/kg). Linder *et al.* (1997b) observed altered spermiation at 10 mg/kg or greater doses in Sprague-Dawley rats exposed to dibromoacetic acid by gavage for 31 or 79 days and seminiferous tubule atrophy at 250 mg/kg per day. The difference in dose response between the present study and that of Linder *et al.* (1997b) might be due to the use of different rat strains (F344/N versus Sprague-Dawley) or different methods of exposure (drinking water versus gavage). The formation of atypical residual bodies was suggested to be a result of impaired degradative function in Sertoli cells. Sperm morphology evaluations in the present study showed reductions in epididymal sperm counts, sperm motility, and spermatid heads at 2,000 mg/L (166 mg/kg) but not at 1,000 mg/L (90 mg/kg). Hypospermia was also diagnosed in the epididymis of rats in the present study. Linder *et al.* (1994b) detected altered sperm morphology and reduced sperm motility with 14 daily gavage doses of 90 mg/kg.

Pituitary gland hypertrophy observed in 2,000 mg/L males in the 3-month study was considered to be secondary to testicular atrophy because a similar response occurs following orchietomy (MacKenzie and Boorman, 1990). In female rats, an increase in the incidence of hematopoietic cell proliferation in the spleen, characterized by increases in myeloid and erythroid precursors, was associated with mild decreases in white blood cell and lymphocyte counts at week 14. Increases in kidney, heart, lung, and thymus weights were not associated with microscopic changes in these organs.

Based on decreased body weight gains, organ weight effects, and the severity of testicular lesions at 2,000 mg/L, drinking water concentrations of dibromoacetic acid selected for the 2-year study were 50, 500, or 1,000 mg/L. In the 2-year study, these exposure concentrations had no effect on survival of male or female rats, while body

weights of the 500 and 1,000 mg/L groups were at most 5% to 15% less than those of controls. These body weight differences were in part due to decreased water consumption and reduced feed intake.

An increase in the incidence of mesotheliomas was observed in the 1,000 mg/L group of male rats. The incidence of 20% in this group far exceeded the historical control rate of mesotheliomas in 2-year drinking water studies in male rats (mean incidence of $6.0\% \pm 4.2\%$). This malignant lesion was observed at multiple sites throughout the abdominal cavity (peritoneum). This response in this study met the criteria for some evidence of carcinogenic activity in male rats. Chemically induced mesotheliomas have been observed predominantly in male rats compared to female rats or mice of either sex. Of over 500 carcinogenicity studies reported by the NTP, 16 agents produced positive evidence of neoplasms in the mesothelium; of those agents, 15 were active in male rats, two in female rats and mice, and one in male mice. Phenoxybenzamine was the only chemical that induced mesotheliomas in the abdominal cavity of both sexes of rats and mice (NCI, 1978). Both mutagenic and nonmutagenic chemicals induced neoplasms in the abdominal cavity mesothelium. There is no apparent relationship between chemical structure and this neoplastic response.

An increased incidence and positive trend of mononuclear cell leukemia was observed in female rats. The incidence in the high dose (1,000 mg/L) female rats (44%) exceeded the historical control rate for 2-year drinking water studies in which animals were given the NTP-2000 diet (mean incidence of $23.5\% \pm 4.4\%$). This response was considered to be some evidence of carcinogenic activity; the incidence in the 1,000 mg/L female rats was also more than two standard deviations greater than the historical rate for 2-year feed studies, which include 510 female rats (mean incidence of $23.8\% \pm 9.1\%$). In male rats, the incidence of mononuclear cell leukemia was increased significantly in the low dose group (50 mg/L) (62% versus 34% in controls), and a marginal increase was observed in the mid-dose group (500 mg/L) (58%); however, because there was no difference in incidence between controls and high-dose males (1,000 mg/L) (26%), the overall response in male rats was not considered to be related to dibromoacetic acid exposure. Pharmacokinetic behavior of dibromoacetate does not explain this unusual dose response.

In female rats, the incidences of hyperplasia of the alveolar epithelium were significantly increased in the 500 and 1,000 mg/L groups; this response was accompanied by a marginal increase in the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) (4% in controls versus 10% in the 1,000 mg/L group). The incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in this group of female rats exceeded the historical control rate for 2-year drinking water studies in which animals were given NTP-2000 diet (mean incidence of $4.0\% \pm 1.6\%$). Because alveolar epithelial hyperplasia is considered to be part of the continuum of proliferative lesions in lung neoplasia, the combined responses indicate a proliferative effect of dibromoacetic acid in the lung.

The only liver lesions observed in the 2-year study in rats were increased incidences of cystic degeneration in all exposed groups of males. This finding is in contrast to the increase in liver neoplasms observed in male F344/N rats exposed to 500 or 1,600 mg/L dichloroacetic acid in drinking water for 100 weeks (DeAngelo *et al.*, 1996). On a molar basis, exposure to 500 mg/L dichloroacetic acid is equivalent to 845 mg/L dibromoacetic acid. Because there was no increase in the incidences of liver neoplasms in male rats given drinking water containing 1,000 mg/L dibromoacetic acid, there appears to be a difference in the hepatocarcinogenic potential of these two dihaloacetic acids in rats.

The incidences of chronic nephropathy were increased in all exposed groups of female rats but not in male rats; the apparent sex difference in identifying this renal effect is likely due to the higher rate of nephropathy in control male rats (88%) that precluded the chance of detecting an increased response. Increases in kidney weights were observed in male and female rats in the present 3-month study and in a two-generation drinking water study (Christian *et al.*, 2002), but no corresponding histopathological changes were noted in either of these studies. The present study indicates that exposure to dibromoacetic acid may exacerbate chronic nephropathy in rats.

In the 3-month drinking water study in mice, dibromoacetic acid caused reductions in body weight gain in the 2,000 mg/L groups of males and females and increases in liver weights at 500 mg/L or greater in both sexes. Although hepatocellular cytoplasmic vacuolization was observed in most mice including controls, the severity of

this lesion was increased in the 1,000 and 2,000 mg/L groups. There was no increase in hepatocyte DNA replication in either sex of mice after 26 days of exposure.

Testicular lesions, similar to those observed in rats, were induced in mice exposed to 1,000 or 2,000 mg/L. These lesions, which had not been reported previously in mice, were characterized as spermatid retention and large atypical residual bodies in seminiferous tubules. The atypical residual bodies in mice were generally larger than those observed in rats (Plates 5 and 6); however, this effect was detected at a lower average daily dose in rats (40 mg/kg) than in mice (115 mg/kg). As noted above for rats, the lesions observed in mice were also probably caused by impaired degradative functions carried out by Sertoli cells. Evaluations of sperm from exposed mice did not demonstrate any effects on morphology, motility, or epididymal counts. Neither testicular atrophy nor decreases in testicular weight were observed in mice exposed to 2,000 mg/L, the concentration of dibromoacetic acid that caused these effects in rats.

Drinking water concentrations of dibromoacetic acid selected for the 2-year toxicology and carcinogenesis study (50, 500, and 1,000 mg/L) were based on decreased body weight gains and the severity of liver lesions at 2,000 mg/L. In the 2-year study, these exposure concentrations had no significant effects on survival, water consumption, or body weight gain in male and female mice.

Exposure of mice to dibromoacetic acid produced significant dose-related increases in the incidences of hepatocellular adenomas and carcinomas in males and females and hepatoblastomas in males. Of particular note is that the increase in hepatocellular neoplasms in male mice was significant even at the lowest exposure concentration of 50 mg/L, which is equivalent to an average daily dose of approximately 4 mg/kg. Although the administration of dichloroacetic acid in drinking water for 2 years also induced hepatocellular neoplasms in male B6C3F₁ mice (DeAngelo *et al.*, 1991), significant increases in neoplasm incidence with dichloroacetic acid were not detected at doses as low as those of dibromoacetic acid that induced significant increases. The potent response observed in this study was not associated with hepatocellular necrosis or regenerative hyperplasia. A substantial

species difference in susceptibility to dibromoacetic acid-induced liver neoplasia is noted by comparing the response in mice to the lack of a response in rats at equivalent exposure concentrations.

Dibromoacetic acid also induced a significant increase in the incidences of alveolar/bronchiolar neoplasms in male mice and a marginal increase in female mice. Also observed in male mice was a dose-related increase in the incidences of alveolar epithelial hyperplasia, a lesion that is considered to be part of the continuum of proliferative changes in lung neoplasia. Thus, the lung neoplastic response in male mice is likely related to dibromoacetic acid exposure, and the response in female mice also may have been related to dibromoacetic acid.

The 2-year studies presented here demonstrate the multiple organ carcinogenicity of dibromoacetic acid in laboratory animals; the primary sites identified in these studies include the abdominal cavity mesothelium of male rats and the liver and lung of mice. The mechanisms of neoplasm induction by dibromoacetic acid or the related dichloroacetic acid are not known. For dichloroacetic acid, it was suggested that the induction of liver tumors in mice is due to selective growth of a phenotypic cell-type that does not respond to mitoinhibitory homeostatic control mechanisms (Carter *et al.*, 2003). Neither hepatocellular necrosis nor regenerative hyperplasia was associated with the development of preneoplastic lesions or liver neoplasms in mice. A similar mechanism of liver neoplasm induction may be operating for dichloroacetic acid and dibromoacetic acid; data on dibromoacetic acid are not sufficient to ascertain any proposed hypothesis. DNA hypomethylation and increased expression of *c-myc* and *IGF-II* genes were suggested as possible early events in the hepatocarcinogenicity of dihaloacetic acids in mice (Pereira *et al.*, 2001; Tao *et al.*, 2004). An early increase in hepatocyte proliferation is not likely involved in the mode of action, because no increases in the DNA labeling index were observed in mice exposed for 26 days, and the slight increase that occurred in male F344/N rats was not accompanied by an increase in liver tumor response. DNA damage due to oxidative stress in the livers of mice exposed to halogenated acetic acids, including dibromoacetic acid (Austin *et al.*, 1996), may contribute to the hepatocarcinogenicity of these chemicals. The carcinogenicity of dibromoacetic acid may also involve a genotoxic mechanism because this agent induces DNA damage in *Escherichia coli* (Giller *et al.*, 1997), mutations in *Salmonella typhimurium* strains TA98 and TA100

(Kargalioglu *et al.*, 2002), and DNA strand breaks in Chinese hamster ovary cells (Plewa *et al.*, 2002). In addition, glyoxylate, a metabolite of dihaloacetate biotransformation, is mutagenic in *S. typhimurium* strains TA97, TA100, and TA104 (Sayato *et al.*, 1987). It is possible that both a genotoxic effect and selective growth of an altered phenotypic cell-type are involved in the carcinogenesis of dibromoacetic acid. For example, increased cell replication rates in preneoplastic hepatic foci and tumors and decreased replication rates in normal hepatocytes of mice exposed to dibromoacetic acid would provide a selective growth advantage to initiated cells. DNA hypomethylation may contribute to the increased expression of proto-oncogenes during the development of liver neoplasms in mice exposed to dichloroacetic acid (Pereira *et al.*, 2001) or dibromoacetic acid (Tao *et al.*, 2004).

The various roles of parent compound and metabolites in the toxicity and carcinogenicity of dihaloacetic acids have not been determined. The major identified metabolites of dihaloacetate biotransformation in rats and mice are glyoxylate, glycolate, and oxalate (Lin *et al.*, 1993; Narayanan *et al.*, 1999). Biotransformation of dihaloacetates to glyoxylate occurs primarily in liver cytosol by a glutathione dependent process (James *et al.*, 1997) catalyzed by GST ζ (Tong *et al.*, 1998a). However, because metabolism via the GST ζ -mediated displacement of a halide by glutathione leads to an irreversible inactivation of this enzyme (Anderson *et al.*, 1999), the rate of metabolic elimination is reduced and the internal dosimetry (or bioavailability) of the parent compound is increased with repeated exposures to dihaloacetic acids. This change in metabolic capability occurs until a new steady state level of GST ζ activity is reached; that level is dependent on the extent of inactivation and degradation of GST ζ in the liver and the rate of resynthesis of this enzyme. Based on plasma-time course data for dibromoacetic acid in female rats and male mice, as well as published physiological and biochemical parameters, the absorption, metabolism, and elimination of this chemical in rats and mice plus the inactivation and resynthesis of GST ζ in the liver were modeled (Appendix M). This physiologically based pharmacokinetic model (PBPK) was used to estimate the area under the concentration (AUC) time course curves of dibromoacetate in the blood and liver, as well as the rate of metabolism of dibromoacetate in the liver via the GST ζ pathway and a nonGST ζ pathway in rats and mice receiving the drinking water concentrations used in the 2-year studies. In rats, the concentrations of dibromoacetate in the blood and liver increase disproportionately with increasing concentrations of dibromoacetic

acid in the drinking water; for example, after 15 months of exposure, the blood AUC increased approximately 600-fold at the 500 mg/L concentration compared to 50 mg/L and increased another 6 fold at the 1,000 mg/L concentration (Figure 6). In contrast, the rate of metabolism of dibromoacetate via the GST ζ pathway is equivalent at exposures of 500 and 1,000 mg/L. The nonlinear relationships between these various internal dose metrics and intake of dibromoacetic acid reflect the consequence of GST ζ inactivation that occurs with dibromoacetate metabolism by this pathway. Because the shape of the dose response curve for mesotheliomas in male rats (neoplasm incidence versus exposure shown in Figure 7) appears to be similar to that of the blood AUC for dibromoacetate but very different from that of the GST ζ metabolic activity, this neoplastic response may be due largely to parent compound.

In mice, the concentrations of dibromoacetate in the blood and liver and the rate of metabolism of dibromoacetate via the GST ζ pathway increase proportionally with increasing concentrations of dibromoacetic acid in the drinking water (Figure 8). Similarly, the incidences of hepatoblastomas in male mice increase proportionally with increasing concentrations of dibromoacetic acid in the drinking water (Figure 9). Thus, the parent compound, metabolites, or both may be important contributors to this neoplastic response. The supralinear-appearing response for hepatocellular adenomas and carcinomas is different than the changes in parent compound concentration in the liver or the GST ζ metabolic activity that occur with increasing concentrations of dibromoacetic acid in the drinking water. For example, at 50 mg/L of dibromoacetic acid, there were relatively low levels of dibromoacetate in the liver and low rates of GST ζ -mediated metabolism, but there was a significant increase in hepatocellular neoplasms. Hence, it appears that the induction of hepatocellular neoplasms in mice may be very sensitive to low tissue levels of dibromoacetate or its metabolites.

The dose response for mononuclear cell leukemia in female rats is consistent with the exposure-related changes in GST ζ -mediated metabolism and the blood levels of parent compound; however, the mononuclear cell leukemia response in male rats (increased incidence at 50 mg/L with no increase at 1,000 mg/L) is not consistent with the dose metrics derived from the PBPK model of dibromoacetic acid.

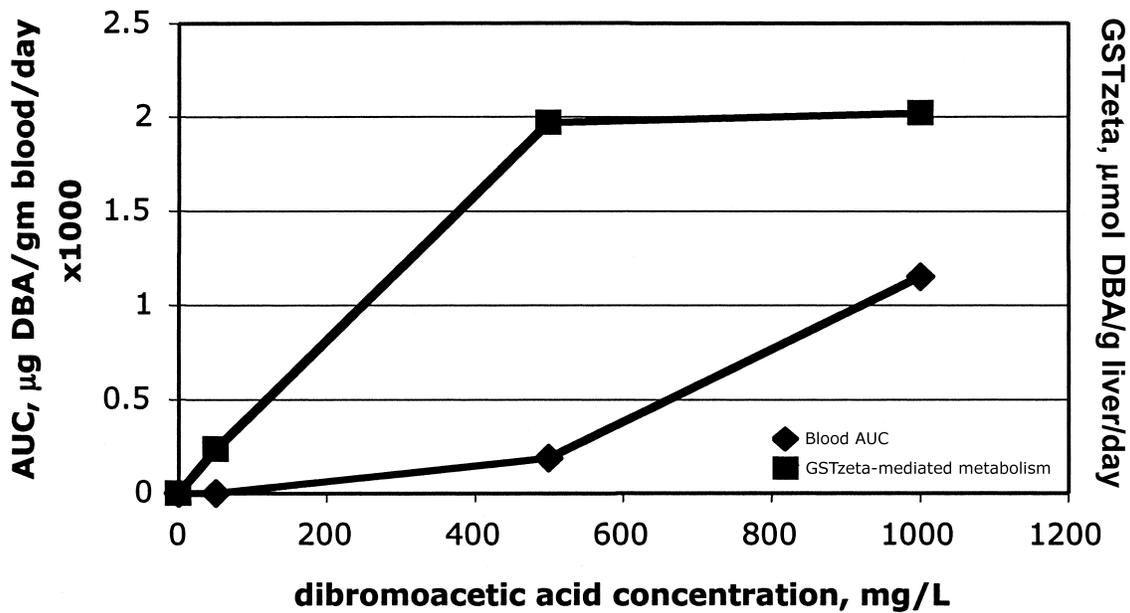


FIGURE 6
PBPK Model-Based Estimates of 24-Hour Blood AUCs for Dibromoacetate (DBA)
and Daily Rates of GSTzeta-Mediated Metabolism in the Liver of Female F344/N Rats
at 15 Months During the 2-Year Drinking Water Study of Dibromoacetic Acid

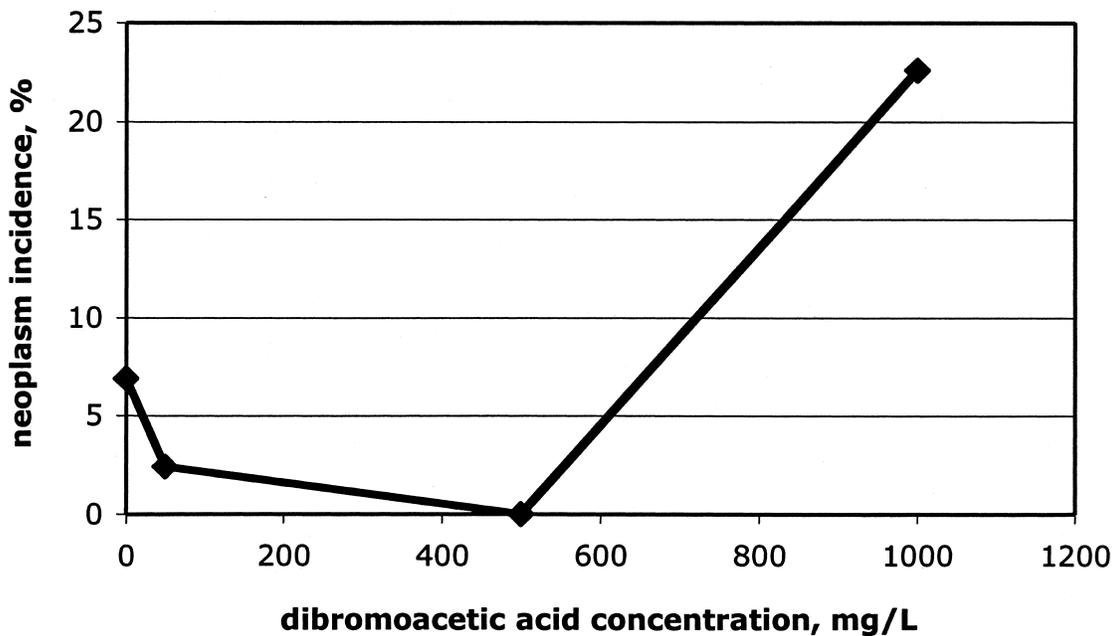


FIGURE 7
Survival-Adjusted (Poly-3) Mesothelioma Incidences in Male F344/N Rats
Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

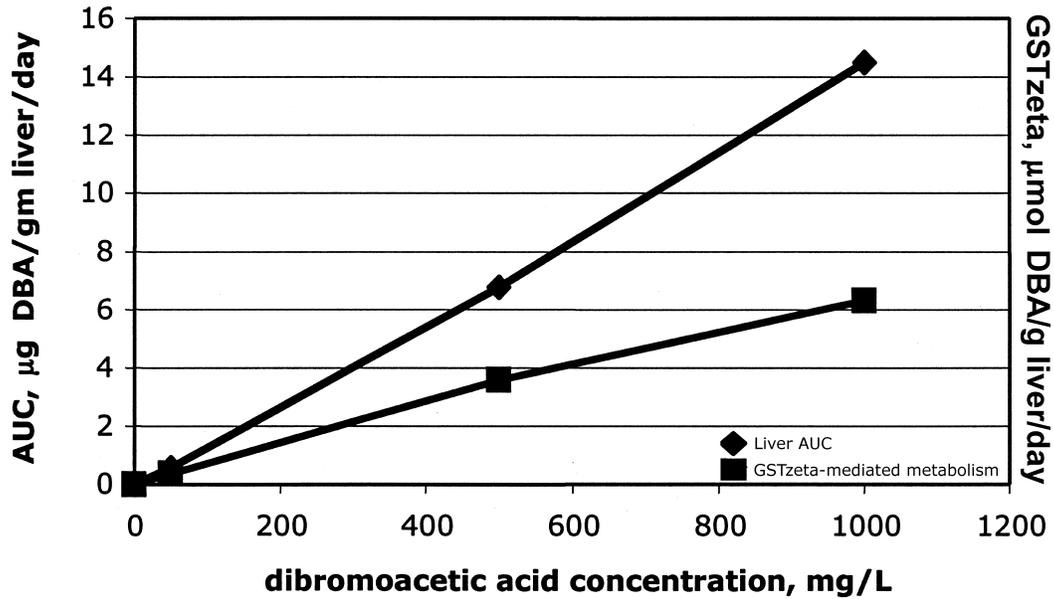


FIGURE 8
PBPK Model-Based Estimates of 24-Hour Liver AUCs for Dibromoacetate (DBA)
and Daily Rates of GSTzeta-Mediated Metabolism in the Liver of Male B6C3F₁ Mice
at 15 Months During the 2-Year Drinking Water Study of Dibromoacetic Acid

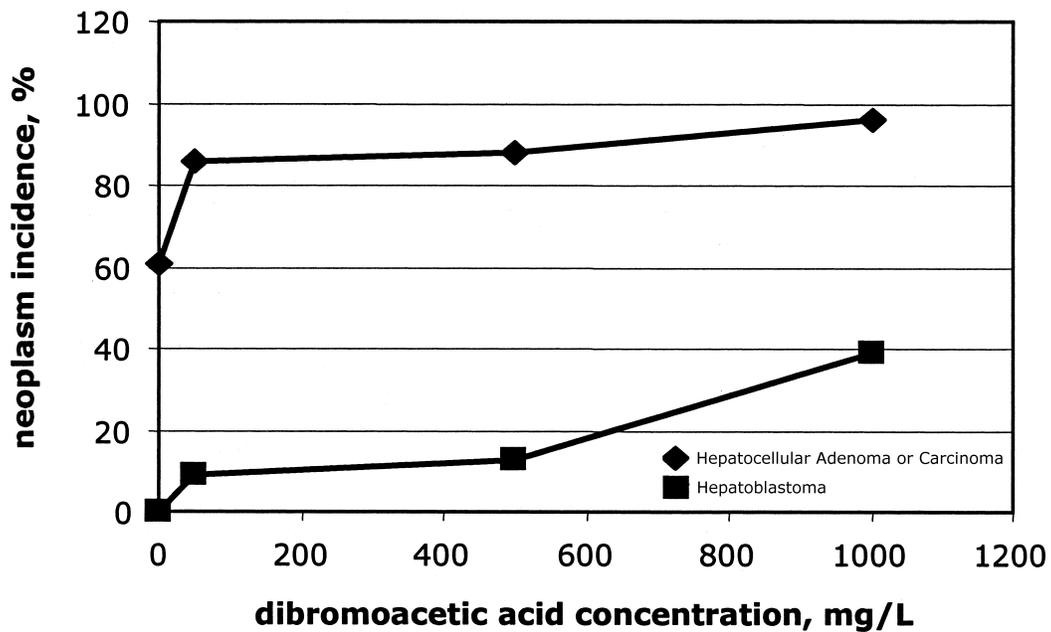


FIGURE 9
Survival-Adjusted (Poly-3) Liver Neoplasm Incidences in Male B6C3F₁ Mice
Exposed to Dibromoacetic Acid in Drinking Water for 2 Years

CONCLUSIONS

Under the conditions of these studies, there was *some evidence of carcinogenic activity** of dibromoacetic acid in male rats based on an increased incidence of malignant mesothelioma. There was *some evidence of carcinogenic activity* of dibromoacetic acid in female rats based on an increased incidence and positive trend of mononuclear cell leukemia. There was *clear evidence of carcinogenic activity* of dibromoacetic acid in male and female mice based on increased incidences of hepatocellular neoplasms and hepatoblastoma (males only). Increased incidences of lung neoplasms in male mice were also considered to be exposure related. The slight increased incidence of lung neoplasms in female mice may have been related to dibromoacetic acid exposure.

Exposure to dibromoacetic acid for two years caused increased incidences of cystic degeneration of the liver in male rats, increased incidences of alveolar epithelial hyperplasia and nephropathy in female rats, and increased incidences of splenic hematopoiesis in male mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11.

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

| | | |
|------------------|--|-------------|
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TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|---------|---------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental death | | | | 1 |
| Moribund | 4 | 15 | 12 | 12 |
| Natural deaths | 12 | 11 | 8 | 9 |
| Survivors | | | | |
| Died last week of study | | | 3 | |
| Terminal sacrifice | 34 | 24 | 27 | 28 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, colon | (48) | (49) | (48) | (50) |
| Polyp adenomatous | | | 1 (2%) | |
| Intestine large, cecum | (45) | (47) | (46) | (47) |
| Intestine small, duodenum | (45) | (48) | (47) | (48) |
| Liver | (50) | (50) | (50) | (50) |
| Hepatocellular carcinoma | | | 1 (2%) | |
| Hepatocellular adenoma | 1 (2%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Histiocytic sarcoma | 1 (2%) | | 1 (2%) | |
| Mesentery | (10) | (14) | (11) | (17) |
| Fibrosarcoma, metastatic, skin | | | 1 (9%) | |
| Histiocytic sarcoma | 1 (10%) | | | |
| Rhabdomyosarcoma | 1 (10%) | | | |
| Oral mucosa | | | (1) | |
| Pharyngeal, squamous cell carcinoma | | | 1 (100%) | |
| Pancreas | (48) | (50) | (48) | (49) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Acinus, adenoma | | 2 (4%) | 2 (4%) | 1 (2%) |
| Salivary glands | (50) | (50) | (49) | (50) |
| Stomach, forestomach | (50) | (49) | (49) | (50) |
| Squamous cell papilloma | 1 (2%) | 1 (2%) | | |
| Stomach, glandular | (50) | (48) | (48) | (50) |
| Tooth | | | (1) | |
| Odontoma | | | 1 (100%) | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (2%) | | |
| Histiocytic sarcoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, lung | | | 1 (2%) | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (49) | (50) |
| Adenoma | | | | 1 (2%) |
| Histiocytic sarcoma | 1 (2%) | | | |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Endocrine System (continued) | | | | |
| Adrenal medulla | (49) | (50) | (49) | (50) |
| Pheochromocytoma malignant | | 2 (4%) | | 1 (2%) |
| Pheochromocytoma complex | | | | 1 (2%) |
| Pheochromocytoma benign | 7 (14%) | 5 (10%) | 7 (14%) | 5 (10%) |
| Bilateral, pheochromocytoma benign | | 1 (2%) | 1 (2%) | 1 (2%) |
| Islets, pancreatic | (48) | (50) | (48) | (50) |
| Adenoma | 5 (10%) | 3 (6%) | 4 (8%) | 1 (2%) |
| Carcinoma | | | 1 (2%) | |
| Parathyroid gland | (50) | (50) | (48) | (47) |
| Adenoma | | | 1 (2%) | |
| Pituitary gland | (50) | (49) | (49) | (50) |
| Pars distalis, adenoma | 23 (46%) | 21 (43%) | 17 (35%) | 8 (16%) |
| Pars intermedia, adenoma | | | 1 (2%) | |
| Thyroid gland | (50) | (50) | (49) | (50) |
| C-cell, adenoma | 6 (12%) | 5 (10%) | 5 (10%) | 7 (14%) |
| C-cell, carcinoma | 1 (2%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Follicular cell, adenoma | 1 (2%) | | 1 (2%) | 2 (4%) |
| General Body System | | | | |
| Peritoneum | (1) | (1) | | (4) |
| Schwannoma malignant, metastatic, peripheral nerve | | 1 (100%) | | |
| Genital System | | | | |
| Epididymis | (50) | (50) | (49) | (49) |
| Preputial gland | (50) | (50) | (50) | (50) |
| Adenoma | 3 (6%) | 5 (10%) | 8 (16%) | 4 (8%) |
| Squamous cell papilloma | 1 (2%) | | | |
| Prostate | (50) | (50) | (50) | (50) |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Testes | (50) | (50) | (49) | (50) |
| Bilateral, interstitial cell, adenoma | 34 (68%) | 42 (84%) | 34 (69%) | 39 (78%) |
| Interstitial cell, adenoma | 7 (14%) | 4 (8%) | 7 (14%) | 7 (14%) |
| Hematopoietic System | | | | |
| Bone marrow | (49) | (49) | (48) | (50) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, lung | | | 1 (2%) | |
| Lymph node | (22) | (26) | (25) | (25) |
| Bronchial, thymoma malignant, metastatic, thymus | | | 1 (4%) | |
| Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (4%) | | |
| Lymph node, mesenteric | (50) | (50) | (49) | (50) |
| Hemangioma | | | 1 (2%) | |
| Histiocytic sarcoma | 1 (2%) | | | |
| Spleen | (50) | (50) | (49) | (49) |
| Histiocytic sarcoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, lung | | | 1 (2%) | |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|---------|----------|----------|------------|
| Hematopoietic System (continued) | | | | |
| Thymus | (49) | (48) | (48) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (2%) | | |
| Histiocytic sarcoma | 1 (2%) | | | |
| Sarcoma | 1 (2%) | | | |
| Thymoma benign | 1 (2%) | | | |
| Thymoma malignant | | | 1 (2%) | |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | | | | 1 (2%) |
| Carcinoma | 1 (2%) | | 1 (2%) | 1 (2%) |
| Fibroadenoma | 4 (8%) | 5 (10%) | 3 (6%) | 5 (10%) |
| Fibroadenoma, multiple | | | 1 (2%) | |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell carcinoma | 1 (2%) | | | |
| Histiocytic sarcoma | 1 (2%) | | | |
| Keratoacanthoma | 3 (6%) | 1 (2%) | 1 (2%) | 2 (4%) |
| Squamous cell carcinoma | 1 (2%) | | | |
| Squamous cell papilloma | 2 (4%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Trichoepithelioma | 1 (2%) | 1 (2%) | | |
| Sebaceous gland, adenoma, multiple | | | | 1 (2%) |
| Subcutaneous tissue, fibroma | 5 (10%) | 4 (8%) | 3 (6%) | 1 (2%) |
| Subcutaneous tissue, fibrosarcoma | | 3 (6%) | 3 (6%) | 2 (4%) |
| Subcutaneous tissue, hemangioma | 1 (2%) | | | |
| Subcutaneous tissue, neural crest tumor | 1 (2%) | | | |
| Subcutaneous tissue, sarcoma | 1 (2%) | | | 1 (2%) |
| Subcutaneous tissue, schwannoma malignant | | | 1 (2%) | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | | | 1 (2%) |
| Vertebra, chordoma | | 1 (2%) | | |
| Skeletal muscle | (2) | (1) | (1) | (3) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (100%) | | |
| Hemangioma | 1 (50%) | | | |
| Histiocytic sarcoma | | | 1 (100%) | |
| Sarcoma | 1 (50%) | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma malignant | | | 1 (2%) | |
| Glioma malignant, mixed cell | | | 1 (2%) | |
| Pineal gland, glioma malignant | 1 (2%) | | | |
| Peripheral nerve | (2) | (1) | (2) | (2) |
| Schwannoma malignant | | 1 (100%) | | |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 2 (4%) | | 4 (8%) | 2 (4%) |
| Alveolar/bronchiolar carcinoma | | 1 (2%) | 1 (2%) | |
| Carcinoma, metastatic, thyroid gland | | 1 (2%) | | |
| Histiocytic sarcoma | 2 (4%) | | 1 (2%) | |
| Schwannoma malignant, metastatic, eye | | 1 (2%) | | |
| Schwannoma malignant, metastatic, heart | | | 1 (2%) | |
| Squamous cell carcinoma | | | 1 (2%) | |
| Mediastinum, sarcoma | 1 (2%) | | | |
| Serosa, thymoma malignant, metastatic, thymus | | | 1 (2%) | |
| Pleura | (1) | | (1) | |
| Histiocytic sarcoma | 1 (100%) | | | |
| Thymoma malignant, metastatic, thymus | | | 1 (100%) | |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Schwannoma malignant | | 1 (2%) | | |
| Harderian gland | (50) | (50) | (49) | (50) |
| Zymbal's gland | (2) | | | (2) |
| Adenoma | 1 (50%) | | | 1 (50%) |
| Carcinoma | | | | 1 (50%) |
| Urinary System | | | | |
| Kidney | (49) | (50) | (49) | (50) |
| Lipoma | | | | 1 (2%) |
| Renal tubule, adenoma | 1 (2%) | | 1 (2%) | |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Carcinoma | 1 (2%) | | | |
| Histiocytic sarcoma | 1 (2%) | | | |
| Papilloma | | 1 (2%) | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | 2 (4%) | | 1 (2%) | |
| Leukemia mononuclear | 17 (34%) | 31 (62%) | 24 (48%) | 13 (26%) |
| Lymphoma malignant lymphocytic | | | | 1 (2%) |
| Mesothelioma malignant | 3 (6%) | 1 (2%) | | 10 (20%) |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|--------|---------|----------|------------|
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | 50 | 50 | 49 | 50 |
| Total primary neoplasms | 145 | 146 | 147 | 127 |
| Total animals with benign neoplasms | 47 | 48 | 45 | 48 |
| Total benign neoplasms | 111 | 103 | 108 | 94 |
| Total animals with malignant neoplasms | 29 | 38 | 35 | 26 |
| Total malignant neoplasms | 33 | 43 | 39 | 33 |
| Total animals with metastatic neoplasms | | 4 | 3 | |
| Total metastatic neoplasms | | 7 | 8 | |
| Total animals with uncertain neoplasms- benign or malignant | 1 | | | |
| Total uncertain neoplasms | 1 | | | |

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

^b 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 Dibromoacetic Acid: 50 mg/L

| Number of Days on Study | 4 | 4 | 4 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | |
|--|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 3 | 4 | 7 | 1 | 1 | 4 | 0 | 1 | 1 | 1 | 2 | 4 | 5 | 5 | 5 | 7 | 7 | 9 | 9 | 9 | 9 | 0 | 0 | 0 | 1 | 2 |
| | 7 | 6 | 1 | 0 | 9 | 7 | 2 | 2 | 5 | 8 | 6 | 1 | 1 | 1 | 1 | 9 | 9 | 1 | 3 | 7 | 1 | 1 | 9 | 0 | 1 | |
| Carcass ID Number | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 6 | 0 | 8 | 9 | 8 | 6 | 5 | 9 | 9 | 9 | 6 | 5 | 5 | 6 | 9 | 6 | 7 | 8 | 5 | 8 | 7 | 7 | 7 | 5 | 9 | |
| | 5 | 0 | 2 | 5 | 4 | 8 | 7 | 1 | 2 | 0 | 2 | 8 | 1 | 7 | 4 | 6 | 3 | 7 | 6 | 5 | 1 | 8 | 9 | 5 | 6 | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, cecum | + | + | + | + | + | + | A | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | |
| Intestine small, duodenum | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | |
| Intestine small, jejunum | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | A | + | + | A | + | A | |
| Intestine small, ileum | + | + | + | + | + | + | A | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mesentery | + | + | + | + | | | | + | + | | | | | | | | + | | + | | | + | | + | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Acinus, adenoma | | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | | | | | | | | | | + | + | | | | | | | | | | + | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pheochromocytoma malignant | X | | | | | | | | | | | | | | | | | | | | | X | | | | |
| Pheochromocytoma benign | | | | | | | | | | | X | | | | | | | | | | | X | | X | X | |
| Bilateral, pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | M | + | + | + | + | + | + | + | |
| Pars distalis, adenoma | | | | | | | | | | | | | | | | | | | | | | | X | | X | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | X | X | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peritoneum | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Schwannoma malignant, metastatic, peripheral nerve | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Coagulating gland | | | | | | | | | | | + | | | | + | | | | | | | | | | | |
| Epididymis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid: 50 mg/L

| | |
|---|---|
| Number of Days on Study | 4 4 4 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 7 |
| | 3 4 7 1 1 4 0 1 1 1 2 4 5 5 5 7 7 9 9 9 9 0 0 0 1 2 |
| | 7 6 1 0 9 7 2 2 5 8 6 1 1 1 1 9 9 1 3 7 1 1 9 0 1 |
| Carcass ID Number | 0 1 0 |
| | 6 0 8 9 8 6 5 9 9 9 6 5 5 6 9 6 7 8 5 8 7 7 7 5 9 |
| | 5 0 2 5 4 8 7 1 2 0 2 8 1 7 4 6 3 7 6 5 1 8 9 5 6 |
| Genital System (continued) | |
| Preputial gland | + |
| Adenoma | |
| | X |
| Prostate | + |
| Seminal vesicle | + |
| Testes | + |
| Bilateral, interstitial cell, adenoma | X |
| Interstitial cell, adenoma | |
| | X X |
| Hematopoietic System | |
| Bone marrow | + + + + + + A + + + + + + + + + + + + + + + + + + |
| Lymph node | + |
| Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung | |
| | X |
| Lymph node, mandibular | M |
| Lymph node, mesenteric | + |
| Spleen | + |
| Thymus | + |
| Alveolar/bronchiolar carcinoma, metastatic, lung | |
| | X |
| Integumentary System | |
| Mammary gland | + |
| Fibroadenoma | |
| | X X |
| Skin | + |
| Keratoacanthoma | |
| | X |
| Squamous cell papilloma | |
| Trichoepithelioma | |
| Subcutaneous tissue, fibroma | |
| | X X |
| Subcutaneous tissue, fibrosarcoma | |
| | X X |
| Musculoskeletal System | |
| Bone | + |
| Vertebra, chordoma | |
| | X |
| Skeletal muscle | |
| | + |
| Alveolar/bronchiolar carcinoma, metastatic, lung | |
| | X |
| Nervous System | |
| Brain | + |
| Peripheral nerve | |
| Schwannoma malignant | |

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid: 50 mg/L

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Days on Study | 4 | 4 | 4 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | |
| | 3 | 4 | 7 | 1 | 1 | 4 | 0 | 1 | 1 | 1 | 2 | 4 | 5 | 5 | 5 | 7 | 7 | 9 | 9 | 9 | 9 | 0 | 0 | 0 | 1 | 2 |
| | 7 | 6 | 1 | 0 | 9 | 7 | 2 | 2 | 5 | 8 | 6 | 1 | 1 | 1 | 1 | 9 | 9 | 1 | 3 | 7 | 1 | 1 | 9 | 0 | 1 | |
| Carcass ID Number | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| | 6 | 0 | 8 | 9 | 8 | 6 | 5 | 9 | 9 | 9 | 6 | 5 | 5 | 6 | 9 | 6 | 7 | 8 | 5 | 8 | 7 | 7 | 7 | 5 | 9 | |
| | 5 | 0 | 2 | 5 | 4 | 8 | 7 | 1 | 2 | 0 | 2 | 8 | 1 | 7 | 4 | 6 | 3 | 7 | 6 | 5 | 1 | 8 | 9 | 5 | 6 | |
| Respiratory System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Carcinoma, metastatic, thyroid gland | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Schwannoma malignant, metastatic, eye | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Nose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Special Senses System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ear | | | | | | | | | | | | | | | | | | | | | | | | | + | |
| Eye | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Schwannoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Harderian gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Urinary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Papilloma | | | | | | | | | | | | | | | | | | | | | | | | | X | |
| Systemic Lesions | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Leukemia mononuclear | X | X | X | X | X | X | X | X | | | | X | | | 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 Dibromoacetic Acid: 500 mg/L

| Number of Days on Study | 0 | 2 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
|--|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2 | 5 | 0 | 2 | 3 | 2 | 6 | 6 | 7 | 8 | 9 | 9 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 |
| | 2 | 3 | 2 | 3 | 7 | 1 | 0 | 0 | 8 | 0 | 0 | 4 | 2 | 7 | 7 | 8 | 9 | 0 | 0 | 1 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | |
| 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 | 1 | 1 | 1 | |
| | 4 | 2 | 0 | 0 | 1 | 2 | 4 | 4 | 1 | 0 | 2 | 3 | 3 | 1 | 2 | 2 | 1 | 1 | 1 | 2 | 0 | 1 | 2 | 2 | 4 | | | | |
| | 9 | 7 | 4 | 9 | 6 | 5 | 4 | 5 | 1 | 5 | 9 | 7 | 6 | 0 | 2 | 8 | 2 | 7 | 8 | 1 | 6 | 9 | 0 | 6 | 3 | | | | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Polyp adenomatous | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine large, cecum | A | + | + | A | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, duodenum | + | + | + | A | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, jejunum | A | + | + | A | A | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Intestine small, ileum | + | + | + | A | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mesentery | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibrosarcoma, metastatic, skin | | | | + | | | + | | | | | | | | + | | + | + | | + | + | + | | + | | | | | |
| Oral mucosa | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pharyngeal, squamous cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | |
| Acinus, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | |
| Tooth | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Odontoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Schwannoma malignant, metastatic, lung | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bilateral, pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | A | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Pars distalis, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pars intermedia, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| C-cell, carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Follicular cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|---------------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Days on Study | 3 | 3 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 |
| | 5 | 8 | 2 | 1 | 1 | 2 | 6 | 7 | 7 | 1 | 1 | 2 | 3 | 3 | 4 | 5 | 5 | 5 | 7 | 7 | 0 | 2 | 2 | 2 | 2 |
| | 6 | 6 | 3 | 2 | 4 | 1 | 1 | 0 | 1 | 0 | 8 | 4 | 1 | 3 | 4 | 1 | 1 | 1 | 4 | 9 | 1 | 3 | 9 | 9 | 9 |
| Carcass ID Number | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | |
| | 8 | 7 | 8 | 8 | 9 | 7 | 9 | 7 | 7 | 6 | 8 | 8 | 9 | 8 | 5 | 5 | 6 | 9 | 6 | 0 | 8 | 6 | 5 | 6 | 6 |
| | 3 | 0 | 2 | 4 | 1 | 5 | 7 | 7 | 8 | 9 | 0 | 7 | 9 | 9 | 4 | 5 | 7 | 5 | 8 | 0 | 6 | 1 | 6 | 0 | 2 |
| Genital System (continued) | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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 | | | | | | | | |
| Hematopoietic System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 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 | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Integumentary System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Keratoacanthoma | | | | | | | | | | | | | | | | | | X | | | | | | X | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Sebaceous gland, adenoma, multiple | | | | | | | | | | | | | X | | | | | | | | | | | | |
| Subcutaneous tissue, fibroma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Subcutaneous tissue, fibrosarcoma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Subcutaneous tissue, sarcoma | | | | | | | | | | X | | | | | | | | | | | | | | | |
| Musculoskeletal System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hemangiosarcoma | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Skeletal muscle | | + | | | + | + | | | | | | | | | | | | | | | | | | | |
| Nervous System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Peripheral nerve | + | | | | | | | | | | | | | | | | | | | | | | | | |
| Spinal cord | + | | | | | | | | | | | | | | | | | | | | | | | | |
| Respiratory System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | X | | |
| Nose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | | | | | | | | | | | | | | | | | | | | | | | | |
|--------------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Days on Study | 3 | 3 | 4 | 5 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 |
| | 5 | 8 | 2 | 1 | 1 | 2 | 6 | 7 | 7 | 1 | 1 | 2 | 3 | 3 | 4 | 5 | 5 | 5 | 7 | 7 | 0 | 2 | 2 | 2 | 2 |
| | 6 | 6 | 3 | 2 | 4 | 1 | 1 | 0 | 1 | 0 | 8 | 4 | 1 | 3 | 4 | 1 | 1 | 1 | 4 | 9 | 1 | 3 | 9 | 9 | 9 |
| Carcass ID Number | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | |
| | 8 | 7 | 8 | 8 | 9 | 7 | 9 | 7 | 7 | 6 | 8 | 8 | 9 | 8 | 5 | 5 | 6 | 9 | 6 | 0 | 8 | 6 | 5 | 6 | 6 |
| | 3 | 0 | 2 | 4 | 1 | 5 | 7 | 7 | 8 | 9 | 0 | 7 | 9 | 9 | 4 | 5 | 7 | 5 | 8 | 0 | 6 | 1 | 6 | 0 | 2 |
| Special Senses System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ear | | | | | | | | | | | | | | | | | | | | | | | | | |
| Eye | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Harderian gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lacrimal gland | | | | | | | | | | | | | | | | | | | | | | | | | |
| Zymbal's gland | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Urinary System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lipoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Systemic Lesions | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lymphoma malignant lymphocytic | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mesothelioma malignant | | | | | | | | | | | | | | | | | | | | | | | | | |

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | |
|--------------------------------|---|-----------------------------|
| Number of Days on Study | 7 | |
| | 2 2 2 3 | |
| | 9 9 9 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 4 4 4 4 4 4 | |
| Carcass ID Number | 1 | Total Tissues/ Tumors |
| | 6 6 6 7 8 8 5 5 5 6 7 7 7 7 7 9 9 9 9 5 5 5 8 9 9 | |
| | 3 4 5 1 1 5 7 8 9 6 2 3 4 6 9 3 4 6 8 1 2 3 8 0 2 | |
| Special Senses System | | |
| Ear | | 1 |
| Eye | + | 50 |
| Harderian gland | + | 50 |
| Lacrimal gland | | 1 |
| Zymbal's gland | | 2 |
| Adenoma | | 1 |
| Carcinoma | | 1 |
| Urinary System | | |
| Kidney | + | 50 |
| Lipoma | | 1 |
| Urinary bladder | + | 50 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Leukemia mononuclear | X X X X X | 13 |
| Lymphoma malignant lymphocytic | | 1 |
| Mesothelioma malignant | | 10 |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|------------|----------------|------------|------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 7/49 (14%) | 6/50 (12%) | 8/49 (16%) | 6/50 (12%) |
| Adjusted rate ^b | 16.4% | 14.2% | 20.0% | 14.5% |
| Terminal rate ^c | 6/34 (18%) | 1/24 (4%) | 5/30 (17%) | 3/28 (11%) |
| First incidence (days) | 711 | 618 | 560 | 624 |
| Poly-3 test ^d | P=0.549N | P=0.507N | P=0.445 | P=0.523N |
| Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma | | | | |
| Overall rate | 7/49 (14%) | 7/50 (14%) | 8/49 (16%) | 8/50 (16%) |
| Adjusted rate | 16.4% | 16.3% | 20.0% | 19.2% |
| Terminal rate | 6/34 (18%) | 1/24 (4%) | 5/30 (17%) | 4/28 (14%) |
| First incidence (days) | 711 | 437 | 560 | 624 |
| Poly-3 test | P=0.371 | P=0.607N | P=0.445 | P=0.481 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate | 2.3% | 2.4% | 7.6% | 2.5% |
| Terminal rate | 1/34 (3%) | 1/24 (4%) | 3/30 (10%) | 0/28 (0%) |
| First incidence (days) | 729 (T) | 729 (T) | 729 (T) | 651 |
| Poly-3 test | P=0.485 | P=0.751 | P=0.274 | P=0.746 |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 2/50 (4%) | 0/50 (0%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted rate | 4.6% | 0.0% | 10.1% | 4.9% |
| Terminal rate | 1/34 (3%) | 0/24 (0%) | 4/30 (13%) | 1/28 (4%) |
| First incidence (days) | 682 | — ^e | 729 (T) | 701 |
| Poly-3 test | P=0.257 | P=0.248N | P=0.295 | P=0.668 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 5/50 (10%) | 2/50 (4%) |
| Adjusted rate | 4.6% | 2.4% | 12.6% | 4.9% |
| Terminal rate | 1/34 (3%) | 0/24 (0%) | 5/30 (17%) | 1/28 (4%) |
| First incidence (days) | 682 | 651 | 729 (T) | 701 |
| Poly-3 test | P=0.331 | P=0.515N | P=0.180 | P=0.668 |
| Mammary Gland: Fibroadenoma | | | | |
| Overall rate | 4/50 (8%) | 5/50 (10%) | 4/50 (8%) | 5/50 (10%) |
| Adjusted rate | 9.2% | 12.0% | 10.1% | 12.4% |
| Terminal rate | 4/34 (12%) | 3/24 (13%) | 4/30 (13%) | 5/28 (18%) |
| First incidence (days) | 729 (T) | 701 | 729 (T) | 729 (T) |
| Poly-3 test | P=0.447 | P=0.474 | P=0.595 | P=0.455 |
| Mammary Gland: Fibroadenoma or Adenoma | | | | |
| Overall rate | 4/50 (8%) | 5/50 (10%) | 4/50 (8%) | 6/50 (12%) |
| Adjusted rate | 9.2% | 12.0% | 10.1% | 14.9% |
| Terminal rate | 4/34 (12%) | 3/24 (13%) | 4/30 (13%) | 6/28 (21%) |
| First incidence (days) | 729 (T) | 701 | 729 (T) | 729 (T) |
| Poly-3 test | P=0.307 | P=0.474 | P=0.595 | P=0.324 |
| Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma | | | | |
| Overall rate | 5/50 (10%) | 5/50 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 11.5% | 12.0% | 12.6% | 17.4% |
| Terminal rate | 5/34 (15%) | 3/24 (13%) | 5/30 (17%) | 7/28 (25%) |
| First incidence (days) | 729 (T) | 701 | 729 (T) | 729 (T) |
| Poly-3 test | P=0.249 | P=0.605 | P=0.575 | P=0.328 |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|-------------|-------------|-------------|------------|
| Pancreatic Islets: Adenoma | | | | |
| Overall rate | 5/48 (10%) | 3/50 (6%) | 4/48 (8%) | 1/50 (2%) |
| Adjusted rate | 11.9% | 7.2% | 10.2% | 2.4% |
| Terminal rate | 4/34 (12%) | 1/24 (4%) | 3/30 (10%) | 0/28 (0%) |
| First incidence (days) | 682 | 679 | 560 | 512 |
| Poly-3 test | P=0.130N | P=0.363N | P=0.547N | P=0.107N |
| Pancreatic Islets: Adenoma or Carcinoma | | | | |
| Overall rate | 5/48 (10%) | 3/50 (6%) | 5/48 (10%) | 1/50 (2%) |
| Adjusted rate | 11.9% | 7.2% | 12.7% | 2.4% |
| Terminal rate | 4/34 (12%) | 1/24 (4%) | 3/30 (10%) | 0/28 (0%) |
| First incidence (days) | 682 | 679 | 560 | 512 |
| Poly-3 test | P=0.157N | P=0.363N | P=0.587 | P=0.107N |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 23/50 (46%) | 21/49 (43%) | 17/49 (35%) | 8/50 (16%) |
| Adjusted rate | 51.0% | 48.9% | 41.0% | 19.3% |
| Terminal rate | 16/34 (47%) | 13/24 (54%) | 11/30 (37%) | 5/28 (18%) |
| First incidence (days) | 591 | 602 | 560 | 512 |
| Poly-3 test | P<0.001N | P=0.507N | P=0.232N | P<0.001N |
| Preputial Gland: Adenoma | | | | |
| Overall rate | 3/50 (6%) | 5/50 (10%) | 8/50 (16%) | 4/50 (8%) |
| Adjusted rate | 6.9% | 12.0% | 20.1% | 9.7% |
| Terminal rate | 3/34 (9%) | 4/24 (17%) | 7/30 (23%) | 2/28 (7%) |
| First incidence (days) | 729 (T) | 679 | 700 | 610 |
| Poly-3 test | P=0.403 | P=0.333 | P=0.072 | P=0.472 |
| Skin: Squamous Cell Papilloma | | | | |
| Overall rate | 2/50 (4%) | 1/50 (2%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 4.6% | 2.4% | 5.0% | 7.4% |
| Terminal rate | 1/34 (3%) | 1/24 (4%) | 2/30 (7%) | 2/28 (7%) |
| First incidence (days) | 629 | 729 (T) | 729 (T) | 723 |
| Poly-3 test | P=0.244 | P=0.519N | P=0.660 | P=0.464 |
| Skin: Keratoacanthoma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 1/50 (2%) | 2/50 (4%) |
| Adjusted rate | 6.9% | 2.4% | 2.5% | 4.9% |
| Terminal rate | 3/34 (9%) | 0/24 (0%) | 1/30 (3%) | 1/28 (4%) |
| First incidence (days) | 729 (T) | 693 | 729 (T) | 651 |
| Poly-3 test | P=0.548N | P=0.320N | P=0.337N | P=0.529N |
| Skin: Squamous Cell Papilloma or Keratoacanthoma | | | | |
| Overall rate | 5/50 (10%) | 2/50 (4%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 11.4% | 4.8% | 5.0% | 12.3% |
| Terminal rate | 4/34 (12%) | 1/24 (4%) | 2/30 (7%) | 3/28 (11%) |
| First incidence (days) | 629 | 693 | 729 (T) | 651 |
| Poly-3 test | P=0.358 | P=0.236N | P=0.256N | P=0.585 |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|--------------|-------------|-------------|
| Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma | | | | |
| Overall rate | 6/50 (12%) | 2/50 (4%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 13.7% | 4.8% | 5.0% | 12.3% |
| Terminal rate | 5/34 (15%) | 1/24 (4%) | 2/30 (7%) | 3/28 (11%) |
| First incidence (days) | 629 | 693 | 729 (T) | 651 |
| Poly-3 test | P=0.463 | P=0.148N | P=0.165N | P=0.551N |
| Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Carcinoma, or Squamous Cell Carcinoma | | | | |
| Overall rate | 7/50 (14%) | 3/50 (6%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 16.0% | 7.2% | 5.0% | 12.3% |
| Terminal rate | 6/34 (18%) | 2/24 (8%) | 2/30 (7%) | 3/28 (11%) |
| First incidence (days) | 629 | 693 | 729 (T) | 651 |
| Poly-3 test | P=0.481N | P=0.177N | P=0.102N | P=0.430N |
| Skin (Subcutaneous Tissue): Fibroma | | | | |
| Overall rate | 5/50 (10%) | 4/50 (8%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate | 11.2% | 9.5% | 7.5% | 2.5% |
| Terminal rate | 2/34 (6%) | 0/24 (0%) | 2/30 (7%) | 1/28 (4%) |
| First incidence (days) | 516 | 651 | 701 | 729 (T) |
| Poly-3 test | P=0.086N | P=0.538N | P=0.423N | P=0.127N |
| Skin (Subcutaneous Tissue): Fibrosarcoma | | | | |
| Overall rate | 0/50 (0%) | 3/50 (6%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 7.1% | 7.4% | 4.9% |
| Terminal rate | 0/34 (0%) | 1/24 (4%) | 2/30 (7%) | 1/28 (4%) |
| First incidence (days) | — | 618 | 402 | 679 |
| Poly-3 test | P=0.357 | P=0.113 | P=0.107 | P=0.222 |
| Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) | 3/50 (6%) |
| Adjusted rate | 2.3% | 7.1% | 7.4% | 7.3% |
| Terminal rate | 1/34 (3%) | 1/24 (4%) | 2/30 (7%) | 1/28 (4%) |
| First incidence (days) | 729 (T) | 618 | 402 | 571 |
| Poly-3 test | P=0.309 | P=0.294 | P=0.281 | P=0.286 |
| Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma | | | | |
| Overall rate | 6/50 (12%) | 6/50 (12%) | 6/50 (12%) | 4/50 (8%) |
| Adjusted rate | 13.4% | 14.1% | 14.8% | 9.7% |
| Terminal rate | 3/34 (9%) | 1/24 (4%) | 4/30 (13%) | 2/28 (7%) |
| First incidence (days) | 516 | 618 | 402 | 571 |
| Poly-3 test | P=0.346N | P=0.585 | P=0.551 | P=0.426N |
| Testes: Adenoma | | | | |
| Overall rate | 41/50 (82%) | 46/50 (92%) | 41/49 (84%) | 46/50 (92%) |
| Adjusted rate | 89.9% | 95.8% | 92.4% | 96.6% |
| Terminal rate | 33/34 (97%) | 24/24 (100%) | 28/30 (93%) | 27/28 (96%) |
| First incidence (days) | 530 | 437 | 437 | 423 |
| Poly-3 test | P=0.228 | P=0.194 | P=0.475 | P=0.149 |
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 6/50 (12%) | 5/50 (10%) | 5/49 (10%) | 7/50 (14%) |
| Adjusted rate | 13.8% | 11.7% | 12.7% | 17.4% |
| Terminal rate | 6/34 (18%) | 2/24 (8%) | 4/30 (13%) | 7/28 (25%) |
| First incidence (days) | 729 (T) | 471 | 701 | 729 (T) |
| Poly-3 test | P=0.314 | P=0.508N | P=0.569N | P=0.444 |

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

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|--------------|----------------|--------------|--------------|
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 7/50 (14%) | 7/50 (14%) | 6/49 (12%) | 8/50 (16%) |
| Adjusted rate | 16.2% | 16.3% | 15.3% | 19.8% |
| Terminal rate | 7/34 (21%) | 3/24 (13%) | 5/30 (17%) | 8/28 (29%) |
| First incidence (days) | 729 (T) | 471 | 701 | 729 (T) |
| Poly-3 test | P=0.378 | P=0.606 | P=0.576N | P=0.439 |
| All Organs: Malignant Mesothelioma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 0/50 (0%) | 10/50 (20%) |
| Adjusted rate | 6.9% | 2.4% | 0.0% | 22.6% |
| Terminal rate | 2/34 (6%) | 1/24 (4%) | 0/30 (0%) | 2/28 (7%) |
| First incidence (days) | 591 | 729 (T) | — | 512 |
| Poly-3 test | P<0.001 | P=0.325N | P=0.137N | P=0.035 |
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 17/50 (34%) | 31/50 (62%) | 24/50 (48%) | 13/50 (26%) |
| Adjusted rate | 37.0% | 66.2% | 56.2% | 30.2% |
| Terminal rate | 11/34 (32%) | 15/24 (63%) | 16/30 (53%) | 5/28 (18%) |
| First incidence (days) | 437 | 446 | 423 | 386 |
| Poly-3 test | P=0.026N | P=0.003 | P=0.051 | P=0.325N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 47/50 (94%) | 48/50 (96%) | 45/50 (90%) | 48/50 (96%) |
| Adjusted rate | 99.0% | 97.6% | 97.9% | 99.5% |
| Terminal rate | 34/34 (100%) | 24/24 (100%) | 30/30 (100%) | 28/28 (100%) |
| First incidence (days) | 516 | 437 | 437 | 423 |
| Poly-3 test | P=0.456 | P=0.630N | P=0.761N | P=0.987 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 29/50 (58%) | 38/50 (76%) | 35/50 (70%) | 26/50 (52%) |
| Adjusted rate | 59.0% | 78.4% | 75.6% | 53.9% |
| Terminal rate | 18/34 (53%) | 17/24 (71%) | 21/30 (70%) | 8/28 (29%) |
| First incidence (days) | 222 | 437 | 253 | 356 |
| Poly-3 test | P=0.084N | P=0.028 | P=0.062 | P=0.380N |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 50/50 (100%) | 50/50 (100%) | 49/50 (98%) | 50/50 (100%) |
| Adjusted rate | 100.0% | 100.0% | 100.0% | 100.0% |
| Terminal rate | 34/34 (100%) | 24/24 (100%) | 30/30 (100%) | 28/28 (100%) |
| First incidence (days) | 222 | 437 | 253 | 356 |
| Poly-3 test | P=1.000N | — ^f | P=1.000N | — |

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, pituitary gland, preputial gland, testis, 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 A4a
Historical Incidence of Malignant Mesothelioma in Control Male F344/N Rats^a

| | Incidence in Controls |
|---|-----------------------|
| Historical Incidence: Drinking Water Studies | |
| Bromodichloromethane | 3/50 |
| Dibromoacetic acid | 3/50 |
| Dipropylene glycol | 6/50 |
| Sodium chlorate | 0/50 |
| Sodium nitrite | 3/50 |
| Overall Historical Incidence: Drinking Water Studies | |
| Total (%) | 15/250 (6.0%) |
| Mean ± standard deviation | 6.0% ± 4.2% |
| Range | 0%-12% |
| Overall Historical Incidence | |
| Total (%) | 57/1,459 (3.9%) |
| Mean ± standard deviation | 3.9% ± 3.0% |
| Range | 0%-12% |

^a Data as of January 26, 2005

TABLE A4b
Historical Incidence of Mononuclear Cell Leukemia in Control Male F344/N Rats^a

| | Incidence in Controls |
|---|-----------------------|
| Historical Incidence: Drinking Water Studies | |
| Bromodichloromethane | 16/50 |
| Dibromoacetic acid | 17/50 |
| Dipropylene glycol | 16/50 |
| Sodium chlorate | 13/50 |
| Sodium nitrite | 17/50 |
| Overall Historical Incidence: Drinking Water Studies | |
| Total (%) | 79/250 (31.6%) |
| Mean ± standard deviation | 31.6% ± 3.3% |
| Range | 26%-34% |
| Overall Historical Incidence | |
| Total (%) | 622/1,459 (42.6%) |
| Mean ± standard deviation | 41.4% ± 12.3% |
| Range | 22%-68% |

^a Data as of January 26, 2005; includes lymphocytic, monocytic, mononuclear, or undifferentiated leukemia

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Accidental death | | | | 1 |
| Moribund sacrifice | 4 | 15 | 12 | 12 |
| Natural death | 12 | 11 | 8 | 9 |
| Survivors | | | | |
| Died last week of study | | | 3 | |
| Terminal sacrifice | 34 | 24 | 27 | 28 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, colon | (48) | (49) | (48) | (50) |
| Edema | | 1 (2%) | 1 (2%) | |
| Inflammation | | 1 (2%) | | |
| Polyp, inflammatory | | | | 1 (2%) |
| Intestine large, cecum | (45) | (47) | (46) | (47) |
| Congestion | | 1 (2%) | | |
| Edema | | 1 (2%) | | |
| Liver | (50) | (50) | (50) | (50) |
| Basophilic focus | 14 (28%) | 4 (8%) | 1 (2%) | 1 (2%) |
| Cholangiofibrosis | 1 (2%) | | | |
| Clear cell focus | 18 (36%) | 19 (38%) | 16 (32%) | 21 (42%) |
| Cyst | 1 (2%) | | | |
| Degeneration, cystic | 3 (6%) | 9 (18%) | 11 (22%) | 15 (30%) |
| Eosinophilic focus | 16 (32%) | 4 (8%) | 3 (6%) | 9 (18%) |
| Hepatodiaphragmatic nodule | 2 (4%) | 1 (2%) | 2 (4%) | 5 (10%) |
| Inflammation, granulomatous | 6 (12%) | 1 (2%) | | 1 (2%) |
| Mixed cell focus | 8 (16%) | 1 (2%) | 5 (10%) | 2 (4%) |
| Necrosis, focal | | | 3 (6%) | 2 (4%) |
| Regeneration | | 1 (2%) | 1 (2%) | |
| Bile duct, hyperplasia | 43 (86%) | 42 (84%) | 35 (70%) | 38 (76%) |
| Centrilobular, necrosis | | 1 (2%) | 1 (2%) | 5 (10%) |
| Hepatocyte, vacuolization cytoplasmic | 14 (28%) | 6 (12%) | 12 (24%) | 12 (24%) |
| Hepatocyte, midzonal, vacuolization cytoplasmic | | | 1 (2%) | |
| Serosa, fibrosis | 1 (2%) | | | |
| Serosa, inflammation | 1 (2%) | | | |
| Mesentery | (10) | (14) | (11) | (17) |
| Accessory spleen | | 1 (7%) | 1 (9%) | |
| Fat, necrosis | 8 (80%) | 7 (50%) | 7 (64%) | 5 (29%) |
| Pancreas | (48) | (50) | (48) | (49) |
| Atrophy | 23 (48%) | 28 (56%) | 25 (52%) | 23 (47%) |
| Cyst | 1 (2%) | 1 (2%) | 1 (2%) | |
| Acinus, hyperplasia, focal | 3 (6%) | 5 (10%) | 1 (2%) | |
| Salivary glands | (50) | (50) | (49) | (50) |
| Inflammation, chronic | | | 1 (2%) | 1 (2%) |

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

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--------------------------------------|----------|----------|----------|------------|
| Alimentary System (continued) | | | | |
| Stomach, forestomach | (50) | (49) | (49) | (50) |
| Edema | 2 (4%) | 5 (10%) | 5 (10%) | 2 (4%) |
| Erosion | | 4 (8%) | 3 (6%) | 1 (2%) |
| Ulcer | 2 (4%) | 5 (10%) | 5 (10%) | 3 (6%) |
| Epithelium, hyperplasia | 7 (14%) | 5 (10%) | 5 (10%) | 1 (2%) |
| Stomach, glandular | (50) | (48) | (48) | (50) |
| Edema | | 5 (10%) | 3 (6%) | 1 (2%) |
| Erosion | | 6 (13%) | 4 (8%) | 1 (2%) |
| Hyperplasia, focal | | | 1 (2%) | |
| Ulcer | | 3 (6%) | 4 (8%) | 2 (4%) |
| Tongue | (1) | | | |
| Hyperplasia | 1 (100%) | | | |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 45 (90%) | 41 (82%) | 43 (86%) | 41 (82%) |
| Thrombosis | | 4 (8%) | 1 (2%) | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (49) | (50) |
| Accessory adrenal cortical nodule | 26 (53%) | 18 (36%) | 20 (41%) | 14 (28%) |
| Degeneration, fatty | 5 (10%) | 12 (24%) | 9 (18%) | 4 (8%) |
| Hyperplasia, focal | 6 (12%) | 4 (8%) | 2 (4%) | 6 (12%) |
| Hypertrophy, focal | 10 (20%) | 12 (24%) | 13 (27%) | 8 (16%) |
| Necrosis | | 1 (2%) | | 1 (2%) |
| Adrenal medulla | (49) | (50) | (49) | (50) |
| Hyperplasia | 6 (12%) | 8 (16%) | 7 (14%) | 8 (16%) |
| Necrosis | | 1 (2%) | | |
| Islets, pancreatic | (48) | (50) | (48) | (50) |
| Hyperplasia | 1 (2%) | | 1 (2%) | 1 (2%) |
| Pituitary gland | (50) | (49) | (49) | (50) |
| Pars distalis, angiectasis | 16 (32%) | 14 (29%) | 12 (27%) | 9 (18%) |
| Pars distalis, cyst | 1 (2%) | 2 (4%) | 5 (10%) | 1 (2%) |
| Pars distalis, hemorrhage | 1 (2%) | 1 (2%) | | 1 (2%) |
| Pars distalis, hyperplasia | | | | 2 (4%) |
| Pars distalis, hyperplasia, focal | 6 (12%) | 10 (20%) | 4 (8%) | 3 (6%) |
| Pars intermedia, angiectasis | | | 1 (2%) | |
| Pars intermedia, cyst | | 1 (2%) | 2 (4%) | |
| Pars intermedia, hyperplasia | | | 1 (2%) | |
| Thyroid gland | (50) | (50) | (49) | (50) |
| Ultimobranchial cyst | 5 (10%) | | 2 (4%) | 3 (6%) |
| C-cell, hyperplasia | 9 (18%) | 13 (26%) | 15 (31%) | 4 (8%) |
| Follicle, cyst | 4 (8%) | 2 (4%) | 2 (4%) | 2 (4%) |
| General Body System | | | | |
| None | | | | |

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--------------------------------------|----------|----------|----------|------------|
| Genital System | | | | |
| Epididymis | (50) | (50) | (49) | (49) |
| Inflammation, chronic | 1 (2%) | | 1 (2%) | 1 (2%) |
| Preputial gland | (50) | (50) | (50) | (50) |
| Cyst | 1 (2%) | 6 (12%) | 5 (10%) | |
| Hyperplasia | | 1 (2%) | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | | 3 (6%) |
| Prostate | (50) | (50) | (50) | (50) |
| Inflammation, chronic | 23 (46%) | 25 (50%) | 29 (58%) | 29 (58%) |
| Seminal vesicle | (50) | (50) | (49) | (50) |
| Inflammation | | | 1 (2%) | |
| Testes | (50) | (50) | (49) | (50) |
| Germinal epithelium, atrophy | 11 (22%) | 24 (48%) | 13 (27%) | 10 (20%) |
| Interstitial cell, hyperplasia | 1 (2%) | 5 (10%) | 4 (8%) | 4 (8%) |
| Hematopoietic System | | | | |
| Bone marrow | (49) | (49) | (48) | (50) |
| Depletion cellular | | | | 1 (2%) |
| Hyperplasia | 2 (4%) | 4 (8%) | 2 (4%) | 5 (10%) |
| Myelofibrosis | | | 3 (6%) | |
| Lymph node | (22) | (26) | (25) | (25) |
| Deep cervical, hyperplasia, lymphoid | | 1 (4%) | | |
| Mediastinal, congestion | 1 (5%) | | 1 (4%) | 2 (8%) |
| Mediastinal, hyperplasia, lymphoid | 8 (36%) | 8 (31%) | 13 (52%) | 14 (56%) |
| Pancreatic, congestion | 1 (5%) | | | |
| Pancreatic, hyperplasia, lymphoid | 2 (9%) | 2 (8%) | 2 (8%) | 2 (8%) |
| Lymph node, mesenteric | (50) | (50) | (49) | (50) |
| Congestion | | | 1 (2%) | 1 (2%) |
| Hyperplasia, lymphoid | | 5 (10%) | 2 (4%) | 2 (4%) |
| Spleen | (50) | (50) | (49) | (49) |
| Fibrosis | 2 (4%) | 2 (4%) | 2 (4%) | |
| Hematopoietic cell proliferation | 2 (4%) | 4 (8%) | 7 (14%) | 5 (10%) |
| Hemorrhage | | | | 1 (2%) |
| Necrosis | | 2 (4%) | 2 (4%) | 2 (4%) |
| Pigmentation | 4 (8%) | 1 (2%) | 4 (8%) | |
| Lymphoid follicle, hyperplasia | 1 (2%) | | | |
| Thymus | (49) | (48) | (48) | (50) |
| Cyst | | | | 2 (4%) |
| Hemorrhage | 1 (2%) | 2 (4%) | 3 (6%) | 2 (4%) |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Cyst | 15 (30%) | 17 (34%) | 17 (34%) | 8 (16%) |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | 4 (8%) | 2 (4%) | 4 (8%) | 1 (2%) |
| Hyperkeratosis | 1 (2%) | | | |
| Hyperplasia | | 1 (2%) | | |
| Inflammation, chronic | | | 2 (4%) | 1 (2%) |

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--------------------------------------|----------|----------|----------|------------|
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Hypertrophy | | 1 (2%) | | |
| Cranium, osteopetrosis | | | | 1 (2%) |
| Skeletal muscle | (2) | (1) | (1) | (3) |
| Hemorrhage | | | | 1 (33%) |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 7 (14%) | 5 (10%) | 7 (14%) | 2 (4%) |
| Hemorrhage | 1 (2%) | 3 (6%) | 3 (6%) | 3 (6%) |
| Necrosis | | 1 (2%) | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | 6 (12%) | 2 (4%) | 6 (12%) | 2 (4%) |
| Hemorrhage | 3 (6%) | 2 (4%) | 3 (6%) | 1 (2%) |
| Infiltration cellular, histiocyte | 31 (62%) | 27 (54%) | 26 (52%) | 28 (56%) |
| Inflammation, chronic | 29 (58%) | 28 (56%) | 27 (54%) | 35 (70%) |
| Inflammation, suppurative | | | | 1 (2%) |
| Alveolar epithelium, hyperplasia | 6 (12%) | 5 (10%) | 10 (20%) | 11 (22%) |
| Nose | (49) | (50) | (49) | (50) |
| Foreign body | 10 (20%) | 8 (16%) | 10 (20%) | 8 (16%) |
| Inflammation, chronic | 14 (29%) | 13 (26%) | 14 (29%) | 14 (28%) |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Cataract | 3 (6%) | | | |
| Inflammation, chronic | 2 (4%) | | | 1 (2%) |
| Harderian gland | (50) | (50) | (49) | (50) |
| Inflammation, chronic | 1 (2%) | | | 1 (2%) |
| Lacrimal gland | | | | (1) |
| Inflammation, suppurative | | | | 1 (100%) |
| Urinary System | | | | |
| Kidney | (49) | (50) | (49) | (50) |
| Cyst | 2 (4%) | | | 1 (2%) |
| Hydronephrosis | 1 (2%) | | 1 (2%) | |
| Infarct | 1 (2%) | 1 (2%) | | 1 (2%) |
| Nephropathy | 43 (88%) | 48 (96%) | 46 (94%) | 47 (94%) |
| Capsule, hemorrhage | | | 1 (2%) | |
| Transitional epithelium, hyperplasia | 3 (6%) | 9 (18%) | 8 (16%) | 3 (6%) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Hemorrhage | | 2 (4%) | | |
| Inflammation, chronic | | | 1 (2%) | |

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR DRINKING WATER STUDY
OF DIBROMOACETIC ACID

| | | |
|-----------------|--|-------------|
| TABLE B1 | Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | B-2 |
| TABLE B2 | Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | B-6 |
| TABLE B3 | Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | B-30 |
| TABLE B4 | Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats | B-33 |
| TABLE B5 | Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | B-34 |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 4 | 5 | 9 | 14 |
| Natural deaths | 11 | 6 | 6 | 4 |
| Survivors | | | | |
| Died last week of study | | 1 | 1 | |
| Terminal sacrifice | 35 | 38 | 34 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, rectum | (49) | (50) | (50) | (48) |
| Polyp adenomatous | | | | 1 (2%) |
| Liver | (50) | (50) | (50) | (50) |
| Hepatocellular carcinoma | | | 1 (2%) | 1 (2%) |
| Hepatocellular adenoma | 2 (4%) | | | 1 (2%) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Serosa, osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Mesentery | (12) | (15) | (15) | (8) |
| Osteosarcoma, metastatic, bone | | | | 1 (13%) |
| Oral mucosa | | (1) | (1) | (1) |
| Pharyngeal, squamous cell papilloma | | 1 (100%) | | 1 (100%) |
| Pancreas | (47) | (50) | (49) | (48) |
| Acinus, adenoma | | | | 1 (2%) |
| Salivary glands | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, mammary gland | | | 1 (2%) | |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Squamous cell papilloma | 1 (2%) | | | |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| Tongue | | | | (1) |
| Squamous cell papilloma | | | | 1 (100%) |
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (2%) | | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (49) |
| Adenoma | 1 (2%) | | | |
| Adrenal medulla | (49) | (50) | (50) | (49) |
| Pheochromocytoma benign | 1 (2%) | 3 (6%) | 2 (4%) | 1 (2%) |
| Parathyroid gland | (50) | (48) | (49) | (47) |
| Adenoma | | | | 1 (2%) |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Pars distalis, adenoma | 25 (50%) | 27 (54%) | 27 (54%) | 22 (45%) |
| Pars distalis, adenoma, multiple | 1 (2%) | 3 (6%) | 1 (2%) | |
| Pars distalis, carcinoma | | 1 (2%) | | 1 (2%) |
| Pars intermedia, adenoma | 1 (2%) | | | |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Endocrine System (continued) | | | | |
| Thyroid gland | (50) | (50) | (50) | (49) |
| Bilateral, C-cell, adenoma | 1 (2%) | 1 (2%) | | |
| C-cell, adenoma | 5 (10%) | 10 (20%) | 5 (10%) | 5 (10%) |
| C-cell, carcinoma | | 1 (2%) | | |
| Follicular cell, adenoma | | | | 2 (4%) |
| Follicular cell, carcinoma | | 1 (2%) | 1 (2%) | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Clitoral gland | (50) | (50) | (48) | (49) |
| Adenoma | 7 (14%) | 10 (20%) | 5 (10%) | 3 (6%) |
| Carcinoma | 1 (2%) | 6 (12%) | 1 (2%) | 5 (10%) |
| Squamous cell papilloma | | | | 1 (2%) |
| Bilateral, adenoma | 1 (2%) | | | |
| Ovary | (50) | (50) | (50) | (50) |
| Uterus | (50) | (50) | (50) | (50) |
| Adenoma | | | | 1 (2%) |
| Deciduoma NOS | | 1 (2%) | | 1 (2%) |
| Polyp stromal | 3 (6%) | 10 (20%) | 7 (14%) | 4 (8%) |
| Bilateral, polyp stromal | | | | 1 (2%) |
| Cervix, polyp stromal | 1 (2%) | | | |
| Cervix, sarcoma stromal | | | 1 (2%) | |
| Vagina | (1) | (4) | (6) | (20) |
| Polyp adenomatous | | | 1 (17%) | |
| Sarcoma stromal | | | 1 (17%) | |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | 1 (2%) | 1 (2%) |
| Lymph node | (13) | (13) | (9) | (21) |
| Mediastinal, carcinoma, metastatic, thyroid gland | | 1 (8%) | | |
| Mediastinal, osteosarcoma, metastatic, bone | | | | 1 (5%) |
| Pancreatic, histiocytic sarcoma | | | | 1 (5%) |
| Lymph node, mandibular | (2) | | (1) | (3) |
| Lymph node, mesenteric | (49) | (50) | (50) | (48) |
| Spleen | (47) | (49) | (50) | (48) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Capsule, osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Thymus | (49) | (49) | (50) | (50) |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Adenoma | | 1 (2%) | | |
| Carcinoma | 2 (4%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Carcinoma, multiple | 1 (2%) | | 1 (2%) | |
| Fibroadenoma | 22 (44%) | 18 (36%) | 11 (22%) | 13 (26%) |
| Fibroadenoma, multiple | 10 (20%) | 15 (30%) | 28 (56%) | 23 (46%) |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Integumentary System (continued) | | | | |
| Skin | (50) | (50) | (50) | (50) |
| Squamous cell papilloma | 1 (2%) | 1 (2%) | | 1 (2%) |
| Squamous cell papilloma, multiple | | | 1 (2%) | |
| Trichoepithelioma | | | | 1 (2%) |
| Pinna, neural crest tumor | | | | 1 (2%) |
| Subcutaneous tissue, fibroma | 1 (2%) | | | |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteoma | | | 1 (2%) | |
| Osteosarcoma | | | | 1 (2%) |
| Cranium, meningioma malignant, metastatic, brain | 1 (2%) | | | |
| Skeletal muscle | | (1) | | (1) |
| Osteosarcoma, metastatic, bone | | | | 1 (100%) |
| Rhabdomyosarcoma | | 1 (100%) | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Astrocytoma malignant | | | | 1 (2%) |
| Carcinoma, metastatic, pituitary gland | | 1 (2%) | | |
| Glioma malignant, mixed cell | | | 1 (2%) | |
| Meningioma malignant | 1 (2%) | | | |
| Spinal cord | (2) | (4) | (1) | (1) |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Alveolar/bronchiolar carcinoma | | 1 (2%) | | 1 (2%) |
| Alveolar/bronchiolar carcinoma, multiple | 1 (2%) | 1 (2%) | | 1 (2%) |
| Carcinoma, metastatic, thyroid gland | | 1 (2%) | | |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (2%) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Pleura | | (1) | | |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | 1 (100%) | | |
| Special Senses System | | | | |
| Harderian gland | (50) | (50) | (50) | (50) |
| Zymbal's gland | (1) | | | (2) |
| Carcinoma | 1 (100%) | | | 2 (100%) |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Renal tubule, adenoma | | 1 (2%) | | |
| Transitional epithelium, carcinoma | | | 1 (2%) | |
| Urinary bladder | (50) | (50) | (50) | (50) |

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Systemic Lesions | | | | |
| Multiple organs ^b | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | 1 (2%) | 1 (2%) |
| Leukemia mononuclear | 11 (22%) | 13 (26%) | 16 (32%) | 22 (44%) |
| Mesothelioma malignant | | | 1 (2%) | |
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | 45 | 49 | 48 | 49 |
| Total primary neoplasms | 103 | 129 | 118 | 126 |
| Total animals with benign neoplasms | 42 | 47 | 43 | 43 |
| Total benign neoplasms | 85 | 102 | 91 | 87 |
| Total animals with malignant neoplasms | 16 | 24 | 20 | 32 |
| Total malignant neoplasms | 18 | 26 | 27 | 37 |
| Total animals with metastatic neoplasms | 1 | 3 | 1 | 2 |
| Total metastatic neoplasms | 1 | 5 | 1 | 7 |
| Total animals with uncertain neoplasms- benign or malignant | | 1 | | 2 |
| Total uncertain neoplasms | | 1 | | 2 |

^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 Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid: 0 mg/L

| Number of Days on Study | 7 | 3 | 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 7 7 7 7 7 7 7 | | | | | | | | | | | | | | | | | | | | | | | | |
|----------------------------------|---|---|---|-----------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----|----|---|
| Carcass ID Number | 2 | 1 2 2 2 2 2 4 4 4 0 0 2 2 3 3 3 3 3 0 0 0 1 3 3 3 4 | 9 0 1 2 3 5 7 8 9 3 5 6 8 1 2 3 4 7 8 9 0 7 8 9 0 | Total Tissues/ Tumors | | | | | | | | | | | | | | | | | | | | | | | |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | | |
| Intestine large, colon | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 | |
| Intestine large, rectum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 | |
| Intestine large, cecum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 44 | |
| Intestine small, duodenum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 | |
| Intestine small, jejunum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 45 | |
| Intestine small, ileum | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 44 | |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 | |
| Mesentery | | + | + | + | | | | | | | | | | | | | | | | | | | | + | + | 12 | |
| Pancreas | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 | |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Squamous cell papilloma | | | | X | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 | |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | | | | | | | | | | | | | + | | | | | | | | | | | + | | 10 | |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Adrenal medulla | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 49 | |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | X | | 1 | |
| Islets, pancreatic | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 47 | |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Pituitary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Pars distalis, adenoma | X | | | | | X | X | | | X | X | | | X | X | | | | | X | X | X | X | | X | 25 | |
| Pars distalis, adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Pars intermedia, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Bilateral, C-cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 | |
| C-cell, adenoma | X | | | | | X | | | | | | | | | | | | | | | | | | X | | 5 | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Adenoma | | | | | | | | | | X | | | | | | | | | | | | | X | X | | X | 7 |
| Carcinoma | | | | | | | | | | | | | | X | | | | | | | | | | | | | 1 |
| Bilateral, adenoma | | | | | | | | | | | | | | | | | | | | | | | | X | | | 1 |

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

| | | |
|--------------------------------|--|--------------------------|
| Number of Days on Study | 7 | |
| | 3 | |
| | 5 5 5 5 5 5 5 5 5 5 6 6 6 6 6 6 6 6 7 7 7 7 7 7 | |
| Carcass ID Number | 2 | Total Tissues/ Tumors |
| | 1 2 2 2 2 2 4 4 4 0 0 2 2 3 3 3 3 3 0 0 0 1 3 3 3 4 | |
| | 9 0 1 2 3 5 7 8 9 3 5 6 8 1 2 3 4 7 8 9 0 7 8 9 0 | |
| Special Senses System | | |
| Ear | | 2 |
| Eye | + | 50 |
| Harderian gland | + | 50 |
| Zymbal's gland | | 1 |
| Carcinoma | | 1 |
| Urinary System | | |
| Kidney | + | 50 |
| Urinary bladder | + | 50 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Leukemia mononuclear | X X X X X | 11 |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
500 mg/L

| | | |
|--------------------------------|---|----------|
| Number of Days on Study | 7 | |
| | 3 | |
| | 5 5 5 5 5 5 5 5 5 5 5 5 5 5 6 6 6 6 7 7 7 7 7 7 7 | |
| Carcass ID Number | 3 | Total |
| | 0 0 0 0 0 1 1 1 1 4 4 4 2 2 2 3 1 2 3 3 3 3 3 3 4 | Tissues/ |
| | 5 6 7 8 9 0 1 2 4 1 2 3 6 8 9 0 6 0 1 3 4 5 7 8 0 | Tumors |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Histiocytic sarcoma | | 1 |
| Leukemia mononuclear | X X X X X X X X X | 16 |
| Mesothelioma malignant | | 1 |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| Number of Days on Study | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
|--|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 9 | 6 | 7 | 8 | 0 | 2 | 2 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 7 | 8 | 8 | 2 | 2 | 2 | 2 | 2 | 2 | 3 |
| | 4 | 5 | 6 | 7 | 0 | 2 | 1 | 2 | 2 | 7 | 1 | 1 | 4 | 4 | 9 | 9 | 2 | 2 | 9 | 9 | 9 | 9 | 9 | 9 | 4 |
| Carcass ID Number | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | 8 | 7 | 5 | 8 | 7 | 5 | 8 | 8 | 0 | 7 | 6 | 7 | 7 | 8 | 5 | 7 | 5 | 8 | 5 | 5 | 6 | 7 | 7 | 7 | 9 |
| | 4 | 7 | 6 | 2 | 9 | 5 | 3 | 7 | 0 | 4 | 5 | 0 | 6 | 0 | 7 | 1 | 2 | 6 | 8 | 9 | 0 | 2 | 3 | 5 | 6 |
| Alimentary System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Esophagus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, colon | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine large, rectum | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Polyp adenomatous | | | | | | | | | | | | | | | | | | | | | | | | | |
| Intestine large, cecum | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, duodenum | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, jejunum | A | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Intestine small, ileum | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Liver | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hepatocellular carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Serosa, osteosarcoma, metastatic, bone | | | | | | | | X | | | | | | | | | | | | | | | | | |
| Mesentery | | | | | + | | | + | | | | | | + | | | | | | | | | | + | |
| Osteosarcoma, metastatic, bone | | | | | | | | X | | | | | | | | | | | | | | | | | |
| Oral mucosa | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pharyngeal, squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pancreas | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Acinus, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Salivary glands | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, forestomach | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Stomach, glandular | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Tongue | | | | | | | | | | | | | | | | | | | | | | | | | + |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Cardiovascular System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Blood vessel | | | | | | | + | + | | | | | + | | | | | | | | | | | | + |
| Heart | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Endocrine System | | | | | | | | | | | | | | | | | | | | | | | | | |
| Adrenal cortex | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adrenal medulla | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Pheochromocytoma benign | | | | | | | | | | | | | | | | | | | | | | | | | |
| Islets, pancreatic | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Parathyroid gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Adenoma | | | | | | | | X | | | | | | | | | | | | | | | | | |
| Pituitary gland | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pars distalis, adenoma | | | | | | | X | | X | | X | X | | | | X | X | X | | X | X | X | | | |
| Pars distalis, carcinoma | | | | | | | | | X | | | | | | | | | | | | | | | | |
| Thyroid gland | + | + | + | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| C-cell, adenoma | | | | | | | X | | | X | X | | | | | | | | | | | | X | | |
| Follicular cell, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | X |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|--|
| Number of Days on Study | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
| | 5 | 9 | 6 | 7 | 8 | 0 | 2 | 2 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 7 | 8 | 8 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | |
| | 4 | 5 | 6 | 7 | 0 | 2 | 1 | 2 | 2 | 7 | 1 | 1 | 4 | 4 | 9 | 9 | 2 | 2 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 4 | |
| Carcass ID Number | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | |
| | 8 | 7 | 5 | 8 | 7 | 5 | 8 | 8 | 0 | 7 | 6 | 7 | 7 | 8 | 5 | 7 | 5 | 8 | 5 | 5 | 6 | 7 | 7 | 7 | 9 | | |
| | 4 | 7 | 6 | 2 | 9 | 5 | 3 | 7 | 0 | 4 | 5 | 0 | 6 | 0 | 7 | 1 | 2 | 6 | 8 | 9 | 0 | 2 | 3 | 5 | 6 | | |
| General Body System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| None | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Clitoral gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | X | | | | | | | | X | X | | | | | | | X | | | | | | |
| Squamous cell papilloma | | | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Ovary | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Uterus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Deciduoma NOS | | | | | | | | | X | | | | | | | | | | | | | | | | | | |
| Polyp stromal | | | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Bilateral, polyp stromal | | | | | | | | | | | | | | | | | | | | | | | | X | | | |
| Vagina | | | + | | | | | + | | + | | + | + | | | + | | | | | | | | + | + | | |
| Hematopoietic System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lymph node | + | | + | | + | + | + | | | + | | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Mediastinal, osteosarcoma, metastatic, bone | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Pancreatic, histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| 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 | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Spleen | + | + | A | + | + | + | + | + | + | A | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Capsule, osteosarcoma, metastatic, bone | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Thymus | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Integumentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Fibroadenoma | | | | X | X | | | | | | | | X | | | | | | | | X | | | | X | | |
| Fibroadenoma, multiple | | | | | | | | | X | | | | | | | | | X | X | | X | X | | X | X | | |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Squamous cell papilloma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Trichoepithelioma | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Pinna, neural crest tumor | | | | | | | | | | | | | | X | | | | | | | | | | | | | |
| Musculoskeletal System | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | |
| Osteosarcoma | | | | | | | | X | | | | | | | | | | | | | | | | | | | |
| Skeletal muscle | | | | | | | | + | | | | | | | | | | | | | | | | | | | |
| Osteosarcoma, metastatic, bone | | | | | | | | X | | | | | | | | | | | | | | | | | | | |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid: 1,000 mg/L

| Number of Days on Study | 7 | 3 | 4 4 4 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 |
|---|---|---|---|
| Carcass ID Number | 3 | 9 9 9 6 6 6 6 7 8 8 8 8 9 5 5 5 6 6 6 6 9 9 9 9 9 | 7 8 9 1 2 3 4 8 1 5 8 9 0 1 3 4 6 7 8 9 1 2 3 4 5 |
| | | | Total Tissues/Tumors |
| General Body System | None | | |
| Genital System | | | |
| Clitoral gland | + M + | | 49 |
| Adenoma | | X | 3 |
| Carcinoma | | X | 5 |
| Squamous cell papilloma | | | 1 |
| Ovary | + | | 50 |
| Uterus | + | | 50 |
| Adenoma | | X | 1 |
| Deciduoma NOS | | | 1 |
| Polyp stromal | | X | 4 |
| Bilateral, polyp stromal | | | 1 |
| Vagina | + | | 20 |
| Hematopoietic System | | | |
| Bone marrow | + | | 50 |
| Histiocytic sarcoma | | X | 1 |
| Lymph node | + M + | | 21 |
| Mediastinal, osteosarcoma, metastatic, bone | | | 1 |
| Pancreatic, histiocytic sarcoma | | X | 1 |
| Lymph node, mandibular | M M M + M | | 3 |
| Lymph node, mesenteric | + | | 48 |
| Spleen | + | | 48 |
| Histiocytic sarcoma | | X | 1 |
| Capsule, osteosarcoma, metastatic, bone | | | 1 |
| Thymus | + | | 50 |
| Integumentary System | | | |
| Mammary gland | + | | 50 |
| Carcinoma | | | 1 |
| Fibroadenoma | | X | 13 |
| Fibroadenoma, multiple | X | | 23 |
| Skin | + | | 50 |
| Squamous cell papilloma | | | 1 |
| Trichoepithelioma | | X | 1 |
| Pinna, neural crest tumor | | | 1 |
| Musculoskeletal System | | | |
| Bone | + | | 50 |
| Osteosarcoma | | | 1 |
| Skeletal muscle | | | 1 |
| Osteosarcoma, metastatic, bone | | | 1 |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | | | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Number of Days on Study | 2 | 3 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 |
| | 5 | 9 | 6 | 7 | 8 | 0 | 2 | 2 | 2 | 3 | 5 | 5 | 6 | 6 | 7 | 7 | 8 | 8 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | 3 |
| | 4 | 5 | 6 | 7 | 0 | 2 | 1 | 2 | 2 | 7 | 1 | 1 | 4 | 4 | 9 | 9 | 2 | 2 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 4 |
| Carcass ID Number | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 4 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| | 8 | 7 | 5 | 8 | 7 | 5 | 8 | 8 | 0 | 7 | 6 | 7 | 7 | 8 | 5 | 7 | 5 | 8 | 5 | 5 | 6 | 7 | 7 | 7 | 9 | 9 |
| | 4 | 7 | 6 | 2 | 9 | 5 | 3 | 7 | 0 | 4 | 5 | 0 | 6 | 0 | 7 | 1 | 2 | 6 | 8 | 9 | 0 | 2 | 3 | 5 | 6 | 6 |
| Nervous System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Astrocytoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Peripheral nerve | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Spinal cord | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Respiratory System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Alveolar/bronchiolar carcinoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Hepatocellular carcinoma, metastatic, liver | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Osteosarcoma, metastatic, bone | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Nose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Special Senses System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ear | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Eye | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Harderian gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Zymbal's gland | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Urinary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Systemic Lesions | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | |

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| Number of Days on Study | 7 | 3 | 4 4 4 5 5 5 5 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|-----------------------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|----|----|
| Carcass ID Number | 3 | 9 9 9 6 6 6 6 7 8 8 8 8 9 5 5 5 6 6 6 6 9 9 9 9 9 | 7 8 9 1 2 3 4 8 1 5 8 9 0 1 3 4 6 7 8 9 1 2 3 4 5 | Total Tissues/ Tumors | | | | | | | | | | | | | | | | | | | | | | |
| Nervous System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Astrocytoma malignant | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Peripheral nerve | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Spinal cord | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Respiratory System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Alveolar/bronchiolar carcinoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Hepatocellular carcinoma, metastatic, liver | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Osteosarcoma, metastatic, bone | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Nose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Special Senses System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Ear | | | | | | | | | | | | | | | | | | | | | | | | | | 3 |
| Eye | | | | | | | | | | | | | | | | | | | | | | | | | | 50 |
| Harderian gland | | | | | | | | | | | | | | | | | | | | | | | | | | 50 |
| Zymbal's gland | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | 2 |
| Urinary System | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Kidney | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Urinary bladder | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Systemic Lesions | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Multiple organs | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | 50 | |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | 1 |
| Leukemia mononuclear | | | | | | | | | | | | | | | | | | | | | | | | | | 22 |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|-------------|
| Adrenal Medulla: Benign Pheochromocytoma | | | | |
| Overall rate ^a | 1/49 (2%) | 3/50 (6%) | 2/50 (4%) | 1/49 (2%) |
| Adjusted rate ^b | 2.3% | 6.5% | 4.5% | 2.4% |
| Terminal rate ^c | 1/35 (3%) | 2/39 (5%) | 2/35 (6%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 720 | 729 (T) | 729 (T) |
| Poly-3 test ^d | P=0.396N | P=0.330 | P=0.509 | P=0.757 |
| Clitoral Gland: Adenoma | | | | |
| Overall rate | 8/50 (16%) | 10/50 (20%) | 5/48 (10%) | 3/49 (6%) |
| Adjusted rate | 18.0% | 21.5% | 11.8% | 7.2% |
| Terminal rate | 7/35 (20%) | 8/39 (21%) | 5/33 (15%) | 3/31 (10%) |
| First incidence (days) | 641 | 682 | 729 (T) | 729 (T) |
| Poly-3 test | P=0.033N | P=0.439 | P=0.306N | P=0.116N |
| Clitoral Gland: Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 6/50 (12%) | 1/48 (2%) | 5/49 (10%) |
| Adjusted rate | 2.3% | 12.7% | 2.4% | 11.7% |
| Terminal rate | 1/35 (3%) | 4/39 (10%) | 1/33 (3%) | 2/31 (7%) |
| First incidence (days) | 729 (T) | 392 | 729 (T) | 580 |
| Poly-3 test | P=0.335 | P=0.068 | P=0.752 | P=0.094 |
| Clitoral Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 9/50 (18%) | 15/50 (30%) | 6/48 (13%) | 8/49 (16%) |
| Adjusted rate | 20.3% | 31.6% | 14.2% | 18.7% |
| Terminal rate | 8/35 (23%) | 11/39 (28%) | 6/33 (18%) | 5/31 (16%) |
| First incidence (days) | 641 | 392 | 729 (T) | 580 |
| Poly-3 test | P=0.143N | P=0.158 | P=0.321N | P=0.533N |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 2/50 (4%) | 3/50 (6%) |
| Adjusted rate | 2.3% | 2.2% | 4.5% | 7.0% |
| Terminal rate | 1/35 (3%) | 1/39 (3%) | 2/35 (6%) | 3/32 (9%) |
| First incidence (days) | 729 (T) | 729 (T) | 729 (T) | 729 (T) |
| Poly-3 test | P=0.137 | P=0.750N | P=0.502 | P=0.295 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) | 5/50 (10%) |
| Adjusted rate | 4.5% | 6.5% | 4.5% | 11.6% |
| Terminal rate | 1/35 (3%) | 3/39 (8%) | 2/35 (6%) | 5/32 (16%) |
| First incidence (days) | 632 | 729 (T) | 729 (T) | 729 (T) |
| Poly-3 test | P=0.161 | P=0.518 | P=0.693 | P=0.201 |
| Mammary Gland: Fibroadenoma | | | | |
| Overall rate | 32/50 (64%) | 33/50 (66%) | 39/50 (78%) | 36/50 (72%) |
| Adjusted rate | 70.1% | 69.7% | 83.8% | 80.2% |
| Terminal rate | 27/35 (77%) | 28/39 (72%) | 31/35 (89%) | 30/32 (94%) |
| First incidence (days) | 602 | 521 | 441 | 577 |
| Poly-3 test | P=0.061 | P=0.576N | P=0.079 | P=0.175 |
| Mammary Gland: Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 6.8% | 2.2% | 4.5% | 2.3% |
| Terminal rate | 3/35 (9%) | 1/39 (3%) | 1/35 (3%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 729 (T) | 640 | 729 (T) |
| Poly-3 test | P=0.371N | P=0.289N | P=0.494N | P=0.315N |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|-------------|
| Mammary Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 2/50 (4%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 6.8% | 4.3% | 4.5% | 2.3% |
| Terminal rate | 3/35 (9%) | 2/39 (5%) | 1/35 (3%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 729 (T) | 640 | 729 (T) |
| Poly-3 test | P=0.271N | P=0.479N | P=0.494N | P=0.315N |
| Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma | | | | |
| Overall rate | 32/50 (64%) | 34/50 (68%) | 40/50 (80%) | 36/50 (72%) |
| Adjusted rate | 70.1% | 71.8% | 85.3% | 80.2% |
| Terminal rate | 27/35 (77%) | 29/39 (74%) | 31/35 (89%) | 30/32 (94%) |
| First incidence (days) | 602 | 521 | 441 | 577 |
| Poly-3 test | P=0.073 | P=0.519 | P=0.052 | P=0.175 |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 26/50 (52%) | 30/50 (60%) | 28/50 (56%) | 22/49 (45%) |
| Adjusted rate | 55.9% | 63.1% | 61.3% | 49.8% |
| Terminal rate | 20/35 (57%) | 24/39 (62%) | 22/35 (63%) | 16/32 (50%) |
| First incidence (days) | 519 | 509 | 622 | 602 |
| Poly-3 test | P=0.197N | P=0.306 | P=0.376 | P=0.352N |
| Pituitary Gland (Pars Distalis): Adenoma or Carcinoma | | | | |
| Overall rate | 26/50 (52%) | 31/50 (62%) | 28/50 (56%) | 23/49 (47%) |
| Adjusted rate | 55.9% | 65.2% | 61.3% | 51.7% |
| Terminal rate | 20/35 (57%) | 25/39 (64%) | 22/35 (63%) | 16/32 (50%) |
| First incidence (days) | 519 | 509 | 622 | 602 |
| Poly-3 test | P=0.214N | P=0.235 | P=0.376 | P=0.421N |
| Thyroid Gland (C-cell): Adenoma | | | | |
| Overall rate | 6/50 (12%) | 11/50 (22%) | 5/50 (10%) | 5/49 (10%) |
| Adjusted rate | 13.5% | 23.8% | 11.3% | 11.6% |
| Terminal rate | 5/35 (14%) | 11/39 (28%) | 5/35 (14%) | 2/32 (6%) |
| First incidence (days) | 595 | 729 (T) | 729 (T) | 602 |
| Poly-3 test | P=0.149N | P=0.160 | P=0.503N | P=0.521N |
| Thyroid Gland (C-cell): Adenoma or Carcinoma | | | | |
| Overall rate | 6/50 (12%) | 12/50 (24%) | 5/50 (10%) | 5/49 (10%) |
| Adjusted rate | 13.5% | 25.9% | 11.3% | 11.6% |
| Terminal rate | 5/35 (14%) | 12/39 (31%) | 5/35 (14%) | 2/32 (6%) |
| First incidence (days) | 595 | 729 (T) | 729 (T) | 602 |
| Poly-3 test | P=0.115N | P=0.108 | P=0.503N | P=0.521N |
| Uterus: Stromal Polyp | | | | |
| Overall rate | 4/50 (8%) | 10/50 (20%) | 7/50 (14%) | 5/50 (10%) |
| Adjusted rate | 9.0% | 21.2% | 15.7% | 11.6% |
| Terminal rate | 2/35 (6%) | 8/39 (21%) | 6/35 (17%) | 4/32 (13%) |
| First incidence (days) | 602 | 521 | 701 | 679 |
| Poly-3 test | P=0.374N | P=0.088 | P=0.257 | P=0.478 |
| Uterus: Stromal Polyp or Stromal Sarcoma | | | | |
| Overall rate | 4/50 (8%) | 10/50 (20%) | 8/50 (16%) | 5/50 (10%) |
| Adjusted rate | 9.0% | 21.2% | 17.7% | 11.6% |
| Terminal rate | 2/35 (6%) | 8/39 (21%) | 6/35 (17%) | 4/32 (13%) |
| First incidence (days) | 602 | 521 | 519 | 679 |
| Poly-3 test | P=0.400N | P=0.088 | P=0.180 | P=0.478 |

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|--------------|--------------|
| All Organs: Mononuclear Cell Leukemia | | | | |
| Overall rate | 11/50 (22%) | 13/50 (26%) | 16/50 (32%) | 22/50 (44%) |
| Adjusted rate | 24.3% | 27.1% | 34.7% | 47.4% |
| Terminal rate | 8/35 (23%) | 8/39 (21%) | 11/35 (31%) | 12/32 (38%) |
| First incidence (days) | 605 | 509 | 519 | 395 |
| Poly-3 test | P=0.006 | P=0.474 | P=0.195 | P=0.016 |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 42/50 (84%) | 47/50 (94%) | 43/50 (86%) | 43/50 (86%) |
| Adjusted rate | 88.1% | 96.9% | 92.4% | 92.6% |
| Terminal rate | 32/35 (91%) | 38/39 (97%) | 35/35 (100%) | 32/32 (100%) |
| First incidence (days) | 519 | 509 | 441 | 566 |
| Poly-3 test | P=0.538 | P=0.085 | P=0.342 | P=0.327 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 16/50 (32%) | 24/50 (48%) | 20/50 (40%) | 32/50 (64%) |
| Adjusted rate | 34.6% | 48.9% | 43.0% | 66.5% |
| Terminal rate | 11/35 (31%) | 17/39 (44%) | 14/35 (40%) | 17/32 (53%) |
| First incidence (days) | 519 | 392 | 519 | 395 |
| Poly-3 test | P=0.004 | P=0.112 | P=0.267 | P<0.001 |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 45/50 (90%) | 49/50 (98%) | 48/50 (96%) | 49/50 (98%) |
| Adjusted rate | 92.8% | 98.0% | 98.9% | 99.9% |
| Terminal rate | 33/35 (94%) | 38/39 (97%) | 35/35 (100%) | 32/32 (100%) |
| First incidence (days) | 519 | 392 | 441 | 395 |
| Poly-3 test | P=0.053 | P=0.219 | P=0.138 | P=0.077 |

(T) Terminal sacrifice

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

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the 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.

TABLE B4
Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats^a

| | Incidence in Controls |
|---|-----------------------|
| Historical Incidence: Drinking Water Studies | |
| Dibromoacetic acid | 11/50 |
| Dipropylene glycol | 10/50 |
| Sodium chlorate | 11/50 |
| Sodium nitrite | 15/50 |
| Overall Historical Incidence: Drinking Water Studies | |
| Total (%) | 47/200 (23.5%) |
| Mean ± standard deviation | 23.5% ± 4.4% |
| Range | 20%-30% |
| Overall Historical Incidence | |
| Total (%) | 383/1,459 (26.3%) |
| Mean ± standard deviation | 26.7% ± 10.5% |
| Range | 12%-52% |

^a Data as of January 26, 2005

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 4 | 5 | 9 | 14 |
| Natural deaths | 11 | 6 | 6 | 4 |
| Survivors | | | | |
| Died last week of study | | 1 | 1 | |
| Terminal sacrifice | 35 | 38 | 34 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, colon | (49) | (49) | (49) | (48) |
| Edema | | | | 2 (4%) |
| Inflammation | | | | 1 (2%) |
| Intestine large, rectum | (49) | (50) | (50) | (48) |
| Inflammation | | 1 (2%) | | |
| Intestine small, ileum | (44) | (47) | (45) | (48) |
| Inflammation, suppurative | | | 1 (2%) | |
| Liver | (50) | (50) | (50) | (50) |
| Basophilic focus | 36 (72%) | 41 (82%) | 29 (58%) | 15 (30%) |
| Clear cell focus | 8 (16%) | 4 (8%) | 14 (28%) | 18 (36%) |
| Degeneration, cystic | | | | 1 (2%) |
| Eosinophilic focus | 4 (8%) | 4 (8%) | 5 (10%) | 9 (18%) |
| Hematopoietic cell proliferation | 1 (2%) | | | |
| Hepatodiaphragmatic nodule | 3 (6%) | 9 (18%) | 2 (4%) | 5 (10%) |
| Inflammation, chronic | 2 (4%) | 3 (6%) | 3 (6%) | 1 (2%) |
| Inflammation, granulomatous | 8 (16%) | | 1 (2%) | 2 (4%) |
| Mixed cell focus | 1 (2%) | 1 (2%) | 1 (2%) | 4 (8%) |
| Necrosis, focal | 1 (2%) | | | 1 (2%) |
| Bile duct, hyperplasia | 17 (34%) | 15 (30%) | 15 (30%) | 19 (38%) |
| Centrilobular, necrosis | 1 (2%) | 1 (2%) | | 1 (2%) |
| Hepatocyte, vacuolization cytoplasmic | 3 (6%) | 2 (4%) | | 1 (2%) |
| Hepatocyte, centrilobular, vacuolization cytoplasmic | 1 (2%) | | | |
| Serosa, fibrosis | 2 (4%) | 2 (4%) | | |
| Mesentery | (12) | (15) | (15) | (8) |
| Accessory spleen | 1 (8%) | | 1 (7%) | |
| Fat, necrosis | 9 (75%) | 12 (80%) | 14 (93%) | 6 (75%) |
| Pancreas | (47) | (50) | (49) | (48) |
| Atrophy | 19 (40%) | 15 (30%) | 20 (41%) | 23 (48%) |
| Cyst | 1 (2%) | | 1 (2%) | 1 (2%) |
| Acinus, hyperplasia, focal | 1 (2%) | 2 (4%) | | 2 (4%) |
| Salivary glands | (50) | (50) | (50) | (50) |
| Inflammation | | | 1 (2%) | 2 (4%) |
| Stomach, forestomach | (50) | (50) | (50) | (50) |
| Edema | 2 (4%) | 5 (10%) | | 1 (2%) |
| Erosion | 1 (2%) | 1 (2%) | | 1 (2%) |
| Ulcer | 3 (6%) | 5 (10%) | | 1 (2%) |
| Epithelium, hyperplasia | | 6 (12%) | | 2 (4%) |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| Edema | 1 (2%) | 2 (4%) | | 2 (4%) |
| Erosion | | 1 (2%) | | 2 (4%) |
| Ulcer | | 1 (2%) | | 2 (4%) |

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

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|-----------------------------------|----------|----------|----------|------------|
| Cardiovascular System | | | | |
| Heart | (50) | (50) | (50) | (50) |
| Cardiomyopathy | 37 (74%) | 29 (58%) | 34 (68%) | 35 (70%) |
| Mineralization | 1 (2%) | | | |
| Thrombosis | 2 (4%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Valve, inflammation, suppurative | | | | 1 (2%) |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (49) |
| Accessory adrenal cortical nodule | 17 (35%) | 8 (16%) | 6 (12%) | 6 (12%) |
| Degeneration, fatty | 14 (29%) | 19 (38%) | 15 (30%) | 14 (29%) |
| Hyperplasia, focal | 8 (16%) | 6 (12%) | 4 (8%) | 5 (10%) |
| Hypertrophy, focal | 18 (37%) | 19 (38%) | 21 (42%) | 17 (35%) |
| Necrosis | 3 (6%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Adrenal medulla | (49) | (50) | (50) | (49) |
| Hyperplasia | 5 (10%) | 1 (2%) | 2 (4%) | 4 (8%) |
| Islets, pancreatic | (47) | (49) | (49) | (48) |
| Metaplasia, hepatocyte | | 1 (2%) | | 3 (6%) |
| Pituitary gland | (50) | (50) | (50) | (49) |
| Pars distalis, angiectasis | 32 (64%) | 24 (48%) | 23 (46%) | 23 (47%) |
| Pars distalis, cyst | 20 (40%) | 24 (48%) | 20 (40%) | 18 (37%) |
| Pars distalis, hyperplasia, focal | 11 (22%) | 9 (18%) | 8 (16%) | 6 (12%) |
| Pars intermedia, angiectasis | | | 1 (2%) | |
| Pars intermedia, cyst | | | | 1 (2%) |
| Thyroid gland | (50) | (50) | (50) | (49) |
| Ultimobranchial cyst | 1 (2%) | | | |
| C-cell, hyperplasia | 23 (46%) | 6 (12%) | 18 (36%) | 18 (37%) |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Clitoral gland | (50) | (50) | (48) | (49) |
| Cyst | 12 (24%) | 4 (8%) | 8 (17%) | 13 (27%) |
| Hyperplasia | 1 (2%) | 4 (8%) | | 2 (4%) |
| Inflammation, chronic | 3 (6%) | 2 (4%) | 5 (10%) | 3 (6%) |
| Ovary | (50) | (50) | (50) | (50) |
| Cyst | 8 (16%) | 9 (18%) | 14 (28%) | 8 (16%) |
| Uterus | (50) | (50) | (50) | (50) |
| Cyst | 3 (6%) | | 1 (2%) | 3 (6%) |
| Hemorrhage | 1 (2%) | | | |
| Hydrometra | 1 (2%) | 2 (4%) | 1 (2%) | |
| Inflammation, chronic | | | 1 (2%) | |
| Cervix, cyst | | | | 1 (2%) |
| Cervix, inflammation, suppurative | | | | 1 (2%) |
| Endometrium, hyperplasia, cystic | 2 (4%) | 1 (2%) | | |
| Vagina | (1) | (4) | (6) | (20) |
| Cyst | | | 3 (50%) | 10 (50%) |
| Inflammation | | 1 (25%) | 1 (17%) | 9 (45%) |

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|------------------------------------|----------|----------|----------|------------|
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Depletion cellular | 1 (2%) | | | |
| Hyperplasia | 5 (10%) | 8 (16%) | 5 (10%) | 3 (6%) |
| Myelofibrosis | | 1 (2%) | | 2 (4%) |
| Lymph node | (13) | (13) | (9) | (21) |
| Deep cervical, hemorrhage | 1 (8%) | | | |
| Deep cervical, hyperplasia | | | | 1 (5%) |
| Mediastinal, congestion | | | 2 (22%) | |
| Mediastinal, hyperplasia, lymphoid | 7 (54%) | 4 (31%) | 3 (33%) | 5 (24%) |
| Pancreatic, hyperplasia, lymphoid | 2 (15%) | 4 (31%) | 2 (22%) | 3 (14%) |
| Pancreatic, pigmentation | 1 (8%) | | | |
| Lymph node, mandibular | (2) | | (1) | (3) |
| Hyperplasia, lymphoid | 1 (50%) | | 1 (100%) | |
| Lymph node, mesenteric | (49) | (50) | (50) | (48) |
| Hyperplasia, lymphoid | 1 (2%) | | | 3 (6%) |
| Hyperplasia, reticulum cell | 1 (2%) | | | |
| Spleen | (47) | (49) | (50) | (48) |
| Fibrosis | | 3 (6%) | 1 (2%) | |
| Hematopoietic cell proliferation | 5 (11%) | 16 (33%) | 7 (14%) | 5 (10%) |
| Necrosis | | 2 (4%) | | |
| Pigmentation | 8 (17%) | 9 (18%) | 6 (12%) | 1 (2%) |
| Lymphoid follicle, hyperplasia | 1 (2%) | | 1 (2%) | 1 (2%) |
| Thymus | (49) | (49) | (50) | (50) |
| Hemorrhage | 2 (4%) | | 1 (2%) | |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Cyst | 45 (90%) | 48 (96%) | 41 (82%) | 37 (74%) |
| Galactocele | | 1 (2%) | 1 (2%) | |
| Hyperplasia | 2 (4%) | 3 (6%) | 4 (8%) | 2 (4%) |
| Hyperplasia, atypical | 5 (10%) | 2 (4%) | 4 (8%) | 4 (8%) |
| Hyperplasia, lobular | | | | 1 (2%) |
| Skin | (50) | (50) | (50) | (50) |
| Cyst epithelial inclusion | | 2 (4%) | | 1 (2%) |
| Hemorrhage | | | | 1 (2%) |
| Inflammation, chronic | 1 (2%) | 3 (6%) | 3 (6%) | |
| Musculoskeletal System | | | | |
| None | | | | |
| Nervous System | | | | |
| Brain | (50) | (50) | (50) | (50) |
| Compression | 6 (12%) | 9 (18%) | 7 (14%) | 5 (10%) |
| Hemorrhage | 1 (2%) | 5 (10%) | 2 (4%) | 3 (6%) |
| Necrosis | | 2 (4%) | | 1 (2%) |

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--------------------------------------|----------|----------|----------|------------|
| Respiratory System | | | | |
| Larynx | | (1) | (1) | |
| Inflammation, chronic, suppurative | | | 1 (100%) | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | 4 (8%) | 2 (4%) | 5 (10%) | 3 (6%) |
| Hemorrhage | 1 (2%) | 1 (2%) | | 1 (2%) |
| Infiltration cellular, histiocyte | 45 (90%) | 47 (94%) | 46 (92%) | 45 (90%) |
| Inflammation, chronic | 31 (62%) | 31 (62%) | 40 (80%) | 36 (72%) |
| Alveolar epithelium, hyperplasia | 3 (6%) | 7 (14%) | 13 (26%) | 14 (28%) |
| Nose | (50) | (50) | (50) | (50) |
| Foreign body | | 2 (4%) | 1 (2%) | 1 (2%) |
| Inflammation, chronic | 2 (4%) | 3 (6%) | 4 (8%) | 6 (12%) |
| Inflammation, suppurative | | | | 1 (2%) |
| Respiratory epithelium, hyperplasia | | | | 1 (2%) |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Cataract | 2 (4%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Inflammation, chronic | 1 (2%) | | | 2 (4%) |
| Urinary System | | | | |
| Kidney | (50) | (50) | (50) | (50) |
| Hydronephrosis | | 1 (2%) | 1 (2%) | |
| Infarct | | 2 (4%) | | 2 (4%) |
| Inflammation, chronic | | | | 2 (4%) |
| Nephropathy | 18 (36%) | 32 (64%) | 37 (74%) | 40 (80%) |
| Renal tubule, hyperplasia | | 1 (2%) | | |
| Transitional epithelium, hyperplasia | | | 2 (4%) | 2 (4%) |
| Urinary bladder | (50) | (50) | (50) | (50) |
| Hemorrhage | | | 1 (2%) | |
| Inflammation, chronic | | | 1 (2%) | |

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE
IN THE 2-YEAR DRINKING WATER STUDY
OF DIBROMOACETIC ACID

| | | |
|------------------|--|-------------|
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TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Other | 1 | | | |
| Moribund | 4 | 6 | 3 | 6 |
| Natural deaths | 14 | 6 | 13 | 13 |
| Survivors | | | | |
| Terminal sacrifice | 31 | 38 | 34 | 31 |
| Animals examined microscopically | 49 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Esophagus | (49) | (50) | (50) | (50) |
| Carcinoma, metastatic, pancreas | | 1 (2%) | | |
| Intestine large, colon | (48) | (50) | (49) | (48) |
| Carcinoma | | | | 1 (2%) |
| Hemangioma | | 1 (2%) | | |
| Intestine small, duodenum | (42) | (50) | (46) | (48) |
| Carcinoma | | | | 1 (2%) |
| Polyp adenomatous | | 1 (2%) | 2 (4%) | 1 (2%) |
| Intestine small, jejunum | (44) | (49) | (46) | (45) |
| Carcinoma | | 2 (4%) | | 3 (7%) |
| Polyp adenomatous | 1 (2%) | 1 (2%) | | |
| Intestine small, ileum | (44) | (49) | (47) | (48) |
| Polyp adenomatous | | 1 (2%) | | |
| Liver | (49) | (50) | (50) | (50) |
| Hemangioma | | | 1 (2%) | |
| Hemangiosarcoma | 1 (2%) | 2 (4%) | | 2 (4%) |
| Hepatoblastoma | | 4 (8%) | 3 (6%) | 16 (32%) |
| Hepatoblastoma, multiple | | | 3 (6%) | 2 (4%) |
| Hepatocellular carcinoma | 11 (22%) | 7 (14%) | 14 (28%) | 11 (22%) |
| Hepatocellular carcinoma, multiple | 3 (6%) | 2 (4%) | 5 (10%) | 15 (30%) |
| Hepatocellular adenoma | 12 (24%) | 20 (40%) | 13 (26%) | 15 (30%) |
| Hepatocellular adenoma, multiple | 6 (12%) | 17 (34%) | 24 (48%) | 27 (54%) |
| Histiocytic sarcoma | | | 1 (2%) | 1 (2%) |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Pheochromocytoma malignant, metastatic, adrenal medulla | 1 (2%) | | | |
| Sarcoma | | 1 (2%) | | |
| Mesentery | (7) | (7) | (10) | (7) |
| Hepatoblastoma, metastatic, liver | | | 1 (10%) | 1 (14%) |
| Osteosarcoma, metastatic, bone | | | 1 (10%) | |
| Sarcoma | | 1 (14%) | | |
| Pancreas | (46) | (50) | (50) | (49) |
| Hepatocellular carcinoma, metastatic, liver | | 1 (2%) | | 1 (2%) |
| Acinus, carcinoma | | 1 (2%) | | |
| Acinus, sarcoma | | 1 (2%) | | |
| Salivary glands | (49) | (50) | (50) | (50) |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Sarcoma | | 1 (2%) | | |
| Squamous cell carcinoma | 1 (2%) | 1 (2%) | | |
| Squamous cell papilloma | | | | 1 (2%) |
| Stomach, glandular | (47) | (50) | (50) | (50) |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|--------|---------|----------|------------|
| Cardiovascular System | | | | |
| Heart | (49) | (50) | (50) | (50) |
| Sarcoma | 1 (2%) | | | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Adenoma | | 1 (2%) | | |
| Hepatocellular carcinoma, metastatic, liver | | | | 2 (4%) |
| Capsule, adenoma | 3 (6%) | 3 (6%) | 2 (4%) | |
| Capsule, carcinoma | | 1 (2%) | | |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Pheochromocytoma malignant | 1 (2%) | | | |
| Islets, pancreatic | (46) | (50) | (50) | (49) |
| Adenoma | | 2 (4%) | 4 (8%) | 3 (6%) |
| Adenoma, multiple | | 1 (2%) | | |
| Thyroid gland | (48) | (50) | (50) | (50) |
| Follicular cell, adenoma | | | 1 (2%) | 1 (2%) |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Coagulating gland | | (1) | (1) | |
| Hemangioma | | | 1 (100%) | |
| Prostate | (48) | (50) | (50) | (49) |
| Carcinoma, metastatic, pancreas | | 1 (2%) | | |
| Testes | (49) | (50) | (50) | (50) |
| Hemangiosarcoma | 1 (2%) | | | |
| Interstitial cell, adenoma | | 2 (4%) | | 1 (2%) |
| Hematopoietic System | | | | |
| Bone marrow | (47) | (50) | (50) | (50) |
| Lymph node | (1) | (2) | (5) | (1) |
| Iliac, carcinoma, metastatic, pancreas | | 1 (50%) | | |
| Renal, carcinoma, metastatic, pancreas | | 1 (50%) | | |
| Lymph node, mandibular | (48) | (50) | (48) | (48) |
| Lymph node, mesenteric | (46) | (49) | (49) | (47) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (2%) |
| Spleen | (47) | (50) | (50) | (50) |
| Hemangiosarcoma | 2 (4%) | 3 (6%) | | |
| Hepatoblastoma, metastatic, liver | | 1 (2%) | | |
| Histiocytic sarcoma | | | 1 (2%) | |
| Thymus | (42) | (45) | (46) | (47) |
| Hepatocellular carcinoma, metastatic, liver | | | | 1 (2%) |
| Integumentary System | | | | |
| Skin | (49) | (50) | (50) | (50) |
| Basal cell carcinoma | | | 1 (2%) | |
| Subcutaneous tissue, fibroma | 1 (2%) | | | |
| Subcutaneous tissue, fibrosarcoma | | 1 (2%) | | |
| Subcutaneous tissue, fibrous histiocytoma | | | | 1 (2%) |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|---------|----------|----------|------------|
| Musculoskeletal System | | | | |
| Bone | (49) | (50) | (50) | (50) |
| Osteosarcoma | | | 1 (2%) | |
| Skeletal muscle | (1) | (1) | | (1) |
| Alveolar/bronchiolar carcinoma, metastatic, lung | | | | 1 (100%) |
| Sarcoma | | 1 (100%) | | |
| Nervous System | | | | |
| None | | | | |
| Respiratory System | | | | |
| Lung | (49) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 7 (14%) | 4 (8%) | 15 (30%) | 8 (16%) |
| Alveolar/bronchiolar adenoma, multiple | | 1 (2%) | 2 (4%) | 4 (8%) |
| Alveolar/bronchiolar carcinoma | 3 (6%) | 7 (14%) | 6 (12%) | 4 (8%) |
| Alveolar/bronchiolar carcinoma, multiple | 2 (4%) | 1 (2%) | 2 (4%) | 3 (6%) |
| Carcinoma, metastatic, pancreas | | 1 (2%) | | |
| Hepatoblastoma, metastatic, liver | | 1 (2%) | 2 (4%) | 6 (12%) |
| Hepatocellular carcinoma, metastatic, liver | 8 (16%) | 5 (10%) | 7 (14%) | 13 (26%) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Osteosarcoma, metastatic, bone | | | 1 (2%) | |
| Pheochromocytoma malignant, metastatic, adrenal medulla | 1 (2%) | | | |
| Bronchus, adenoma | 1 (2%) | | 1 (2%) | |
| Mediastinum, hepatoblastoma, metastatic, liver | | | | 2 (4%) |
| Special Senses System | | | | |
| Harderian gland | (49) | (50) | (50) | (50) |
| Adenoma | 3 (6%) | 4 (8%) | 5 (10%) | 6 (12%) |
| Adenoma, multiple | | 1 (2%) | | 1 (2%) |
| Urinary System | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Renal tubule, adenoma | 1 (2%) | 1 (2%) | | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (49) | (50) | (50) | (50) |
| Histiocytic sarcoma | | | 1 (2%) | 1 (2%) |
| Lymphoma malignant | | 5 (10%) | 3 (6%) | 2 (4%) |

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|--------|---------|----------|------------|
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | 37 | 44 | 49 | 50 |
| Total primary neoplasms | 61 | 103 | 110 | 130 |
| Total animals with benign neoplasms | 25 | 38 | 42 | 46 |
| Total benign neoplasms | 35 | 61 | 71 | 68 |
| Total animals with malignant neoplasms | 23 | 28 | 32 | 43 |
| Total malignant neoplasms | 26 | 42 | 39 | 62 |
| Total animals with metastatic neoplasms | 9 | 7 | 10 | 21 |
| Total metastatic neoplasms | 10 | 13 | 13 | 28 |

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

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid: 0 mg/L

| | | |
|--------------------------------|---|----------------------|
| Number of Days on Study | 7 | |
| | 3 | |
| | 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 4 4 4 4 4 4 4 4 4 | |
| Carcass ID Number | 0 | Total Tissues/Tumors |
| | 1 2 3 3 4 0 0 1 1 2 3 3 4 4 4 0 0 0 1 1 2 2 3 3 4 | |
| | 2 6 2 3 8 2 6 5 6 0 4 9 3 5 6 1 8 9 4 9 3 7 1 5 0 | |
| Special Senses System | | |
| Eye | + | 49 |
| Harderian gland | + | 49 |
| Adenoma | X | 3 |
| Urinary System | | |
| Kidney | + | 49 |
| Renal tubule, adenoma | | 1 |
| Urinary bladder | + | 49 |
| Systemic Lesions | | |
| Multiple organs | + | 49 |

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid: 500 mg/L

| Number of Days on Study | 3 | 4 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 6 | 3 | 2 | 7 | 7 | 9 | 0 | 2 | 2 | 3 | 5 | 5 | 9 | 9 | 0 | 1 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 2 | 3 | |
| | 7 | 1 | 1 | 3 | 6 | 6 | 4 | 5 | 6 | 1 | 3 | 4 | 1 | 9 | 8 | 4 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 9 | 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 | 1 | 1 | |
| | 1 | 4 | 0 | 4 | 3 | 1 | 0 | 4 | 4 | 1 | 2 | 2 | 0 | 3 | 4 | 3 | 0 | 0 | 1 | 2 | 2 | 2 | 2 | 3 | 4 | 0 | | |
| | 5 | 6 | 7 | 0 | 1 | 0 | 6 | 1 | 3 | 9 | 2 | 8 | 2 | 8 | 2 | 5 | 3 | 4 | 7 | 4 | 5 | 6 | 0 | 7 | 8 | | | |
| Genital System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Coagulating gland | | | | | | | | | | | | | | | | | | | | | | | | | | | | + |
| Hemangioma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Epididymis | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Preputial gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Prostate | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Seminal vesicle | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Testes | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Hematopoietic System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone marrow | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph node | + | | | | | | | | + | | | + | | | | | | | | | | | | | | | | + |
| Lymph node, mandibular | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Lymph node, mesenteric | I | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Spleen | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Histiocytic sarcoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Thymus | + | + | + | + | M | + | + | + | + | + | M | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Integumentary System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Mammary gland | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Skin | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Basal cell carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Musculoskeletal System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Bone | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Osteosarcoma | | | | X | | | | | | | | | | | | | | | | | | | | | | | | |
| Nervous System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Brain | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Peripheral nerve | | | | | | | | | | | | | | | | | | | | | | | | | | | | + |
| Spinal cord | | | | | | | | | | | | | | | | | | | | | | | | | | | | + |
| Respiratory System | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| Lung | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Alveolar/bronchiolar adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Alveolar/bronchiolar adenoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Alveolar/bronchiolar carcinoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Alveolar/bronchiolar carcinoma, multiple | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Hepatoblastoma, metastatic, liver | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Hepatocellular carcinoma, metastatic, liver | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Osteosarcoma, metastatic, bone | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Bronchus, adenoma | | | | | | | | | | | | | | | | | | | | | | | | | | | | X |
| Nose | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| Trachea | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid:
1,000 mg/L

| | | |
|--------------------------------|---|----------------------|
| Number of Days on Study | 7 | |
| | 3 | |
| | 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 4 4 4 4 | |
| Carcass ID Number | 1 | Total Tissues/Tumors |
| | 6 7 7 8 8 9 5 5 6 7 7 7 8 8 8 8 9 9 9 9 5 5 7 7 8 | |
| | 0 4 5 3 8 0 2 3 4 0 2 3 1 2 4 5 3 5 7 9 1 8 1 6 6 | |
| Special Senses System | | |
| Eye | + | 50 |
| Harderian gland | + | 50 |
| Adenoma | X X X X X | 6 |
| Adenoma, multiple | X | 1 |
| Urinary System | | |
| Kidney | + | 50 |
| Urinary bladder | + | 49 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Histiocytic sarcoma | | 1 |
| Lymphoma malignant | X X | 2 |

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|----------------|
| Adrenal Cortex: Adenoma | | | | |
| Overall rate ^a | 3/49 (6%) | 4/50 (8%) | 2/50 (4%) | 0/50 (0%) |
| Adjusted rate ^b | 7.1% | 8.7% | 4.6% | 0.0% |
| Terminal rate ^c | 3/31 (10%) | 3/38 (8%) | 2/34 (6%) | 0/31 (0%) |
| First incidence (days) | 729 (T) | 659 | 729 (T) | — ^e |
| Poly-3 test ^d | P=0.042N | P=0.549 | P=0.484N | P=0.113N |
| Harderian Gland: Adenoma | | | | |
| Overall rate | 3/50 (6%) | 5/50 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 7.1% | 10.9% | 11.4% | 16.0% |
| Terminal rate | 3/31 (10%) | 4/38 (11%) | 4/34 (12%) | 6/31 (19%) |
| First incidence (days) | 729 (T) | 721 | 714 | 706 |
| Poly-3 test | P=0.151 | P=0.404 | P=0.375 | P=0.169 |
| Harderian Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 3/50 (6%) | 5/50 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 7.1% | 10.9% | 11.4% | 16.0% |
| Terminal rate | 3/31 (10%) | 4/38 (11%) | 4/34 (12%) | 6/31 (19%) |
| First incidence (days) | 729 (T) | 721 | 714 | 706 |
| Poly-3 test | P=0.151 | P=0.404 | P=0.375 | P=0.169 |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 18/49 (37%) | 37/50 (74%) | 37/50 (74%) | 42/50 (84%) |
| Adjusted rate | 41.6% | 78.0% | 80.1% | 88.7% |
| Terminal rate | 14/31 (45%) | 31/38 (82%) | 29/34 (85%) | 29/31 (94%) |
| First incidence (days) | 596 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P<0.001 | P<0.001 | P<0.001 |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 14/49 (29%) | 9/50 (18%) | 19/50 (38%) | 26/50 (52%) |
| Adjusted rate | 31.3% | 19.4% | 40.7% | 54.9% |
| Terminal rate | 6/31 (19%) | 6/38 (16%) | 10/34 (29%) | 14/31 (45%) |
| First incidence (days) | 451 | 659 | 573 | 476 |
| Poly-3 test | P<0.001 | P=0.141N | P=0.236 | P=0.016 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 28/49 (57%) | 41/50 (82%) | 42/50 (84%) | 47/50 (94%) |
| Adjusted rate | 61.2% | 85.8% | 88.3% | 96.0% |
| Terminal rate | 17/31 (55%) | 33/38 (87%) | 30/34 (88%) | 30/31 (97%) |
| First incidence (days) | 451 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.004 | P<0.001 | P<0.001 |
| Liver: Hepatoblastoma | | | | |
| Overall rate | 0/49 (0%) | 4/50 (8%) | 6/50 (12%) | 18/50 (36%) |
| Adjusted rate | 0.0% | 8.7% | 13.3% | 39.1% |
| Terminal rate | 0/31 (0%) | 3/38 (8%) | 3/34 (9%) | 9/31 (29%) |
| First incidence (days) | — | 717 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.072 | P=0.019 | P<0.001 |
| Liver: Hepatocellular Carcinoma or Hepatoblastoma | | | | |
| Overall rate | 14/49 (29%) | 11/50 (22%) | 23/50 (46%) | 37/50 (74%) |
| Adjusted rate | 31.3% | 23.7% | 48.9% | 75.5% |
| Terminal rate | 6/31 (19%) | 8/38 (21%) | 13/34 (38%) | 20/31 (65%) |
| First incidence (days) | 451 | 659 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.281N | P=0.063 | P<0.001 |

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|-------------|-------------|----------------|-------------|
| Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma | | | | |
| Overall rate | 28/49 (57%) | 41/50 (82%) | 43/50 (86%) | 48/50 (96%) |
| Adjusted rate | 61.2% | 85.8% | 90.4% | 97.4% |
| Terminal rate | 17/31 (55%) | 33/38 (87%) | 31/34 (91%) | 30/31 (97%) |
| First incidence (days) | 451 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.004 | P<0.001 | P<0.001 |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 7/49 (14%) | 5/50 (10%) | 17/50 (34%) | 12/50 (24%) |
| Adjusted rate | 16.3% | 10.8% | 38.4% | 26.6% |
| Terminal rate | 5/31 (16%) | 4/38 (11%) | 15/34 (44%) | 8/31 (26%) |
| First incidence (days) | 482 | 596 | 604 | 489 |
| Poly-3 test | P=0.019 | P=0.326N | P=0.016 | P=0.178 |
| Lung: Alveolar/bronchiolar Carcinoma | | | | |
| Overall rate | 5/49 (10%) | 8/50 (16%) | 8/50 (16%) | 7/50 (14%) |
| Adjusted rate | 11.7% | 17.3% | 18.2% | 15.9% |
| Terminal rate | 3/31 (10%) | 6/38 (16%) | 6/34 (18%) | 5/31 (16%) |
| First incidence (days) | 662 | 691 | 699 | 652 |
| Poly-3 test | P=0.436 | P=0.329 | P=0.294 | P=0.400 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 12/49 (24%) | 12/50 (24%) | 22/50 (44%) | 17/50 (34%) |
| Adjusted rate | 27.7% | 25.7% | 49.4% | 37.3% |
| Terminal rate | 8/31 (26%) | 9/38 (24%) | 18/34 (53%) | 11/31 (36%) |
| First incidence (days) | 482 | 596 | 604 | 489 |
| Poly-3 test | P=0.066 | P=0.513N | P=0.027 | P=0.226 |
| Pancreatic Islets: Adenoma | | | | |
| Overall rate | 0/46 (0%) | 3/50 (6%) | 4/50 (8%) | 3/49 (6%) |
| Adjusted rate | 0.0% | 6.5% | 9.1% | 7.0% |
| Terminal rate | 0/31 (0%) | 2/38 (5%) | 3/34 (9%) | 3/31 (10%) |
| First incidence (days) | — | 721 | 596 | 729 (T) |
| Poly-3 test | P=0.211 | P=0.144 | P=0.073 | P=0.131 |
| Small Intestine (Jejunum): Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 2/50 (4%) | 0/50 (0%) | 3/50 (6%) |
| Adjusted rate | 0.0% | 4.4% | 0.0% | 6.9% |
| Terminal rate | 0/31 (0%) | 2/38 (5%) | 0/34 (0%) | 3/31 (10%) |
| First incidence (days) | — | 729 (T) | — | 729 (T) |
| Poly-3 test | P=0.157 | P=0.256 | — ^f | P=0.124 |
| Small Intestine (Duodenum, Jejunum): Carcinoma | | | | |
| Overall rate | 0/50 (0%) | 2/50 (4%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate | 0.0% | 4.4% | 0.0% | 9.2% |
| Terminal rate | 0/31 (0%) | 2/38 (5%) | 0/34 (0%) | 4/31 (13%) |
| First incidence (days) | — | 729 (T) | — | 729 (T) |
| Poly-3 test | P=0.059 | P=0.256 | — | P=0.064 |
| Small Intestine (Duodenum, Jejunum, Ileum): Adenomatous Polyp | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 2.4% | 6.5% | 4.6% | 2.3% |
| Terminal rate | 1/31 (3%) | 3/38 (8%) | 2/34 (6%) | 0/31 (0%) |
| First incidence (days) | 729 (T) | 729 (T) | 729 (T) | 489 |
| Poly-3 test | P=0.371N | P=0.336 | P=0.512 | P=0.751N |

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|--------------|
| Spleen: Hemangiosarcoma | | | | |
| Overall rate | 2/47 (4%) | 3/50 (6%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 4.9% | 6.5% | 0.0% | 0.0% |
| Terminal rate | 2/30 (7%) | 3/38 (8%) | 0/34 (0%) | 0/31 (0%) |
| First incidence (days) | 729 (T) | 729 (T) | — | — |
| Poly-3 test | P=0.039N | P=0.555 | P=0.221N | P=0.222N |
| All Organs: Hemangiosarcoma | | | | |
| Overall rate | 4/50 (8%) | 4/50 (8%) | 0/50 (0%) | 2/50 (4%) |
| Adjusted rate | 9.5% | 8.7% | 0.0% | 4.6% |
| Terminal rate | 4/31 (13%) | 4/38 (11%) | 0/34 (0%) | 2/31 (7%) |
| First incidence (days) | 729 (T) | 729 (T) | — | 729 (T) |
| Poly-3 test | P=0.127N | P=0.597N | P=0.056N | P=0.323N |
| All Organs: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 4/50 (8%) | 5/50 (10%) | 2/50 (4%) | 2/50 (4%) |
| Adjusted rate | 9.5% | 10.9% | 4.6% | 4.6% |
| Terminal rate | 4/31 (13%) | 5/38 (13%) | 1/34 (3%) | 2/31 (7%) |
| First incidence (days) | 729 (T) | 729 (T) | 699 | 729 (T) |
| Poly-3 test | P=0.138N | P=0.552 | P=0.320N | P=0.323N |
| All Organs: Malignant Lymphoma | | | | |
| Overall rate | 0/50 (0%) | 5/50 (10%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate | 0.0% | 10.9% | 6.6% | 4.6% |
| Terminal rate | 0/31 (0%) | 5/38 (13%) | 0/34 (0%) | 2/31 (7%) |
| First incidence (days) | — | 729 (T) | 367 | 729 (T) |
| Poly-3 test | P=0.518N | P=0.038 | P=0.133 | P=0.244 |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 25/50 (50%) | 38/50 (76%) | 42/50 (84%) | 46/50 (92%) |
| Adjusted rate | 56.7% | 79.6% | 90.4% | 94.7% |
| Terminal rate | 19/31 (61%) | 31/38 (82%) | 32/34 (94%) | 31/31 (100%) |
| First incidence (days) | 482 | 573 | 573 | 469 |
| Poly-3 test | P<0.001 | P=0.012 | P<0.001 | P<0.001 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 23/50 (46%) | 28/50 (56%) | 32/50 (64%) | 43/50 (86%) |
| Adjusted rate | 50.9% | 59.5% | 64.6% | 87.2% |
| Terminal rate | 12/31 (39%) | 22/38 (58%) | 17/34 (50%) | 25/31 (81%) |
| First incidence (days) | 451 | 596 | 367 | 469 |
| Poly-3 test | P<0.001 | P=0.267 | P=0.125 | P<0.001 |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 37/50 (74%) | 44/50 (88%) | 49/50 (98%) | 50/50 (100%) |
| Adjusted rate | 79.1% | 91.6% | 98.0% | 100.0% |
| Terminal rate | 23/31 (74%) | 35/38 (92%) | 33/34 (97%) | 31/31 (100%) |
| First incidence (days) | 451 | 573 | 367 | 469 |
| Poly-3 test | P<0.001 | P=0.067 | P=0.002 | P<0.001 |

(T) Terminal sacrifice

^a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, 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

^f Value of statistic cannot be computed.

TABLE C4a
Historical Incidence of Neoplasms in the Liver of Control Male B6C3F₁ Mice^a

| Study | Incidence in Controls | | | |
|---|------------------------|--------------------------|-------------------------------------|-----------------|
| | Hepatocellular Adenoma | Hepatocellular Carcinoma | Hepatocellular Adenoma or Carcinoma | Hepatoblastoma |
| Historical Incidence: Drinking Water Studies | | | | |
| Dibromoacetic acid | 18/49 | 14/49 | 28/49 | 0/49 |
| Dipropylene glycol | 17/50 | 14/50 | 29/50 | 0/50 |
| Sodium chlorate | 30/48 | 20/48 | 41/48 | 6/48 |
| Sodium nitrite | 19/50 | 9/50 | 24/50 | 5/50 |
| Overall Historical Incidence: Drinking Water Studies | | | | |
| Total (%) | 84/197 (42.6%) | 57/197 (28.9%) | 122/197 (61.9%) | 11/197 (5.6%) |
| Mean ± standard deviation | 34.3% ± 22.3% | 23.3% ± 15.5% | 49.7% ± 31.1% | 4.5% ± 6.2% |
| Range | 34%-63% | 18%-42% | 48%-85% | 0%-13% |
| Overall Historical Incidence | | | | |
| Total (%) | 490/1,506 (32.5%) | 344/1,506 (22.8%) | 745/1,506 (49.5%) | 22/1,506 (1.5%) |
| Mean ± standard deviation | 32.6% ± 12.7% | 22.9% ± 10.0% | 49.5% ± 17.8% | 1.5% ± 3.1% |
| Range | 12%-63% | 8%-46% | 20%-85% | 0%-13% |

^a Data as of January 26, 2005

TABLE C4b
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Male B6C3F₁ Mice^a

| Study | Incidence in Controls | | |
|---|-----------------------|-------------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence: Drinking Water Studies | | | |
| Dibromoacetic acid | 7/49 | 5/49 | 12/49 |
| Dipropylene glycol | 3/50 | 3/50 | 6/50 |
| Sodium chlorate | 6/50 | 4/50 | 10/50 |
| Sodium nitrite | 10/50 | 4/50 | 13/50 |
| Overall Historical Incidence: Drinking Water Studies | | | |
| Total (%) | 26/199 (13.1%) | 16/199 (8.0%) | 41/199 (20.6%) |
| Mean ± standard deviation | 10.5% ± 7.7% | 6.4% ± 3.9% | 16.5% ± 10.7% |
| Range | 6%-20% | 6%-10% | 12%-26% |
| Overall Historical Incidence | | | |
| Total (%) | 258/1,507 (17.1%) | 151/1,507 (10.0%) | 385/1,507 (25.6%) |
| Mean ± standard deviation | 16.7% ± 7.3% | 9.9% ± 5.0% | 25.1% ± 9.4% |
| Range | 4%-28% | 4%-24% | 12%-44% |

^a Data as of January 26, 2005

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---------------------------------------|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Other | 1 | | | |
| Moribund | 4 | 6 | 3 | 6 |
| Natural deaths | 14 | 6 | 13 | 13 |
| Survivors | | | | |
| Terminal sacrifice | 31 | 38 | 34 | 31 |
| Animals examined microscopically | 49 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, cecum | (43) | (49) | (48) | (46) |
| Edema | 2 (5%) | 2 (4%) | 3 (6%) | 1 (2%) |
| Hyperplasia, lymphoid | | | 1 (2%) | |
| Intestine small, duodenum | (42) | (50) | (46) | (48) |
| Hyperplasia, lymphoid | | 1 (2%) | | |
| Intestine small, jejunum | (44) | (49) | (46) | (45) |
| Hyperplasia, lymphoid | | | 2 (4%) | 2 (4%) |
| Liver | (49) | (50) | (50) | (50) |
| Angiectasis | | | 1 (2%) | |
| Basophilic focus | 2 (4%) | | 1 (2%) | 2 (4%) |
| Clear cell focus | 10 (20%) | 13 (26%) | 23 (46%) | 13 (26%) |
| Congestion | 1 (2%) | | | |
| Cyst | | 1 (2%) | | 1 (2%) |
| Eosinophilic focus | 15 (31%) | 10 (20%) | 15 (30%) | 17 (34%) |
| Hematopoietic cell proliferation | | | | 1 (2%) |
| Hemorrhage | 1 (2%) | | | |
| Hepatodiaphragmatic nodule | | 1 (2%) | | |
| Infarct | | | 1 (2%) | |
| Infiltration cellular, mixed cell | 2 (4%) | 4 (8%) | 2 (4%) | 2 (4%) |
| Mixed cell focus | 3 (6%) | 7 (14%) | 8 (16%) | 4 (8%) |
| Necrosis, focal | 8 (16%) | 7 (14%) | 3 (6%) | 5 (10%) |
| Tension lipidosis | | 1 (2%) | 1 (2%) | 2 (4%) |
| Bile duct, cyst | | | 1 (2%) | |
| Bile duct, cyst multilocular | | | 1 (2%) | |
| Centrilobular, necrosis | 4 (8%) | 4 (8%) | | 5 (10%) |
| Hepatocyte, vacuolization cytoplasmic | 8 (16%) | 9 (18%) | 4 (8%) | 10 (20%) |
| Kupffer cell, pigmentation | 1 (2%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Mesentery | (7) | (7) | (10) | (7) |
| Inflammation, chronic | | | 1 (10%) | |
| Fat, necrosis | 5 (71%) | 6 (86%) | 7 (70%) | 4 (57%) |
| Pancreas | (46) | (50) | (50) | (49) |
| Necrosis | | 1 (2%) | | |
| Acinus, cytoplasmic alteration | 2 (4%) | 2 (4%) | 2 (4%) | |
| Salivary glands | (49) | (50) | (50) | (50) |
| Atrophy | 1 (2%) | | | 2 (4%) |
| Hyperplasia, lymphoid | 3 (6%) | 4 (8%) | 6 (12%) | 1 (2%) |

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

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--------------------------------------|----------|----------|----------|------------|
| Alimentary System (continued) | | | | |
| Stomach, forestomach | (49) | (50) | (50) | (50) |
| Diverticulum | | 2 (4%) | 1 (2%) | |
| Erosion | 1 (2%) | | 1 (2%) | 2 (4%) |
| Inflammation, chronic | 3 (6%) | 1 (2%) | 3 (6%) | 6 (12%) |
| Ulcer | 2 (4%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Epithelium, hyperplasia | 4 (8%) | 3 (6%) | 4 (8%) | 7 (14%) |
| Epithelium, hyperplasia, focal | | | 1 (2%) | 1 (2%) |
| Stomach, glandular | (47) | (50) | (50) | (50) |
| Erosion | 2 (4%) | 2 (4%) | 1 (2%) | |
| Glands, hyperplasia | | 1 (2%) | | |
| Tooth | (5) | (6) | | |
| Malformation | 5 (100%) | 6 (100%) | | |
| Cardiovascular System | | | | |
| Heart | (49) | (50) | (50) | (50) |
| Cardiomyopathy | 1 (2%) | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | | | |
| Mineralization | 3 (6%) | | 2 (4%) | 1 (2%) |
| Myocardium, necrosis | | 1 (2%) | | |
| Endocrine System | | | | |
| Adrenal cortex | (49) | (50) | (50) | (50) |
| Accessory adrenal cortical nodule | 7 (14%) | 5 (10%) | 4 (8%) | 6 (12%) |
| Hyperplasia, focal | 4 (8%) | 7 (14%) | 5 (10%) | 1 (2%) |
| Hypertrophy | 1 (2%) | | | 1 (2%) |
| Hypertrophy, focal | 12 (24%) | 11 (22%) | 13 (26%) | 6 (12%) |
| Capsule, hyperplasia | 7 (14%) | 4 (8%) | 1 (2%) | 5 (10%) |
| Adrenal medulla | (49) | (50) | (50) | (50) |
| Hyperplasia | 1 (2%) | 1 (2%) | | 1 (2%) |
| Islets, pancreatic | (46) | (50) | (50) | (49) |
| Atrophy | | | | 1 (2%) |
| Hyperplasia | 34 (74%) | 32 (64%) | 26 (52%) | 13 (27%) |
| Parathyroid gland | (45) | (47) | (49) | (47) |
| Cyst | | 2 (4%) | 3 (6%) | 4 (9%) |
| Pituitary gland | (45) | (50) | (48) | (48) |
| Pars distalis, cyst | 7 (16%) | 7 (14%) | 7 (15%) | 5 (10%) |
| Pars distalis, hyperplasia, focal | 1 (2%) | | 1 (2%) | |
| Thyroid gland | (48) | (50) | (50) | (50) |
| Degeneration, cystic | 5 (10%) | 8 (16%) | 3 (6%) | 6 (12%) |
| Follicular cell, hyperplasia | 3 (6%) | | | |
| General Body System | | | | |
| None | | | | |
| Genital System | | | | |
| Epididymis | (49) | (50) | (50) | (50) |
| Atypia cellular | 5 (10%) | 4 (8%) | 2 (4%) | 6 (12%) |
| Granuloma sperm | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Spermatocele | 1 (2%) | | | 1 (2%) |

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|------------------------------------|----------|----------|----------|------------|
| Genital System (continued) | | | | |
| Preputial gland | (49) | (50) | (50) | (50) |
| Cyst | 25 (51%) | 26 (52%) | 33 (66%) | 33 (66%) |
| Inflammation, chronic | 21 (43%) | 30 (60%) | 24 (48%) | 28 (56%) |
| Prostate | (48) | (50) | (50) | (49) |
| Inflammation, chronic | | 4 (8%) | | 1 (2%) |
| Seminal vesicle | (49) | (50) | (50) | (50) |
| Degeneration | 3 (6%) | 5 (10%) | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | | | |
| Testes | (49) | (50) | (50) | (50) |
| Germinal epithelium, atrophy | 9 (18%) | 4 (8%) | 4 (8%) | 8 (16%) |
| Hematopoietic System | | | | |
| Bone marrow | (47) | (50) | (50) | (50) |
| Angiectasis | | | | 1 (2%) |
| Depletion cellular | 1 (2%) | | | |
| Hyperplasia | 22 (47%) | 22 (44%) | 28 (56%) | 32 (64%) |
| Lymph node | (1) | (2) | (5) | (1) |
| Bronchial, hyperplasia, lymphoid | | | 1 (20%) | |
| Iliac, ectasia | | 1 (50%) | | |
| Inguinal, hyperplasia, lymphoid | | | 1 (20%) | |
| Inguinal, pigmentation | | 1 (50%) | | |
| Mediastinal, hyperplasia, lymphoid | 1 (100%) | | 1 (20%) | |
| Mediastinal, pigmentation | | | | 1 (100%) |
| Pancreatic, hyperplasia, lymphoid | | | 1 (20%) | |
| Lymph node, mandibular | (48) | (50) | (48) | (48) |
| Atrophy | 5 (10%) | 2 (4%) | 3 (6%) | 7 (15%) |
| Hematopoietic cell proliferation | 1 (2%) | | | |
| Hemorrhage | 1 (2%) | | 1 (2%) | |
| Hyperplasia, lymphoid | 15 (31%) | 14 (28%) | 6 (13%) | 7 (15%) |
| Pigmentation | 7 (15%) | 8 (16%) | 1 (2%) | 7 (15%) |
| Lymph node, mesenteric | (46) | (49) | (49) | (47) |
| Atrophy | 4 (9%) | 2 (4%) | 1 (2%) | 9 (19%) |
| Hematopoietic cell proliferation | 1 (2%) | 1 (2%) | 3 (6%) | 3 (6%) |
| Hemorrhage | 8 (17%) | 5 (10%) | 9 (18%) | 7 (15%) |
| Hyperplasia, lymphoid | 2 (4%) | 8 (16%) | 3 (6%) | 6 (13%) |
| Hyperplasia, plasma cell | | | 1 (2%) | |
| Spleen | (47) | (50) | (50) | (50) |
| Angiectasis | | | | 1 (2%) |
| Hematopoietic cell proliferation | 18 (38%) | 20 (40%) | 28 (56%) | 38 (76%) |
| Pigmentation | | 1 (2%) | 1 (2%) | 1 (2%) |
| Lymphoid follicle, atrophy | 4 (9%) | 7 (14%) | 3 (6%) | 8 (16%) |
| Lymphoid follicle, hyperplasia | 3 (6%) | 6 (12%) | 6 (12%) | 6 (12%) |
| Thymus | (42) | (45) | (46) | (47) |
| Atrophy | 14 (33%) | 16 (36%) | 12 (26%) | 22 (47%) |
| Hyperplasia, lymphoid | | 1 (2%) | 1 (2%) | 1 (2%) |
| Integumentary System | | | | |
| Skin | (49) | (50) | (50) | (50) |
| Cyst epithelial inclusion | | 2 (4%) | | |
| Ulcer | 3 (6%) | 2 (4%) | | 3 (6%) |
| Epidermis, hyperplasia | 3 (6%) | 2 (4%) | | 3 (6%) |

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|-----------------------------------|---------|----------|----------|------------|
| Musculoskeletal System | | | | |
| Bone | (49) | (50) | (50) | (50) |
| Hyperostosis | | 1 (2%) | | |
| Cranium, hyperostosis | 1 (2%) | | | 1 (2%) |
| Skeletal muscle | (1) | (1) | | (1) |
| Atrophy | | 1 (100%) | | |
| Nervous System | | | | |
| Peripheral nerve | (2) | (2) | (1) | |
| Atrophy | | 1 (50%) | 1 (100%) | |
| Spinal cord | (2) | (2) | (1) | |
| Gliosis | 1 (50%) | | | |
| Respiratory System | | | | |
| Lung | (49) | (50) | (50) | (50) |
| Edema | 2 (4%) | | 1 (2%) | 2 (4%) |
| Hemorrhage | 2 (4%) | | | 4 (8%) |
| Hyperplasia, lymphoid | 3 (6%) | | 1 (2%) | |
| Infiltration cellular, histiocyte | 2 (4%) | 6 (12%) | 3 (6%) | 6 (12%) |
| Metaplasia, osseous | 1 (2%) | 2 (4%) | | 1 (2%) |
| Alveolar epithelium, hyperplasia | 2 (4%) | 6 (12%) | 6 (12%) | 7 (14%) |
| Bronchiole, hyperplasia | | | | 1 (2%) |
| Nose | (48) | (50) | (50) | (50) |
| Foreign body | | | 1 (2%) | |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 3 (6%) | |
| Special Senses System | | | | |
| Eye | (49) | (50) | (50) | (50) |
| Atrophy | | 1 (2%) | | |
| Cataract | | 1 (2%) | 1 (2%) | 5 (10%) |
| Inflammation, chronic | | | 2 (4%) | 2 (4%) |
| Bilateral, cataract | | | | 1 (2%) |
| Cornea, hyperplasia | | | 1 (2%) | 1 (2%) |
| Harderian gland | (49) | (50) | (50) | (50) |
| Cyst | | 1 (2%) | | |
| Hyperplasia, focal | 5 (10%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Lacrimal gland | | (1) | | |
| Cyst | | 1 (100%) | | |

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Urinary System | | | | |
| Kidney | (49) | (50) | (50) | (50) |
| Cyst | 9 (18%) | 13 (26%) | 14 (28%) | 11 (22%) |
| Hydronephrosis | 4 (8%) | 2 (4%) | | 4 (8%) |
| Hyperplasia, lymphoid | 5 (10%) | 5 (10%) | 6 (12%) | 5 (10%) |
| Infarct | 3 (6%) | 7 (14%) | 4 (8%) | 5 (10%) |
| Inflammation, suppurative | | 1 (2%) | | 1 (2%) |
| Metaplasia, osseous | 6 (12%) | 3 (6%) | 2 (4%) | 2 (4%) |
| Nephropathy | 36 (73%) | 44 (88%) | 42 (84%) | 39 (78%) |
| Papilla, necrosis | | | | 1 (2%) |
| Renal tubule, accumulation, hyaline droplet | | | 1 (2%) | |
| Renal tubule, dilatation | 1 (2%) | 3 (6%) | | 1 (2%) |
| Renal tubule, hyperplasia | 1 (2%) | 1 (2%) | 1 (2%) | 3 (6%) |
| Renal tubule, necrosis | 1 (2%) | | | |
| Renal tubule, pigmentation | 1 (2%) | 3 (6%) | 2 (4%) | 7 (14%) |
| Transitional epithelium, hyperplasia | | | 1 (2%) | |
| Urethra | | (3) | | |
| Angiectasis | | 3 (100%) | | |
| Inflammation, chronic active | | 3 (100%) | | |
| Urinary bladder | (49) | (50) | (50) | (49) |
| Edema | | | 1 (2%) | |
| Hemorrhage | | 1 (2%) | | |
| Hyperplasia, lymphoid | | 1 (2%) | 1 (2%) | |
| Inflammation, chronic | | | | 1 (2%) |
| Transitional epithelium, hyperplasia | | | 1 (2%) | 1 (2%) |

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE
IN THE 2-YEAR DRINKING WATER STUDY
OF DIBROMOACETIC ACID

| | | |
|------------------|--|-------------|
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TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 2 | 2 | 5 | 7 |
| Natural deaths | 10 | 13 | 13 | 11 |
| Survivors | | | | |
| Died last week of study | | | 1 | |
| Terminal sacrifice | 38 | 35 | 31 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, cecum | (47) | (46) | (49) | (46) |
| Intestine small, duodenum | (46) | (48) | (48) | (45) |
| Polyp adenomatous | | 2 (4%) | | |
| Intestine small, jejunum | (46) | (47) | (49) | (43) |
| Carcinoma | | | | 1 (2%) |
| Sarcoma, metastatic, skin | | 1 (2%) | | |
| Intestine small, ileum | (45) | (47) | (47) | (46) |
| Fibrosarcoma, metastatic, tissue NOS | | | | 1 (2%) |
| Liver | (49) | (50) | (50) | (49) |
| Hemangioma | 1 (2%) | | 1 (2%) | |
| Hemangiosarcoma | 1 (2%) | 1 (2%) | 2 (4%) | |
| Hepatoblastoma | 1 (2%) | | 1 (2%) | |
| Hepatoblastoma, multiple | | | | 1 (2%) |
| Hepatocellular carcinoma | 2 (4%) | 2 (4%) | 7 (14%) | 5 (10%) |
| Hepatocellular carcinoma, multiple | 1 (2%) | 1 (2%) | 5 (10%) | 3 (6%) |
| Hepatocellular adenoma | 8 (16%) | 12 (24%) | 9 (18%) | 9 (18%) |
| Hepatocellular adenoma, multiple | 11 (22%) | 14 (28%) | 23 (46%) | 26 (53%) |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Ito cell tumor malignant | | | | 1 (2%) |
| Sarcoma | 1 (2%) | | | |
| Sarcoma, metastatic, skin | | 1 (2%) | | |
| Mesentery | (28) | (20) | (16) | (19) |
| Hemangioma | 1 (4%) | | | |
| Hemangiosarcoma | | | 1 (6%) | 1 (5%) |
| Leiomyosarcoma | | | 1 (6%) | |
| Sarcoma | 1 (4%) | | | |
| Sarcoma, metastatic, pancreas | | | 1 (6%) | |
| Sarcoma, metastatic, skin | | 1 (5%) | | |
| Schwannoma malignant, metastatic, skin | | 1 (5%) | | |
| Oral mucosa | | (1) | | |
| Squamous cell carcinoma | | 1 (100%) | | |
| Pancreas | (49) | (49) | (49) | (47) |
| Histiocytic sarcoma | | | | 1 (2%) |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Acinus, carcinoma | | | | 1 (2%) |
| Acinus, sarcoma | | | 1 (2%) | |
| Salivary glands | (50) | (49) | (50) | (50) |
| Stomach, forestomach | (49) | (50) | (50) | (49) |
| Squamous cell carcinoma | | | 1 (2%) | 1 (2%) |
| Squamous cell papilloma | 1 (2%) | 1 (2%) | 2 (4%) | |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|---------|---------|----------|------------|
| Alimentary System (continued) | | | | |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| Carcinoma | 1 (2%) | | | |
| Schwannoma malignant, metastatic, skin | | 1 (2%) | | |
| Serosa, sarcoma, metastatic, skin | | 1 (2%) | | |
| Tongue | (2) | | | |
| Squamous cell carcinoma | 1 (50%) | | | |
| Squamous cell papilloma | 1 (50%) | | | |
| Cardiovascular System | | | | |
| Heart | (50) | (49) | (50) | (50) |
| Hemangiosarcoma | | 1 (2%) | | |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | | | |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Leiomyosarcoma, metastatic, mesentery | | | 1 (2%) | |
| Sarcoma, metastatic, pancreas | | | 1 (2%) | |
| Capsule, adenoma | | | 1 (2%) | |
| Extra adrenal tissue, sarcoma | 1 (2%) | | | |
| Adrenal medulla | (50) | (50) | (50) | (49) |
| Pheochromocytoma benign | 1 (2%) | | 1 (2%) | 1 (2%) |
| Islets, pancreatic | (49) | (49) | (49) | (46) |
| Adenoma | 1 (2%) | | 2 (4%) | 2 (4%) |
| Carcinoma | | | 1 (2%) | |
| Pituitary gland | (47) | (49) | (49) | (49) |
| Pars distalis, adenoma | 2 (4%) | 4 (8%) | | 1 (2%) |
| Pars intermedia, adenoma | 1 (2%) | | 2 (4%) | |
| Thyroid Gland | (50) | (50) | (50) | (50) |
| Follicular cell, adenoma | 3 (6%) | | | |
| General Body System | | | | |
| Tissue NOS | | | | (2) |
| Fibrosarcoma | | | | 1 (50%) |
| Schwannoma malignant | | | | 1 (50%) |
| Genital System | | | | |
| Ovary | (49) | (47) | (47) | (49) |
| Cystadenoma | | 2 (4%) | 1 (2%) | |
| Hemangioma | | | 1 (2%) | |
| Histiocytic sarcoma | | | | 1 (2%) |
| Uterus | (50) | (50) | (50) | (50) |
| Hemangioma | 1 (2%) | | | |
| Histiocytic sarcoma | | | 1 (2%) | |
| Leiomyoma | | | | 1 (2%) |
| Polyp stromal | 1 (2%) | 1 (2%) | | |
| Sarcoma stromal | | | | 1 (2%) |
| Cervix, carcinoma | | | 1 (2%) | |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|--------|---------|----------|------------|
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hemangiosarcoma | | 1 (2%) | | |
| Lymph node | (12) | (13) | (11) | (8) |
| Fibrosarcoma, metastatic, skin | | 1 (8%) | | |
| Iliac, osteosarcoma, metastatic, bone | | | | 1 (13%) |
| Iliac, sarcoma, metastatic, skin | 1 (8%) | 1 (8%) | | |
| Inguinal, fibrosarcoma, metastatic, skin | | 1 (8%) | | |
| Mediastinal, neurofibrosarcoma, metastatic, skin | | 1 (8%) | | |
| Mediastinal, sarcoma, metastatic, skin | | | | 1 (13%) |
| Pancreatic, carcinoma, metastatic, pancreas | | | | 1 (13%) |
| Pancreatic, leiomyosarcoma, metastatic, mesentery | | | 1 (9%) | |
| Renal, leiomyosarcoma, metastatic, mesentery | | | 1 (9%) | |
| Lymph node, mandibular | (48) | (49) | (47) | (50) |
| Histiocytic sarcoma | | 1 (2%) | | 1 (2%) |
| Lymph node, mesenteric | (49) | (49) | (48) | (45) |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | | | |
| Histiocytic sarcoma | | 1 (2%) | | 1 (2%) |
| Spleen | (50) | (50) | (50) | (48) |
| Histiocytic sarcoma | | 1 (2%) | | 1 (2%) |
| Thymus | (44) | (47) | (47) | (48) |
| Hepatocellular carcinoma, metastatic, liver | 1 (2%) | | | |
| Histiocytic sarcoma | | | | 1 (2%) |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Carcinoma | | 1 (2%) | 1 (2%) | |
| Fibroadenoma | | 1 (2%) | | |
| Skin | (50) | (50) | (50) | (50) |
| Basal cell carcinoma | | | | 1 (2%) |
| Squamous cell carcinoma | | 1 (2%) | | |
| Subcutaneous tissue, fibrosarcoma | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Subcutaneous tissue, fibrous histiocytoma | 3 (6%) | | | |
| Subcutaneous tissue, hemangioma | | 1 (2%) | | 1 (2%) |
| Subcutaneous tissue, hemangiopericytoma | | 1 (2%) | | |
| Subcutaneous tissue, hemangiosarcoma | | 1 (2%) | | |
| Subcutaneous tissue, neurofibroma | | | | 1 (2%) |
| Subcutaneous tissue, neurofibrosarcoma | | 1 (2%) | | |
| Subcutaneous tissue, osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Subcutaneous tissue, sarcoma | 1 (2%) | 1 (2%) | 1 (2%) | 4 (8%) |
| Subcutaneous tissue, sarcoma, metastatic, tissue NOS | 1 (2%) | | | |
| Subcutaneous tissue, schwannoma malignant | | 1 (2%) | | 1 (2%) |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Osteosarcoma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Skeletal muscle | (3) | (4) | (2) | (5) |
| Carcinoma, metastatic, harderian gland | | 1 (25%) | | |
| Fibrosarcoma, metastatic, skin | | | 1 (50%) | |
| Hemangiosarcoma | | | | 1 (20%) |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|----------|----------|------------|
| Musculoskeletal System (continued) | | | | |
| Skeletal muscle (continued) | (3) | (4) | (2) | (5) |
| Hepatocellular carcinoma, metastatic, liver | 1 (33%) | | | |
| Rhabdomyosarcoma | | | | 1 (20%) |
| Sarcoma | 1 (33%) | 1 (25%) | | 2 (40%) |
| Sarcoma, metastatic, pancreas | | | 1 (50%) | |
| Sarcoma, metastatic, skin | 1 (33%) | | | |
| Schwannoma malignant, metastatic, skin | | 1 (25%) | | |
| Nervous System | | | | |
| None | | | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Alveolar/bronchiolar adenoma | 1 (2%) | 3 (6%) | 3 (6%) | 6 (12%) |
| Alveolar/bronchiolar carcinoma | 1 (2%) | 2 (4%) | 2 (4%) | 2 (4%) |
| Carcinoma, metastatic, harderian gland | | 1 (2%) | | |
| Carcinoma, metastatic, uterus | | | 1 (2%) | |
| Fibrosarcoma, metastatic, skin | 1 (2%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Hepatocellular carcinoma, metastatic, liver | 3 (6%) | 1 (2%) | 3 (6%) | 4 (8%) |
| Histiocytic sarcoma | | 1 (2%) | | |
| Neurofibrosarcoma | | 1 (2%) | | |
| Osteosarcoma, metastatic, bone | | | | 1 (2%) |
| Sarcoma, metastatic, skin | 1 (2%) | 1 (2%) | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, harderian gland | | 1 (2%) | | |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Carcinoma, metastatic, harderian gland | | 1 (2%) | | |
| Harderian gland | (49) | (50) | (50) | (50) |
| Adenoma | 9 (18%) | 4 (8%) | 6 (12%) | 8 (16%) |
| Adenoma, multiple | | | 2 (4%) | |
| Carcinoma | 1 (2%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Urinary System | | | | |
| Kidney | (49) | (50) | (50) | (49) |
| Urinary bladder | (50) | (49) | (50) | (49) |
| Fibrosarcoma, metastatic, tissue NOS | | | | 1 (2%) |
| Leiomyosarcoma, metastatic, mesentery | | | 1 (2%) | |
| Systemic Lesions | | | | |
| Multiple organs ^b | (50) | (50) | (50) | (50) |
| Histiocytic sarcoma | | 1 (2%) | 1 (2%) | 1 (2%) |
| Lymphoma malignant | 27 (54%) | 24 (48%) | 17 (34%) | 14 (28%) |

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|--------|---------|----------|------------|
| Neoplasm Summary | | | | |
| Total animals with primary neoplasms ^c | 42 | 43 | 46 | 48 |
| Total primary neoplasms | 88 | 93 | 102 | 104 |
| Total animals with benign neoplasms | 29 | 33 | 41 | 38 |
| Total benign neoplasms | 43 | 46 | 54 | 56 |
| Total animals with malignant neoplasms | 36 | 37 | 34 | 31 |
| Total malignant neoplasms | 45 | 47 | 48 | 48 |
| Total animals with metastatic neoplasms | 5 | 7 | 7 | 10 |
| Total metastatic neoplasms | 12 | 20 | 13 | 13 |

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

^b Number of animals with any tissue examined microscopically

^c Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid: 0 mg/L

| Number of Days on Study | 7 | |
|---|---|----------------------|
| | 3 | |
| | 5 5 5 5 5 5 5 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 | |
| Carcass ID Number | 2 | Total Tissues/Tumors |
| | 2 2 2 2 4 4 4 3 3 4 0 0 0 0 0 0 0 0 2 2 2 3 4 4 4 4 | |
| | 1 3 4 5 6 7 8 7 8 0 1 2 5 6 7 8 9 6 7 9 0 1 2 4 5 | |
| Nervous System | | |
| Brain | + | 50 |
| Respiratory System | | |
| Lung | + | 50 |
| Alveolar/bronchiolar adenoma | | 1 |
| Alveolar/bronchiolar carcinoma | | 1 |
| Fibrosarcoma, metastatic, skin | | 1 |
| Hepatocellular carcinoma, metastatic, liver | | 3 |
| Sarcoma, metastatic, skin | | 1 |
| Nose | + | 50 |
| Trachea | + | 50 |
| Special Senses System | | |
| Eye | + | 50 |
| Harderian gland | + | 49 |
| Adenoma | | 9 |
| Carcinoma | | 1 |
| Urinary System | | |
| Kidney | + | 49 |
| Urinary bladder | + | 50 |
| Systemic Lesions | | |
| Multiple organs | + | 50 |
| Lymphoma malignant | X | 27 |

TABLE D3

Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|--------------------------|-------------|--------------------------|-------------|
| Harderian Gland: Adenoma | | | | |
| Overall rate ^a | 9/50 (18%) | 4/50 (8%) | 8/50 (16%) | 8/50 (16%) |
| Adjusted rate ^b | 19.3% | 8.6% | 17.8% | 17.6% |
| Terminal rate ^c | 6/38 (16%) | 2/35 (6%) | 5/32 (16%) | 5/32 (16%) |
| First incidence (days) | 591 | 487 | 622 | 688 |
| Poly-3 test ^d | P=0.334 | P=0.116N | P=0.532N | P=0.525N |
| Harderian Gland: Adenoma or Carcinoma | | | | |
| Overall rate | 10/50 (20%) | 5/50 (10%) | 10/50 (20%) | 9/50 (18%) |
| Adjusted rate | 21.4% | 10.7% | 22.1% | 19.7% |
| Terminal rate | 7/38 (18%) | 2/35 (6%) | 6/32 (19%) | 5/32 (16%) |
| First incidence (days) | 591 | 487 | 622 | 659 |
| Poly-3 test | P=0.324 | P=0.129N | P=0.568 | P=0.521N |
| Liver: Hepatocellular Adenoma | | | | |
| Overall rate | 19/49 (39%) | 26/50 (52%) | 32/50 (64%) | 35/49 (71%) |
| Adjusted rate | 41.4% | 56.8% | 69.7% | 75.9% |
| Terminal rate | 18/38 (47%) | 22/35 (63%) | 26/32 (81%) | 28/32 (88%) |
| First incidence (days) | 653 | 694 | 573 | 636 |
| Poly-3 test | P<0.001 | P=0.098 | P=0.004 | P<0.001 |
| Liver: Hepatocellular Carcinoma | | | | |
| Overall rate | 3/49 (6%) | 3/50 (6%) | 12/50 (24%) | 8/49 (16%) |
| Adjusted rate | 6.5% | 6.6% | 26.7% | 17.7% |
| Terminal rate | 1/38 (3%) | 2/35 (6%) | 9/32 (28%) | 7/32 (22%) |
| First incidence (days) | 658 | 694 | 617 | 671 |
| Poly-3 test | P=0.019 | P=0.659 | P=0.009 | P=0.094 |
| Liver: Hepatocellular Adenoma or Carcinoma | | | | |
| Overall rate | 22/49 (45%) ^e | 28/50 (56%) | 37/50 (74%) ^e | 37/49 (76%) |
| Adjusted rate | 47.6% | 61.2% | 79.5% | 79.8% |
| Terminal rate | 19/38 (50%) | 24/35 (69%) | 28/32 (88%) | 29/32 (91%) |
| First incidence (days) | 653 | 694 | 573 | 636 |
| Poly-3 test | P<0.001 | P=0.131 | P<0.001 | P<0.001 |
| Liver: Hepatocellular Carcinoma or Hepatoblastoma | | | | |
| Overall rate | 4/49 (8%) | 3/50 (6%) | 13/50 (26%) | 9/49 (18%) |
| Adjusted rate | 8.7% | 6.6% | 28.8% | 19.9% |
| Terminal rate | 2/38 (5%) | 2/35 (6%) | 9/32 (28%) | 8/32 (25%) |
| First incidence (days) | 658 | 694 | 617 | 671 |
| Poly-3 test | P=0.015 | P=0.504N | P=0.012 | P=0.109 |
| Lung: Alveolar/bronchiolar Adenoma | | | | |
| Overall rate | 1/50 (2%) | 3/50 (6%) | 3/50 (6%) | 6/50 (12%) |
| Adjusted rate | 2.2% | 6.5% | 6.7% | 13.2% |
| Terminal rate | 1/38 (3%) | 2/35 (6%) | 1/32 (3%) | 4/32 (13%) |
| First incidence (days) | 729 (T) | 490 | 553 | 636 |
| Poly-3 test | P=0.044 | P=0.307 | P=0.297 | P=0.054 |
| Lung: Alveolar/bronchiolar Adenoma or Carcinoma | | | | |
| Overall rate | 2/50 (4%) | 5/50 (10%) | 5/50 (10%) | 7/50 (14%) |
| Adjusted rate | 4.4% | 10.8% | 11.0% | 15.4% |
| Terminal rate | 2/38 (5%) | 3/35 (9%) | 2/32 (6%) | 5/32 (16%) |
| First incidence (days) | 729 (T) | 490 | 553 | 636 |
| Poly-3 test | P=0.095 | P=0.219 | P=0.210 | P=0.076 |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|-----------|----------------|-----------|------------|
| Pancreatic Islets: Adenoma or Carcinoma | | | | |
| Overall rate | 1/49 (2%) | 0/49 (0%) | 3/49 (6%) | 2/46 (4%) |
| Adjusted rate | 2.2% | 0.0% | 6.7% | 4.7% |
| Terminal rate | 1/38 (3%) | 0/35 (0%) | 1/32 (3%) | 2/32 (6%) |
| First incidence (days) | 729 (T) | — ^f | 553 | 729 (T) |
| Poly-3 test | P=0.158 | P=0.503N | P=0.300 | P=0.478 |
| Pituitary Gland (Pars Distalis): Adenoma | | | | |
| Overall rate | 2/47 (4%) | 4/49 (8%) | 0/49 (0%) | 1/49 (2%) |
| Adjusted rate | 4.6% | 9.0% | 0.0% | 2.3% |
| Terminal rate | 1/36 (3%) | 3/34 (9%) | 0/31 (0%) | 1/31 (3%) |
| First incidence (days) | 720 | 694 | — | 729 (T) |
| Poly-3 test | P=0.114N | P=0.347 | P=0.239N | P=0.496N |
| Skin (Subcutaneous Tissue): Fibrous Histiocytoma | | | | |
| Overall rate | 3/50 (6%) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 6.5% | 0.0% | 0.0% | 0.0% |
| Terminal rate | 3/38 (8%) | 0/35 (0%) | 0/32 (0%) | 0/32 (0%) |
| First incidence (days) | 729 (T) | — | — | — |
| Poly-3 test | P=0.092N | P=0.121N | P=0.125N | P=0.122N |
| Skin (Subcutaneous Tissue): Sarcoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 1/50 (2%) | 4/50 (8%) |
| Adjusted rate | 2.2% | 2.2% | 2.2% | 8.8% |
| Terminal rate | 1/38 (3%) | 0/35 (0%) | 0/32 (0%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 710 | 659 | 682 |
| Poly-3 test | P=0.063 | P=0.759 | P=0.754 | P=0.175 |
| Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma | | | | |
| Overall rate | 4/50 (8%) | 3/50 (6%) | 3/50 (6%) | 6/50 (12%) |
| Adjusted rate | 8.7% | 6.5% | 6.7% | 13.2% |
| Terminal rate | 3/38 (8%) | 0/35 (0%) | 1/32 (3%) | 2/32 (6%) |
| First incidence (days) | 710 | 490 | 573 | 682 |
| Poly-3 test | P=0.210 | P=0.497N | P=0.511N | P=0.361 |
| Stomach (Forestomach): Squamous Cell Papilloma or Carcinoma | | | | |
| Overall rate | 1/50 (2%) | 1/50 (2%) | 3/50 (6%) | 1/50 (2%) |
| Adjusted rate | 2.2% | 2.2% | 6.8% | 2.2% |
| Terminal rate | 1/38 (3%) | 1/35 (3%) | 2/32 (6%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 729 (T) | 718 | 729 (T) |
| Poly-3 test | P=0.503 | P=0.758 | P=0.293 | P=0.757 |
| Thyroid Gland (Follicular Cell): Adenoma | | | | |
| Overall rate | 3/50 (6%) | 0/50 (0%) | 0/50 (0%) | 0/50 (0%) |
| Adjusted rate | 6.5% | 0.0% | 0.0% | 0.0% |
| Terminal rate | 2/38 (5%) | 0/35 (0%) | 0/32 (0%) | 0/32 (0%) |
| First incidence (days) | 714 | — | — | — |
| Poly-3 test | P=0.091N | P=0.121N | P=0.126N | P=0.122N |
| All Organs: Hemangioma | | | | |
| Overall rate | 3/50 (6%) | 1/50 (2%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate | 6.5% | 2.2% | 4.5% | 2.2% |
| Terminal rate | 3/38 (8%) | 1/35 (3%) | 1/32 (3%) | 1/32 (3%) |
| First incidence (days) | 729 (T) | 729 (T) | 659 | 729 (T) |
| Poly-3 test | P=0.353N | P=0.309N | P=0.514N | P=0.312N |

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|-------------|-------------|-------------|-------------|
| All Organs: Hemangioma or Hemangiosarcoma | | | | |
| Overall rate | 4/50 (8%) | 3/50 (6%) | 4/50 (8%) | 2/50 (4%) |
| Adjusted rate | 8.7% | 6.6% | 8.9% | 4.4% |
| Terminal rate | 3/38 (8%) | 3/35 (9%) | 1/32 (3%) | 1/32 (3%) |
| First incidence (days) | 653 | 729 (T) | 617 | 629 |
| Poly-3 test | P=0.331N | P=0.509N | P=0.629 | P=0.345N |
| All Organs: Malignant Lymphoma | | | | |
| Overall rate | 27/50 (54%) | 24/50 (48%) | 17/50 (34%) | 14/50 (28%) |
| Adjusted rate | 57.8% | 51.7% | 37.5% | 30.3% |
| Terminal rate | 25/38 (66%) | 21/35 (60%) | 12/32 (38%) | 11/32 (34%) |
| First incidence (days) | 474 | 490 | 618 | 430 |
| Poly-3 test | P=0.002N | P=0.348N | P=0.036N | P=0.005N |
| All Organs: Benign Neoplasms | | | | |
| Overall rate | 29/50 (58%) | 33/50 (66%) | 41/50 (82%) | 38/50 (76%) |
| Adjusted rate | 60.9% | 69.0% | 85.5% | 82.2% |
| Terminal rate | 23/38 (61%) | 25/35 (71%) | 28/32 (88%) | 30/32 (94%) |
| First incidence (days) | 563 | 487 | 553 | 636 |
| Poly-3 test | P=0.005 | P=0.267 | P=0.004 | P=0.015 |
| All Organs: Malignant Neoplasms | | | | |
| Overall rate | 36/50 (72%) | 37/50 (74%) | 34/50 (68%) | 32/50 (64%) |
| Adjusted rate | 74.6% | 76.9% | 70.8% | 66.0% |
| Terminal rate | 29/38 (76%) | 27/35 (77%) | 19/32 (59%) | 18/32 (56%) |
| First incidence (days) | 474 | 383 | 573 | 430 |
| Poly-3 test | P=0.124N | P=0.491 | P=0.421N | P=0.240N |
| All Organs: Benign or Malignant Neoplasms | | | | |
| Overall rate | 42/50 (84%) | 43/50 (86%) | 46/50 (92%) | 48/50 (96%) |
| Adjusted rate | 85.1% | 88.0% | 94.6% | 97.9% |
| Terminal rate | 31/38 (82%) | 31/35 (89%) | 30/32 (94%) | 31/32 (97%) |
| First incidence (days) | 474 | 383 | 553 | 430 |
| Poly-3 test | P=0.008 | P=0.452 | P=0.103 | P=0.025 |

(T) Terminal sacrifice

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

^b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

^c Observed incidence at terminal kill

^d Beneath the control incidence is the P value associated with the trend test. Beneath the exposed 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 One hepatoblastoma occurred in an animal that also had a hepatocellular adenoma

^f Not applicable; no neoplasms in animal group

TABLE D4a
Historical Incidence of Hepatocellular Neoplasms in Control Female B6C3F₁ Mice^a

| Study | Incidence in Controls | | |
|---|-----------------------|------------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence: Drinking Water Studies | | | |
| Bromodichloromethane | 24/50 | 13/50 | 30/50 |
| Dibromoacetic acid | 19/49 | 3/49 | 22/49 |
| Dipropylene glycol | 11/50 | 7/50 | 17/50 |
| Sodium chlorate | 30/49 | 3/49 | 31/49 |
| Sodium nitrite | 9/50 | 2/50 | 10/50 |
| Overall Historical Incidence: Drinking Water Studies | | | |
| Total (%) | 93/248 (37.5%) | 28/248 (11.3%) | 110/248 (44.4%) |
| Mean ± standard deviation | 37.6% ± 18.0% | 11.3% ± 9.1% | 44.4% ± 18.1% |
| Range | 18%-61% | 4%-26% | 20%-63% |
| Overall Historical Incidence | | | |
| Total (%) | 312/1,549 (20.1%) | 128/1,549 (8.3%) | 408/1,549 (26.3%) |
| Mean ± standard deviation | 21.2% ± 13.4% | 8.7% ± 5.8% | 27.7% ± 15.5% |
| Range | 6%-61% | 0%-26% | 8%-63% |

^a Data as of January 26, 2005

TABLE D4b
Historical Incidence of Alveolar/bronchiolar Neoplasms in Control Female B6C3F₁ Mice^a

| Study | Incidence in Controls | | |
|---|-----------------------|-----------------|----------------------|
| | Adenoma | Carcinoma | Adenoma or Carcinoma |
| Historical Incidence: Drinking Water Studies | | | |
| Bromodichloromethane | 6/50 | 1/50 | 6/50 |
| Dibromoacetic acid | 1/50 | 1/50 | 2/50 |
| Dipropylene glycol | 2/50 | 2/50 | 3/50 |
| Sodium chlorate | 3/50 | 1/50 | 4/50 |
| Sodium nitrite | 1/50 | 0/50 | 1/50 |
| Overall Historical Incidence: Drinking Water Studies | | | |
| Total (%) | 13/250 (5.2%) | 5/250 (2.0%) | 16/250 (6.4%) |
| Mean ± standard deviation | 5.2% ± 4.2% | 2.0% ± 1.4% | 6.4% ± 3.9% |
| Range | 2%-12% | 0%-4% | 2%-12% |
| Overall Historical Incidence | | | |
| Total (%) | 80/1,552 (5.2%) | 40/1,552 (2.6%) | 117/1,552 (7.5%) |
| Mean ± standard deviation | 5.1% ± 3.5% | 2.5% ± 2.6% | 7.4% ± 3.8% |
| Range | 0%-12% | 0%-12% | 0%-14% |

^a Data as of January 26, 2005

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---------------------------------------|----------|----------|----------|------------|
| Disposition Summary | | | | |
| Animals initially in study | 50 | 50 | 50 | 50 |
| Early deaths | | | | |
| Moribund | 2 | 2 | 5 | 7 |
| Natural deaths | 10 | 13 | 13 | 11 |
| Survivors | | | | |
| Died last week of study | | | 1 | |
| Terminal sacrifice | 38 | 35 | 31 | 32 |
| Animals examined microscopically | 50 | 50 | 50 | 50 |
| Alimentary System | | | | |
| Intestine large, rectum | (49) | (49) | (48) | (49) |
| Ulcer | | | 1 (2%) | |
| Intestine large, cecum | (47) | (46) | (49) | (46) |
| Edema | 2 (4%) | 1 (2%) | | 3 (7%) |
| Inflammation, chronic | | 1 (2%) | | 1 (2%) |
| Epithelium, hyperplasia | | | 1 (2%) | |
| Intestine small, duodenum | (46) | (48) | (48) | (45) |
| Epithelium, hyperplasia | | 1 (2%) | | |
| Intestine small, jejunum | (46) | (47) | (49) | (43) |
| Hyperplasia, lymphoid | | 2 (4%) | | 2 (5%) |
| Intestine small, ileum | (45) | (47) | (47) | (46) |
| Inflammation, chronic | | 1 (2%) | | |
| Liver | (49) | (50) | (50) | (49) |
| Angiectasis | 1 (2%) | 2 (4%) | | |
| Basophilic focus | | 1 (2%) | 3 (6%) | 3 (6%) |
| Clear cell focus | 3 (6%) | 1 (2%) | 8 (16%) | 5 (10%) |
| Eosinophilic focus | 7 (14%) | 6 (12%) | 10 (20%) | 9 (18%) |
| Hematopoietic cell proliferation | 2 (4%) | 4 (8%) | 2 (4%) | 2 (4%) |
| Hepatodiaphragmatic nodule | | | | 2 (4%) |
| Hyperplasia, lymphoid | 2 (4%) | 1 (2%) | | |
| Infarct | | | | 1 (2%) |
| Infiltration cellular, mixed cell | 7 (14%) | 7 (14%) | 4 (8%) | 9 (18%) |
| Inflammation, chronic active | | | 1 (2%) | |
| Mixed cell focus | 2 (4%) | 6 (12%) | 3 (6%) | 1 (2%) |
| Necrosis, focal | 3 (6%) | 2 (4%) | 3 (6%) | 2 (4%) |
| Tension lipidosis | 1 (2%) | 3 (6%) | | |
| Centrilobular, necrosis | | 1 (2%) | 4 (8%) | 4 (8%) |
| Hepatocyte, vacuolization cytoplasmic | 2 (4%) | 7 (14%) | 4 (8%) | 2 (4%) |
| Kupffer cell, pigmentation | 3 (6%) | | | 3 (6%) |
| Mesentery | (28) | (20) | (16) | (19) |
| Fat, necrosis | 22 (79%) | 18 (90%) | 11 (69%) | 13 (68%) |
| Pancreas | (49) | (49) | (49) | (47) |
| Atrophy | | 1 (2%) | 1 (2%) | 3 (6%) |
| Cyst | | | 1 (2%) | 3 (6%) |
| Inflammation, chronic | | | | 1 (2%) |
| Acinus, cytoplasmic alteration | 1 (2%) | | | |
| Salivary glands | (50) | (49) | (50) | (50) |
| Hyperplasia, lymphoid | 15 (30%) | 13 (27%) | 16 (32%) | 10 (20%) |

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

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Alimentary System (continued) | | | | |
| Stomach, forestomach | (49) | (50) | (50) | (49) |
| Erosion | | 1 (2%) | | |
| Inflammation, chronic | 1 (2%) | 2 (4%) | 2 (4%) | 1 (2%) |
| Ulcer | 1 (2%) | 1 (2%) | 1 (2%) | 3 (6%) |
| Epithelium, hyperplasia | 4 (8%) | 5 (10%) | | 4 (8%) |
| Stomach, glandular | (49) | (50) | (50) | (49) |
| Erosion | | 1 (2%) | | 1 (2%) |
| Pylorus, epithelium, hyperplasia, atypical | | 1 (2%) | | |
| Tooth | | | (1) | |
| Malformation | | | 1 (100%) | |
| Cardiovascular System | | | | |
| Blood vessel | (1) | (1) | (1) | |
| Hypertrophy | | | 1 (100%) | |
| Inflammation, chronic active | | | 1 (100%) | |
| Heart | (50) | (49) | (50) | (50) |
| Cardiomyopathy | | | 1 (2%) | 1 (2%) |
| Inflammation, chronic | | 1 (2%) | | 1 (2%) |
| Inflammation, chronic active | | | | 1 (2%) |
| Mineralization | | 1 (2%) | 1 (2%) | 2 (4%) |
| Endocrine System | | | | |
| Adrenal cortex | (50) | (50) | (50) | (49) |
| Accessory adrenal cortical nodule | 9 (18%) | 11 (22%) | 6 (12%) | 9 (18%) |
| Hyperplasia, focal | | 2 (4%) | | 1 (2%) |
| Hypertrophy, focal | | 1 (2%) | | |
| Capsule, hyperplasia | | 1 (2%) | | 1 (2%) |
| Adrenal medulla | (50) | (50) | (50) | (49) |
| Hyperplasia | | 2 (4%) | 1 (2%) | 1 (2%) |
| Islets, pancreatic | (49) | (49) | (49) | (46) |
| Hyperplasia | 2 (4%) | 5 (10%) | 3 (6%) | 3 (7%) |
| Parathyroid gland | (45) | (49) | (47) | (49) |
| Cyst | 2 (4%) | 1 (2%) | 1 (2%) | 3 (6%) |
| Pituitary gland | (47) | (49) | (49) | (49) |
| Pars distalis, angiectasis | 2 (4%) | 3 (6%) | 1 (2%) | 1 (2%) |
| Pars distalis, cyst | 2 (4%) | 2 (4%) | 1 (2%) | 1 (2%) |
| Pars distalis, hyperplasia, focal | 4 (9%) | 9 (18%) | 4 (8%) | 7 (14%) |
| Pars intermedia, hyperplasia, focal | 1 (2%) | | | |
| Thyroid gland | (50) | (50) | (50) | (50) |
| Degeneration, cystic | 10 (20%) | 26 (52%) | 16 (32%) | 14 (28%) |
| Follicular cell, hyperplasia | 8 (16%) | 1 (2%) | 1 (2%) | 2 (4%) |
| General Body System | | | | |
| None | | | | |

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|----------|----------|----------|------------|
| Genital System | | | | |
| Clitoral gland | (50) | (48) | (48) | (49) |
| Cyst | | 1 (2%) | | |
| Ovary | (49) | (47) | (47) | (49) |
| Angiectasis | 5 (10%) | 3 (6%) | 9 (19%) | 7 (14%) |
| Cyst | 14 (29%) | 12 (26%) | 14 (30%) | 22 (45%) |
| Corpus luteum, hyperplasia | 1 (2%) | 1 (2%) | | |
| Granulosa cell, hyperplasia | 1 (2%) | | | |
| Interstitial cell, hyperplasia | | | | 1 (2%) |
| Uterus | (50) | (50) | (50) | (50) |
| Angiectasis | | 4 (8%) | 1 (2%) | 2 (4%) |
| Hyperplasia, cystic | 44 (88%) | 42 (84%) | 44 (88%) | 39 (78%) |
| Inflammation, chronic | 1 (2%) | 1 (2%) | 1 (2%) | |
| Metaplasia, squamous | 1 (2%) | | 1 (2%) | 1 (2%) |
| Hematopoietic System | | | | |
| Bone marrow | (50) | (50) | (50) | (50) |
| Hyperplasia | 20 (40%) | 26 (52%) | 24 (48%) | 20 (40%) |
| Infiltration cellular, mast cell | | 1 (2%) | | |
| Lymph node | (12) | (13) | (11) | (8) |
| Hyperplasia, lymphoid | | 1 (8%) | | |
| Bronchial, hemorrhage | | 1 (8%) | | |
| Bronchial, hyperplasia, lymphoid | | 1 (8%) | | |
| Deep cervical, hemorrhage | | | | 1 (13%) |
| Iliac, hematopoietic cell proliferation | | 1 (8%) | | |
| Iliac, hemorrhage | 1 (8%) | | | 1 (13%) |
| Iliac, hyperplasia, lymphoid | 1 (8%) | 1 (8%) | 1 (9%) | 2 (25%) |
| Iliac, pigmentation | 1 (8%) | | | |
| Inguinal, hematopoietic cell proliferation | | 1 (8%) | | |
| Inguinal, hyperplasia, lymphoid | | 2 (15%) | | |
| Mediastinal, hemorrhage | 1 (8%) | | | |
| Mediastinal, hyperplasia, lymphoid | 1 (8%) | 1 (8%) | 1 (9%) | 1 (13%) |
| Mediastinal, pigmentation | 1 (8%) | | | |
| Pancreatic, hyperplasia, lymphoid | | | 1 (9%) | |
| Renal, hyperplasia, lymphoid | 1 (8%) | | 1 (9%) | 1 (13%) |
| Lymph node, mandibular | (48) | (49) | (47) | (50) |
| Atrophy | | 1 (2%) | 1 (2%) | 1 (2%) |
| Hematopoietic cell proliferation | 1 (2%) | 1 (2%) | | 2 (4%) |
| Hemorrhage | | 2 (4%) | 2 (4%) | 1 (2%) |
| Hyperplasia, lymphoid | 13 (27%) | 6 (12%) | 8 (17%) | 9 (18%) |
| Pigmentation | 18 (38%) | 25 (51%) | 17 (36%) | 23 (46%) |
| Lymph node, mesenteric | (49) | (49) | (48) | (45) |
| Angiectasis | | | 1 (2%) | |
| Atrophy | | | 2 (4%) | 1 (2%) |
| Ectasia | | | 1 (2%) | |
| Hematopoietic cell proliferation | 2 (4%) | | 2 (4%) | 1 (2%) |
| Hemorrhage | 1 (2%) | 1 (2%) | 1 (2%) | 5 (11%) |
| Hyperplasia, lymphoid | 8 (16%) | 9 (18%) | 7 (15%) | 5 (11%) |
| Pigmentation | | | 1 (2%) | 1 (2%) |
| Spleen | (50) | (50) | (50) | (48) |
| Hematopoietic cell proliferation | 34 (68%) | 42 (84%) | 35 (70%) | 34 (71%) |
| Necrosis | 1 (2%) | | | |
| Pigmentation | 5 (10%) | 5 (10%) | 1 (2%) | 2 (4%) |
| Lymphoid follicle, atrophy | | 1 (2%) | 2 (4%) | 2 (4%) |
| Lymphoid follicle, hyperplasia | 8 (16%) | 11 (22%) | 14 (28%) | 12 (25%) |

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|--|---------|----------|----------|------------|
| Hematopoietic System (continued) | | | | |
| Thymus | (44) | (47) | (47) | (48) |
| Atrophy | 8 (18%) | 9 (19%) | 11 (23%) | 13 (27%) |
| Hemorrhage | 1 (2%) | | | |
| Hyperplasia, lymphoid | | 3 (6%) | 3 (6%) | 2 (4%) |
| Integumentary System | | | | |
| Mammary gland | (50) | (50) | (50) | (50) |
| Hyperplasia | 5 (10%) | 6 (12%) | 2 (4%) | 5 (10%) |
| Skin | (50) | (50) | (50) | (50) |
| Edema | | 1 (2%) | 1 (2%) | 1 (2%) |
| Erosion | | | 1 (2%) | |
| Ulcer | 2 (4%) | 1 (2%) | 1 (2%) | 1 (2%) |
| Epidermis, hyperplasia | 2 (4%) | 1 (2%) | 2 (4%) | 1 (2%) |
| Musculoskeletal System | | | | |
| Bone | (50) | (50) | (50) | (50) |
| Myelofibrosis | 1 (2%) | 2 (4%) | 1 (2%) | 2 (4%) |
| Skeletal muscle | (3) | (4) | (2) | (5) |
| Inflammation, chronic active | | | | 1 (20%) |
| Nervous System | | | | |
| Brain | (50) | (49) | (50) | (50) |
| Compression | 1 (2%) | 1 (2%) | 1 (2%) | |
| Hemorrhage | | 2 (4%) | | |
| Necrosis | | 1 (2%) | 1 (2%) | |
| Peripheral nerve | | (1) | (1) | (1) |
| Atrophy | | | | 1 (100%) |
| Spinal cord | | (1) | (1) | (1) |
| Necrosis | | 1 (100%) | | |
| Respiratory System | | | | |
| Lung | (50) | (50) | (50) | (50) |
| Congestion | | | 2 (4%) | |
| Hemorrhage | | 2 (4%) | 2 (4%) | 3 (6%) |
| Hyperplasia, lymphoid | 5 (10%) | 5 (10%) | 1 (2%) | 3 (6%) |
| Infiltration cellular, histiocyte | 1 (2%) | 2 (4%) | 2 (4%) | 4 (8%) |
| Alveolar epithelium, hyperplasia | 3 (6%) | 3 (6%) | | 4 (8%) |
| Perivascular, inflammation, chronic active | | | | 1 (2%) |
| Nose | (50) | (50) | (50) | (50) |
| Inflammation, chronic | | | | 1 (2%) |
| Special Senses System | | | | |
| Eye | (50) | (50) | (50) | (50) |
| Atrophy | | | 1 (2%) | |
| Cataract | | | 2 (4%) | 2 (4%) |
| Inflammation, chronic | 2 (4%) | 2 (4%) | 3 (6%) | 3 (6%) |
| Cornea, hyperplasia | 1 (2%) | | 4 (8%) | |
| Cornea, mineralization | | | | 1 (2%) |
| Harderian gland | (49) | (50) | (50) | (50) |
| Hyperplasia, focal | 2 (4%) | 4 (8%) | 4 (8%) | 2 (4%) |

TABLE D5
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| | 0 mg/L | 50 mg/L | 500 mg/L | 1,000 mg/L |
|---|----------|---------|----------|------------|
| Urinary System | | | | |
| Kidney | (49) | (50) | (50) | (49) |
| Cyst | | 1 (2%) | | 1 (2%) |
| Hydronephrosis | | | 1 (2%) | 1 (2%) |
| Hyperplasia, lymphoid | 7 (14%) | 2 (4%) | 5 (10%) | 8 (16%) |
| Infarct | 3 (6%) | 8 (16%) | 6 (12%) | 8 (16%) |
| Nephropathy | 14 (29%) | 8 (16%) | 15 (30%) | 12 (24%) |
| Renal tubule, accumulation, hyaline droplet | 1 (2%) | 1 (2%) | 2 (4%) | 2 (4%) |
| Renal tubule, dilatation | | 2 (4%) | 1 (2%) | 1 (2%) |
| Renal tubule, necrosis | | 1 (2%) | | 1 (2%) |
| Renal tubule, pigmentation | 1 (2%) | | 3 (6%) | 1 (2%) |
| Urinary bladder | (50) | (49) | (50) | (49) |
| Edema | 2 (4%) | | | |
| Hyperplasia, lymphoid | 3 (6%) | 5 (10%) | 3 (6%) | 2 (4%) |
| Transitional epithelium, hyperplasia | | | 1 (2%) | |

APPENDIX E

GENETIC TOXICOLOGY

| | |
|--|-----|
| <i>SALMONELLA TYPHIMURIUM</i> MUTAGENICITY TEST PROTOCOL | E-2 |
| MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL | E-2 |
| EVALUATION PROTOCOL | E-3 |
| RESULTS | E-3 |
| TABLE E1 Mutagenicity of Dibromoacetic Acid in <i>Salmonella typhimurium</i> | E-4 |
| TABLE E2 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Dibromoacetic Acid in Drinking Water for 3 Months | E-5 |

GENETIC TOXICOLOGY

***SALMONELLA TYPHIMURIUM* MUTAGENICITY TEST PROTOCOL**

Testing was performed as reported by Zeiger *et al.* (1992). Dibromoacetic acid was sent to the laboratory as a coded aliquot from Research Triangle Institute (Research Triangle Park, NC). It was incubated with the *Salmonella typhimurium* tester strains TA98 and TA100 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 five doses of dibromoacetic acid. In the absence of toxicity, 10,000 µg/plate was selected as the high dose. All positive trials were repeated under the conditions that elicited the positive response.

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

MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the termination of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of up to 10 animals per exposure group. In addition, the percentage of polychromatic erythrocytes (PCEs) in a population of 1,000 erythrocytes was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposed 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 exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results from 3-month studies were accepted without repeat tests because additional test data could not be obtained. 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.

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

Dibromoacetic acid, tested over a concentration range of 33 to 10,000 $\mu\text{g}/\text{plate}$, was mutagenic in *S. typhimurium* strain TA100 with and without 30% rat or hamster liver metabolic activation enzymes (S9); no activity was detected in strain TA98, with or without rat or hamster liver S9 enzymes (Table E1). Increased frequencies of micronucleated NCEs were observed in peripheral blood samples from male B6C3F₁ mice administered 125 to 2,000 mg dibromoacetic acid/L in drinking water for 3 months; no increases in NCEs were seen in female mice similarly exposed (Table E2). No evidence of bone marrow toxicity, as measured by the percentage of immature (polychromatic) erythrocytes among total erythrocytes, was observed in either male or female mice.

TABLE E1
Mutagenicity of Dibromoacetic Acid in *Salmonella typhimurium*^a

| Strain | Dose (µg/plate) | Revertants/Plate ^b | | | | | |
|-------------------------------|--------------------|-------------------------------|--------------|------------------|------------|--------------|------------|
| | | -S9 | | + 30% hamster S9 | | +30% rat S9 | |
| | | Trial 1 | Trial 2 | Trial 1 | Trial 2 | Trial 1 | Trial 2 |
| TA100 | 0 | 148 ± 5.0 | 108 ± 5.9 | 144 ± 9.5 | 125 ± 6.8 | 165 ± 2.8 | 149 ± 7.0 |
| | 33 | 140 ± 8.8 | 126 ± 3.4 | | 141 ± 9.8 | | 164 ± 9.1 |
| | 100 | 167 ± 4.9 | 154 ± 7.4 | 212 ± 6.1 | 180 ± 4.2 | 253 ± 9.5 | 289 ± 10.5 |
| | 333 | 261 ± 11.5 | 196 ± 4.5 | 339 ± 14.5 | 334 ± 27.1 | 356 ± 6.6 | 351 ± 19.3 |
| | 1,000 | 469 ± 14.5 | 360 ± 21.4 | 459 ± 16.4 | 388 ± 15.3 | 445 ± 20.9 | 434 ± 26.6 |
| | 3,333 | 981 ± 20.3 | 770 ± 23.3 | 1,041 ± 63.5 | 754 ± 17.7 | 930 ± 28.1 | 802 ± 24.1 |
| | 10,000 | | | 1,361 ± 37.3 | | 1,294 ± 29.4 | |
| | Trial Summary | Positive | Positive | Positive | Positive | Positive | Positive |
| Positive control ^c | 1,187 ± 15.9 | 1,352 ± 27.4 | 1,250 ± 62.8 | 763 ± 25.2 | 838 ± 37.4 | 673 ± 38.4 | |
| TA98 | 0 | 22 ± 1.8 | | 23 ± 3.2 | | 20 ± 2.1 | |
| | 33 | 22 ± 1.7 | | | | | |
| | 100 | 18 ± 1.8 | | 18 ± 2.0 | | 22 ± 3.2 | |
| | 333 | 22 ± 3.3 | | 22 ± 3.8 | | 16 ± 2.9 | |
| | 1,000 | 19 ± 3.5 | | 19 ± 1.2 | | 25 ± 0.6 | |
| | 3,333 | 23 ± 4.5 | | 21 ± 1.7 | | 18 ± 2.1 | |
| | 10,000 | | | 23 ± 1.5 | | 17 ± 1.2 | |
| | Trial Summary | Negative | | Negative | | Negative | |
| Positive control | 422 ± 9.0 | | 904 ± 33.8 | | 513 ± 31.9 | | |

^a Study was performed at SRI International. The detailed protocol is presented by Zeiger *et al.* (1992). 0 µg/plate was the solvent control.

^b Revertants are presented as mean ± standard error from three plates.

^c The positive controls in the absence of metabolic activation were sodium azide (TA100) and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Dibromoacetic Acid in Drinking Water for 3 Months^a

| Compound | Exposure Concentration (mg/L) | Number of Mice with Erythrocytes Scored | Micronucleated NCEs/ 1,000 NCEs ^b | P-Value ^c | PCEs ^b (%) |
|--------------------|-------------------------------|---|---|----------------------|-----------------------|
| Male | | | | | |
| Tap water | 0 | 10 | 1.80 ± 0.33 | | 3.0 ± 0.2 |
| Dibromoacetic acid | 125 | 10 | 2.30 ± 0.47 | 0.2172 | 2.8 ± 0.1 |
| | 250 | 10 | 3.30 ± 0.56 | 0.0177 | 2.8 ± 0.1 |
| | 500 | 10 | 3.20 ± 0.42 | 0.0237 | 2.7 ± 0.1 |
| | 1,000 | 9 | 4.00 ± 0.50 | 0.0022 | 2.8 ± 0.2 |
| | 2,000 | 10 | 3.60 ± 0.56 | 0.0071 | 3.0 ± 0.1 |
| | | | P=0.010 ^d | | |
| Female | | | | | |
| Tap water | 0 | 10 | 2.30 ± 0.56 | | 2.8 ± 0.1 |
| Dibromoacetic acid | 125 | 10 | 2.10 ± 0.43 | 0.6186 | 2.4 ± 0.1 |
| | 250 | 10 | 1.70 ± 0.30 | 0.8289 | 2.6 ± 0.1 |
| | 500 | 10 | 2.10 ± 0.28 | 0.6186 | 2.8 ± 0.1 |
| | 1,000 | 10 | 2.20 ± 0.44 | 0.5593 | 2.7 ± 0.1 |
| | 2,000 | 10 | 2.20 ± 0.29 | 0.5593 | 2.7 ± 0.1 |
| | | | P=0.397 | | |

^a Study was performed at ILS, Inc. The detailed protocol presented by MacGregor, *et al.* (1990). PCE=polychromatic erythrocyte; NCE=normochromatic erythrocyte.

^b Mean ± standard error

^c Pairwise comparison with the untreated control group; significant at P≤0.005 (ILS, 1990)

^d Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX F CLINICAL PATHOLOGY RESULTS

| | | |
|-----------------|--|------------|
| TABLE F1 | Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid | F-2 |
| TABLE F2 | Hematology Data for Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid | F-7 |

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|---|--------------|--------------|--------------|--------------|--------------|----------------|
| Male | | | | | | |
| Hematology | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 9 |
| Hematocrit (%) | | | | | | |
| Day 4 | 45.3 ± 0.8 | 42.5 ± 0.9 | 44.2 ± 0.7 | 43.7 ± 0.5 | 45.1 ± 0.9 | 45.7 ± 0.6 |
| Day 21 | 46.2 ± 0.7 | 45.5 ± 0.6 | 45.6 ± 0.6 | 45.3 ± 0.5 | 45.5 ± 0.6 | 42.8 ± 0.6** |
| Week 14 | 46.8 ± 0.3 | 45.7 ± 0.5 | 45.4 ± 0.6 | 45.3 ± 0.5* | 45.5 ± 0.3 | 45.3 ± 0.3 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 14.7 ± 0.3 | 13.9 ± 0.3 | 14.5 ± 0.2 | 14.3 ± 0.2 | 14.7 ± 0.3 | 15.1 ± 0.2 |
| Day 21 | 15.4 ± 0.2 | 15.0 ± 0.2 | 15.2 ± 0.2 | 15.0 ± 0.2 | 15.0 ± 0.2 | 14.2 ± 0.2** |
| Week 14 | 15.4 ± 0.1 | 15.1 ± 0.2 | 15.0 ± 0.1* | 15.1 ± 0.1 | 15.1 ± 0.1 | 15.0 ± 0.1 |
| Erythrocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 7.30 ± 0.12 | 6.90 ± 0.15 | 7.20 ± 0.11 | 7.11 ± 0.11 | 7.49 ± 0.16 | 7.66 ± 0.12 |
| Day 21 | 7.65 ± 0.13 | 7.49 ± 0.11 | 7.49 ± 0.10 | 7.42 ± 0.06 | 7.63 ± 0.10 | 7.26 ± 0.11 |
| Week 14 | 9.08 ± 0.08 | 8.86 ± 0.10 | 8.78 ± 0.11 | 8.80 ± 0.09 | 8.85 ± 0.07 | 8.89 ± 0.06 |
| Reticulocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 8.28 ± 0.32 | 8.08 ± 0.23 | 8.38 ± 0.27 | 7.99 ± 0.30 | 7.35 ± 0.30* | 5.79 ± 0.22** |
| Day 21 | 4.79 ± 0.10 | 5.10 ± 0.10 | 5.05 ± 0.13 | 5.04 ± 0.17 | 4.69 ± 0.13 | 4.52 ± 0.16 |
| Week 14 | 2.53 ± 0.06 | 2.86 ± 0.09* | 2.69 ± 0.06 | 2.76 ± 0.05 | 2.64 ± 0.07 | 2.68 ± 0.04 |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 62.0 ± 0.3 | 61.6 ± 0.3 | 61.5 ± 0.4 | 61.4 ± 0.2 | 60.3 ± 0.5* | 59.7 ± 0.2** |
| Day 21 | 60.5 ± 0.3 | 60.8 ± 0.5 | 60.9 ± 0.4 | 61.1 ± 0.4 | 59.7 ± 0.3 | 59.0 ± 0.3* |
| Week 14 | 51.6 ± 0.2 | 51.5 ± 0.2 | 51.7 ± 0.1 | 51.5 ± 0.2 | 51.5 ± 0.2 | 50.9 ± 0.2 |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 20.1 ± 0.1 | 20.1 ± 0.1 | 20.1 ± 0.1 | 20.0 ± 0.1 | 19.7 ± 0.2 | 19.8 ± 0.1* |
| Day 21 | 20.1 ± 0.1 | 20.1 ± 0.2 | 20.4 ± 0.1 | 20.3 ± 0.1 | 19.7 ± 0.1* | 19.6 ± 0.1** |
| Week 14 | 17.0 ± 0.1 | 17.0 ± 0.1 | 17.1 ± 0.1 | 17.2 ± 0.1 | 17.0 ± 0.1 | 16.9 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 32.5 ± 0.2 | 32.7 ± 0.1 | 32.7 ± 0.2 | 32.7 ± 0.2 | 32.7 ± 0.2 | 33.1 ± 0.2 |
| Day 21 | 33.3 ± 0.2 | 33.1 ± 0.2 | 33.4 ± 0.1 | 33.2 ± 0.2 | 33.0 ± 0.1 | 33.2 ± 0.2 |
| Week 14 | 33.0 ± 0.2 | 33.0 ± 0.2 | 33.0 ± 0.2 | 33.4 ± 0.2 | 33.1 ± 0.2 | 33.2 ± 0.2 |
| Platelets (10 ³ /μL) | | | | | | |
| Day 4 | 875.8 ± 15.7 | 920.8 ± 25.7 | 909.1 ± 13.9 | 939.0 ± 26.3 | 868.7 ± 18.5 | 828.5 ± 24.5 |
| Day 21 | 869.2 ± 18.6 | 883.7 ± 13.5 | 859.6 ± 17.0 | 894.3 ± 19.7 | 836.8 ± 9.9 | 742.2 ± 14.8** |
| Week 14 | 636.1 ± 9.9 | 629.0 ± 14.2 | 634.7 ± 12.6 | 646.5 ± 8.9 | 610.8 ± 8.5 | 572.2 ± 12.3** |
| Leukocytes (10 ³ /μL) | | | | | | |
| Day 4 | 8.09 ± 0.41 | 8.06 ± 0.43 | 8.23 ± 0.29 | 8.53 ± 0.33 | 8.35 ± 0.36 | 8.68 ± 0.70 |
| Day 21 | 9.48 ± 0.39 | 9.79 ± 0.41 | 9.55 ± 0.30 | 9.35 ± 0.50 | 9.11 ± 0.30 | 9.36 ± 0.25 |
| Week 14 | 10.66 ± 0.44 | 10.20 ± 0.45 | 10.39 ± 0.45 | 10.39 ± 0.46 | 9.83 ± 0.23 | 10.36 ± 0.37 |
| Segmented neutrophils (10 ³ /μL) | | | | | | |
| Day 4 | 1.13 ± 0.04 | 1.13 ± 0.05 | 1.13 ± 0.05 | 1.15 ± 0.04 | 1.16 ± 0.08 | 1.09 ± 0.09 |
| Day 21 | 0.99 ± 0.03 | 0.97 ± 0.03 | 0.85 ± 0.03 | 0.92 ± 0.04 | 0.90 ± 0.06 | 1.02 ± 0.04 |
| Week 14 | 1.21 ± 0.07 | 1.26 ± 0.03 | 1.12 ± 0.05 | 1.13 ± 0.04 | 1.15 ± 0.04 | 1.38 ± 0.09 |
| Lymphocytes (10 ³ /μL) | | | | | | |
| Day 4 | 6.55 ± 0.36 | 6.46 ± 0.40 | 6.65 ± 0.25 | 6.90 ± 0.31 | 6.73 ± 0.29 | 7.06 ± 0.56 |
| Day 21 | 8.08 ± 0.36 | 8.36 ± 0.38 | 8.27 ± 0.26 | 7.99 ± 0.44 | 7.76 ± 0.28 | 7.87 ± 0.19 |
| Week 14 | 8.46 ± 0.34 | 8.04 ± 0.39 | 8.27 ± 0.39 | 8.18 ± 0.40 | 7.78 ± 0.18 | 7.98 ± 0.26 |
| Activated lymphocytes (10 ³ /μL) | | | | | | |
| Day 4 | 0.26 ± 0.03 | 0.31 ± 0.03 | 0.30 ± 0.03 | 0.33 ± 0.02 | 0.33 ± 0.02 | 0.37 ± 0.04** |
| Day 21 | 0.27 ± 0.03 | 0.31 ± 0.03 | 0.30 ± 0.03 | 0.29 ± 0.03 | 0.32 ± 0.02 | 0.35 ± 0.02 |
| Week 14 | 0.81 ± 0.05 | 0.72 ± 0.07 | 0.81 ± 0.06 | 0.87 ± 0.05 | 0.72 ± 0.04 | 0.85 ± 0.07 |

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|------------------------------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Male (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| Day 4 | 10 | 10 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 10 | 10 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 9 |
| Monocytes ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.11 ± 0.02 | 0.11 ± 0.01 | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.09 ± 0.01 | 0.10 ± 0.02 |
| Day 21 | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.10 ± 0.02 | 0.08 ± 0.02 |
| Week 14 | 0.09 ± 0.01 | 0.10 ± 0.02 | 0.11 ± 0.04 | 0.13 ± 0.03 | 0.10 ± 0.01 | 0.07 ± 0.01 |
| Basophils ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.013 ± 0.004 | 0.014 ± 0.003 | 0.014 ± 0.003 | 0.014 ± 0.002 | 0.015 ± 0.004 | 0.017 ± 0.004 |
| Day 21 | 0.013 ± 0.002 | 0.014 ± 0.003 | 0.014 ± 0.003 | 0.015 ± 0.003 | 0.016 ± 0.002 | 0.016 ± 0.002 |
| Week 14 | 0.026 ± 0.004 | 0.017 ± 0.004 | 0.022 ± 0.004 | 0.030 ± 0.004 | 0.022 ± 0.003 | 0.022 ± 0.004 |
| Eosinophils ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.03 ± 0.00 | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.01 |
| Day 21 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.04 ± 0.00* |
| Week 14 | 0.07 ± 0.01 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.05 ± 0.01* | 0.05 ± 0.00 |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 12.5 ± 0.3 | 11.8 ± 0.5 | 12.1 ± 0.4 | 13.0 ± 0.3 | 13.5 ± 0.2 | 13.8 ± 0.6 |
| Day 21 | 11.8 ± 0.3 | 11.8 ± 0.4 | 12.8 ± 0.4 | 11.6 ± 0.2 | 13.3 ± 0.4** | 14.0 ± 0.3** |
| Week 14 | 16.9 ± 0.5 | 12.0 ± 0.5** | 14.3 ± 0.9 | 14.4 ± 0.5 | 12.3 ± 0.5** | 13.1 ± 0.6** |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.45 ± 0.02 | 0.42 ± 0.01 | 0.44 ± 0.02 | 0.43 ± 0.02 | 0.42 ± 0.01 | 0.41 ± 0.01 |
| Day 21 | 0.55 ± 0.02 | 0.54 ± 0.02 | 0.53 ± 0.02 | 0.52 ± 0.01 | 0.48 ± 0.01** | 0.49 ± 0.01** |
| Week 14 | 0.55 ± 0.02 | 0.55 ± 0.02 | 0.54 ± 0.02 | 0.55 ± 0.02 | 0.55 ± 0.02 | 0.51 ± 0.01 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.5 ± 0.1 | 5.3 ± 0.1 | 5.3 ± 0.1 | 5.4 ± 0.1 | 5.2 ± 0.0* | 4.9 ± 0.1** |
| Day 21 | 6.3 ± 0.1 | 6.5 ± 0.1 | 6.6 ± 0.1* | 6.5 ± 0.1 | 6.5 ± 0.1 | 6.0 ± 0.1 |
| Week 14 | 6.7 ± 0.0 | 6.8 ± 0.1 | 6.9 ± 0.1* | 6.9 ± 0.1* | 7.0 ± 0.1** | 6.9 ± 0.1** |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.0 ± 0.1 | 3.9 ± 0.1 | 4.0 ± 0.1 | 4.0 ± 0.1 | 3.9 ± 0.0 | 3.8 ± 0.1* |
| Day 21 | 4.5 ± 0.0 | 4.7 ± 0.1 | 4.7 ± 0.1* | 4.7 ± 0.1 | 4.7 ± 0.1* | 4.5 ± 0.1 |
| Week 14 | 4.8 ± 0.0 | 4.9 ± 0.1* | 5.1 ± 0.1** | 5.0 ± 0.1** | 5.1 ± 0.0** | 5.2 ± 0.1** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 80 ± 2 | 77 ± 2 | 77 ± 3 | 78 ± 2 | 87 ± 1* | 85 ± 2 |
| Day 21 | 54 ± 1 | 52 ± 1 | 55 ± 3 | 47 ± 1** | 47 ± 1** | 47 ± 2** |
| Week 14 | 83 ± 4 | 47 ± 2** | 62 ± 7** | 52 ± 3** | 43 ± 1** | 49 ± 2** |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 745 ± 17 | 754 ± 14 | 769 ± 25 | 805 ± 22* | 818 ± 17* | 788 ± 17* |
| Day 21 | 556 ± 8 | 509 ± 11** | 515 ± 16* | 516 ± 11* | 504 ± 19** | 479 ± 7** |
| Week 14 | 200 ± 10 | 182 ± 4 | 201 ± 10 | 174 ± 9 | 186 ± 6 | 192 ± 6 |
| Creatine kinase (IU/L) | | | | | | |
| Day 4 | 537 ± 31 | 497 ± 61 | 472 ± 36 | 410 ± 26 | 564 ± 65 | 426 ± 17 |
| Day 21 | 306 ± 26 | 256 ± 20 | 281 ± 16 | 318 ± 36 | 354 ± 52 | 255 ± 14 |
| Week 14 | 306 ± 29 | 207 ± 21 | 223 ± 28 | 282 ± 15 | 219 ± 23 | 194 ± 17* |

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|---|--------------------------|--------------|--------------|--------------|--------------|----------------|
| Male (continued) | | | | | | |
| Clinical Chemistry | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 7 ± 0 | 8 ± 0 | 9 ± 0* | 9 ± 0** | 10 ± 0** | 11 ± 0** |
| Day 21 | 9 ± 1 | 9 ± 1 | 9 ± 1 | 10 ± 1 | 11 ± 1 | 12 ± 1** |
| Week 14 | 9 ± 1 | 8 ± 1 | 10 ± 1 | 8 ± 1 | 9 ± 1 | 10 ± 1 |
| Bile acids (μmol/L) | | | | | | |
| Day 4 | 34.9 ± 3.1 | 33.9 ± 2.2 | 35.9 ± 2.2 | 38.0 ± 2.7 | 52.8 ± 3.3** | 53.2 ± 2.9** |
| Day 21 | 24.9 ± 1.5 | 26.2 ± 1.2 | 31.7 ± 1.4** | 26.0 ± 0.9 | 29.0 ± 1.7 | 24.2 ± 1.1 |
| Week 14 | 20.4 ± 2.1 | 16.6 ± 1.7 | 13.6 ± 0.7* | 18.5 ± 1.7 | 17.8 ± 1.9 | 16.9 ± 1.5 |
| Female | | | | | | |
| Hematology | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 9 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | | | | | | |
| Day 4 | 43.5 ± 0.6 | 44.5 ± 0.5 | 45.1 ± 0.7 | 45.8 ± 1.2 | 45.5 ± 0.7 | 46.6 ± 1.0 |
| Day 21 | 46.2 ± 0.3 | 45.4 ± 0.4 | 44.9 ± 0.5 | 46.8 ± 0.7 | 44.2 ± 0.4** | 43.4 ± 0.8** |
| Week 14 | 42.4 ± 0.3 | 42.9 ± 0.4 | 42.7 ± 0.3 | 42.4 ± 0.5 | 41.5 ± 0.5 | 40.9 ± 0.5 |
| Hemoglobin (g/dL) | | | | | | |
| Day 4 | 14.3 ± 0.2 | 14.6 ± 0.2 | 14.8 ± 0.3 | 15.2 ± 0.4 | 15.1 ± 0.3 | 15.4 ± 0.3 |
| Day 21 | 15.6 ± 0.1 | 15.3 ± 0.2 | 15.3 ± 0.2 | 15.7 ± 0.2 | 15.0 ± 0.2* | 14.7 ± 0.2** |
| Week 14 | 13.7 ± 0.1 | 13.9 ± 0.1 | 13.9 ± 0.1 | 13.8 ± 0.1 | 13.5 ± 0.2 | 13.2 ± 0.1* |
| Erythrocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 7.53 ± 0.11 | 7.71 ± 0.12 | 7.76 ± 0.15 | 7.96 ± 0.19 | 7.90 ± 0.14 | 8.23 ± 0.16** |
| Day 21 | 7.98 ± 0.07 | 7.77 ± 0.06 | 7.70 ± 0.10 | 8.08 ± 0.13 | 7.73 ± 0.13 | 7.79 ± 0.15 |
| Week 14 | 8.07 ± 0.05 | 8.11 ± 0.05 | 8.13 ± 0.05 | 8.13 ± 0.10 | 8.06 ± 0.09 | 7.97 ± 0.08 |
| Reticulocytes (10 ⁶ /μL) | | | | | | |
| Day 4 | 6.49 ± 0.32 | 6.40 ± 0.33 | 6.38 ± 0.26 | 6.13 ± 0.43 | 5.77 ± 0.34 | 4.52 ± 0.28** |
| Day 21 | 2.87 ± 0.16 _b | 3.04 ± 0.17 | 2.85 ± 0.14 | 2.90 ± 0.16 | 2.74 ± 0.10 | 2.88 ± 0.16 |
| Week 14 | 2.30 ± 0.08 _b | 2.28 ± 0.03 | 2.16 ± 0.05 | 2.30 ± 0.08 | 2.21 ± 0.06 | 2.75 ± 0.08* |
| Mean cell volume (fL) | | | | | | |
| Day 4 | 57.8 ± 0.3 | 57.8 ± 0.4 | 58.2 ± 0.3 | 57.5 ± 0.2 | 57.7 ± 0.3 | 56.7 ± 0.5 |
| Day 21 | 57.9 ± 0.3 | 58.4 ± 0.5 | 58.4 ± 0.2 | 58.0 ± 0.5 | 57.2 ± 0.6 | 55.7 ± 0.3** |
| Week 14 | 52.5 ± 0.2 | 53.0 ± 0.2 | 52.6 ± 0.2 | 52.1 ± 0.2 | 51.5 ± 0.2* | 51.3 ± 0.2** |
| Mean cell hemoglobin (pg) | | | | | | |
| Day 4 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 19.0 ± 0.1 | 18.7 ± 0.1 |
| Day 21 | 19.6 ± 0.1 | 19.7 ± 0.1 | 19.9 ± 0.1 | 19.5 ± 0.1 | 19.4 ± 0.1 | 18.9 ± 0.1** |
| Week 14 | 17.0 ± 0.1 | 17.1 ± 0.1 | 17.2 ± 0.1 | 17.0 ± 0.1 | 16.7 ± 0.1* | 16.6 ± 0.1* |
| Mean cell hemoglobin concentration (g/dL) | | | | | | |
| Day 4 | 32.8 ± 0.1 | 32.8 ± 0.2 | 32.7 ± 0.1 | 33.1 ± 0.2 | 33.0 ± 0.2 | 33.0 ± 0.2 |
| Day 21 | 33.8 ± 0.1 | 33.7 ± 0.2 | 34.1 ± 0.1 | 33.6 ± 0.2 | 33.9 ± 0.2 | 33.9 ± 0.2 |
| Week 14 | 32.4 ± 0.2 | 32.3 ± 0.3 | 32.6 ± 0.2 | 32.7 ± 0.2 | 32.5 ± 0.3 | 32.4 ± 0.2 |
| Platelets (10 ³ /μL) | | | | | | |
| Day 4 | 766.0 ± 19.4 | 802.3 ± 23.6 | 800.1 ± 24.8 | 794.9 ± 25.8 | 820.2 ± 21.1 | 744.6 ± 12.5 |
| Day 21 | 722.9 ± 19.2 | 815.3 ± 24.9 | 803.8 ± 14.8 | 806.1 ± 25.1 | 754.4 ± 22.4 | 705.3 ± 19.8 |
| Week 14 | 690.2 ± 15.6 | 661.5 ± 10.5 | 689.0 ± 15.4 | 674.7 ± 10.7 | 660.1 ± 13.9 | 608.2 ± 10.9** |

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--|---------------|---------------|---------------|---------------|---------------|---------------|
| Female (continued) | | | | | | |
| Hematology (continued) | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 9 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Leukocytes ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 9.28 ± 0.33 | 9.82 ± 0.34 | 9.99 ± 0.53 | 9.61 ± 0.41 | 9.60 ± 0.34 | 9.55 ± 0.48 |
| Day 21 | 9.95 ± 0.35 | 9.24 ± 0.52 | 9.52 ± 0.41 | 9.87 ± 0.66 | 9.46 ± 0.47 | 9.15 ± 0.35 |
| Week 14 | 8.43 ± 0.23 | 8.06 ± 0.25 | 7.52 ± 0.32* | 7.49 ± 0.45 | 6.70 ± 0.54** | 6.97 ± 0.34** |
| Segmented neutrophils ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 1.08 ± 0.05 | 1.11 ± 0.06 | 1.15 ± 0.06 | 1.19 ± 0.08 | 1.22 ± 0.04 | 1.17 ± 0.14 |
| Day 21 | 0.98 ± 0.07 | 0.92 ± 0.05 | 0.90 ± 0.04 | 1.09 ± 0.10 | 1.02 ± 0.06 | 1.13 ± 0.11 |
| Week 14 | 1.41 ± 0.10 | 1.21 ± 0.06 | 1.18 ± 0.05 | 1.06 ± 0.08* | 1.01 ± 0.08** | 1.25 ± 0.08 |
| Lymphocytes ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 7.73 ± 0.30 | 8.22 ± 0.33 | 8.32 ± 0.47 | 7.92 ± 0.33 | 7.87 ± 0.31 | 7.82 ± 0.40 |
| Day 21 | 8.53 ± 0.29 | 7.87 ± 0.46 | 8.17 ± 0.37 | 8.31 ± 0.54 | 7.92 ± 0.40 | 7.52 ± 0.31 |
| Week 14 | 6.31 ± 0.13 | 6.18 ± 0.24 | 5.69 ± 0.26 | 5.75 ± 0.34 | 5.00 ± 0.44** | 5.00 ± 0.26** |
| Activated lymphocytes ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.29 ± 0.01 | 0.32 ± 0.03 | 0.34 ± 0.02 | 0.33 ± 0.03 | 0.35 ± 0.02 | 0.39 ± 0.02** |
| Day 21 | 0.29 ± 0.02 | 0.30 ± 0.03 | 0.30 ± 0.02 | 0.33 ± 0.03 | 0.38 ± 0.05 | 0.38 ± 0.03 |
| Week 14 | 0.56 ± 0.06 | 0.53 ± 0.03 | 0.51 ± 0.04 | 0.55 ± 0.05 | 0.58 ± 0.05 | 0.61 ± 0.04 |
| Monocytes ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.13 ± 0.01 | 0.12 ± 0.01 | 0.12 ± 0.02 | 0.13 ± 0.01 | 0.11 ± 0.01 | 0.10 ± 0.01 |
| Day 21 | 0.09 ± 0.01 | 0.09 ± 0.01 | 0.09 ± 0.01 | 0.08 ± 0.01 | 0.08 ± 0.01 | 0.06 ± 0.01 |
| Week 14 | 0.09 ± 0.01 | 0.07 ± 0.00* | 0.08 ± 0.01* | 0.07 ± 0.01** | 0.06 ± 0.00** | 0.06 ± 0.01** |
| Basophils ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.011 ± 0.002 | 0.016 ± 0.002 | 0.016 ± 0.002 | 0.016 ± 0.002 | 0.016 ± 0.003 | 0.022 ± 0.004 |
| Day 21 | 0.015 ± 0.002 | 0.015 ± 0.003 | 0.013 ± 0.002 | 0.014 ± 0.002 | 0.018 ± 0.004 | 0.013 ± 0.002 |
| Week 14 | 0.018 ± 0.004 | 0.014 ± 0.002 | 0.009 ± 0.003 | 0.010 ± 0.003 | 0.010 ± 0.001 | 0.008 ± 0.002 |
| Eosinophils ($10^3/\mu\text{L}$) | | | | | | |
| Day 4 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.04 ± 0.00 | 0.03 ± 0.00 | 0.04 ± 0.01 | 0.04 ± 0.00 |
| Day 21 | 0.05 ± 0.01 | 0.04 ± 0.01 | 0.04 ± 0.00 | 0.05 ± 0.01 | 0.04 ± 0.00 | 0.05 ± 0.00 |
| Week 14 | 0.05 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.00 |
| Clinical Chemistry | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Urea nitrogen (mg/dL) | | | | | | |
| Day 4 | 10.2 ± 0.6 | 11.2 ± 0.3 | 11.1 ± 0.4 | 12.0 ± 0.4* | 11.6 ± 0.5 | 12.5 ± 0.6** |
| Day 21 | 14.0 ± 0.3 | 13.5 ± 0.5 | 13.1 ± 0.5 | 13.0 ± 0.5 | 12.5 ± 0.3 | 14.1 ± 0.4 |
| Week 14 | 13.0 ± 0.5 | 13.3 ± 0.5 | 13.8 ± 0.4 | 12.7 ± 0.4 | 12.8 ± 0.5 | 12.2 ± 0.5 |
| Creatinine (mg/dL) | | | | | | |
| Day 4 | 0.49 ± 0.01 | 0.49 ± 0.01 | 0.51 ± 0.01 | 0.49 ± 0.01 | 0.45 ± 0.02 | 0.48 ± 0.01 |
| Day 21 | 0.48 ± 0.01 | 0.45 ± 0.02 | 0.45 ± 0.02 | 0.48 ± 0.02 | 0.44 ± 0.02 | 0.46 ± 0.02 |
| Week 14 | 0.59 ± 0.01 | 0.59 ± 0.02 | 0.59 ± 0.01 | 0.63 ± 0.03 | 0.57 ± 0.02 | 0.57 ± 0.02 |
| Total protein (g/dL) | | | | | | |
| Day 4 | 5.6 ± 0.1 | 5.6 ± 0.1 | 5.7 ± 0.1 | 5.6 ± 0.1 | 5.3 ± 0.1 | 5.2 ± 0.1* |
| Day 21 | 6.2 ± 0.1 | 6.3 ± 0.1 | 6.3 ± 0.1 | 6.6 ± 0.1* | 6.7 ± 0.1** | 6.4 ± 0.1* |
| Week 14 | 6.7 ± 0.1 | 7.1 ± 0.1* | 7.4 ± 0.1** | 7.3 ± 0.1** | 7.5 ± 0.1** | 7.4 ± 0.1** |

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|---------------------------------|------------|-------------|-------------|-----------------------|--------------|--------------|
| Female (continued) | | | | | | |
| Clinical Chemistry (continued) | | | | | | |
| Day 4 | 10 | 9 | 10 | 10 | 10 | 10 |
| Day 21 | 10 | 10 | 10 | 8 | 9 | 10 |
| Week 14 | 10 | 10 | 10 | 10 | 10 | 10 |
| Albumin (g/dL) | | | | | | |
| Day 4 | 4.1 ± 0.1 | 4.3 ± 0.1 | 4.3 ± 0.1 | 4.3 ± 0.1 | 4.0 ± 0.1 | 4.0 ± 0.1 |
| Day 21 | 4.6 ± 0.1 | 4.8 ± 0.1* | 4.8 ± 0.1* | 4.9 ± 0.1** | 5.1 ± 0.1** | 5.0 ± 0.1** |
| Week 14 | 5.2 ± 0.2 | 5.6 ± 0.1* | 5.9 ± 0.1** | 5.8 ± 0.1** | 6.0 ± 0.1** | 5.9 ± 0.1** |
| Alanine aminotransferase (IU/L) | | | | | | |
| Day 4 | 60 ± 5 | 63 ± 2 | 67 ± 2 | 69 ± 3 | 67 ± 7* | 68 ± 6 |
| Day 21 | 41 ± 3 | 36 ± 1** | 38 ± 1* | 38 ± 1* | 29 ± 2** | 32 ± 2** |
| Week 14 | 40 ± 1 | 40 ± 2 | 37 ± 1 | 35 ± 2 | 34 ± 3** | 36 ± 3* |
| Alkaline phosphatase (IU/L) | | | | | | |
| Day 4 | 550 ± 13 | 596 ± 24 | 582 ± 15 | 642 ± 25** | 674 ± 31** | 613 ± 26** |
| Day 21 | 374 ± 10 | 355 ± 8 | 341 ± 12* | 356 ± 10 | 317 ± 9** | 302 ± 10** |
| Week 14 | 165 ± 8 | 162 ± 7 | 162 ± 6 | 152 ± 5 | 149 ± 5 | 142 ± 6* |
| Creatine kinase (IU/L) | | | | | | |
| Day 4 | 646 ± 33 | 635 ± 33 | 603 ± 36 | 750 ± 53 ^b | 629 ± 32 | 631 ± 33 |
| Day 21 | 530 ± 74 | 449 ± 28 | 477 ± 62 | 475 ± 35 ^b | 440 ± 30 | 473 ± 39 |
| Week 14 | 287 ± 32 | 212 ± 25 | 250 ± 30 | 253 ± 19 | 220 ± 25 | 187 ± 21 |
| Sorbitol dehydrogenase (IU/L) | | | | | | |
| Day 4 | 7 ± 0 | 7 ± 1 | 8 ± 0 | 8 ± 1 ^b | 9 ± 1* | 8 ± 1* |
| Day 21 | 8 ± 0 | 8 ± 0 | 8 ± 1 | 8 ± 0 ^b | 9 ± 0* | 9 ± 0** |
| Week 14 | 7 ± 0 | 7 ± 0 | 8 ± 0 | 8 ± 0 | 8 ± 0 | 8 ± 1 |
| Bile acids (µmol/L) | | | | | | |
| Day 4 | 21.5 ± 2.0 | 22.9 ± 2.2 | 25.1 ± 2.1 | 25.9 ± 1.9 | 32.9 ± 3.3** | 44.8 ± 4.8** |
| Day 21 | 24.0 ± 2.4 | 17.5 ± 0.9* | 20.3 ± 1.2 | 20.4 ± 1.6 | 18.2 ± 0.9 | 18.1 ± 0.7 |
| Week 14 | 22.9 ± 2.1 | 25.6 ± 2.9 | 20.7 ± 2.7 | 22.5 ± 2.8 | 23.0 ± 3.1 | 24.9 ± 2.8 |

* 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 F2
Hematology Data for Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|---|---------------------------|---------------|----------------------------|--------------------------|--------------------------|--------------------------|
| Male | | | | | | |
| n | 10 | 10 | 10 | 10 | 9 | 10 |
| Hematocrit (%) | 48.5 ± 0.7 | 48.7 ± 0.7 | 48.7 ± 0.9 | 49.5 ± 0.7 | 51.0 ± 1.1 | 49.9 ± 0.8 |
| Hemoglobin (g/dL) | 16.7 ± 0.2 | 16.6 ± 0.2 | 16.5 ± 0.3 | 16.7 ± 0.3 | 17.1 ± 0.3 | 16.8 ± 0.3 |
| Erythrocytes (10 ⁶ /μL) | 10.97 ± 0.15 ^b | 10.93 ± 0.16 | 10.99 ± 0.21 | 11.09 ± 0.18 | 11.46 ± 0.24 | 11.39 ± 0.17 |
| Reticulocytes (10 ⁶ /μL) | 3.97 ± 0.10 ^b | 3.94 ± 0.09 | 3.94 ± 0.11 | 4.02 ± 0.09 | 4.14 ± 0.12 | 4.37 ± 0.12 ^b |
| Mean cell volume (fL) | 44.2 ± 0.1 | 44.6 ± 0.2 | 44.4 ± 0.2 | 44.6 ± 0.1 | 44.5 ± 0.2 | 43.8 ± 0.1 |
| Mean cell hemoglobin (pg) | 15.2 ± 0.1 | 15.2 ± 0.1 | 15.0 ± 0.1 | 15.1 ± 0.1 | 15.0 ± 0.1 | 14.8 ± 0.1** |
| Mean cell hemoglobin concentration (g/dL) | 34.4 ± 0.2 | 34.0 ± 0.1 | 33.9 ± 0.2 | 33.7 ± 0.1** | 33.6 ± 0.2** | 33.8 ± 0.2** |
| Platelets (10 ³ /μL) | 844.9 ± 49.3 | 859.5 ± 20.1 | 848.7 ± 33.6 | 819.2 ± 44.2 | 716.0 ± 67.6 | 712.9 ± 51.4* |
| Leukocytes (10 ⁷ /μL) | 6.90 ± 0.32 | 5.85 ± 0.28 | 5.71 ± 0.34 | 5.58 ± 0.50 | 5.90 ± 0.45 | 5.32 ± 0.45* |
| Segmented | | | | | | |
| neutrophils (10 ³ /μL) | 0.90 ± 0.11 | 0.68 ± 0.04* | 0.61 ± 0.07* | 0.59 ± 0.05** | 0.68 ± 0.11** | 0.54 ± 0.08 |
| Lymphocytes (10 ⁷ /μL) | 5.79 ± 0.25 | 5.02 ± 0.25 | 4.97 ± 0.29 | 4.84 ± 0.44 | 5.08 ± 0.38 | 4.64 ± 0.37 |
| Activated | | | | | | |
| lymphocytes (10 ³ /μL) | 0.07 ± 0.01 | 0.06 ± 0.00 | 0.06 ± 0.01 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.05 ± 0.01 |
| Monocytes (10 ³ /μL) | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.04 ± 0.00* | 0.05 ± 0.00 | 0.05 ± 0.01 | 0.05 ± 0.01 |
| Basophils (10 ³ /μL) | 0.004 ± 0.002 | 0.004 ± 0.002 | 0.005 ± 0.002 ^b | 0.003 ± 0.002 | 0.004 ± 0.002 | 0.005 ± 0.002 |
| Eosinophils (10 ³ /μL) | 0.06 ± 0.03 | 0.03 ± 0.01 | 0.02 ± 0.01 ^b | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.04 ± 0.01 |
| Female | | | | | | |
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Hematocrit (%) | 50.4 ± 0.4 | 49.6 ± 0.5 | 47.9 ± 0.7 | 50.6 ± 0.9 | 50.4 ± 1.0 | 50.5 ± 1.0 |
| Hemoglobin (g/dL) | 16.4 ± 0.1 | 16.0 ± 0.2 | 15.6 ± 0.2 | 16.5 ± 0.2 | 16.6 ± 0.3 | 16.5 ± 0.2 |
| Erythrocytes (10 ⁶ /μL) | 10.47 ± 0.09 | 10.24 ± 0.14 | 9.96 ± 0.14 | 10.55 ± 0.18 | 10.46 ± 0.19 | 10.51 ± 0.19 |
| Reticulocytes (10 ⁶ /μL) | 3.37 ± 0.13 | 3.12 ± 0.17 | 3.26 ± 0.12 ^b | 3.37 ± 0.12 ^b | 3.42 ± 0.18 ^b | 3.81 ± 0.14 |
| Mean cell volume (fL) | 48.1 ± 0.2 | 48.4 ± 0.3 | 48.1 ± 0.2 | 48.0 ± 0.2 | 48.2 ± 0.2 | 48.1 ± 0.2 |
| Mean cell hemoglobin (pg) | 15.6 ± 0.1 | 15.7 ± 0.1 | 15.7 ± 0.1 | 15.6 ± 0.1 | 15.8 ± 0.1 | 15.7 ± 0.1 |
| Mean cell hemoglobin concentration (g/dL) | 32.5 ± 0.1 | 32.4 ± 0.2 | 32.7 ± 0.2 | 32.6 ± 0.2 | 32.9 ± 0.2 | 32.7 ± 0.3 |
| Platelets (10 ³ /μL) | 767.0 ± 26.9 | 853.2 ± 44.5 | 906.1 ± 38.2 | 788.3 ± 48.2 | 648.0 ± 54.1 | 663.8 ± 49.6 |
| Leukocytes (10 ⁷ /μL) | 4.56 ± 0.48 | 4.30 ± 0.37 | 3.56 ± 0.48 | 4.31 ± 0.30 | 3.78 ± 0.32 | 4.46 ± 0.55 |
| Segmented | | | | | | |
| neutrophils (10 ³ /μL) | 0.54 ± 0.06 | 0.47 ± 0.04 | 0.43 ± 0.07 | 0.47 ± 0.07 | 0.34 ± 0.04 | 0.48 ± 0.08 |
| Lymphocytes (10 ⁷ /μL) | 3.81 ± 0.41 | 3.63 ± 0.31 | 2.93 ± 0.42 | 3.62 ± 0.23 | 3.25 ± 0.28 | 3.76 ± 0.46 |
| Activated | | | | | | |
| lymphocytes (10 ³ /μL) | 0.10 ± 0.01 | 0.11 ± 0.01 | 0.12 ± 0.02 | 0.11 ± 0.01 | 0.10 ± 0.01 | 0.12 ± 0.02 |
| Monocytes (10 ³ /μL) | 0.04 ± 0.01 | 0.03 ± 0.01 | 0.03 ± 0.00 | 0.03 ± 0.00 | 0.02 ± 0.00 | 0.03 ± 0.00 |
| Basophils (10 ³ /μL) | 0.000 ± 0.000 | 0.003 ± 0.002 | 0.000 ± 0.000 | 0.003 ± 0.002 | 0.002 ± 0.001 | 0.006 ± |
| Eosinophils (10 ³ /μL) | 0.07 ± 0.01 | 0.06 ± 0.01 | 0.05 ± 0.01 | 0.07 ± 0.01 | 0.05 ± 0.01 | 0.07 ± 0.01 |

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

**P ≤ 0.01

^a Mean ± standard error. Statistical tests were performed on unrounded data.

^b n=9

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

| | | |
|-----------------|--|------------|
| TABLE G1 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Drinking Water Studies of Dibromoacetic Acid | G-2 |
| TABLE G2 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Studies of Dibromoacetic Acid | G-3 |
| TABLE G3 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Drinking Water Studies of Dibromoacetic Acid | G-4 |
| TABLE G4 | Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Studies of Dibromoacetic Acid | G-5 |

TABLE G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Drinking Water Studies
of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|----------------------|----------------|------------------|------------------|------------------|------------------|------------------|
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Male | | | | | | |
| Necropsy body weight | 161 ± 4 | 169 ± 6 | 167 ± 8 | 167 ± 5 | 171 ± 4 | 166 ± 5 |
| Heart | | | | | | |
| Absolute | 0.594 ± 0.014 | 0.560 ± 0.021 | 0.576 ± 0.024 | 0.556 ± 0.007 | 0.566 ± 0.015 | 0.508 ± 0.020* |
| Relative | 3.693 ± 0.035 | 3.307 ± 0.029** | 3.468 ± 0.082** | 3.337 ± 0.080** | 3.309 ± 0.081** | 3.055 ± 0.040** |
| R. Kidney | | | | | | |
| Absolute | 0.690 ± 0.030 | 0.762 ± 0.047 | 0.776 ± 0.044 | 0.790 ± 0.020 | 0.814 ± 0.019* | 0.780 ± 0.017 |
| Relative | 4.287 ± 0.138 | 4.489 ± 0.150 | 4.656 ± 0.076 | 4.739 ± 0.134* | 4.757 ± 0.059* | 4.706 ± 0.108 |
| Liver | | | | | | |
| Absolute | 7.520 ± 0.070 | 9.246 ± 0.509** | 8.882 ± 0.364** | 9.870 ± 0.311** | 10.486 ± 0.253** | 10.474 ± 0.436** |
| Relative | 46.843 ± 1.043 | 54.545 ± 1.807** | 53.603 ± 2.171** | 59.080 ± 0.537** | 61.342 ± 1.696** | 62.987 ± 1.014** |
| Lung | | | | | | |
| Absolute | 1.112 ± 0.107 | 1.114 ± 0.123 | 1.046 ± 0.068 | 0.948 ± 0.014 | 1.150 ± 0.129 | 0.884 ± 0.046 |
| Relative | 6.891 ± 0.584 | 6.634 ± 0.828 | 6.268 ± 0.172 | 5.693 ± 0.174 | 6.674 ± 0.610 | 5.326 ± 0.239 |
| R. Testis | | | | | | |
| Absolute | 1.005 ± 0.030 | 1.002 ± 0.018 | 1.006 ± 0.038 | 0.956 ± 0.043 | 0.944 ± 0.036 | 0.891 ± 0.038 |
| Relative | 6.250 ± 0.157 | 5.937 ± 0.150 | 6.059 ± 0.126 | 5.730 ± 0.267* | 5.511 ± 0.097** | 5.362 ± 0.121** |
| Thymus | | | | | | |
| Absolute | 0.380 ± 0.016 | 0.392 ± 0.012 | 0.363 ± 0.008 | 0.382 ± 0.014 | 0.369 ± 0.024 | 0.339 ± 0.007 |
| Relative | 2.372 ± 0.123 | 2.320 ± 0.050 | 2.196 ± 0.070 | 2.290 ± 0.065 | 2.155 ± 0.119 | 2.046 ± 0.050* |
| Female | | | | | | |
| Necropsy body weight | 120 ± 3 | 121 ± 2 | 129 ± 2 | 125 ± 3 | 118 ± 4 | 124 ± 4 |
| Heart | | | | | | |
| Absolute | 0.458 ± 0.017 | 0.442 ± 0.011 | 0.480 ± 0.016 | 0.438 ± 0.008 | 0.408 ± 0.016 | 0.430 ± 0.016 |
| Relative | 3.811 ± 0.148 | 3.642 ± 0.081 | 3.726 ± 0.094 | 3.509 ± 0.077* | 3.448 ± 0.048* | 3.468 ± 0.064* |
| R. Kidney | | | | | | |
| Absolute | 0.532 ± 0.021 | 0.582 ± 0.024 | 0.598 ± 0.017 | 0.602 ± 0.026 | 0.550 ± 0.022 | 0.600 ± 0.020 |
| Relative | 4.419 ± 0.116 | 4.787 ± 0.124 | 4.645 ± 0.110 | 4.806 ± 0.088 | 4.653 ± 0.143 | 4.852 ± 0.163 |
| Liver | | | | | | |
| Absolute | 5.148 ± 0.158 | 5.812 ± 0.255* | 6.518 ± 0.148** | 6.392 ± 0.196** | 6.258 ± 0.268** | 7.430 ± 0.232** |
| Relative | 42.783 ± 0.708 | 47.819 ± 1.574** | 50.632 ± 0.908** | 51.104 ± 0.497** | 52.862 ± 0.815** | 59.969 ± 0.640** |
| Lung | | | | | | |
| Absolute | 0.936 ± 0.103 | 0.920 ± 0.065 | 0.864 ± 0.046 | 0.770 ± 0.024 | 0.850 ± 0.057 | 0.752 ± 0.025 |
| Relative | 7.849 ± 1.020 | 7.593 ± 0.567 | 6.712 ± 0.347 | 6.172 ± 0.223 | 7.189 ± 0.435 | 6.075 ± 0.156 |
| Thymus | | | | | | |
| Absolute | 0.319 ± 0.015 | 0.335 ± 0.015 | 0.340 ± 0.011 | 0.303 ± 0.006 | 0.302 ± 0.009 | 0.293 ± 0.007 |
| Relative | 2.649 ± 0.086 | 2.760 ± 0.100 | 2.638 ± 0.085 | 2.436 ± 0.097 | 2.558 ± 0.052 | 2.364 ± 0.035* |

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

** P≤0.01

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

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Studies of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|----------------------|----------------|------------------|------------------|------------------|------------------|------------------|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Male | | | | | | |
| Necropsy body weight | 333 ± 5 | 345 ± 6 | 350 ± 4 | 348 ± 4 | 346 ± 6 | 311 ± 3* |
| Heart | | | | | | |
| Absolute | 0.867 ± 0.013 | 0.870 ± 0.012 | 0.870 ± 0.009 | 0.853 ± 0.016 | 0.868 ± 0.019 | 0.773 ± 0.012** |
| Relative | 2.609 ± 0.040 | 2.527 ± 0.033 | 2.488 ± 0.031* | 2.454 ± 0.031** | 2.509 ± 0.022 | 2.485 ± 0.028* |
| R. Kidney | | | | | | |
| Absolute | 1.018 ± 0.023 | 1.097 ± 0.032* | 1.076 ± 0.023 | 1.125 ± 0.023** | 1.173 ± 0.020** | 1.162 ± 0.021** |
| Relative | 3.062 ± 0.063 | 3.177 ± 0.043 | 3.072 ± 0.038 | 3.236 ± 0.044* | 3.394 ± 0.044** | 3.737 ± 0.063** |
| Liver | | | | | | |
| Absolute | 10.737 ± 0.370 | 12.334 ± 0.319** | 13.273 ± 0.323** | 13.282 ± 0.238** | 14.186 ± 0.384** | 15.032 ± 0.307** |
| Relative | 32.306 ± 1.125 | 35.734 ± 0.369** | 37.935 ± 0.879** | 38.218 ± 0.464** | 41.013 ± 0.819** | 48.321 ± 0.812** |
| Lung | | | | | | |
| Absolute | 1.382 ± 0.034 | 1.402 ± 0.035 | 1.477 ± 0.064 | 1.393 ± 0.040 | 1.398 ± 0.027 | 1.307 ± 0.038 |
| Relative | 4.165 ± 0.134 | 4.063 ± 0.046 | 4.229 ± 0.202 | 4.007 ± 0.095 | 4.045 ± 0.061 | 4.197 ± 0.092 |
| R. Testis | | | | | | |
| Absolute | 1.421 ± 0.014 | 1.442 ± 0.022 | 1.411 ± 0.028 | 1.444 ± 0.026 | 1.414 ± 0.018 | 0.682 ± 0.078** |
| Relative | 4.279 ± 0.067 | 4.190 ± 0.070 | 4.033 ± 0.073 | 4.158 ± 0.078 | 4.092 ± 0.046 | 2.193 ± 0.250** |
| Thymus | | | | | | |
| Absolute | 0.266 ± 0.014 | 0.242 ± 0.010 | 0.249 ± 0.006 | 0.258 ± 0.010 | 0.263 ± 0.014 | 0.214 ± 0.008** |
| Relative | 0.802 ± 0.046 | 0.702 ± 0.029 | 0.712 ± 0.021 | 0.744 ± 0.035 | 0.760 ± 0.035 | 0.688 ± 0.022 |
| Female | | | | | | |
| Necropsy body weight | 196 ± 3 | 193 ± 3 | 196 ± 2 | 191 ± 3 | 190 ± 3 | 170 ± 2** |
| Heart | | | | | | |
| Absolute | 0.568 ± 0.019 | 0.562 ± 0.008 | 0.565 ± 0.013 | 0.567 ± 0.009 | 0.560 ± 0.008 | 0.509 ± 0.008** |
| Relative | 2.903 ± 0.094 | 2.918 ± 0.043 | 2.883 ± 0.040 | 2.972 ± 0.052 | 2.957 ± 0.038 | 2.994 ± 0.043 |
| R. Kidney | | | | | | |
| Absolute | 0.651 ± 0.010 | 0.665 ± 0.011 | 0.669 ± 0.012 | 0.697 ± 0.013* | 0.734 ± 0.010** | 0.714 ± 0.015** |
| Relative | 3.328 ± 0.046 | 3.450 ± 0.042 | 3.417 ± 0.051 | 3.649 ± 0.049** | 3.876 ± 0.045** | 4.197 ± 0.063** |
| Liver | | | | | | |
| Absolute | 6.099 ± 0.149 | 6.544 ± 0.132* | 7.366 ± 0.117** | 7.479 ± 0.141** | 7.917 ± 0.171** | 8.188 ± 0.156** |
| Relative | 31.158 ± 0.626 | 33.946 ± 0.494** | 37.608 ± 0.306** | 39.141 ± 0.350** | 41.746 ± 0.407** | 48.141 ± 0.716** |
| Lung | | | | | | |
| Absolute | 1.000 ± 0.023 | 0.949 ± 0.022 | 0.999 ± 0.026 | 0.952 ± 0.022 | 0.975 ± 0.028 | 0.903 ± 0.020* |
| Relative | 5.121 ± 0.151 | 4.928 ± 0.118 | 5.096 ± 0.086 | 4.986 ± 0.100 | 5.138 ± 0.096 | 5.312 ± 0.113 |
| Thymus | | | | | | |
| Absolute | 0.218 ± 0.006 | 0.199 ± 0.005 | 0.211 ± 0.007 | 0.207 ± 0.003 | 0.195 ± 0.006** | 0.174 ± 0.003** |
| Relative | 1.113 ± 0.023 | 1.034 ± 0.032 | 1.077 ± 0.032 | 1.085 ± 0.018 | 1.025 ± 0.024 | 1.024 ± 0.017 |

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

** $P \leq 0.01$

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

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|----------------------|----------------|----------------|----------------|-----------------|------------------|------------------|
| n | 5 | 5 | 5 | 5 | 5 | 5 |
| Male | | | | | | |
| Necropsy body weight | 25.2 ± 0.4 | 24.4 ± 0.6 | 25.5 ± 0.4 | 26.4 ± 0.4 | 25.7 ± 0.3 | 24.5 ± 0.4 |
| Heart | | | | | | |
| Absolute | 0.128 ± 0.006 | 0.126 ± 0.004 | 0.126 ± 0.002 | 0.126 ± 0.002 | 0.120 ± 0.004 | 0.114 ± 0.002 |
| Relative | 5.096 ± 0.264 | 5.154 ± 0.078 | 4.957 ± 0.154 | 4.784 ± 0.118 | 4.676 ± 0.207 | 4.653 ± 0.106 |
| R. Kidney | | | | | | |
| Absolute | 0.248 ± 0.011 | 0.242 ± 0.009 | 0.252 ± 0.006 | 0.246 ± 0.004 | 0.252 ± 0.006 | 0.222 ± 0.007 |
| Relative | 9.864 ± 0.462 | 9.896 ± 0.191 | 9.907 ± 0.268 | 9.340 ± 0.207 | 9.810 ± 0.245 | 9.067 ± 0.352 |
| Liver | | | | | | |
| Absolute | 1.308 ± 0.022 | 1.240 ± 0.070 | 1.344 ± 0.032 | 1.420 ± 0.044 | 1.498 ± 0.060* | 1.624 ± 0.048** |
| Relative | 52.092 ± 1.683 | 50.604 ± 1.837 | 52.834 ± 1.441 | 53.929 ± 1.974 | 58.351 ± 2.575 | 66.362 ± 2.626** |
| Lung | | | | | | |
| Absolute | 0.170 ± 0.003 | 0.156 ± 0.005 | 0.174 ± 0.010 | 0.160 ± 0.004 | 0.176 ± 0.005 | 0.156 ± 0.002 |
| Relative | 6.763 ± 0.160 | 6.381 ± 0.124 | 6.834 ± 0.386 | 6.078 ± 0.219 | 6.853 ± 0.224 | 6.373 ± 0.181 |
| R. Testis | | | | | | |
| Absolute | 0.102 ± 0.004 | 0.103 ± 0.002 | 0.095 ± 0.004 | 0.102 ± 0.002 | 0.106 ± 0.001 | 0.096 ± 0.002 |
| Relative | 4.057 ± 0.107 | 4.237 ± 0.094 | 3.737 ± 0.109 | 3.879 ± 0.072 | 4.121 ± 0.094 | 3.916 ± 0.071 |
| Thymus | | | | | | |
| Absolute | 0.048 ± 0.002 | 0.047 ± 0.003 | 0.040 ± 0.006 | 0.043 ± 0.003 | 0.034 ± 0.003** | 0.031 ± 0.002** |
| Relative | 1.896 ± 0.095 | 1.906 ± 0.107 | 1.580 ± 0.238 | 1.646 ± 0.096 | 1.310 ± 0.118** | 1.276 ± 0.091** |
| Female | | | | | | |
| Necropsy body weight | 20.5 ± 0.4 | 20.5 ± 0.3 | 20.6 ± 0.3 | 21.0 ± 0.4 | 21.4 ± 0.3 | 20.9 ± 0.4 |
| Heart | | | | | | |
| Absolute | 0.104 ± 0.002 | 0.102 ± 0.004 | 0.110 ± 0.005 | 0.110 ± 0.003 | 0.112 ± 0.004 | 0.104 ± 0.004 |
| Relative | 5.068 ± 0.132 | 4.988 ± 0.191 | 5.337 ± 0.214 | 5.240 ± 0.135 | 5.229 ± 0.167 | 4.975 ± 0.195 |
| R. Kidney | | | | | | |
| Absolute | 0.154 ± 0.004 | 0.158 ± 0.004 | 0.162 ± 0.007 | 0.160 ± 0.004 | 0.164 ± 0.004 | 0.170 ± 0.005 |
| Relative | 7.501 ± 0.169 | 7.725 ± 0.182 | 7.861 ± 0.265 | 7.616 ± 0.106 | 7.657 ± 0.163 | 8.131 ± 0.261 |
| Liver | | | | | | |
| Absolute | 1.004 ± 0.007 | 0.994 ± 0.022 | 1.026 ± 0.040 | 1.136 ± 0.048* | 1.198 ± 0.049** | 1.302 ± 0.034** |
| Relative | 48.952 ± 1.037 | 48.636 ± 1.410 | 49.802 ± 1.437 | 54.011 ± 1.385* | 55.868 ± 1.761** | 62.200 ± 0.662** |
| Lung | | | | | | |
| Absolute | 0.150 ± 0.006 | 0.156 ± 0.007 | 0.152 ± 0.007 | 0.146 ± 0.002 | 0.152 ± 0.008 | 0.180 ± 0.025 |
| Relative | 7.295 ± 0.212 | 7.622 ± 0.308 | 7.378 ± 0.260 | 6.955 ± 0.076 | 7.097 ± 0.366 | 8.596 ± 1.189 |
| Thymus | | | | | | |
| Absolute | 0.065 ± 0.005 | 0.058 ± 0.002 | 0.056 ± 0.006 | 0.057 ± 0.003 | 0.052 ± 0.003* | 0.047 ± 0.001** |
| Relative | 3.155 ± 0.192 | 2.838 ± 0.119 | 2.720 ± 0.279 | 2.713 ± 0.099 | 2.430 ± 0.150** | 2.229 ± 0.047** |

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

** P≤0.01

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

TABLE G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 125 mg/L | 250 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|----------------------|----------------|----------------|----------------|------------------|------------------|------------------|
| n | 10 | 10 | 10 | 10 | 10 | 10 |
| Male | | | | | | |
| Necropsy body weight | 39.4 ± 1.2 | 40.3 ± 0.7 | 40.9 ± 0.5 | 41.1 ± 0.9 | 38.9 ± 1.0 | 36.2 ± 1.2 |
| Heart | | | | | | |
| Absolute | 0.152 ± 0.002 | 0.165 ± 0.004 | 0.168 ± 0.004 | 0.160 ± 0.007 | 0.145 ± 0.004 | 0.138 ± 0.004* |
| Relative | 3.882 ± 0.114 | 4.101 ± 0.071 | 4.111 ± 0.101 | 3.891 ± 0.135 | 3.744 ± 0.130 | 3.842 ± 0.163 |
| R. Kidney | | | | | | |
| Absolute | 0.297 ± 0.007 | 0.286 ± 0.003 | 0.305 ± 0.006 | 0.297 ± 0.005 | 0.293 ± 0.008 | 0.286 ± 0.004 |
| Relative | 7.588 ± 0.259 | 7.122 ± 0.139 | 7.462 ± 0.149 | 7.251 ± 0.170 | 7.556 ± 0.197 | 7.985 ± 0.305 |
| Liver | | | | | | |
| Absolute | 1.630 ± 0.059 | 1.760 ± 0.048 | 1.795 ± 0.047 | 1.835 ± 0.076* | 1.873 ± 0.065** | 2.097 ± 0.064** |
| Relative | 41.363 ± 0.898 | 43.726 ± 0.896 | 43.863 ± 0.956 | 44.545 ± 1.116 | 48.202 ± 1.418** | 58.280 ± 1.979** |
| Lung | | | | | | |
| Absolute | 0.294 ± 0.011 | 0.303 ± 0.003 | 0.301 ± 0.009 | 0.297 ± 0.013 | 0.286 ± 0.015 | 0.296 ± 0.014 |
| Relative | 7.515 ± 0.348 | 7.549 ± 0.156 | 7.366 ± 0.234 | 7.258 ± 0.340 | 7.394 ± 0.467 | 8.319 ± 0.581 |
| R. Testis | | | | | | |
| Absolute | 0.121 ± 0.002 | 0.121 ± 0.003 | 0.126 ± 0.002 | 0.123 ± 0.001 | 0.118 ± 0.003 | 0.115 ± 0.002 |
| Relative | 3.093 ± 0.108 | 2.981 ± 0.048 | 3.089 ± 0.064 | 2.994 ± 0.073 | 3.052 ± 0.116 | 3.196 ± 0.100 |
| Thymus | | | | | | |
| Absolute | 0.041 ± 0.002 | 0.041 ± 0.002 | 0.042 ± 0.002 | 0.040 ± 0.003 | 0.038 ± 0.002 | 0.035 ± 0.002 |
| Relative | 1.030 ± 0.033 | 1.028 ± 0.065 | 1.071 ± 0.062 | 0.970 ± 0.069 | 0.977 ± 0.056 | 0.975 ± 0.037 |
| Female | | | | | | |
| Necropsy body wt | 30.5 ± 1.0 | 30.6 ± 1.1 | 31.6 ± 0.9 | 31.0 ± 1.1 | 31.0 ± 0.4 | 27.4 ± 0.7 |
| Heart | | | | | | |
| Absolute | 0.123 ± 0.004 | 0.126 ± 0.003 | 0.122 ± 0.004 | 0.123 ± 0.003 | 0.123 ± 0.003 | 0.117 ± 0.002 |
| Relative | 4.049 ± 0.127 | 4.147 ± 0.103 | 3.880 ± 0.140 | 4.021 ± 0.172 | 3.975 ± 0.101 | 4.298 ± 0.111 |
| R. Kidney | | | | | | |
| Absolute | 0.182 ± 0.006 | 0.185 ± 0.003 | 0.189 ± 0.006 | 0.190 ± 0.005 | 0.193 ± 0.006 | 0.191 ± 0.005 |
| Relative | 5.984 ± 0.183 | 6.109 ± 0.190 | 6.004 ± 0.190 | 6.165 ± 0.147 | 6.225 ± 0.165 | 7.034 ± 0.290** |
| Liver | | | | | | |
| Absolute | 1.191 ± 0.050 | 1.236 ± 0.067 | 1.346 ± 0.058 | 1.390 ± 0.051* | 1.551 ± 0.043** | 1.670 ± 0.063** |
| Relative | 39.111 ± 1.371 | 40.284 ± 1.023 | 42.544 ± 1.173 | 45.050 ± 1.465** | 50.031 ± 1.196** | 61.041 ± 1.571** |
| Lung | | | | | | |
| Absolute | 0.213 ± 0.008 | 0.214 ± 0.012 | 0.200 ± 0.013 | 0.240 ± 0.009 | 0.260 ± 0.013* | 0.247 ± 0.013* |
| Relative | 7.052 ± 0.370 | 7.121 ± 0.538 | 6.390 ± 0.443 | 7.822 ± 0.362 | 8.389 ± 0.415* | 9.097 ± 0.540** |
| Thymus | | | | | | |
| Absolute | 0.045 ± 0.002 | 0.039 ± 0.002 | 0.046 ± 0.002 | 0.043 ± 0.003 | 0.049 ± 0.002 | 0.037 ± 0.002 |
| Relative | 1.478 ± 0.075 | 1.292 ± 0.080 | 1.470 ± 0.066 | 1.401 ± 0.070 | 1.589 ± 0.063 | 1.365 ± 0.066 |

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

** P≤0.01

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

APPENDIX H

REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

| | | |
|-----------------|---|------------------|
| TABLE H1 | Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid | H-2 |
| TABLE H2 | Estrous Cycle Characterization for Female Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid | H-2 |
| TABLE H3 | Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid | H-3 |
| TABLE H4 | Estrous Cycle Characterization for Female Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid | H-3 |

TABLE H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--|-----------------|-----------------|-----------------|-------------------|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body weight | 333 ± 5 | 348 ± 4 | 346 ± 6 | 311 ± 3** |
| L. Cauda | 0.1603 ± 0.0062 | 0.1541 ± 0.0053 | 0.1671 ± 0.0064 | 0.1015 ± 0.0069** |
| L. Epididymis | 0.5124 ± 0.0138 | 0.5067 ± 0.0114 | 0.5416 ± 0.0197 | 0.3363 ± 0.0160** |
| L. Testis | 1.5090 ± 0.0174 | 1.5810 ± 0.0194 | 1.5455 ± 0.0198 | 0.7008 ± 0.0706** |
| Spermatid measurements | | | | |
| Spermatid heads (10 ⁷ /g testis) | 122.71 ± 4.36 | 135.36 ± 4.43 | 140.10 ± 5.77 | 26.44 ± 14.75* |
| Spermatid heads (10 ⁷ /testis) | 168.50 ± 6.70 | 193.00 ± 5.26 | 198.13 ± 8.34 | 24.94 ± 14.83* |
| Spermatid heads (10 ⁷ /cauda) | 112.64 ± 7.24 | 102.52 ± 7.29 | 106.67 ± 5.37 | 0.47 ± 0.29** |
| Epididymal spermatozoal measurements | | | | |
| Motility (%) | 75.41 ± 1.14 | 76.10 ± 2.22 | 75.76 ± 1.82 | 13.09 ± 8.80** |
| Concentration (10 ⁷ /g cauda epididymal tissue) | 708 ± 46 | 673 ± 55 | 650 ± 49 | 4 ± 3** |

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

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's (body weights), Williams' (tissue weights), or Shirley's (epididymal spermatozoal measurements) test

^a Data are presented as mean ± standard error.

TABLE H2
Estrous Cycle Characterization for Female Rats in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|-----------------------------|-------------|-------------|-------------|--------------|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt | 196 ± 3 | 192 ± 3 | 190 ± 3 | 170 ± 2** |
| Estrous cycle length (days) | 4.55 ± 0.16 | 4.90 ± 0.07 | 4.85 ± 0.17 | 5.00 ± 0.00* |

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test (estrous cycle length)

** Significantly different ($P \leq 0.01$) from the control group by Williams' test (body weight)

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error.

Please provide data for estrous stages.

TABLE H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|--|-----------------|------------------|-----------------|-----------------|
| n | 10 | 10 | 10 | 10 |
| Weights (g) | | | | |
| Necropsy body weight | 39.4 ± 1.2 | 41.1 ± 0.9 | 38.9 ± 1.0 | 36.2 ± 1.2 |
| L. Cauda | 0.0277 ± 0.0011 | 0.274 ± 0.0011 | 0.275 ± 0.0013 | 0.260 ± 0.0014 |
| L. Epididymis | 0.0585 ± 0.0020 | 0.0615 ± 0.0034 | 0.593 ± 0.0019 | 0.0553 ± 0.0028 |
| L. Testis | 0.1188 ± 0.0022 | 0.1259 ± 0.0018* | 0.1191 ± 0.0026 | 0.1144 ± 0.0014 |
| Spermatid measurements | | | | |
| Spermatid heads (10 ⁷ /g testis) | 201.47 ± 5.97 | 173.67 ± 7.03* | 188.94 ± 5.29 | 190.43 ± 8.79 |
| Spermatid heads (10 ⁷ /testis) | 22.31 ± 0.74 | 20.14 ± 0.75 | 20.65 ± 0.71 | 20.23 ± 0.94 |
| Spermatid heads (10 ⁷ /cauda) | 21.66 ± 2.69 | 21.49 ± 1.16 | 21.56 ± 1.20 | 19.79 ± 2.18 |
| Epididymal spermatozoal measurements | | | | |
| Motility (%) | 69.51 ± 1.88 | 70.50 ± 2.43 | 69.47 ± 2.19 | 63.80 ± 2.66 |
| Concentration (10 ⁷ /g cauda epididymal tissue) | 802 ± 102 | 791 ± 47 | 804 ± 66 | 806 ± 132 |

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's (tissue weights) or Dunn's (spermatid measurements) test

^a Data are presented as mean ± standard error. Differences from the control group are not significant by Dunn's test (epididymal spermatozoal measurements).

TABLE H4
Estrous Cycle Characterization for Female Mice in the 3-Month Drinking Water Study of Dibromoacetic Acid^a

| | 0 mg/L | 500 mg/L | 1,000 mg/L | 2,000 mg/L |
|-----------------------------|-------------|-------------|-------------|-------------|
| n | 10 | 10 | 10 | 10 |
| Necropsy body wt | 31 ± 1 | 31 ± 1 | 31 ± 0 | 27 ± 1* |
| Estrous cycle length (days) | 4.67 ± 0.50 | 5.09 ± 0.75 | 4.28 ± 0.25 | 4.35 ± 0.21 |

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

^a Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test.

Please provide data for estrous stages.

APPENDIX I

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

| | |
|--|-------------|
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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF DIBROMOACETIC ACID

Dibromoacetic acid was obtained from Fluka (Buchs, Switzerland) in one lot (46019/1 55196). Lot 46019/1 55196 was used in the 2-week, 3-month and 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory at Battelle (Columbus, OH) and by the study laboratory at Southern Research Institute (Birmingham, AL). Karl Fischer titration, elemental analysis, and melting point determination were performed by Galbraith Laboratories, Inc. (Knoxville, TN).

The chemical, a clumped white powder or moist white crystalline solid, was identified as dibromoacetic acid by the study laboratory using infrared spectroscopy and by the analytical chemistry laboratory using infrared, ultraviolet/visible, proton and carbon-13 nuclear magnetic resonance spectroscopy. In addition, the study laboratory made a comparison of infrared and proton nuclear magnetic resonance spectra to that of a frozen reference from the same lot and a reference sample manufactured by Aldrich Chemical Co. (Milwaukee, WI). All spectra were consistent with the literature spectra for dibromoacetic acid (*Aldrich*, 1985, 1992). Representative infrared and nuclear magnetic resonance spectra are presented in Figures I1, I2, and I3.

The purity of lot 46019/1 55196 was determined by the analytical laboratory using functional group titration, ion chromatography, and high performance liquid chromatography (HPLC) and by the study laboratory using HPLC. HPLC (Waters, Milford, MA) was performed with a Prodigy 5 ODS-3, 150 mm × 4.6 mm, (5 μm particle size) column (Phenomenex, Torrance, CA), an Inertsil ODS-2 guard cartridge, and an ultraviolet detector at 220 nm; mobile phases A: 15 mM H₃PO₄ and B: 30 mM H₃PO₄:acetonitrile (1:1), at a flow rate of 1 mL/minute; beginning with 100% A linear to 100% B in 20 minutes (19 minutes for stability analysis; 5 minutes for standard addition), held 15 minutes, then linear to 100% A in 5 minutes, held 25 minutes (20 minutes for stability analysis; 10 minutes for standard addition). Ion chromatography (IC) (Dionex, Sunnyvale, CA) was performed using an Ionpac As11 column (250 mm × 4 mm), an Ionpac As11 guard column (50 mm), mobile phases of A: 4mM NaOH and B: 32 mM NaOH, with a flow rate of 1.5 mL/minute, and a suppressed conductivity detector. The mobile phase gradient was held at 100% A for 9.9 minutes, then linear to 100% B in 6 seconds, held 13.9 minutes, then linear to 100% A in 6 seconds, and held for 2 minutes.

Karl Fischer titration indicated 0.27% water. Elemental analyses for carbon, hydrogen, and bromine were in agreement with the theoretical values for dibromoacetic acid. The melting point determination was slightly higher (38.6° C) than that given by the manufacturer's certificate of analysis and Materials Safety Data Sheet (32° to 38° C); no reference was found in the literature. Functional group titration indicated a purity of greater than 100%, which was consistent with the manufacturer's certificate of analysis. IC indicated one major peak and two impurities with a combined area of 3.4% (3.2% and 0.27%). Using HPLC and standard addition, the 3.2% impurity was found to have the same retention time as monobromoacetic acid (MBA); comparison with HPLC results (0.4%) suggest that IC results may overestimate the amount of MBA. HPLC indicated one major peak and one impurity with a relative peak area of 0.34%. The overall purity was determined to be greater than 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using HPLC. These studies indicated that dibromoacetic acid was stable for up to 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature, protected from light, in sealed amber glass containers.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared once during the 2-week studies, four times during the 3-month studies, and approximately every 2 weeks throughout the 2-year studies. The dose formulations were prepared by mixing dibromoacetic acid with tap water (Table I1). Formulations were adjusted to pH 5 with 0.1 N sodium hydroxide and stored in sealed opaque glass or Nalgene[®] containers at 5° C for at least 42 days.

Homogeneity studies of 125 and 2,000 mg/mL formulations were conducted by the study laboratory and stability studies of a 10 µg/mL formulation (pH 5) were conducted by the analytical study laboratory using IC by the system previously described except for a change to an isocratic mobile phase of 4mM NaOH for 25 minutes. Homogeneity was confirmed, and stability was confirmed for at least 42 days when stored at 5° C in sealed opaque glass or Nalgene[®] containers, and at animal room conditions for at least 3 hours.

Periodic analyses of the dose formulations of dibromoacetic acid were conducted by the study laboratory using IC according to the previously described method. During the 2-week studies, the dose formulations were analyzed once; all five of the dose formulations for rats and mice were within 10% of the target concentrations, with no value greater than 10% of the target concentration (Table I2). Animal room samples of these dose formulations were also analyzed; all 23 samples analyzed were within 10% of the target concentrations. During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 20 of the dose formulations used for rats and mice were within 10% of the target concentrations, with no value greater than 8% of the target concentration (Table I3). Animal room samples of these dose formulations were also analyzed; 21 of 25 samples were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 7 to 8 weeks (Table I4). All 34 of the dose formulations analyzed were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; 23 of 24 samples were within 10% of the target concentrations.

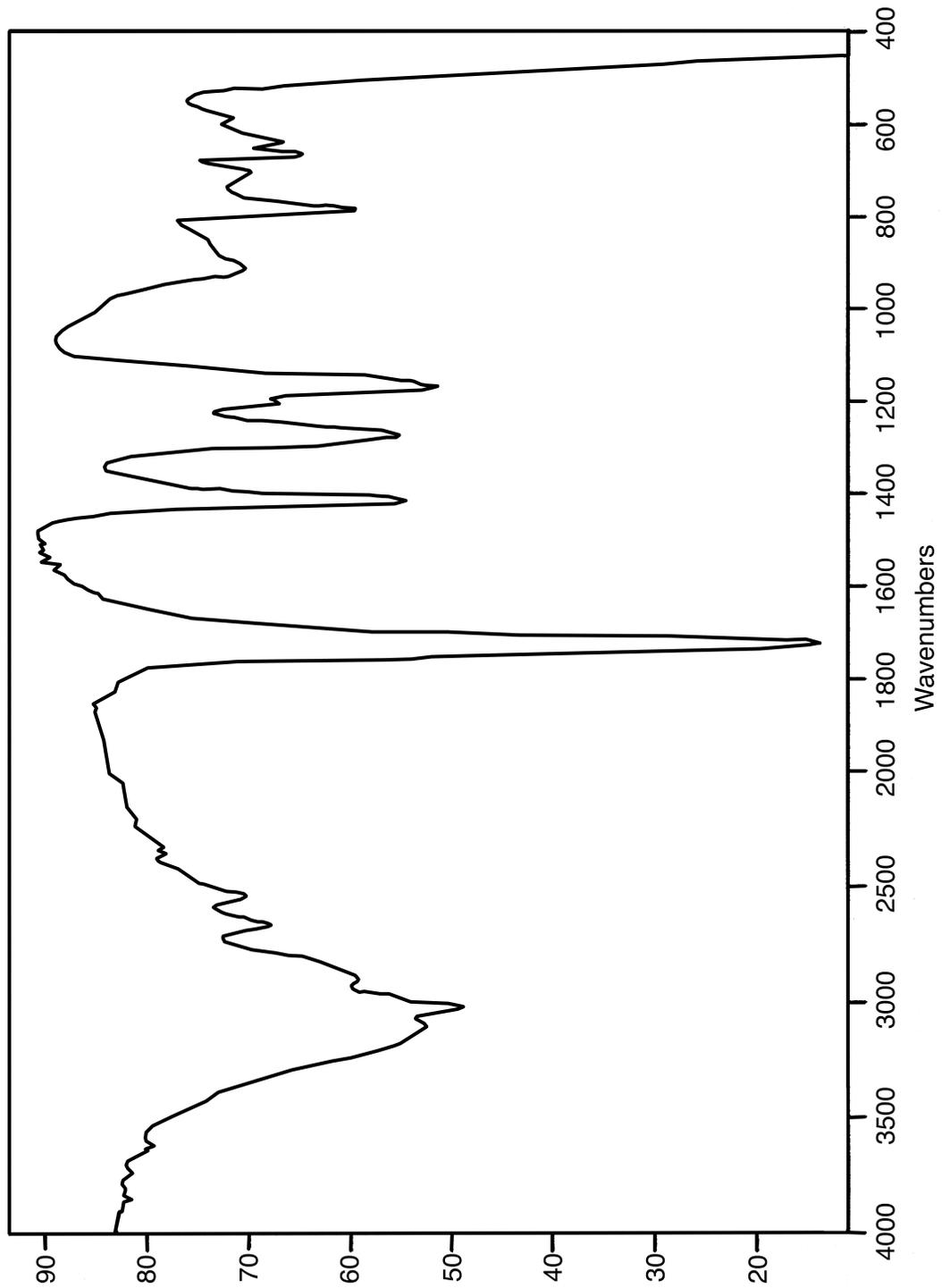


FIGURE II
Infrared Absorption Spectrum of Dibromoacetic Acid

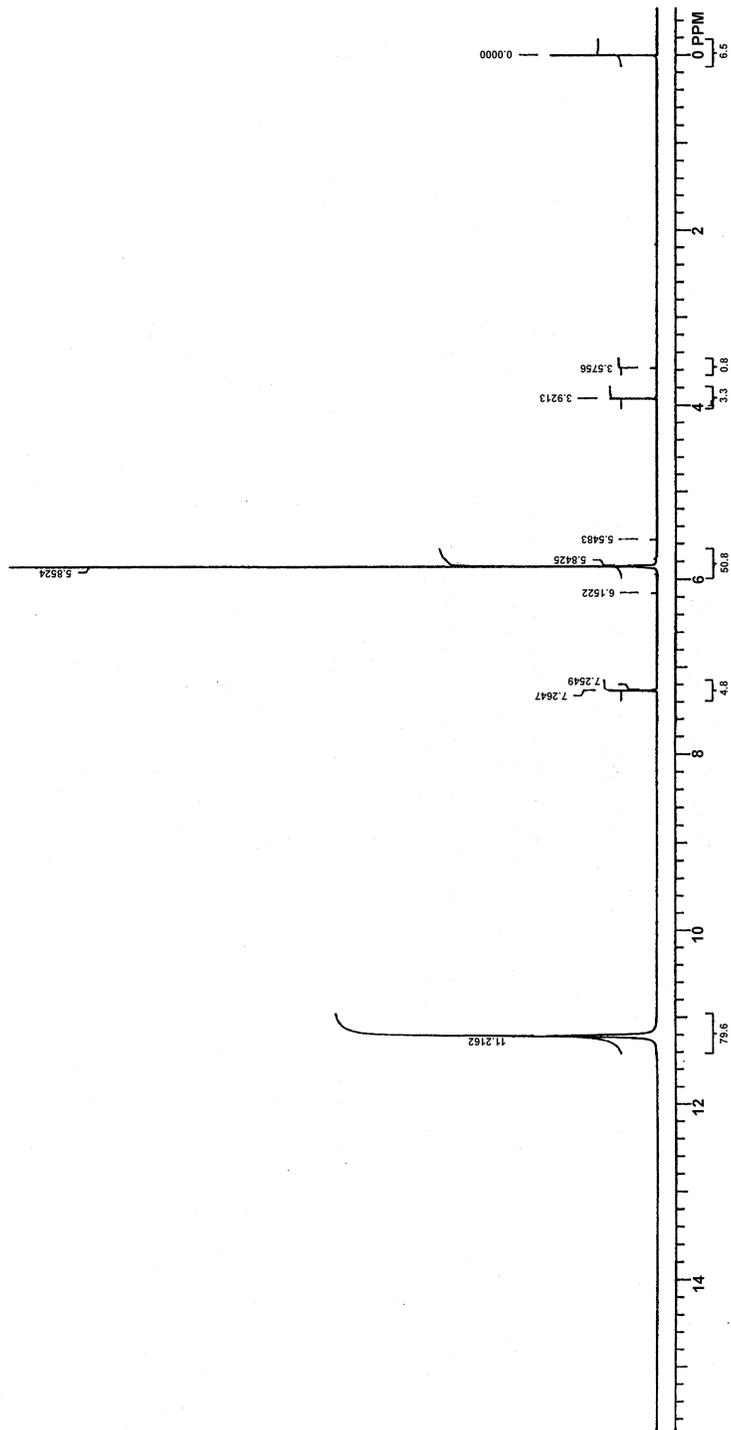


FIGURE I2
Proton Nuclear Magnetic Resonance Spectrum of Dibromoacetic Acid

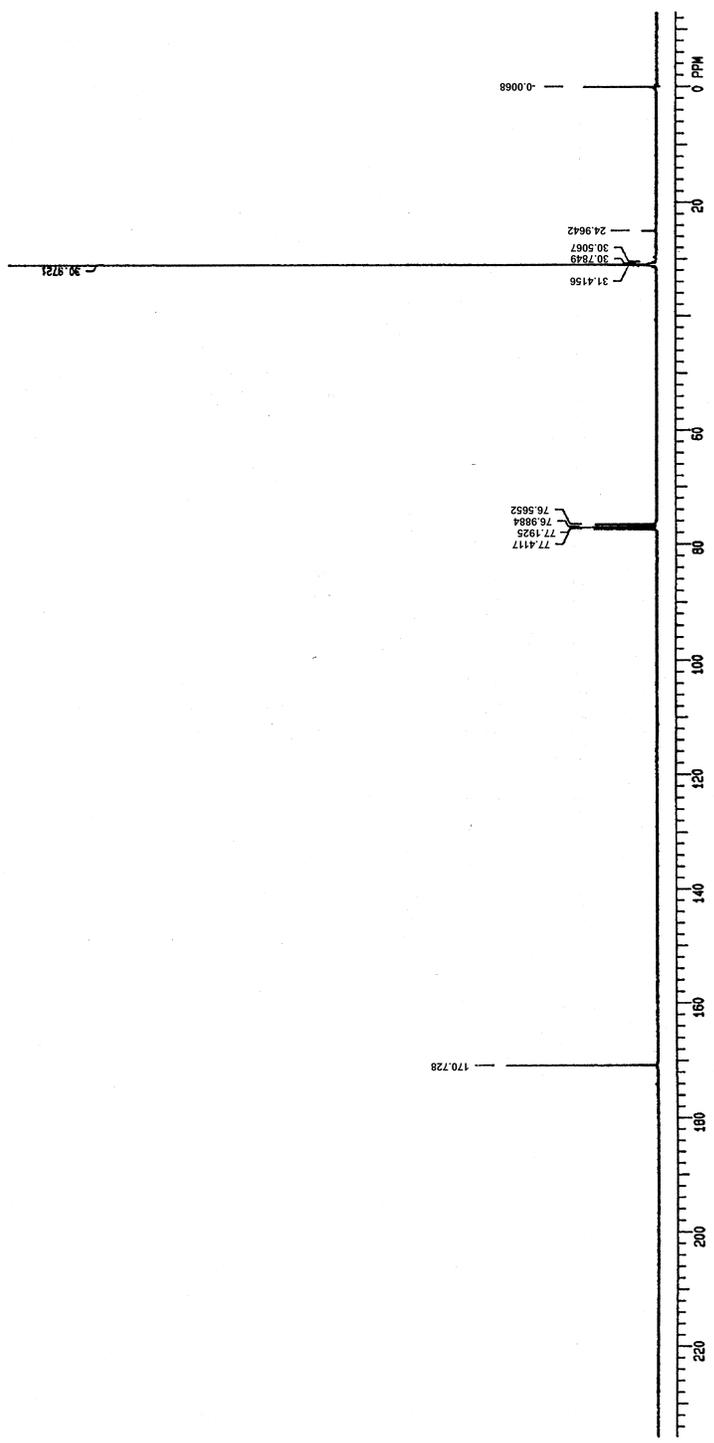


FIGURE I3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Dibromoacetic Acid

TABLE II
Preparation and Storage of Dose Formulations in the Drinking Water Studies of Dibromoacetic Acid

Preparation

A premix solution was prepared by adding the appropriate amount of dibromoacetic acid and water to a flask and mixing with a stir bar until dibromoacetic acid was in solution. The premix was added to a partially filled mixing tank, the premix container rinsed (5 times), and the rinsate added to the tank and mixed for up to 2 minutes. The tank was filled to the desired volume and mixed for up to 5 minutes. While mixing, the pH of the solution was adjusted to pH 5 by the addition of 1 N sodium hydroxide or hydrochloric acid as necessary, after which the formulation was mixed for at least 10 minutes.

Chemical Lot Number

46019/1 55196

Maximum Storage Time

42 days

Storage Conditions

Stored in Nalgene® containers at 5° C

Study Laboratory

Southern Research Institute (Birmingham, AL)

TABLE I2
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 2-Week Drinking Water Studies of Dibromoacetic Acid

| Date Prepared | Date Analyzed | Target Concentration (mg/L) | Determined Concentration ^a (mg/L) | Difference from Target (%) |
|----------------------------|----------------------|-----------------------------|--|----------------------------|
| Rats and Mice | | | | |
| November 13, 1998 | November 16-18, 1998 | 125 | 137 | +10 |
| | | 250 | 267 | +7 |
| | | 500 | 504 | +1 |
| | | 1,000 | 972 | -3 |
| | | 2,000 | 2,038 | +2 |
| Animal Room Samples | | | | |
| Rats | | | | |
| November 13, 1998 | December 1-2, 1998 | 125 | 129 | +3 |
| | | 250 | 247 | -1 |
| | | 500 | 522 | +4 |
| | | 1,000 | 1,035 | +4 |
| | | 2,000 | 2,068 | +3 |
| Mice | | | | |
| November 13, 1998 | December 1-2, 1998 | 125 | 119 | -5 |
| | | 125 | 130 | +4 |
| | | 125 | 112 | -10 |
| | | 125 | 118 | -6 |
| | | 250 | 261 | +4 |
| | | 250 | 246 | -2 |
| | | 500 | 532 | +6 |
| | | 500 | 507 | +1 |
| | | 500 | 470 | -6 |
| | | 500 | 479 | -4 |
| | | 1,000 | 1,066 | +7 |
| | | 1,000 | 964 | -4 |
| | | 1,000 | 1,008 | +1 |
| | | 1,000 | 912 | -9 |
| | | 2,000 | 1,900 | -5 |
| 2,000 | 1,953 | -2 | | |
| 2,000 | 2,003 | -2 | | |
| 2,000 | 2,075 | +4 | | |

^a Results of duplicate analyses

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 3-Month Drinking Water Studies of Dibromoacetic Acid

| Date Prepared | Date Analyzed | Target Concentration (mg/L) | Determined Concentration ^a (mg/L) | Difference from Target (%) |
|----------------------------|----------------------|-----------------------------|--|----------------------------|
| Rats and Mice | | | | |
| February 10, 1999 | February 11-12, 1999 | 125 | 119 | -5 |
| | | 250 | 248 | -1 |
| | | 500 | 489 | -2 |
| | | 1,000 | 973 | -3 |
| | | 2,000 | 1,841 | -8 |
| February 11, 1999 | February 11-12, 1999 | 125 | 121 | -3 |
| | | 250 | 234 | -6 |
| | | 500 | 493 | -1 |
| | | 1,000 | 1,033 | +3 |
| | | 2,000 | 1,889 | -6 |
| March 10, 1999 | March 11-12, 1999 | 125 | 141 ^b | +12 |
| | | 250 | 279 ^b | +12 |
| | | 500 | 546 | +9 |
| | | 1,000 | 1,059 ^b | +6 |
| | | 2,000 | 2,271 ^b | +14 |
| March 12, 1999 | March 12-13, 1999 | 125 | 124 | -1 |
| | | 250 | 249 | 0 |
| | | 2,000 | 1,897 | -5 |
| May 5, 1999 | May 5-8, 1999 | 125 | 120 | -4 |
| | | 250 | 243 | -3 |
| | | 500 | 491 | -2 |
| | | 1,000 | 967 | -3 |
| | | 2,000 | 1,935 | -3 |
| Animal Room Samples | | | | |
| Rats | | | | |
| February 10, 1999 | March 16-18, 1999 | 125 | 106 | -15 |
| | | 250 | 243 | -3 |
| | | 500 | 480 | -4 |
| | | 1,000 | 947 | -5 |
| | | 2,000 | 1,877 | -6 |
| March 10, 1999 | April 13-14, 1999 | 500 | 554 | +11 |
| | | 1,000 | 1,128 | +13 |
| March 12, 1999 | April 13-14, 1999 | 125 | 136 | +9 |
| | | 250 | 280 | +12 |
| | | 2000 | 2,095 | +5 |
| May 5, 1999 | May 19-20, 1999 | 125 | 129 | +3 |
| | | 250 | 247 | -1 |
| | | 500 | 488 | -2 |
| | | 1,000 | 1,060 | +6 |
| | | 2,000 | 1,993 | 0 |

TABLE I3
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 3-Month Drinking Water Studies of Dibromoacetic Acid

| Date Prepared | Date Analyzed | Target Concentration (mg/L) | Determined Concentration ^a (mg/L) | Difference from Target (%) |
|--|-------------------|-----------------------------|--|----------------------------|
| Animal Room Samples (continued) | | | | |
| Mice | | | | |
| February 10, 1999 | March 16-18, 1999 | 125 | 118 | -6 |
| | | 250 | 240 | -4 |
| | | 500 | 485 | -3 |
| | | 1,000 | 989 | -1 |
| | | 2,000 | 2,009 | 0 |
| March 10, 1999 | April 13-14, 1999 | 500 | 516 | +3 |
| | | 1,000 | 921 | -8 |
| March 12, 1999 | April 13-14, 1999 | 125 | 123 | -2 |
| | | 250 | 243 | -3 |
| | | 2000 | 1,889 | -6 |
| May 5, 1999 | May 19-20, 1999 | 125 | 122 | -2 |
| | | 250 | 246 | -1 |
| | | 500 | 500 | 0 |
| | | 1,000 | 999 | 0 |
| | | 2,000 | 1999 | 0 |

- ^a Results of duplicate analyses
^b Not used in study
^c Results of remix

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 2-Year Drinking Water Studies of Dibromoacetic Acid

| Date Prepared | Date Analyzed | Target Concentration (mg/L) | Determined Concentration ^a (mg/L) | Difference from Target (%) |
|----------------------------|----------------------|-----------------------------|--|----------------------------|
| Rats and Mice | | | | |
| February 15, 2000 | February 15-16, 2000 | 50 | 50 | 0 |
| | | 500 | 514 | +3 |
| | | 1,000 | 1,009 | +1 |
| April 11, 2000 | April 13-14, 2000 | 50 | 50 | 0 |
| | | 500 | 486 | -3 |
| | | 1,000 | 981 | -2 |
| June 20, 2000 | June 21-22, 2000 | 50 | 55 | +10 ^b |
| | | 500 | 551 | +10 ^b |
| | | 1,000 | 1,092 | +9 |
| June 26, 2000 ^c | June 27, 2000 | 500 | 507 | +1 ^c |
| August 29, 2000 | August 30-31, 2000 | 50 | 50 | 0 |
| | | 500 | 486 | -3 |
| | | 1,000 | 953 | -5 |
| November 7, 2000 | November 8-9, 2000 | 50 | 49 | -2 |
| | | 500 | 493 | -1 |
| | | 1,000 | 1,016 | +2 |
| January 17, 2001 | January 18-20, 2001 | 50 | 50 | 0 |
| | | 500 | 499 | 0 |
| | | 1,000 | 970 | -3 |
| March 27, 2001 | March 28-29, 2001 | 50 | 48 | -4 |
| | | 500 | 494 | -1 |
| | | 1,000 | 982 | -2 |
| June 6, 2001 | June 7-8, 2001 | 50 | 50 | 0 |
| | | 500 | 505 | +1 |
| | | 1,000 | 1,030 | +3 |
| August 15, 2001 | August 16, 2001 | 50 | 50 | 0 |
| | | 500 | 503 | +1 |
| | | 1,000 | 976 | -2 |
| October 24, 2001 | October 25-26, 2001 | 50 | 50 | -1 |
| | | 500 | 506 | +1 |
| | | 1,000 | 1,015 | +2 |
| January 2, 2002 | January 4-8, 2002 | 50 | 50 | 0 |
| | | 500 | 501 | 0 |
| | | 1,000 | 1,024 | +2 |

TABLE I4
Results of Analyses of Dose Formulations Administered to Rats and Mice
in the 2-Year Drinking Water Studies of Dibromoacetic Acid

| Date Prepared | Date Analyzed | Target Concentration (mg/L) | Determined Concentration ^a (mg/L) | Difference from Target (%) |
|----------------------------|-----------------------|-----------------------------|--|----------------------------|
| Animal Room Samples | | | | |
| Rats | | | | |
| February 15, 2000 | March 27-28, 2000 | 50 | 52 | +4 |
| | | 500 | 507 | +1 |
| | | 1,000 | 1,027 | +3 |
| August 29, 2000 | September 25-26, 2000 | 50 | 48 | -4 |
| | | 500 | 498 | 0 |
| | | 1,000 | 985 | -2 |
| March 27, 2001 | April 17-18, 2001 | 50 | 51 | +2 |
| | | 500 | 512 | +2 |
| | | 1,000 | 1,105 | +11 |
| October 24, 2001 | November 19-20, 2001 | 50 | 49 | -2 |
| | | 500 | 503 | +1 |
| | | 1,000 | 991 | -1 |
| Mice | | | | |
| February 15, 2000 | March 27-28, 2000 | 50 | 51 | +2 |
| | | 500 | 499 | 0 |
| | | 1,000 | 1,049 | +5 |
| August 29, 2000 | September 25-26, 2000 | 50 | 49 | -2 |
| | | 500 | 488 | -2 |
| | | 1,000 | 998 | 0 |
| March 27, 2001 | April 17-18, 2001 | 50 | 52 | +4 |
| | | 500 | 549 | +10 |
| | | 1,000 | 1,082 | +8 |
| October 24, 2001 | November 19-20, 2001 | 50 | 49 | -2 |
| | | 500 | 495 | -1 |
| | | 1,000 | 974 | -3 |

^a Results of duplicate analyses

^b Not used

^c Results of remix

APPENDIX J
WATER AND COMPOUND CONSUMPTION
IN THE 2-YEAR DRINKING WATER STUDIES
OF DIBROMOACETIC ACID

| | | |
|-----------------|---|------------|
| TABLE J1 | Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | J-2 |
| TABLE J2 | Water and Compound Consumption by Female Rats in the 2-Year Drinking Water Study of Dibromoacetic Acid | J-3 |
| TABLE J3 | Water and Compound Consumption by Male Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid | J-4 |
| TABLE J4 | Water and Compound Consumption by Female Mice in the 2-Year Drinking Water Study of Dibromoacetic Acid | J-5 |

TABLE J1
Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| Week | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 mg/L | | |
|-----------------------|-------------------------------|-----------------------|------------------|-----------------------|------------------------------|------------------|-----------------------|-----------------|------------------|-----------------------|-----------------|
| | 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) |
| 1 | 15.8 | 108 | 16.4 | 110 | 8 | 16.7 | 110 | 76 | 17.1 | 108 | 158 |
| 2 | 18.0 | 140 | 17.9 | 143 | 6 | 17.9 | 145 | 62 | 18.8 | 142 | 132 |
| 3 | 18.6 | 174 | 18.2 | 173 | 5 | 18.2 | 179 | 51 | 18.7 | 178 | 106 |
| 4 | 18.4 | 203 | 18.1 | 205 | 4 | 18.2 | 206 | 44 | 18.2 | 206 | 88 |
| 5 | 19.2 | 227 | 18.9 | 227 | 4 | 18.3 | 229 | 40 | 18.3 | 230 | 80 |
| 6 | 18.6 | 254 | 18.3 | 253 | 4 | 18.2 | 255 | 36 | 18.2 | 255 | 71 |
| 7 | 18.7 | 271 | 18.3 | 268 | 3 | 17.8 | 268 | 33 | 17.9 | 272 | 66 |
| 8 | 18.3 | 286 | 17.9 | 283 | 3 | 17.0 | 283 | 30 | 17.4 | 285 | 61 |
| 9 | 18.1 | 303 | 18.8 | 296 | 3 | 17.8 | 296 | 30 | 17.0 | 299 | 57 |
| 10 | 18.2 | 319 | 17.7 | 314 | 3 | 17.2 | 311 | 28 | 16.8 | 313 | 54 |
| 11 | 17.3 | 325 | 17.3 | 319 | 3 | 16.5 | 314 | 26 | 16.4 | 316 | 52 |
| 12 | 17.0 | 337 | 17.2 | 329 | 3 | 16.2 | 323 | 25 | 15.7 | 325 | 48 |
| 13 | 16.8 | 350 | 17.4 | 338 | 3 | 16.3 | 330 | 25 | 16.0 | 331 | 48 |
| 17 | 17.6 | 374 | 17.6 | 368 | 2 | 16.4 | 360 | 23 | 16.7 | 357 | 47 |
| 21 | 15.8 | 398 | 16.1 | 392 | 2 | 15.4 | 382 | 20 | 15.3 | 377 | 41 |
| 25 | 16.7 | 418 | 17.3 | 413 | 2 | 17.0 | 401 | 21 | 16.0 | 398 | 40 |
| 29 | 16.3 | 434 | 16.5 | 430 | 2 | 16.0 | 416 | 19 | 15.6 | 413 | 38 |
| 33 | 15.9 | 452 | 15.6 | 445 | 2 | 14.8 | 432 | 17 | 15.0 | 427 | 35 |
| 37 | 15.6 | 467 | 15.3 | 458 | 2 | 15.2 | 442 | 17 | 14.4 | 438 | 33 |
| 41 | 16.4 | 473 | 16.2 | 467 | 2 | 15.9 | 452 | 18 | 15.4 | 445 | 35 |
| 45 | 15.9 | 484 | 15.8 | 475 | 2 | 15.6 | 462 | 17 | 14.8 | 452 | 33 |
| 49 | 16.4 | 494 | 16.7 | 484 | 2 | 15.7 | 468 | 17 | 15.2 | 457 | 33 |
| 53 | 15.3 | 503 | 15.4 | 491 | 2 | 15.3 | 475 | 16 | 14.6 | 462 | 32 |
| 57 | 18.7 | 509 | 18.9 | 498 | 2 | 18.0 | 483 | 19 | 16.9 | 467 | 36 |
| 61 | 16.7 | 516 | 17.0 | 501 | 2 | 16.0 | 485 | 17 | 14.5 | 467 | 31 |
| 65 | 15.4 | 519 | 15.3 | 501 | 2 | 14.9 | 485 | 15 | 13.9 | 462 | 30 |
| 69 | 15.7 | 522 | 15.2 | 507 | 2 | 14.8 | 489 | 15 | 13.9 | 467 | 30 |
| 73 | 16.3 | 523 | 15.5 | 504 | 2 | 15.2 | 491 | 16 | 14.0 | 469 | 30 |
| 77 | 16.1 | 522 | 16.5 | 508 | 2 | 16.6 | 486 | 17 | 14.0 | 462 | 30 |
| 81 | 16.1 | 522 | 16.8 | 509 | 2 | 15.2 | 487 | 16 | 14.0 | 457 | 31 |
| 85 | 17.1 | 512 | 17.7 | 506 | 2 | 16.8 | 474 | 18 | 15.0 | 449 | 33 |
| 89 | 18.3 | 516 | 18.7 | 503 | 2 | 18.0 | 479 | 19 | 16.0 | 441 | 36 |
| 93 | 17.5 | 521 | 17.1 | 492 | 2 | 15.9 | 477 | 17 | 14.3 | 450 | 32 |
| 97 | 16.7 | 515 | 16.8 | 496 | 2 | 16.3 | 463 | 18 | 15.0 | 445 | 34 |
| 101 | 18.1 | 509 | 18.7 | 504 | 2 | 17.1 | 457 | 19 | 14.9 | 435 | 34 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 17.9 | 254 | 17.9 | 251 | 4 | 17.4 | 250 | 39 | 17.4 | 251 | 79 |
| 14-52 | 16.3 | 444 | 16.3 | 437 | 2 | 15.8 | 424 | 19 | 15.4 | 418 | 37 |
| 53-101 | 16.8 | 516 | 16.9 | 502 | 2 | 16.2 | 479 | 17 | 14.7 | 456 | 32 |

^a Grams of water consumed per animal per day

^b Milligrams of dibromoacetic acid consumed per kilogram body weight per day

TABLE J2
Water and Compound Consumption by Female Rats in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| Week | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 mg/L | | |
|-----------------------|-------------------------------|-----------------------|------------------|-----------------------|------------------------------|------------------|-----------------------|-----------------|------------------|-----------------------|-----------------|
| | 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) |
| 1 | 12.4 | 92 | 12.3 | 92 | 7 | 12.8 | 92 | 69 | 13.3 | 95 | 140 |
| 2 | 13.3 | 112 | 13.1 | 114 | 6 | 13.4 | 116 | 58 | 13.7 | 119 | 116 |
| 3 | 13.1 | 126 | 12.6 | 126 | 5 | 12.8 | 130 | 49 | 12.6 | 132 | 96 |
| 4 | 13.3 | 140 | 12.1 | 140 | 4 | 13.0 | 145 | 45 | 12.1 | 147 | 82 |
| 5 | 13.4 | 152 | 13.2 | 150 | 4 | 13.1 | 154 | 43 | 12.8 | 156 | 82 |
| 6 | 13.8 | 160 | 12.7 | 159 | 4 | 13.0 | 163 | 40 | 12.5 | 163 | 77 |
| 7 | 13.1 | 167 | 12.5 | 166 | 4 | 12.6 | 170 | 37 | 12.5 | 171 | 73 |
| 8 | 13.3 | 171 | 12.5 | 170 | 4 | 12.1 | 174 | 35 | 11.8 | 173 | 68 |
| 9 | 12.3 | 176 | 12.8 | 174 | 4 | 12.3 | 178 | 35 | 12.0 | 179 | 67 |
| 10 | 12.4 | 181 | 11.7 | 181 | 3 | 11.4 | 184 | 31 | 11.7 | 183 | 64 |
| 11 | 11.9 | 185 | 11.3 | 183 | 3 | 11.1 | 184 | 30 | 10.8 | 184 | 59 |
| 12 | 11.9 | 190 | 11.1 | 187 | 3 | 10.9 | 189 | 29 | 10.7 | 189 | 57 |
| 13 | 11.3 | 192 | 11.3 | 189 | 3 | 10.7 | 190 | 28 | 10.0 | 189 | 53 |
| 17 | 12.1 | 207 | 11.9 | 204 | 3 | 11.3 | 205 | 28 | 10.9 | 207 | 52 |
| 21 | 10.8 | 219 | 10.5 | 219 | 2 | 9.7 | 219 | 22 | 9.3 | 215 | 43 |
| 25 | 11.6 | 220 | 11.6 | 218 | 3 | 10.8 | 220 | 25 | 9.9 | 218 | 46 |
| 29 | 11.4 | 229 | 11.2 | 225 | 3 | 9.9 | 228 | 22 | 9.9 | 225 | 44 |
| 33 | 10.9 | 239 | 10.8 | 235 | 2 | 10.1 | 236 | 21 | 9.5 | 236 | 40 |
| 37 | 11.0 | 244 | 10.6 | 242 | 2 | 9.5 | 242 | 20 | 8.8 | 241 | 37 |
| 41 | 11.1 | 251 | 10.9 | 251 | 2 | 10.4 | 250 | 21 | 9.8 | 243 | 40 |
| 45 | 10.9 | 258 | 10.8 | 257 | 2 | 10.4 | 252 | 21 | 9.8 | 249 | 39 |
| 49 | 11.6 | 265 | 11.4 | 265 | 2 | 10.7 | 259 | 21 | 10.1 | 254 | 40 |
| 53 | 10.9 | 276 | 10.9 | 276 | 2 | 10.1 | 272 | 21 | 9.6 | 260 | 37 |
| 57 | 13.9 | 287 | 13.9 | 286 | 2 | 13.0 | 278 | 23 | 12.0 | 268 | 45 |
| 61 | 11.8 | 294 | 11.9 | 295 | 2 | 11.6 | 288 | 20 | 10.2 | 272 | 38 |
| 65 | 11.4 | 306 | 10.6 | 305 | 2 | 10.2 | 292 | 18 | 10.0 | 281 | 36 |
| 69 | 11.6 | 312 | 11.3 | 313 | 2 | 11.2 | 298 | 19 | 10.4 | 288 | 36 |
| 73 | 12.2 | 317 | 11.4 | 319 | 2 | 11.2 | 307 | 18 | 10.1 | 292 | 35 |
| 77 | 13.0 | 326 | 12.1 | 324 | 2 | 11.7 | 316 | 19 | 11.5 | 294 | 39 |
| 81 | 13.1 | 332 | 12.9 | 329 | 2 | 12.3 | 317 | 19 | 10.8 | 296 | 36 |
| 85 | 14.0 | 334 | 14.1 | 333 | 2 | 13.4 | 316 | 21 | 11.8 | 301 | 39 |
| 89 | 15.5 | 342 | 15.4 | 338 | 2 | 14.0 | 324 | 22 | 12.8 | 304 | 42 |
| 93 | 13.8 | 346 | 13.7 | 347 | 2 | 12.6 | 326 | 19 | 10.7 | 307 | 35 |
| 97 | 14.2 | 350 | 12.7 | 347 | 2 | 13.6 | 332 | 21 | 10.8 | 303 | 36 |
| 101 | 15.8 | 351 | 14.1 | 340 | 2 | 14.3 | 336 | 21 | 11.7 | 306 | 38 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 12.7 | 157 | 12.2 | 156 | 4 | 12.2 | 159 | 41 | 12.0 | 160 | 80 |
| 14-52 | 11.3 | 237 | 11.1 | 235 | 2 | 10.3 | 235 | 22 | 9.8 | 232 | 42 |
| 53-101 | 13.2 | 321 | 12.7 | 319 | 2 | 12.3 | 308 | 20 | 11.0 | 290 | 38 |

^a Grams of water consumed per animal per day

^b Milligrams of dibromoacetic acid consumed per kilogram body weight per day

TABLE J3
Water and Compound Consumption by Male Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| Week | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 mg/L | | |
|-----------------------|-------------------------------|-----------------------|------------------|-----------------------|------------------------------|------------------|-----------------------|-----------------|------------------|-----------------------|-----------------|
| | 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) |
| 1 | 3.8 | 21.9 | 4.1 | 22.1 | 9 | 4.1 | 22.4 | 92 | 4.0 | 22.5 | 177 |
| 2 | 4.0 | 24.2 | 4.1 | 24.4 | 8 | 4.0 | 24.9 | 80 | 4.0 | 24.7 | 162 |
| 3 | 4.2 | 25.7 | 4.2 | 25.9 | 8 | 3.9 | 26.3 | 74 | 3.9 | 26.2 | 149 |
| 4 | 4.3 | 27.1 | 4.3 | 27.4 | 8 | 4.0 | 27.8 | 73 | 3.9 | 27.5 | 143 |
| 5 | 4.2 | 28.9 | 4.4 | 29.2 | 8 | 3.9 | 29.3 | 66 | 3.8 | 29.1 | 130 |
| 6 | 4.0 | 30.2 | 4.2 | 30.6 | 7 | 3.7 | 30.6 | 61 | 3.7 | 30.1 | 122 |
| 7 | 4.0 | 31.9 | 4.0 | 32.4 | 6 | 3.5 | 32.2 | 55 | 3.7 | 31.9 | 116 |
| 8 | 4.0 | 33.4 | 4.0 | 33.8 | 6 | 3.4 | 33.4 | 52 | 3.3 | 33.1 | 101 |
| 9 | 4.0 | 34.8 | 4.1 | 35.1 | 6 | 3.6 | 34.4 | 52 | 3.7 | 33.9 | 109 |
| 10 | 3.8 | 35.9 | 3.9 | 36.3 | 5 | 3.8 | 35.6 | 54 | 3.7 | 35.2 | 106 |
| 11 | 3.7 | 37.4 | 3.7 | 37.9 | 5 | 3.5 | 36.9 | 48 | 3.3 | 36.6 | 91 |
| 12 | 3.7 | 38.3 | 3.8 | 39.1 | 5 | 3.5 | 38.3 | 45 | 3.6 | 37.8 | 94 |
| 13 | 3.8 | 39.7 | 3.8 | 40.2 | 5 | 3.4 | 39.3 | 44 | 3.2 | 38.9 | 83 |
| 17 | 3.4 | 43.7 | 3.4 | 44.8 | 4 | 3.0 | 43.0 | 35 | 3.1 | 42.7 | 74 |
| 21 | 3.8 | 47.0 | 3.7 | 47.8 | 4 | 3.5 | 46.8 | 38 | 3.4 | 46.5 | 72 |
| 25 | 3.8 | 49.4 | 3.8 | 49.6 | 4 | 3.5 | 48.9 | 36 | 3.2 | 49.3 | 65 |
| 29 | 4.0 | 50.0 | 4.2 | 50.5 | 4 | 3.8 | 49.8 | 38 | 3.8 | 50.0 | 76 |
| 33 | 3.6 | 50.9 | 3.9 | 51.3 | 4 | 3.5 | 50.5 | 35 | 3.4 | 50.8 | 67 |
| 37 | 3.9 | 52.2 | 4.5 | 52.6 | 4 | 3.8 | 51.7 | 37 | 3.5 | 51.8 | 68 |
| 41 | 3.8 | 52.1 | 4.1 | 52.9 | 4 | 3.8 | 52.2 | 36 | 3.5 | 52.2 | 66 |
| 45 | 4.0 | 52.2 | 4.5 | 52.5 | 4 | 4.0 | 52.3 | 38 | 3.9 | 52.3 | 75 |
| 49 | 4.3 | 52.1 | 4.8 | 53.0 | 5 | 4.4 | 52.4 | 42 | 4.0 | 52.8 | 75 |
| 53 | 4.4 | 52.2 | 4.8 | 53.3 | 5 | 4.2 | 52.6 | 40 | 3.9 | 53.1 | 73 |
| 57 | 4.5 | 51.1 | 4.6 | 52.7 | 4 | 4.4 | 52.6 | 42 | 4.1 | 52.8 | 78 |
| 61 | 5.1 | 52.0 | 5.4 | 53.3 | 5 | 5.2 | 53.1 | 49 | 5.1 | 53.1 | 95 |
| 65 | 4.6 | 52.5 | 4.9 | 54.0 | 5 | 4.6 | 53.4 | 43 | 4.3 | 53.8 | 80 |
| 69 | 4.3 | 51.7 | 4.5 | 54.0 | 4 | 4.5 | 53.3 | 42 | 4.0 | 52.6 | 77 |
| 73 | 4.3 | 52.1 | 4.6 | 54.1 | 4 | 4.4 | 53.0 | 41 | 4.3 | 52.6 | 81 |
| 77 | 4.6 | 51.6 | 4.6 | 53.8 | 4 | 4.6 | 52.9 | 44 | 4.5 | 52.0 | 86 |
| 81 | 4.7 | 51.8 | 4.8 | 53.8 | 5 | 4.7 | 52.1 | 45 | 4.6 | 52.0 | 89 |
| 85 | 4.8 | 50.7 | 4.9 | 52.8 | 5 | 5.0 | 51.9 | 49 | 4.8 | 50.5 | 95 |
| 89 | 4.6 | 48.7 | 4.5 | 51.7 | 4 | 5.0 | 51.5 | 48 | 4.9 | 50.3 | 97 |
| 93 | 4.2 | 48.1 | 4.5 | 51.0 | 4 | 5.0 | 51.6 | 48 | 5.0 | 49.3 | 101 |
| 97 | 4.1 | 47.3 | 4.2 | 49.9 | 4 | 4.9 | 50.5 | 49 | 4.7 | 48.3 | 98 |
| 101 | 4.4 | 46.7 | 4.7 | 50.0 | 5 | 5.6 | 51.1 | 54 | 5.3 | 46.6 | 117 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 4.0 | 31.5 | 4.0 | 31.9 | 7 | 3.7 | 31.7 | 61 | 3.7 | 31.3 | 122 |
| 14-52 | 3.9 | 49.9 | 4.1 | 50.6 | 4 | 3.7 | 49.8 | 37 | 3.5 | 49.8 | 72 |
| 53-101 | 4.5 | 50.5 | 4.7 | 52.6 | 5 | 4.8 | 52.3 | 46 | 4.6 | 51.3 | 90 |

^a Grams of water consumed per animal per day

^b Milligrams of dibromoacetic acid consumed per kilogram body weight per day

TABLE J4
Water and Compound Consumption by Female Mice in the 2-Year Drinking Water Study
of Dibromoacetic Acid

| Week | 0 mg/L | | 50 mg/L | | | 500 mg/L | | | 1,000 mg/L | | |
|-----------------------|-------------------------------|-----------------------|------------------|-----------------------|------------------------------|------------------|-----------------------|-----------------|------------------|-----------------------|-----------------|
| | 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) |
| 1 | 3.1 | 18.4 | 3.3 | 18.6 | 9 | 3.2 | 18.8 | 85 | 3.0 | 18.5 | 164 |
| 2 | 3.4 | 19.4 | 4.1 | 19.3 | 11 | 3.3 | 19.7 | 84 | 3.2 | 19.7 | 163 |
| 3 | 3.7 | 20.5 | 4.2 | 20.4 | 10 | 3.9 | 20.8 | 93 | 3.5 | 20.7 | 169 |
| 4 | 3.5 | 21.2 | 3.8 | 21.1 | 9 | 3.9 | 21.5 | 90 | 3.4 | 21.3 | 161 |
| 5 | 4.0 | 22.2 | 4.0 | 22.2 | 9 | 3.6 | 22.7 | 80 | 3.2 | 22.5 | 141 |
| 6 | 3.9 | 23.4 | 4.3 | 23.3 | 9 | 3.5 | 23.9 | 72 | 3.4 | 23.5 | 144 |
| 7 | 3.7 | 24.3 | 3.4 | 24.2 | 7 | 3.5 | 24.7 | 72 | 3.3 | 24.4 | 137 |
| 8 | 3.6 | 24.6 | 3.6 | 24.9 | 7 | 3.4 | 25.4 | 68 | 3.2 | 24.9 | 131 |
| 9 | 3.6 | 25.7 | 3.6 | 25.7 | 7 | 3.7 | 26.3 | 71 | 3.6 | 25.8 | 138 |
| 10 | 3.9 | 26.7 | 4.0 | 26.1 | 8 | 3.3 | 27.4 | 61 | 3.3 | 26.7 | 125 |
| 11 | 3.5 | 28.0 | 3.5 | 27.8 | 6 | 3.3 | 28.3 | 59 | 3.1 | 27.8 | 112 |
| 12 | 3.8 | 28.4 | 4.1 | 28.3 | 7 | 3.3 | 29.4 | 56 | 3.3 | 27.9 | 117 |
| 13 | 4.0 | 29.8 | 4.0 | 29.0 | 7 | 3.5 | 30.0 | 59 | 2.8 | 29.2 | 96 |
| 17 | 3.2 | 34.8 | 3.2 | 34.0 | 5 | 3.0 | 34.7 | 44 | 2.9 | 33.0 | 87 |
| 21 | 4.6 | 38.9 | 3.5 | 38.2 | 5 | 3.3 | 38.4 | 43 | 3.0 | 36.3 | 81 |
| 25 | 3.9 | 44.0 | 3.2 | 44.0 | 4 | 3.3 | 43.5 | 38 | 2.7 | 42.1 | 64 |
| 29 | 3.5 | 47.6 | 3.0 | 47.9 | 3 | 2.7 | 46.7 | 29 | 2.6 | 45.0 | 58 |
| 33 | 3.0 | 49.7 | 2.4 | 50.4 | 2 | 3.4 | 49.2 | 35 | 3.1 | 47.1 | 66 |
| 37 | 2.5 | 53.5 | 2.6 | 54.6 | 2 | 2.7 | 52.4 | 26 | 2.6 | 50.4 | 51 |
| 41 | 2.6 | 56.2 | 2.8 | 56.2 | 3 | 2.8 | 54.3 | 26 | 2.6 | 52.4 | 49 |
| 45 | 2.9 | 57.0 | 2.8 | 57.9 | 2 | 2.7 | 55.5 | 25 | 3.0 | 53.9 | 56 |
| 49 | 3.4 | 58.0 | 2.7 | 59.1 | 2 | 2.8 | 57.3 | 25 | 2.7 | 55.6 | 48 |
| 53 | 2.8 | 60.1 | 3.2 | 60.4 | 3 | 2.7 | 59.2 | 23 | 2.6 | 57.3 | 45 |
| 57 | 2.6 | 60.9 | 2.8 | 60.6 | 2 | 2.9 | 59.8 | 24 | 2.8 | 58.2 | 48 |
| 61 | 3.3 | 63.4 | 3.5 | 62.1 | 3 | 3.5 | 61.2 | 29 | 3.3 | 60.0 | 55 |
| 65 | 3.1 | 63.5 | 2.8 | 62.3 | 2 | 2.7 | 62.0 | 22 | 2.5 | 61.0 | 41 |
| 69 | 2.5 | 64.1 | 2.7 | 62.7 | 2 | 2.7 | 62.5 | 21 | 2.4 | 61.2 | 39 |
| 73 | 2.6 | 63.8 | 3.0 | 63.1 | 2 | 2.9 | 62.9 | 23 | 2.7 | 61.1 | 44 |
| 77 | 2.7 | 64.3 | 2.9 | 63.6 | 2 | 3.1 | 63.3 | 24 | 2.6 | 62.0 | 41 |
| 81 | 2.9 | 63.2 | 3.2 | 63.1 | 3 | 3.3 | 61.3 | 27 | 2.7 | 60.7 | 44 |
| 85 | 3.0 | 63.1 | 3.0 | 61.8 | 3 | 3.1 | 61.8 | 25 | 2.9 | 59.2 | 49 |
| 89 | 3.2 | 63.2 | 3.2 | 62.5 | 3 | 3.4 | 61.4 | 27 | 2.8 | 60.4 | 46 |
| 93 | 3.2 | 63.6 | 3.2 | 63.9 | 3 | 3.9 | 61.9 | 32 | 3.3 | 60.9 | 54 |
| 97 | 3.1 | 60.8 | 3.0 | 62.1 | 2 | 3.8 | 59.2 | 32 | 3.3 | 58.3 | 57 |
| 101 | 4.0 | 59.0 | 3.3 | 59.5 | 3 | 4.0 | 57.9 | 35 | 3.8 | 56.6 | 67 |
| Mean for weeks | | | | | | | | | | | |
| 1-13 | 3.7 | 24.0 | 3.8 | 23.9 | 8 | 3.5 | 24.5 | 73 | 3.3 | 24.1 | 138 |
| 14-52 | 3.3 | 48.9 | 2.9 | 49.1 | 3 | 3.0 | 48.0 | 32 | 2.8 | 46.2 | 62 |
| 53-101 | 3.0 | 62.5 | 3.1 | 62.1 | 3 | 3.2 | 61.1 | 27 | 2.9 | 59.8 | 48 |

^a Grams of water consumed per animal per day

^b Milligrams of dibromoacetic acid consumed per kilogram body weight per day

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

| | | |
|-----------------|---|------------|
| TABLE K1 | Ingredients of NTP-2000 Rat and Mouse Ration | K-2 |
| TABLE K2 | Vitamins and Minerals in NTP-2000 Rat and Mouse Ration | K-2 |
| TABLE K3 | Nutrient Composition of NTP-2000 Rat and Mouse Ration | K-3 |
| TABLE K4 | Contaminant Levels in NTP-2000 Rat and Mouse Ration | K-4 |

TABLE K1
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 K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration^a

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

^a Per kilogram of finished product

TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

| Nutrient | Mean ± Standard Deviation | Range | Number of Samples |
|--|---------------------------------------|---------------|-------------------|
| Protein (% by weight) | 14.1 ± 0.67 | 13.2 – 15.7 | 23 |
| Crude fat (% by weight) | 8.1 ± 0.29 | 7.6 – 8.6 | 23 |
| Crude fiber (% by weight) | 9.1 ± 0.58 | 8.0 – 10.5 | 23 |
| Ash (% by weight) | 5.2 ± 0.27 | 4.8 – 5.8 | 23 |
| 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 |
| Tryptophan | 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) | 4,705 ± 799 | 3,060 – 6,810 | 23 |
| Vitamin D (IU/kg) | 1,000 ^a (from formulation) | | |
| α-Tocopherol (ppm) | 84.3 ± 17.06 | 52.0 – 110.0 | 12 |
| Thiamine (ppm) | 7.1 ± 0.86 | 6.0 – 8.8 | 23 |
| 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) | 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) | 3,094 ± 292 | 2,700 – 3,790 | 12 |
| Minerals | | | |
| Calcium (%) | 1.039 ± 0.047 | 0.964 – 1.140 | 23 |
| Phosphorus (%) | 0.606 ± 0.037 | 0.552 – 0.701 | 23 |
| 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 K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration^a

| | Mean ± Standard Deviation ^b | Range | Number of Samples |
|---|--|---------------|-------------------|
| Contaminants | | | |
| Arsenic (ppm) | 0.21 ± 0.023 | 0.16 – 0.25 | 23 |
| Cadmium (ppm) | 0.04 ± 0.005 | 0.04 – 0.06 | 23 |
| Lead (ppm) | 0.09 ± 0.099 | 0.05 – 0.54 | 23 |
| Mercury (ppm) | < 0.02 | | 23 |
| Selenium (ppm) | 0.23 ± 0.057 | 0.14 – 0.36 | 23 |
| Aflatoxins (ppb) | < 5.00 | | 23 |
| Nitrate nitrogen (ppm) ^c | 11.7 ± 3.52 | 6.85 – 21.1 | 23 |
| Nitrite nitrogen (ppm) ^c | < 0.61 | | 23 |
| BHA (ppm) ^d | < 1.0 | | 23 |
| BHT (ppm) ^d | < 1.0 | | 23 |
| Aerobic plate count (CFU/gm) | 14 ± 14 | 10 – 70 | 23 |
| Coliform (MPN/gm) | 2.8 ± 1.3 | 0.0 – 3.6 | 23 |
| <i>Escherichia coli</i> (MPN/gm) | < 10 | | 23 |
| <i>Salmonella</i> (MPN/gm) | Negative | | 23 |
| Total nitrosoamines (ppb) ^e | 4.7 ± 1.19 | 3.1 – 7.5 | 23 |
| <i>N</i> -Nitrosodimethylamine (ppb) ^e | 2.1 ± 0.57 | 1.0 – 3.2 | 23 |
| <i>N</i> -Nitrosopyrrolidine (ppb) ^e | 2.6 ± 1.13 | 1.0 – 5.1 | 23 |
| Pesticides (ppm) | | | |
| α-BHC | <0.01 | | 23 |
| β-BHC | <0.02 | | 23 |
| γ-BHC | <0.01 | | 23 |
| δ-BHC | <0.01 | | 23 |
| Heptachlor | <0.01 | | 23 |
| Aldrin | <0.01 | | 23 |
| Heptachlor epoxide | <0.01 | | 23 |
| DDE | <0.01 | | 23 |
| DDD | <0.01 | | 23 |
| DDT | <0.01 | | 23 |
| HCB | <0.01 | | 23 |
| Mirex | <0.01 | | 23 |
| Methoxychlor | <0.05 | | 23 |
| Dieldrin | <0.01 | | 23 |
| Endrin | <0.01 | | 23 |
| Telodrin | <0.01 | | 23 |
| Chlordane | <0.05 | | 23 |
| Toxaphene | <0.10 | | 23 |
| Estimated PCBs | <0.20 | | 23 |
| Ronnel | <0.01 | | 23 |
| Ethion | <0.02 | | 23 |
| Trithion | <0.05 | | 23 |
| Diazinon | <0.10 | | 23 |
| Methyl chlorpyrifos | 0.153 ± 0.095 | 0.020 – 0.418 | 23 |
| Methyl parathion | <0.02 | | 23 |
| Ethyl parathion | <0.02 | | 23 |
| Malathion | 0.179 ± 0.143 | 0.020 – 0.557 | 23 |
| Endosulfan I | <0.01 | | 23 |
| Endosulfan II | <0.01 | | 23 |
| Endosulfane sulfate | <0.03 | | 23 |

^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 L

SENTINEL ANIMAL PROGRAM

METHODS L-2
RESULTS L-3

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 mice during the 3-month and 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 Corporation (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

3-Month Study

ELISA

Mycoplasma arthritidis

Study termination

Mycoplasma pulmonis

Study termination

PVM (pneumonia virus of mice)

Study termination

RCV/SDA

(rat coronavirus/sialodacryoadenitis virus)

Study termination

Sendai

Study termination

Immunofluorescence Assay

Parvovirus

Study termination

2-Year Study

ELISA

M. arthritidis

Study termination

M. pulmonis

Study termination

PVM

6, 12, and 18 months, study termination

RCV/SDA

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

M. arthritidis

Study termination

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

ELISA

| | |
|--|-------------------|
| Ectromelia virus | Study termination |
| EDIM (epizootic diarrhea of infant mice) | Study termination |
| GDVII (mouse encephalomyelitis virus) | Study termination |
| LCM (lymphocytic choriomeningitis virus) | Study termination |
| Mouse adenoma virus-FL | Study termination |
| MHV (mouse hepatitis virus) | Study termination |
| <i>M. arthritidis</i> | Study termination |
| <i>M. pulmonis</i> | Study termination |
| PVM | Study termination |
| Reovirus 3 | Study termination |
| Sendai | Study termination |

Immunofluorescence Assay

| | |
|------------------------------|-------------------|
| MCMV (mouse cytomegalovirus) | Study termination |
| Parvovirus | Study termination |

2-Year Study

ELISA

| | |
|------------------------|---|
| Ectromelia virus | 6, 12, and 18 months, study termination |
| EDIM | 6, 12, and 18 months, study termination |
| GDVII | 6, 12, and 18 months, study termination |
| LCM | 6, 12, and 18 months, study termination |
| Mouse adenoma virus-FL | 6, 12, and 18 months, study termination |
| MHV | 6, 12, and 18 months, study termination |
| <i>M. arthritidis</i> | Study termination |
| <i>M. pulmonis</i> | 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

| | |
|------------------------|---|
| EDIM | 12 months, study termination |
| GDVII | Study termination |
| LCM | 12 and 18 months |
| Mouse adenoma virus-FL | 6 and 18 months |
| MCMV | Study termination |
| MHV | Study termination |
| PVM | 12 months |
| Parvovirus | 6, 12, and 18 months, study termination |

RESULTS

All test results were negative.

APPENDIX M

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

| | |
|---|------|
| INTRODUCTION | M-2 |
| MODEL DEVELOPMENT | M-2 |
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PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model was developed to describe the uptake, distribution, and metabolism of dibromoacetic acid in female F344/N rats and male B6C3F₁ mice. This PBPK model was based on a published model for dichloroacetic acid (Keys *et al.*, 2004). A key feature of the dichloroacetic acid model is the inclusion of suicide inhibition in the description of dichloroacetic acid metabolism. Parameters fit to data in the dichloroacetic acid model that may be different for dibromoacetic acid were estimated by fitting model predictions to data from concurrent single-dose NTP toxicokinetic studies.

MODEL DEVELOPMENT

All features of Keys' model for dichloroacetic acid were retained for the current dibromoacetic acid model. The dibromoacetic acid model (Figure M1) has four flow-limited tissue compartments: liver, kidney, slowly perfused tissue, and rapidly perfused tissue. Keys *et al.* (2004) had an interest in modeling dichloroacetic acid that had been metabolically derived from intravenously administered trichloroethylene, and they provided no description of oral absorption kinetics. When these authors modeled drinking water studies, they assumed that 100 percent of the dose reached the liver instantaneously. Evaluation of Keys' model with dichloroacetic acid gavage data suggested that inclusion of oral absorption kinetics would be necessary for gavage study simulation. A stomach compartment was added with a linear uptake rate to the liver.

Urinary elimination was modeled using published values for urine flow to describe the rate of removal of dibromoacetic acid from the kidney tubule region. Published values for glomerular filtration were used to describe the distribution of dibromoacetic acid into kidney tissue and tubule regions (Davies and Morris, 1993). There was also saturable reabsorption from the kidney tubule region into kidney tissue. Keys' model includes glutathione s-transferase_{zeta}-mediated metabolism with suicide inhibition as well as a linear, non-glutathione s-transferase_{zeta}-mediated pathway. For dichloroacetic acid, the glutathione s-transferase_{zeta} pathway leads to glyoxylate, while the metabolite resulting from the second pathway has not been determined (Keys *et al.*, 2004). The dibromoacetic acid model includes both pathways.

The physiological parameters in Table M1 were taken from Brown *et al.* (1997). Due to similarities of structure, behavior, and octanol:water partition coefficients for the haloacetates, the partition coefficients for dibromoacetic acid (Table M2) were those provided for dichloroacetic acid in Keys' model.

The NTP toxicokinetic data used for parameter estimation included blood concentrations of dibromoacetic acid and urine content of dibromoacetic acid and its metabolites glyoxylic acid and oxalic acid. In these studies, blood and urine samples were collected from female F344/N rats administered single intravenous injections of 10 or 110 mg dibromoacetic acid/kg body weight or single gavage doses of 10, 40, or 100 mg/kg; similar samples were collected from male B6C3F₁ mice administered single intravenous injections of 100 mg/kg or single gavage doses of 100, 200, or 400 mg/kg. For rats and mice, samples were collected from three animals per timepoint for blood concentration analysis and from three groups of three animals each for urine concentration analysis.

There were seven unknown parameters in the model that had very little information to suggest the correct order of magnitude. Therefore, the values of the parameters were first found using a differential evolution optimization algorithm (ICSI, 1995). This type of algorithm has the ability to search across the global parameter space without being restricted to a local minimum. A cost function computed the sum of squared errors between the simulated results and experimental measurements for blood and urine. Another cost function computed the natural logarithm of the sum of squared errors, but the results from both cost functions were similar. The differential evolution algorithm was run for at least 200 generations. The best parameters from the differential evolution algorithm were then used as the initial conditions in the constrained optimization routine in MATLAB[®] (The Math Works, Inc., Natick, MA) to find the final parameter values (Table M3).

Definitions of Abbreviations A_i = Amount in tissue compartment i (mg) V_i = Volume of tissue compartment i (L) C_i = Concentration in tissue compartment i (mg/L) $C_{v,i}$ = Concentration in venous blood of tissue compartment i (mg/L) Q_i = Blood flow rate for tissue compartment i ; if $i=urine$, then urine flow rate (L/hour) P_i = Tissue compartment i : blood partition coefficient k_{abs} = Linear rate of absorption from stomach (hour⁻¹) V_{max} = Maximum metabolism rate (mg/hour) K_m = Michaelis-Menten constant associated with metabolism (mg/L) k_f = Linear rate of metabolism (hour⁻¹) k_d = Inhibition constant (hour⁻¹) k_s = Rate of resynthesis (mg/hour²) k_{de} = Degradation rate (hour⁻¹) GFR = Glomerular filtration rate (L/hour) T_{max} = Maximum reabsorption rate (mg/hour) K_t = Michaelis-Menten constant associated with tubular reabsorption (mg/L)**Model Equations**

Amount in stomach compartment:

$$\frac{dA_{stomach}}{dt} = dose_{oral} + dose_{drink} - k_{abs} A_{stomach}$$

Amount in rapidly/slowly perfused tissue compartments:

$$\frac{dA_{tissue}}{dt} = Q_{tissue} (C_{arterial} - C_{v,tissue})$$

Rate of metabolism:

$$\frac{dA_M}{dt} = \frac{V_{max} C_{v,l}}{K_m + C_{v,l}} + k_f A_l$$

Change in maximum rate of glutathione s-transferase_{zeta} metabolism:

$$\frac{dV_{max}}{dt} = -k_d \frac{V_{max} C_{v,l}}{K_m + C_{v,l}} + k_s - k_{de} V_{max} : V_{max}(0) = V_{max0}$$

Amount in liver tissue compartment:

$$\frac{dA_l}{dt} = Q_l (C_{arterial} - C_{v,l}) + \frac{dA_{stomach}}{dt}$$

Amount in kidney tubule compartment:

$$\frac{dA_{kidTub}}{dt} = GFR \cdot C_{v,k} - \frac{C_{kidTub} T_{max}}{C_{kidTub} + K_t} - \frac{dA_{urine}}{dt}$$

Amount in kidney tissue compartment:

$$\frac{dA_k}{dt} = Q_k (C_{arterial} - C_{v,k}) + \frac{C_{kidTub} T_{max}}{C_{kidTub} + K_t} - GFR \cdot C_{v,k}$$

Amount eliminated through urine:

$$\frac{dA_{urine}}{dt} = Q_{urine} C_{kidTub}$$

Concentration in tissue compartment i :

$$C_i = \frac{A_i}{V_i}$$

Concentration in venous blood of tissue compartment i :

$$C_{v,i} = \frac{C_i}{P_i}$$

Resynthesis rate:

$$k_s = k_{de} \cdot V_{max0}$$

RESULTS

Figures M2 through M10 show the results of simulations performed with the PBPK model for dibromoacetic acid compared to the experimental data from the NTP toxicokinetic studies. Each figure represents one study and shows separate plots for blood concentration, urinary elimination, and the response of V_{max} over 48 hours. PBPK model predictions for blood concentrations of dibromoacetic acid and V_{max} during 2-week drinking water studies in rodents exposed to 50, 500, or 1,000 mg/L are shown in Figures M11 (F344/N rats) and M12 (B6C3F₁ mice).

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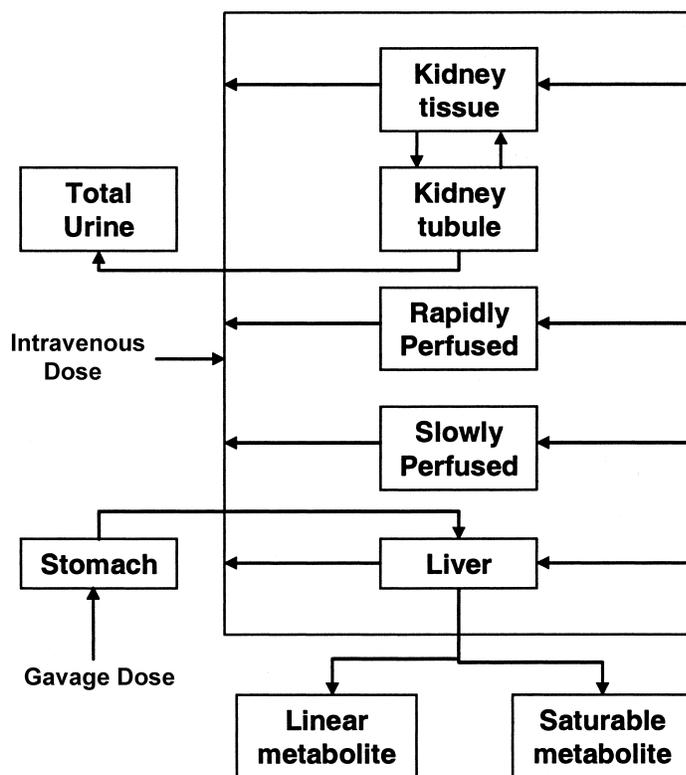


FIGURE M1
Physiologically Based Pharmacokinetic Model for F344/N Rats and B6C3F₁ Mice
Exposed to Dibromoacetic Acid by Single-Dose Intravenous Injection or Oral Gavage

TABLE M1
Physiological Parameters for F344/N Rats and B6C3F₁ Mice
for the Physiologically Based Pharmacokinetic Model of Dibromoacetic Acid^a

| | Female F344/N Rats | Male B6C3F ₁ Mice |
|--|--------------------|------------------------------|
| Parameter | | |
| Body weight (kg) | 0.1798 | 0.02782 |
| Cardiac output (L/hour per kg ^{0.75} body weight) | 14.1 | 16.5 |
| Urine flow (L/hour per kg body weight) ^b | 0.00833 | 0.00208 |
| Glomerular filtration (L/hour per kg body weight) ^b | 0.3144 | 0.84 |
| Tissue Volume as Fraction of Body Weight | | |
| Kidney tissue | 0.0073 | 0.0167 |
| Kidney tubule | 0.000073 | 0.000167 |
| Liver | 0.0366 | 0.0549 |
| Rapidly perfused tissue | 0.1561 | 0.1283 |
| Slowly perfused tissue | 0.62 | 0.62 |
| Tissue Blood Flow as Fraction of Cardiac Output | | |
| Kidney | 0.141 | 0.091 |
| Liver | 0.183 | 0.161 |
| Rapidly perfused tissue | 0.266 | 0.338 |
| Slowly perfused tissue | 0.41 | 0.41 |

^a Except as noted, parameter estimates were determined from Brown *et al.* (1997).

^b Parameter estimate was taken from Davies and Morris (1993).

TABLE M2
Partition Coefficients for Dibromoacetic Acid
for the Physiologically Based Pharmacokinetic Model of Dibromoacetic Acid^a

| Tissue | Partition Coefficient |
|-------------------------|-----------------------|
| Kidney | 0.14 |
| Liver | 1.08 |
| Rapidly perfused tissue | 1.08 |
| Slowly perfused tissue | 0.37 |

^a All coefficients are expressed as tissue:blood ratios and are the same as those provided for dichloroacetic acid by Keys *et al.* (2004).

TABLE M3

Parameter Estimates for F344/N Rats and B6C3F₁ Mice
from the Physiologically Based Pharmacokinetic Model of Dibromoacetic Acid

| | Female F344/N Rats | Male B6C3F ₁ Mice |
|--|----------------------|------------------------------|
| k_{abs} (hour ⁻¹) | 2.8766 | 2.0472 |
| V_{max0c} (mg/hour per kg ^{0.75} body weight) | 74.3186 | 133.6532 |
| K_m (mg/L) | 15.0064 | 33.7813 |
| k_{fc} (hour ⁻¹ /kg ^{-0.25} body weight) | 0.2910 | 0.0280 |
| k_d (hour ⁻¹) | 1.3166 | 0.2265 |
| k_{de} (hour ⁻¹) | 0.00875 ^a | 0.00875 ^a |
| T_{max} (mg/hour) | 28.5329 | 61.4492 |
| K_t (mg/L) | 61.5938 | 272.9621 |

^a Value taken from Anderson *et al.* (1999).

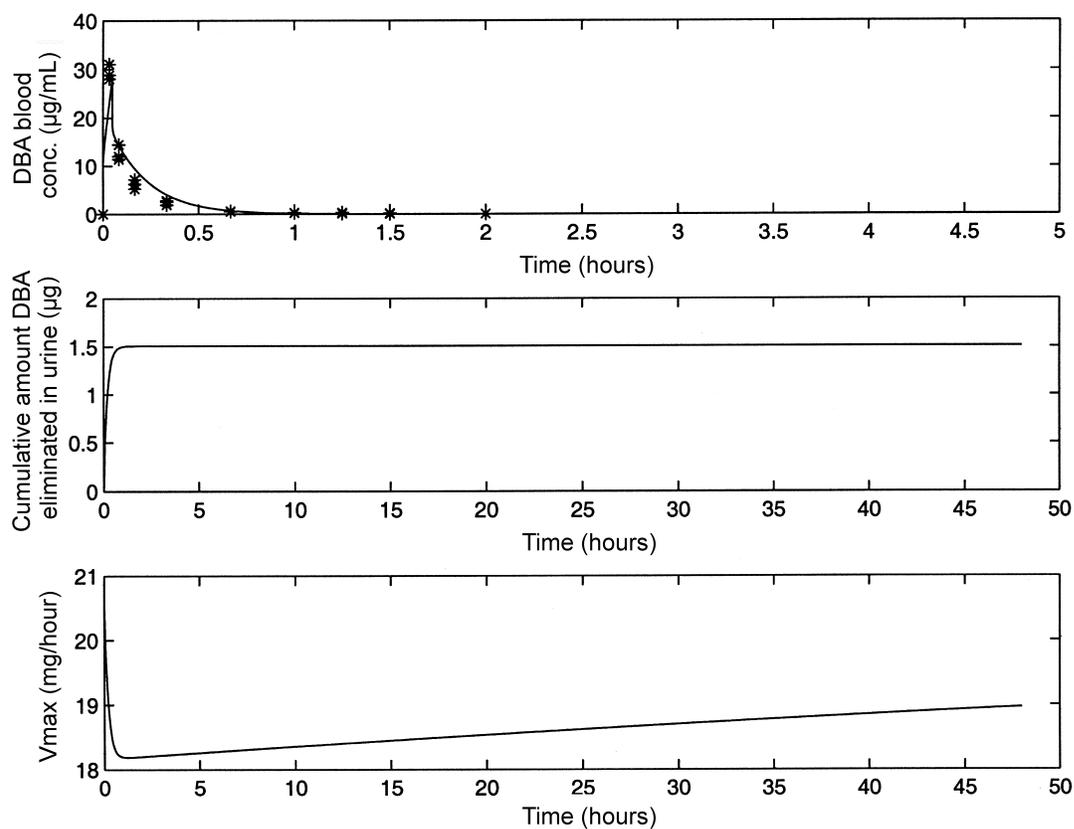


FIGURE M2
Data (Stars) and PBPK Model Predictions (Lines) for Female F344/N Rats
Administered a Single Intravenous Injection of 10 mg/kg Dibromoacetic Acid
Data are presented as the mean of three samples per timepoint.

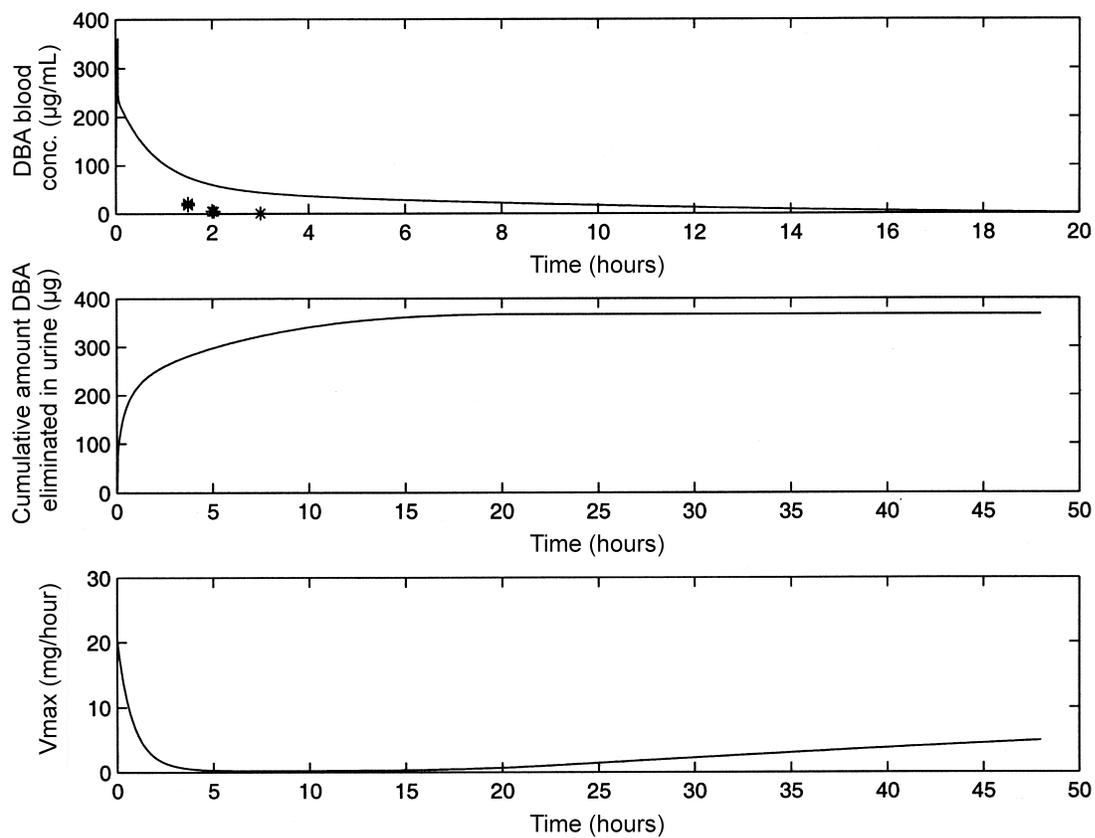


FIGURE M3
Data (Stars) and PBPK Model Predictions (Lines) for Female F344/N Rats
Administered a Single Intravenous Injection of 110 mg/kg Dibromoacetic Acid
 Data are presented as the mean of three samples per timepoint.

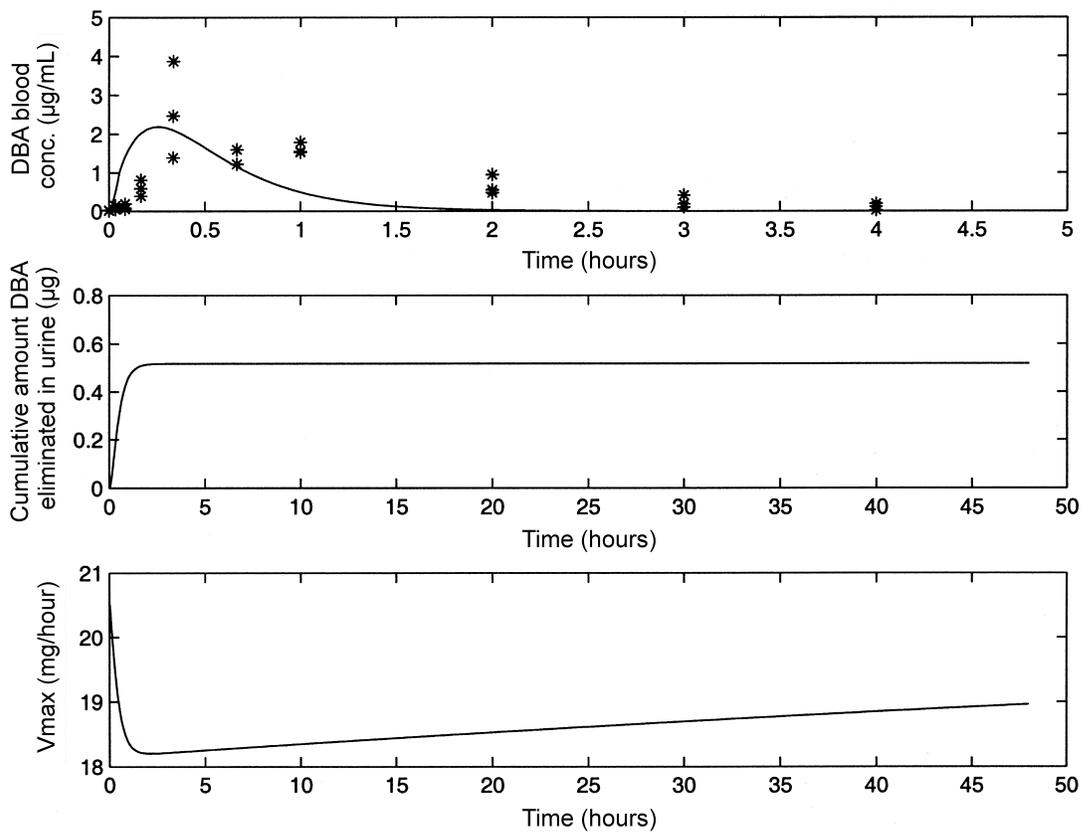


FIGURE M4
Data (Stars) and PBPK Model Predictions (Lines) for Female F344/N Rats
Administered a Single Gavage Dose of 10 mg/kg Dibromoacetic Acid
Data are presented as the mean of three samples per timepoint.

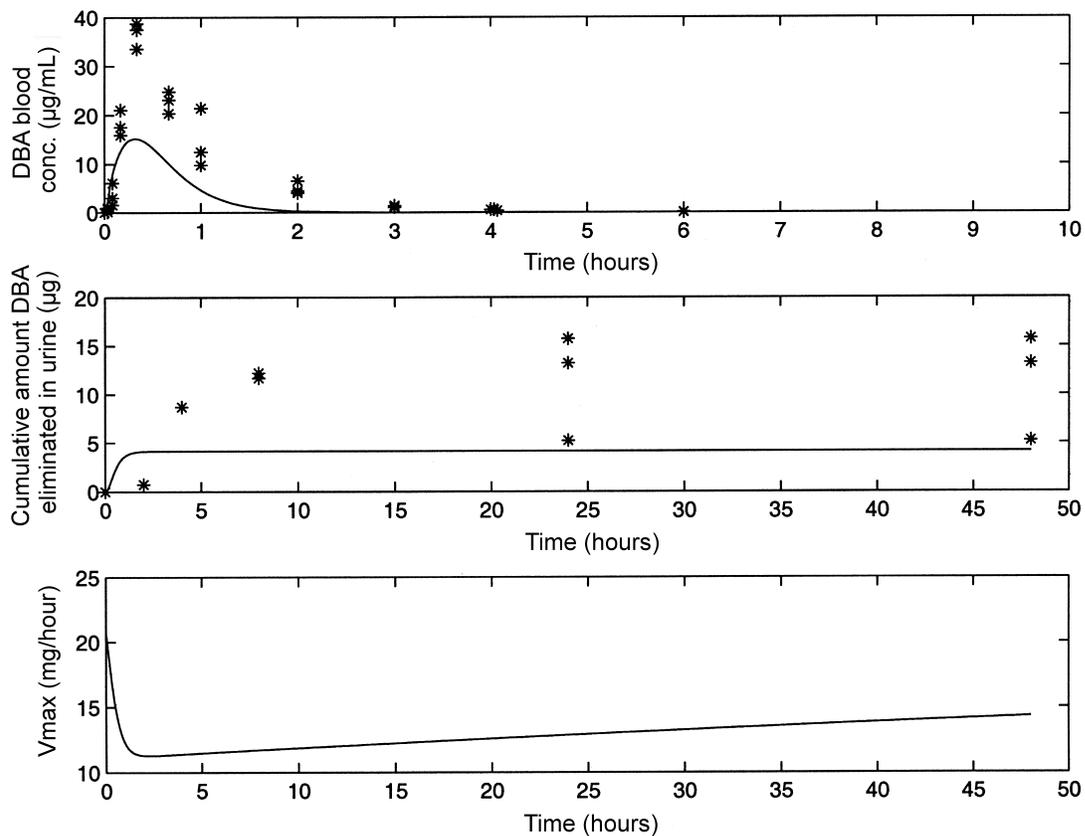


FIGURE M5
Data (Stars) and PBPK Model Predictions (Lines) for Female F344/N Rats
Administered a Single Gavage Dose of 40 mg/kg Dibromoacetic Acid
 Data are presented as the mean of three samples per timepoint.

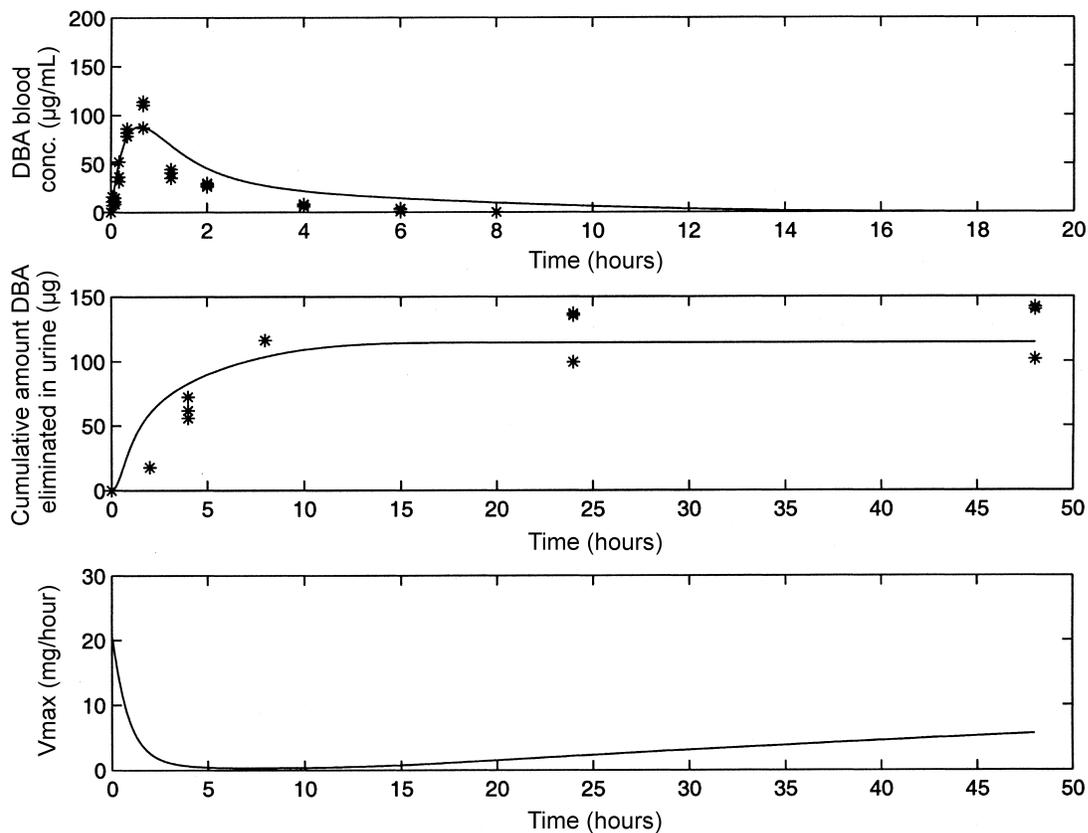


FIGURE M6
Data (Stars) and PBPK Model Predictions (Lines) for Female F344/N Rats
Administered a Single Gavage Dose of 100 mg/kg Dibromoacetic Acid
Data are presented as the mean of three samples per timepoint.

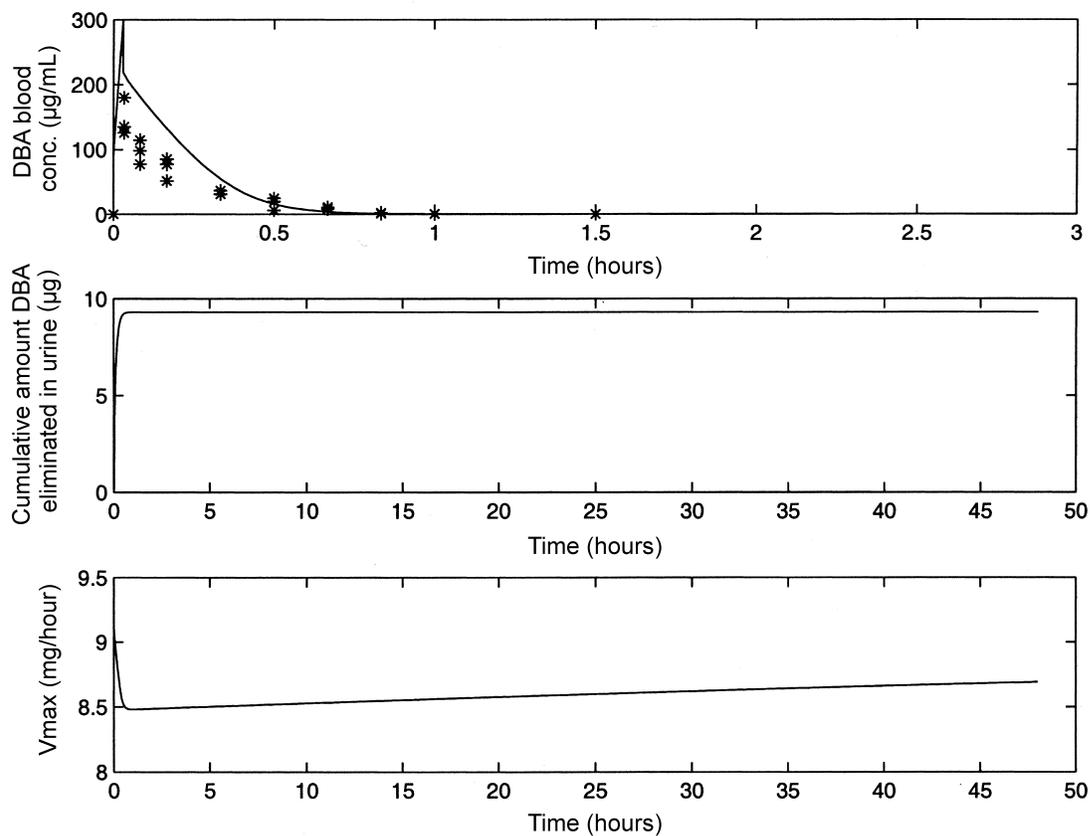


FIGURE M7
Data (Stars) and PBPK Model Predictions (Lines) for Male B6C3F₁ Mice
Administered a Single Intravenous Injection of 100 mg/kg Dibromoacetic Acid
 Data are presented as the mean of three samples per timepoint.

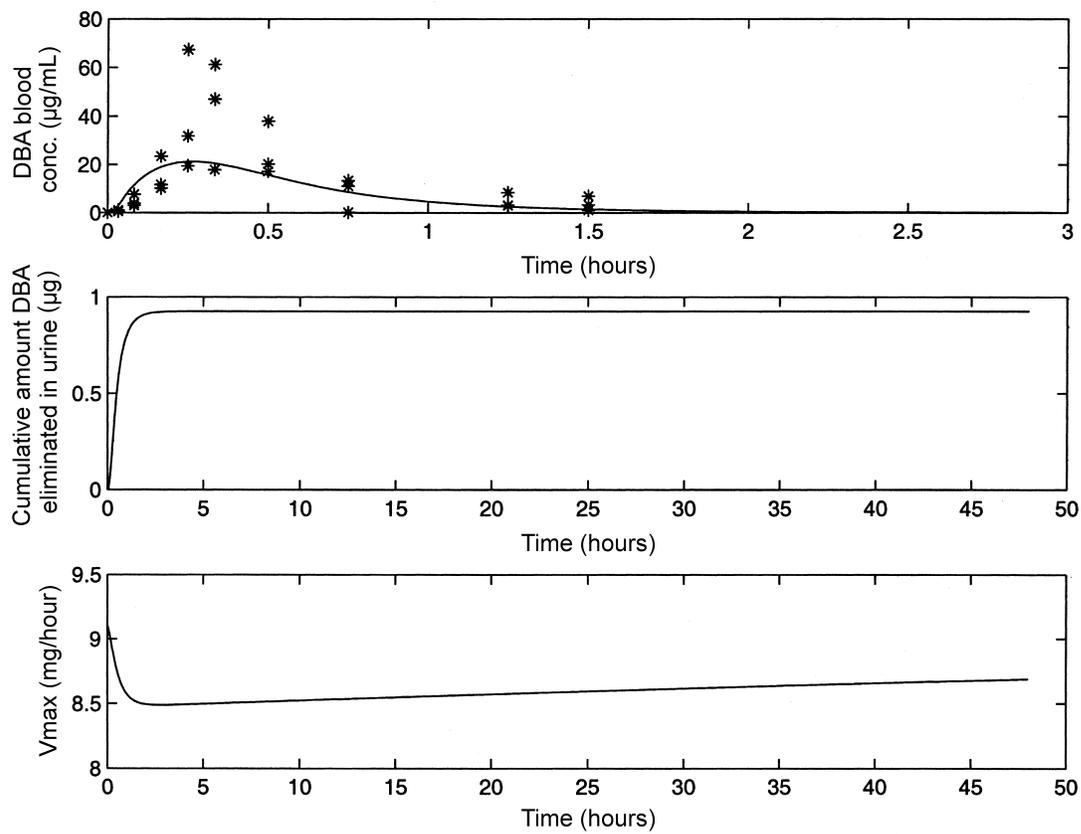


FIGURE M8
Data (Stars) and PBPK Model Predictions (Lines) for Male B6C3F₁ Mice
Administered a Single Gavage Dose of 100 mg/kg Dibromoacetic Acid
Data are presented as the mean of three samples per timepoint.

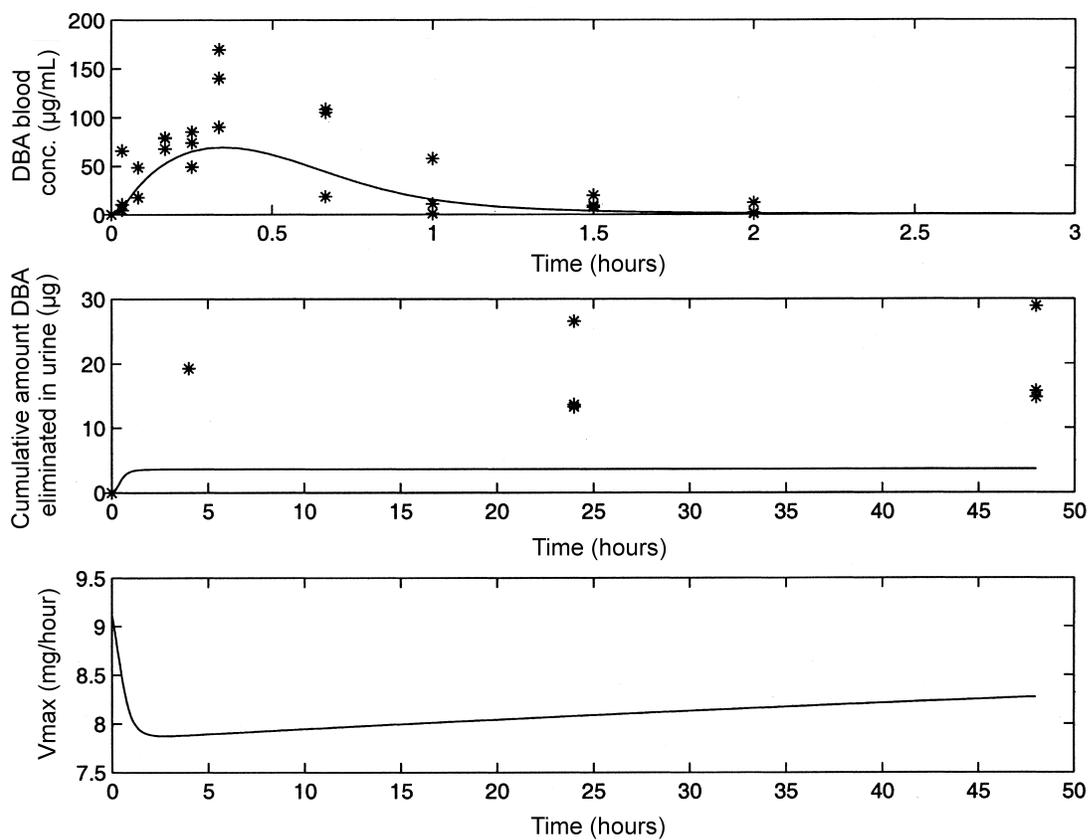


FIGURE M9
Data (Stars) and PBPK Model Predictions (Lines) for Male B6C3F₁ Mice
Administered a Single Gavage Dose of 200 mg/kg Dibromoacetic Acid
 Data are presented as the mean of three samples per timepoint.

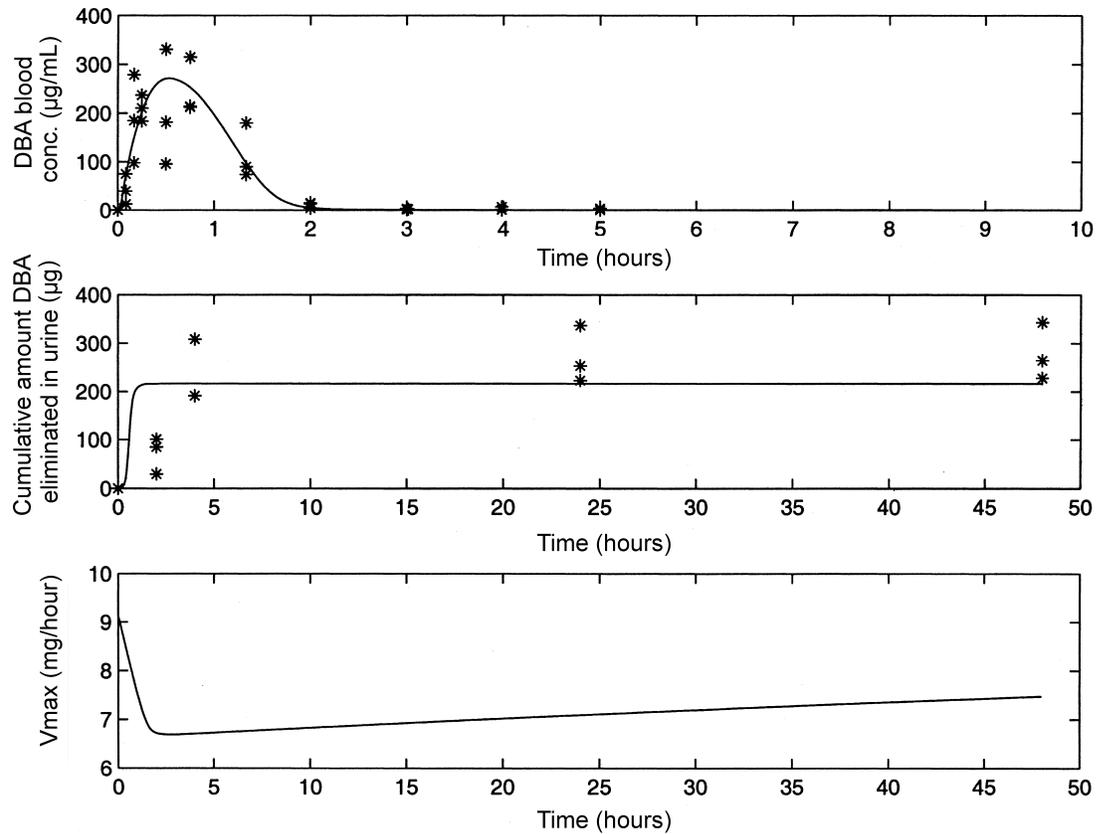


FIGURE M10
Data (Stars) and PBPK Model Predictions (Lines) for Male B6C3F₁ Mice
Administered a Single Gavage Dose of 400 mg/kg Dibromoacetic Acid
Data are presented as the mean of three samples per timepoint.

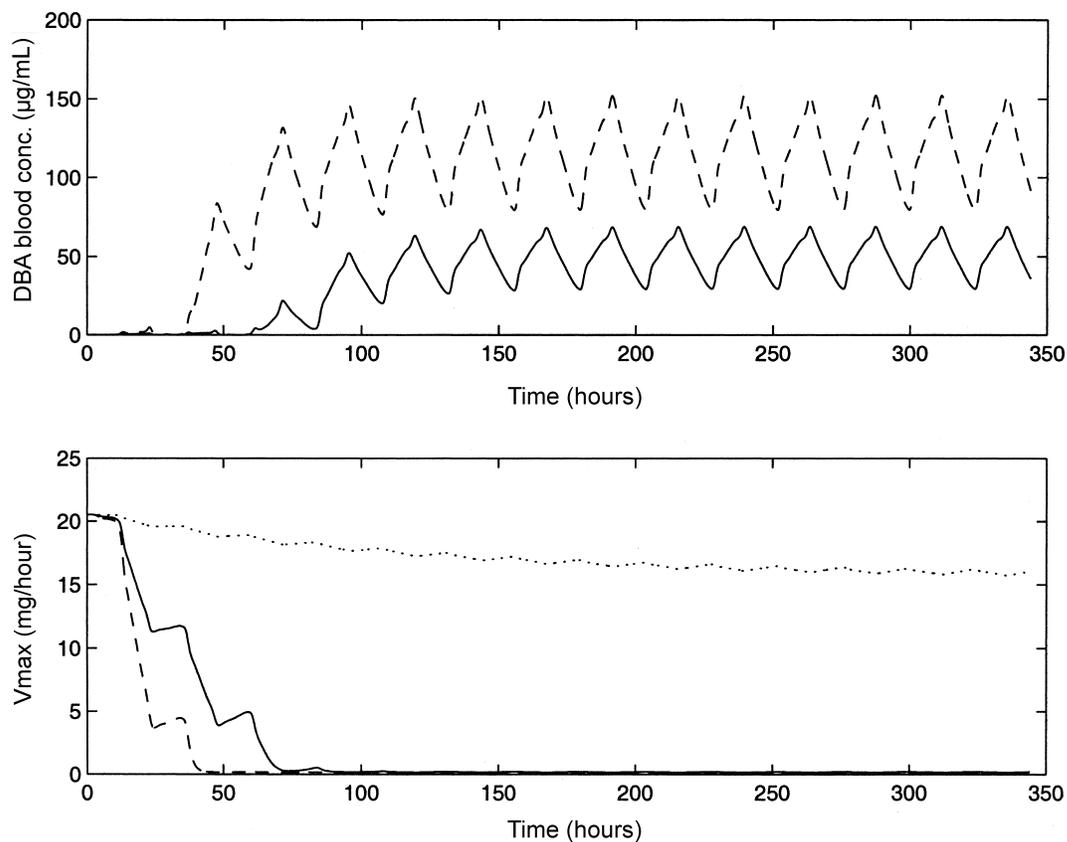


FIGURE M11
PBPK Model Predictions for Female F344/N Rats Administered 50 (Dotted Line), 500 (Solid Lines), or 1,000 (Dashed Lines) mg/L Dibromoacetic Acid in Drinking Water for 2 Weeks

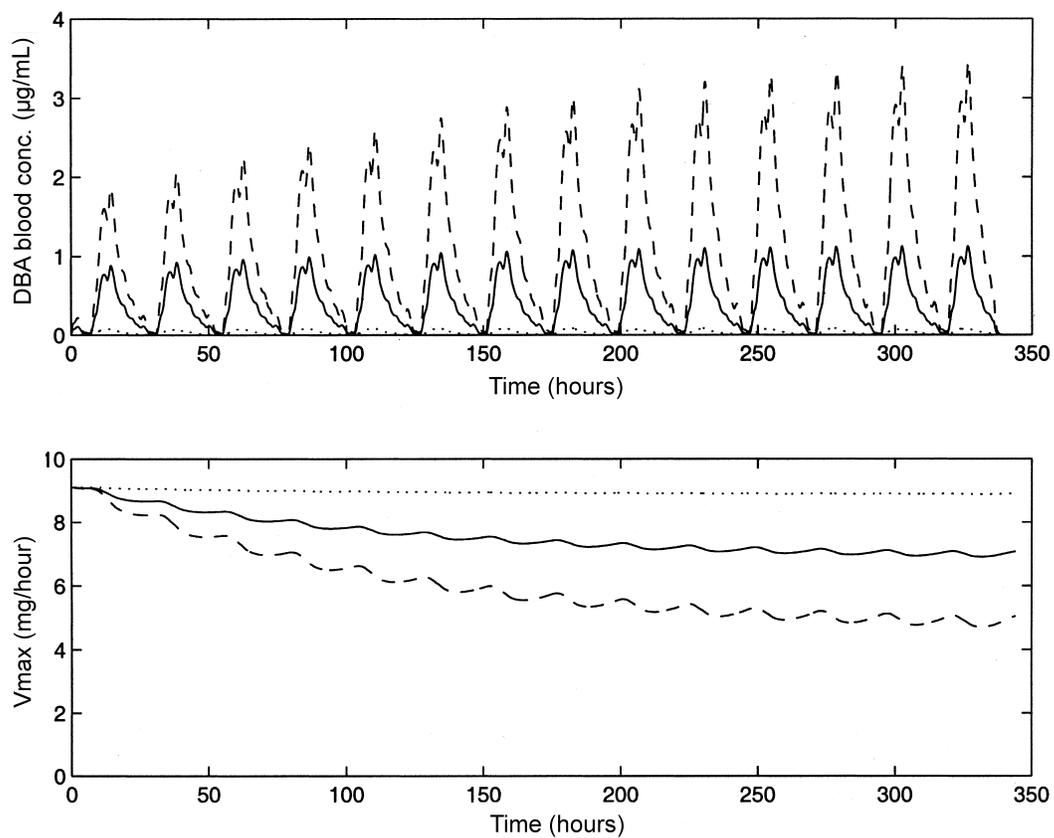


FIGURE M12
PBPK Model Predictions for Male B6C3F₁ Mice Administered 50 (Dotted Lines), 500 (Solid Lines), or 1,000 (Dashed Lines) mg/L Dibromoacetic Acid in Drinking Water for 2 Weeks

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

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

INTRODUCTION

Single-dose intravenous injection and gavage toxicokinetic studies were designed to estimate toxicokinetic parameters for the elimination of dibromoacetic acid from the plasma of F344/N rats and B6C3F₁ mice. Male and female F344/N rats received a single intravenous injection of 25 mg dibromoacetic acid/kg body weight or a single gavage dose of 25, 50, or 125 mg/kg. Male and female B6C3F₁ mice received a single intravenous injection of 70 mg/kg or a single gavage dose of 70, 175, or 350 mg/kg. Post-dose plasma samples were analyzed for dibromoacetic acid, and the results were used to calculate toxicokinetic parameters.

MATERIALS AND METHODS

Dibromoacetic acid (lot 46019/1 55196) was obtained from Fluka Chemie AG (Buchs, Switzerland), homogenized, repackaged in the original bottles, and stored at room temperature. The material was analyzed for identity and purity; the results and analytical systems are described in Appendix I.

On the day of dosing, the animals were 14 to 15 weeks old. For the gavage studies, male and female F344/N rats ranged in weight from 256.4 to 333.1 and 129.4 to 201.2 grams, respectively, and male and female B6C3F₁ mice ranged in weight from 21.0 to 35.1 and 14.0 to 29.4 grams, respectively. All doses of dibromoacetic acid were formulated in normal saline.

After dosing, animals were anesthetized with CO₂/O₂ prior to bleeding. Blood samples were collected using the retroorbital puncture method for rats and cardiac puncture for mice. Three rats and three mice were bled at each timepoint. Only one sample was collected from each mouse, but rats were rebled twice with at least one hour between sample collections. Blood samples of approximately 1 mL (rats) and the maximum volume obtainable for mice (approximately 0.4 to 1 mL) were placed into individual tubes containing EDTA as an anticoagulant. Whole blood samples were gently rocked by hand to ensure adequate mixing with the anticoagulant and then placed on wet ice. Approximately 60 minutes after collection, the whole blood was centrifuged, and the plasma was transferred to a plastic storage vial. The plasma was stored at -70° C until analyzed.

For analysis, plasma samples were thawed to room temperature. Aliquots of 200 µL of plasma were combined with 50 µL of working internal standard solution (40 µg dichloroacetic acid/mL) and 500 µL of 12% boron trifluoride-methanol complex; the mixture was sealed and heated at approximately 115° C for 45 minutes. Two mL of dichloromethane and 1 mL of deionized water were added, and then the mixture was tumbled for 30 minutes and centrifuged at 1,500 rpm for 5 minutes. The lower (dichloromethane) layer was transferred to autosampler vials, and the vials were sealed. Chromatography was performed on a gas chromatography system (Agilent, Palo Alto, CA) equipped with an electron capture detector and an RTX-5 column (30 m × 0.53 mm ID, 1.5 µm film; Supelco, Inc., Bellefonte, PA). The temperature program was 50° C for 1 minute, increased to 150° C at 12° C/minute, and then increased to 300° C at 70° C/minute. Sample concentrations of dibromoacetic acid were calculated using 1/y-weighted quadratic regression analysis of instrument response to calibration standards prepared in blank F344/N rat plasma.

The analytical method for determining derivatized dibromoacetic acid in plasma samples was validated within a range of 0.1 to 2 µg/mL (low range) and 2 to 100 µg/mL (high range). The limit of quantitation was 0.1 µg/mL. Precision, based on standard deviation of quality control samples, was less than or equal to 15%. Accuracy, based on percent relative errors in the determined versus the prepared concentration of calibration standards, was less than or equal to 15% except at the limit of quantitation, where less than or equal to 20% was acceptable.

Toxicokinetics

Dibromoacetic acid plasma concentration versus time data were evaluated using WinNONLIN[®] (version 2.1; Scientific Consulting, Inc., Freeman, SD). A one-compartment model with no lag time and first-order absorption and elimination was used to fit the data:

$$C(t) = D \cdot K_{01} / V / (K_{01} - K_{10}) \cdot [\exp(K_{10} \cdot t) - \exp(K_{01} \cdot t)]$$

In this model, C(t) is the plasma concentration at time t, D is dose, V is volume of distribution, K₀₁ is the absorption rate constant, and K₁₀ is the elimination rate constant. These parameters were estimated by nonlinear regression using a least-squares method and a weighting factor (1/y² predicted).

AUC (area under the plasma concentration versus time curve) values were calculated using the trapezoidal rule:

$$AUC_t = \Sigma [(C_{n-1} + C_n) / 2 \times (t_n - t_{n-1})]$$

AUC extrapolated to infinity was calculated as:

$$AUC_{\infty} = AUC_t + (C_f / K_{10})$$

Clearance was calculated as D/AUC_∞ and the half-lives for the absorption and elimination phases were calculated as 0.693/K₀₁ and 0.693/K₁₀, respectively.

RESULTS AND DISCUSSION

Dibromoacetic acid was rapidly absorbed when administered by gavage in an aqueous formulation to male and female F344/N rats and B6C3F₁ mice such that measurable concentrations of dibromoacetic acid were observed within 5 minutes of administration (Figures N1 and N2). The administration of dibromoacetic acid in an aqueous solution averted any dissolution phase, which is the rate-limiting step for solid dosage formulations such as dosed feed, and thereby precluded the presence of a lag time in the plasma concentration time profile. A one-compartment model with no lag phase and first-order absorption and elimination provided the best fit to the plasma concentration versus time profiles. The absorption rate constant and absorption half-life values observed with gavage administration very closely resembled the elimination rate constant and elimination half-life values observed with intravenous administration. In addition, the elimination rate constant and elimination half-life values observed with gavage administration were 4- to 5-fold greater than those associated with intravenous administration, confirming flip-flop kinetics. Flip-flop kinetics are commonly observed with test articles that undergo very fast elimination and indicate that the toxicokinetic parameters determined for the absorption phases are actually estimates of the elimination phase and vice versa.

Based on the flip-flop model findings for rats, the absorption rate constant values ranged from 0.87 to 0.62 hours⁻¹ for males and 0.80 to 0.71 hours⁻¹ for females. Corresponding absorption half-life values for all dose groups with both sexes ranged from 0.8 to 1.2 hours (Table N1). Elimination rate constants ranged from 4.1 to 6.2 hours⁻¹ for males and 10.0 to 7.0 hours⁻¹ for females. Corresponding elimination half-lives were similar across both sexes and all dose groups and ranged from 0.1 to 0.2 hours. Bioavailability, from comparison of the AUCs for intravenous and gavage administration, ranged from approximately 43% to 61% for males and 64% to 91% for females.

Very similar observations to those in rats were made with the mouse plasma concentration versus time profiles. Absorption rate constants for male mice ranged from 1.9 to 0.40 hours⁻¹ and ranged from 2.1 to 0.35 hours⁻¹ in females based on the flip-flop model. Corresponding absorption half-life values ranged from 0.4 to 1.8 hours for males and 0.3 to 2.0 hours for females and increased with increasing dose (Table N2). Elimination rate constants ranged from 4.9 to 11.0 hours⁻¹ for males (not consistent with dose) and were 8.6 to 17.1 hours⁻¹ in females.

Across both sexes and all dose groups, the elimination half-lives ranged from 0.04 to 0.12 hours. Bioavailability in mice was approximately 11% to 44 % and increased with dose.

In the current toxicokinetic studies in rats and mice, volume of distribution, clearance, and AUC_{∞} values as typically calculated were invalid because these parameters are a function of the elimination phase, which is not the terminal linear phase in these studies. These values, therefore, are not reported for these studies. AUC values not affected by the extrapolation of the terminal linear phase increased with dose in both sexes of both species.

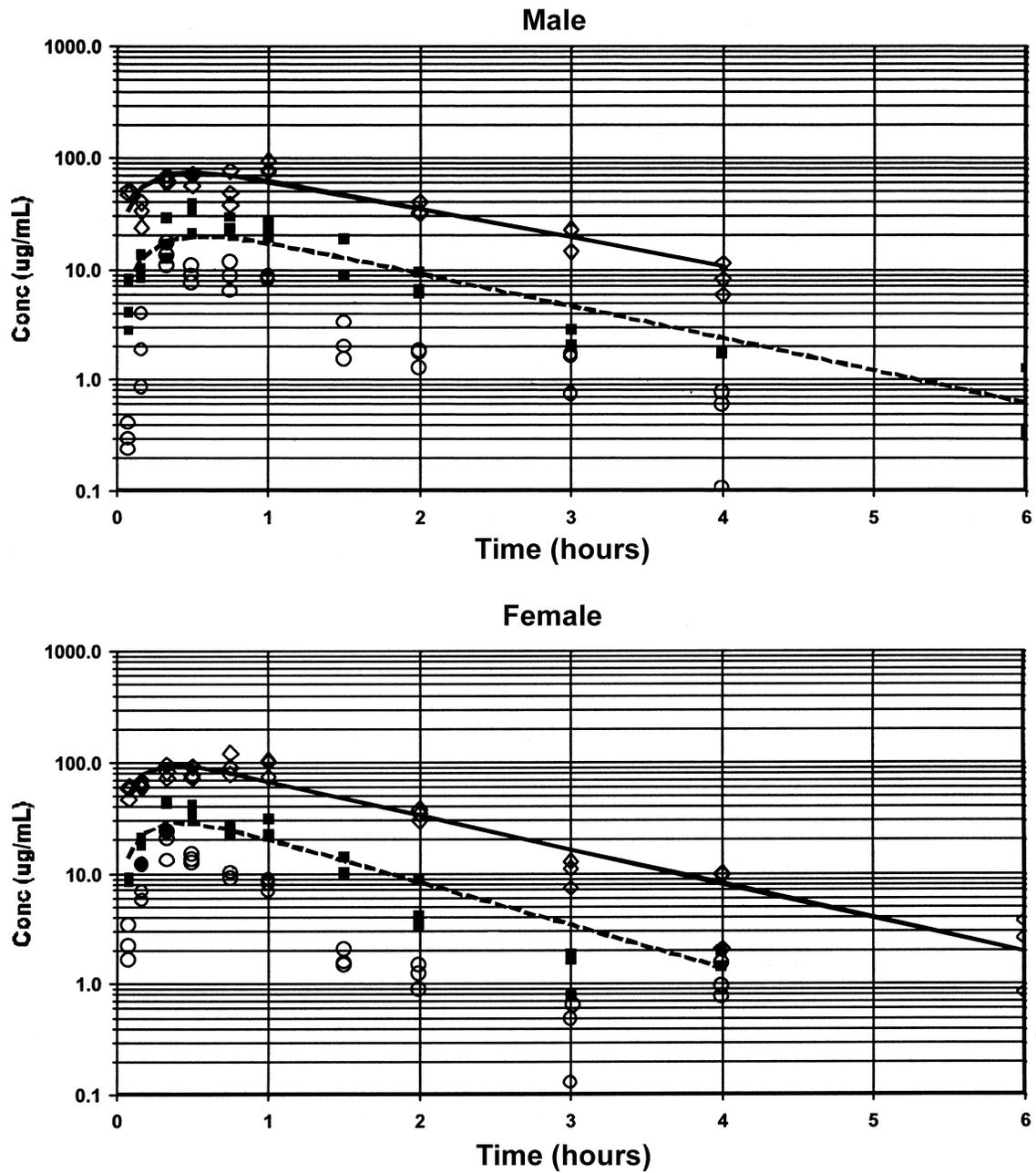


FIGURE N1
Plasma Concentrations of Dibromoacetic Acid in Male and Female F344/N Rats
After a Single Gavage Dose of 25, 50, or 125 mg/kg Dibromoacetic Acid
 Lines represent the best-fit curves (WinNONLIN[®]) plotted through the observed data points;
 data points represent plasma samples from individual rats.
 (Observed data: ○ – 25 mg/kg; ■ – 50 mg/kg; ◇ – 125 mg/kg)

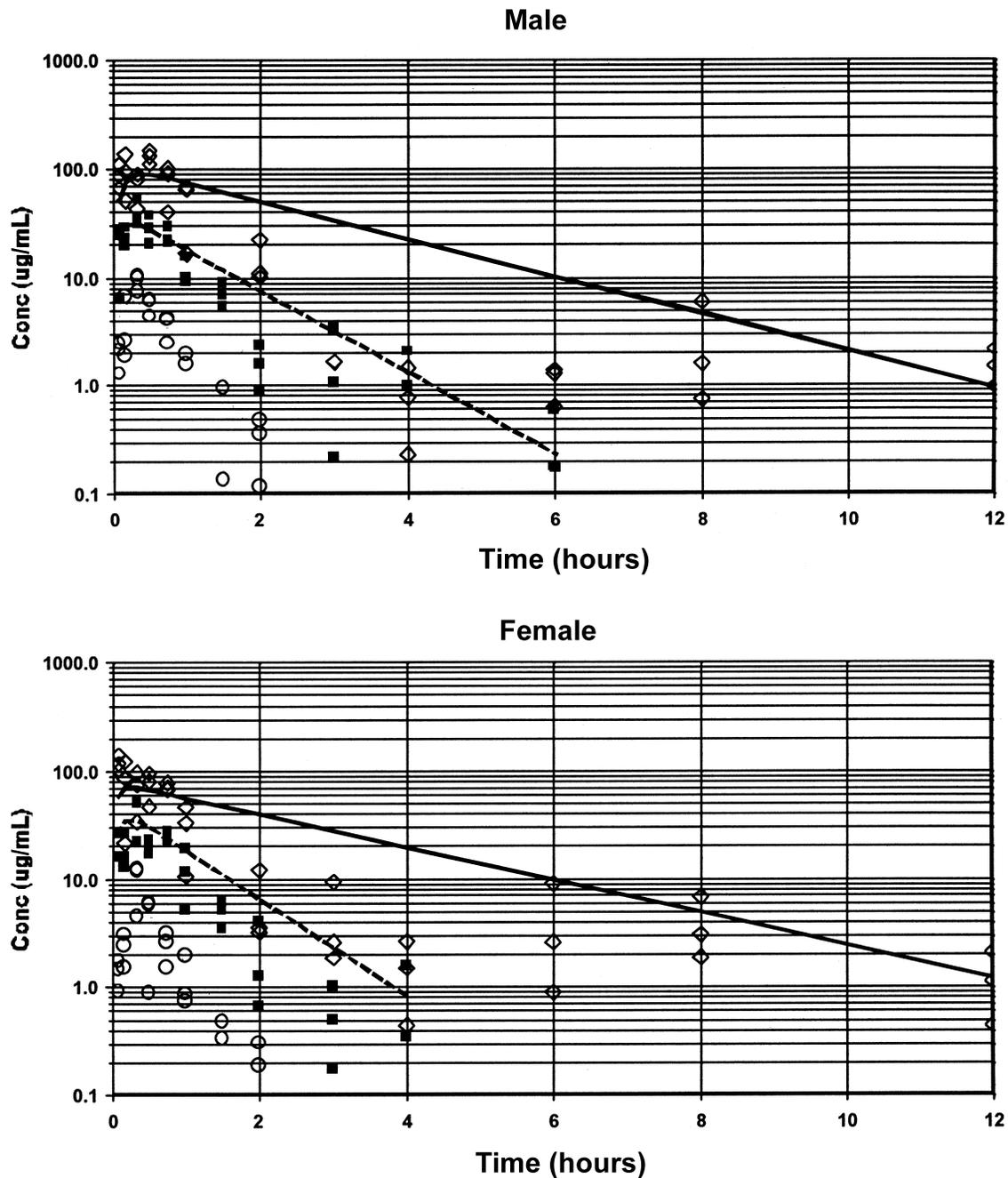


FIGURE N2

**Plasma Concentrations of Dibromoacetic Acid in Male and Female B6C3F₁ Mice
After a Single Gavage Dose of 70, 175, or 350 mg/kg Dibromoacetic Acid**

Lines represent the best-fit curves (WinNONLIN[®]) plotted through the observed data points;
data points represent plasma samples from individual mice.

(Observed data: ○ – 70 mg/kg; ■ – 175 mg/kg; ◇ – 350 mg/kg)

TABLE N1
Toxicokinetic Parameter Estimates for the Elimination of Dibromoacetic Acid from the Plasma of F344/N Rats
After a Single Gavage Dose of Dibromoacetic Acid^a

| Parameter | 25 mg/kg | 50 mg/kg | 125 mg/kg |
|--|---------------|---------------|---------------|
| Male | | | |
| C _{max} (µg/mL) | 10.5 ± 1.3 | 24.2 ± 2.4 | 77.5 ± 5.9 |
| C _{max} /dose | 0.42 ± 0.052 | 0.48 ± 0.048 | 0.62 ± 0.00 |
| AUC _{0-1 hour} [(µg • hours)/mL] | 7.77 | 20.6 | 54.7 |
| AUC _{0-1 hour} /dose | 0.31 | 0.41 | 0.44 |
| Absorption half-life (hours) ^b | 0.168 ± 0.064 | 0.156 ± 0.046 | 0.112 ± 0.028 |
| Elimination half-life (hours) ^b | 0.798 ± 0.117 | 0.945 ± 0.079 | 1.19 ± 1.09 |
| Female | | | |
| C _{max} (µg/mL) | 14.9 ± 2.0 | 29.2 ± 2.7 | 94.9 ± 7.7 |
| C _{max} /dose | 0.59 ± 0.080 | 0.58 ± 0.054 | 0.76 ± 0.062 |
| AUC _{0-1 hour} [(µg • hours)/mL] | 10.8 | 26.3 | 76.8 |
| AUC _{0-1 hour} /dose | 0.43 | 0.53 | 0.61 |
| Absorption half-life (hours) ^b | 0.069 ± 0.036 | 0.130 ± 0.037 | 0.098 ± 0.027 |
| Elimination half-life (hours) ^b | 0.869 ± 0.11 | 0.771 ± 0.071 | 0.982 ± 0.059 |

^a Data are presented as mean (AUCs) or mean ± standard error. C_{max} = maximum plasma concentration; AUC_{0-1 hour} = area under the plasma concentration versus time curve from time 0 to 1 hour

^b Due to flip-flop kinetics, absorption half-life estimates are actually elimination half-life estimates and vice versa.

TABLE N2
Toxicokinetic Parameter Estimates for the Elimination of Dibromoacetic Acid from the Plasma of B6C3F₁ Mice
After a Single Gavage Dose of Dibromoacetic Acid^a

| Parameter | 70 mg/kg | 175 mg/kg | 350 mg/kg |
|---|---------------|---------------|---------------|
| Male | | | |
| C _{max} (µg/mL) | 6.04 ± 0.75 | 32.2 ± 4.1 | 91.1 ± 13.9 |
| C _{max} /dose | 0.086 ± 0.011 | 0.184 ± 0.023 | 0.26 ± 0.040 |
| AUC _{0-20 minutes} [(µg • hours)/mL] | 1.42 | 8.03 | 24.3 |
| AUC _{0-20 minutes} /dose | 0.020 | 0.046 | 0.069 |
| Absorption half-life (hours) ^b | 0.118 ± 0.046 | 0.063 ± 0.031 | 0.087 ± 0.048 |
| Elimination half-life (hours) ^b | 0.359 ± 0.058 | 0.798 ± 0.056 | 1.75 ± 0.16 |
| Female | | | |
| C _{max} (µg/mL) | 6.75 ± 1.21 | 36.4 ± 5.12 | 72.2 ± 10.7 |
| C _{max} /dose | 0.373 ± 0.017 | 0.208 ± 0.029 | 0.206 ± 0.031 |
| AUC _{0-20 minutes} [(µg • hours)/mL] | 1.22 | 7.43 | 24.7 |
| AUC _{0-20 minutes} /dose | 0.017 | 0.042 | 0.070 |
| Absorption half-life (hours) ^b | 0.081 ± 0.041 | 0.065 ± 0.035 | 0.040 ± 0.037 |
| Elimination half-life (hours) ^b | 0.330 ± 0.051 | 0.671 ± 0.066 | 1.99 ± 0.20 |

^a Data are presented as mean (AUCs) or mean ± standard error. C_{max} = maximum plasma concentration; AUC_{0-20 minutes} = area under the plasma concentration versus time curve from time 0 to 20 minutes

^b Due to flip-flop kinetics, absorption half-life estimates are actually elimination half-life estimates and vice versa.

