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

NTP Technical Report on the Toxicology and Carcinogenesis Studies of Bromochloroacetic Acid (CAS No. 5589-96-8) in F344/N Rats and B6C3F1 Mice (Drinking Water Studies)

NTP TR 549

FEBRUARY 2009

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF BROMOCHLOROACETIC ACID

(CAS NO. 5589-96-8)

IN F344/N RATS AND B6C3F1 MICE

(DRINKING WATER STUDIES)

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

February 2009

NTP TR 549

NIH Publication No. 09-5890

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

FOREWORD

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

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

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CONTENTS

SUMMARY

Background

Bromochloroacetic acid occurs as a by-product of water disinfection. We studied the effects of bromochloroacetic acid in drinking water on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods

We gave drinking water containing 250, 500, or 1,000 mg of bromochloroacetic acid per liter of water to groups of 50 male and female rats and mice for 2 years. Control animals received the same tap water with no chemical added. At the end of the study tissues from more than 40 sites were examined for every animal.

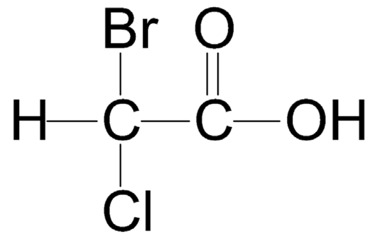
Results

Survival was similar for rats and female mice receiving bromochloroacetic acid and the controls; survival of 1,000 mg/L male mice was less. Male rats receiving bromochloroacetic acid had increased rates of malignant mesotheliomas. Adenomas of the large intestine were seen in both male and female rats receiving the highest concentration of bromochloroacetic acid. Exposed female rats also had increased incidences of multiple fibroadenomas of the mammary gland. Slightly increased incidences of liver hepatocellular adenomas in male and female rats and pancreatic islet adenomas in male rats were also observed in exposed animals. Male and female mice exposed to bromochloroacetic acid had increased rates of a variety of liver cancers.

Conclusions

We conclude that bromochloroacetic acid in the drinking water caused mesothelioma in male rats, multiple fibroadenomas of the mammary gland in female rats, and adenomas of the large intestine in both male and female rats. Adenomas of the liver in male and female rats and of the pancreatic islets in male rats may also have been related to bromochloroacetic acid exposure. We conclude that bromochloroacetic acid caused liver cancer in male and female mice.

ABSTRACT



BROMOCHLOROACETIC ACID

CAS No. 5589-96-8

Chemical Formula: $C_2H_2BrClO_2$ Molecular Weight: 173.40

Synonyms: Acetic acid; bromochloro (9CI); bromochloroacetate; bromochloroethanoic acid

Bromochloroacetic acid is a water disinfection by-product. Bromochloroacetic 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 bromochloroacetic acid (greater than 95% pure) in drinking water for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium, Escherichia coli,* and peripheral blood erythrocytes of exposed mice.

2-Week Study in Rats

Groups of five male and five female rats were exposed to drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg/L bromochloroacetic acid for 2 weeks (equivalent to average daily doses of approximately 9, 18, 35, 75, or 140 mg bromochloroacetic acid/kg body weight to males and 8, 17, 35, 70, or 130 mg/kg to females). All rats survived to the end of the study. Mean body weights of exposed males and females were similar to those of the controls. Water consumption by exposed and control groups was similar. Right kidney weights of 1,000 mg/L males were significantly increased. No exposure-related gross or histopathologic lesions were observed.

2-Week Study in Mice

Groups of five male and five female mice were exposed to drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg/L bromochloroacetic acid for 2 weeks (equivalent to average daily doses of approximately 10, 20, 40, 80, or 170 mg/kg to males and 9, 17, 40, 75, or 155 mg/kg to females). All mice survived to the end of the study. Mean body weights of 250 mg/L males were significantly greater than those of the controls. Water consumption by exposed and control groups was similar. No gross or histopathologic lesions related to exposure to bromochloroacetic acid were observed.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg/L bromochloroacetic acid for 3 months (equivalent to average daily doses of approximately 5, 10, 20, 40, or 75 mg/kg to males and 5, 10, 20, 40, or 85 mg/kg to females). All rats survived to the end of

the study. Mean body weights of exposed male and female rats were similar to those of the controls. Water consumption by exposed and control groups was similar. Liver weights of 500 and 1,000 mg/L males and females and kidney weights of 1,000 mg/L males were significantly increased. In the liver, there were significantly increased incidences of cytoplasmic vacuolization in 1,000 mg/L males and females.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to drinking water containing 0, 62.5, 125, 250, 500, or 1,000 mg/L bromochloroacetic acid for 3 months (equivalent to average daily doses of approximately 8, 16, 32, 65, or 125 mg/kg to males and 8, 17, 35, 70, or 140 mg/kg to females). All mice survived to the end of the study. Mean body weight gains of females exposed to 250 mg/L or greater were significantly decreased. Water consumption by exposed and control groups was similar. Liver weights of 1,000 mg/L males and all exposed groups of females were significantly increased. All males and females exposed to 500 or 1,000 mg/L had periportal cytoplasmic vacuolization. In the spleen, there were increased incidences of hematopoietic cell proliferation in 62.5, 125, and 250 mg/L males and 125 and 1,000 mg/L females.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to drinking water containing 0, 250, 500, or 1,000 mg/L bromochloroacetic acid for 2 years (equivalent to average daily doses of approximately 10, 20, or 40 mg/kg to males and 13, 25, or 50 mg/kg to females). Survival of exposed rats was similar to that of the control groups. Mean body weights of 500 mg/L males were 8% less than the control group after week 81, and those of 1,000 mg/L males were 10% less than the control group after week 69. Mean body weights of 1,000 mg/L females were 10% less than the control group after week 85. Water consumption by exposed and control groups was similar.

The incidences of malignant mesothelioma in all exposed groups of male rats exceeded the historical control ranges, and the incidence in the 500 mg/L group was significantly increased. Positive trends in the incidences of adenoma of the large intestine (colon or rectum) occurred in male and female rats, and the incidence in 1,000 mg/L females was significantly increased. Although the incidences of mammary gland fibroadenoma were not significantly increased in exposed female rats, the incidences of multiple fibroadenomas of the mammary gland were increased in 500 and 1,000 mg/L females. The incidence of pancreatic islet adenoma was significantly increased in 500 mg/L males. The incidences of hepatocellular adenoma occurred with a positive trend in females; the incidences in 500 mg/L males and 1,000 mg/L males and females exceeded the historical control ranges.

In the liver, the incidences of eosinophilic focus in 500 mg/L females and 1,000 mg/L males and females and of mixed cell focus in 1,000 mg/L females were significantly increased. In the lung, the incidence of alveolar epithelium hyperplasia was significantly increased in 1,000 mg/L females

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to drinking water containing 0, 250, 500, or 1,000 mg/L bromochloroacetic acid for 2 years (equivalent to average daily doses of approximately 25, 50, or 90 mg/kg to males and 15, 30, or 60 mg/kg to females). Survival of 1,000 mg/L males was significantly less than that of the control group. Mean body weights of 1,000 mg/L males were 12% less than the control group after week 97, and those of 1,000 mg/L females were 8% less than the control group after week 21. Water consumption by exposed and control groups was similar.

The incidences of hepatocellular adenoma in 250 and 500 mg/L males and all exposed groups of females, hepatocellular carcinoma in 500 and 1,000 mg/L males and 500 mg/L females, hepatocellular adenoma or carcinoma (combined) in all exposed groups of males and females, and hepatoblastoma in all exposed groups of males were significantly increased. The incidences of hepatocyte cytoplasmic vacuolization in all exposed groups, eosinophilic focus in 500 and 1,000 mg/L females, and centrilobular necrosis in 1,000 mg/L males were significantly increased.

The incidences of hematopoietic cell proliferation of the spleen were significantly increased in 500 and 1,000 mg/L males, and the incidence of bone marrow hyperplasia was significantly increased in 1,000 mg/L males.

Genetic Toxicology

In two different bacterial mutagenicity assays, bromochloroacetic acid was positive in *Salmonella typhimurium* strain TA100, in tests conducted with and without exogenous metabolic activation enzymes (S9); no mutagenicity was detected in strain TA98 or in *Escherichia coli* WP2 *uvrA*/pKM101, with or without S9. No significant increases in the frequency of micronucleated erythrocytes were observed in blood samples of male or female mice exposed to bromochloroacetic acid for 3 months in drinking water, indicating no induction of chromosomal damage in proerythrocytes under these conditions in mice.

Conclusions

Under the conditions of these 2-year studies, there was *clear evidence of carcinogenic activity** of bromochloroacetic acid in male F344/N rats based on increased incidences of malignant mesotheliomas and adenomas of the large intestine. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in female F344/N rats based on increased incidences of adenomas of the large intestine; increased incidences of multiple fibroadenomas of the mammary gland in female rats were also considered to be exposure related. Increased incidences of pancreatic islet adenomas in male rats and of hepatocellular adenomas in male and female rats may have been related to bromochloroacetic acid exposure. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms and hepatoblastoma (males only).

Exposure to bromochloroacetic acid for 2 years resulted in increased incidences of nonneoplastic lesions in the liver of male rats, liver and lung of female rats, and liver of male and female mice.

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

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in drinking water	0, 250, 500, or 1,000 mg/L	0, 250, 500, or 1,000 mg/L	0, 250, 500, or 1,000 mg/L	0, 250, 500, or 1,000 mg/L
Body weights	500 mg/L group 8% less than the control group after week 81; 1,000 mg/L group 10% less than the control group after week 69	1,000 mg/L group 10% less than the control group after week 85	1,000 mg/L group 12% less than the control group after week 97	1,000 mg/L group 8% less than the control group after week 21
Survival rates	31/50, 26/50, 25/50, 29/50	34/50, 31/50, 37/50, 35/50	38/50, 35/50, 30/50, 21/50	36/50, 42/50, 32/50, 40/50
Nonneoplastic effects	Liver: eosinophilic focus (2/50, 5/50, 4/50, 8/50)	Liver: eosinophilic focus (1/50, 6/50, 9/50, 15/50); mixed cell focus (1/50, 4/50, 6/50, 10/50) Lung: alveolar epithelium hyperplasia (5/50, 7/50, 8/50, 18/50)	Liver: hepatocyte cytoplasmic vacuolization (3/50, 12/50, 17/50, 19/50)	Liver: hepatocyte cytoplasmic vacuolization (3/50, 11/50, 27/50, 42/50); eosinophilic focus (13/50, 22/50, 31/50, 24/50)
Neoplastic effects	Malignant mesothelioma: (1/50, 5/50, 10/50, 6/50) Large intestine: adenoma (0/50, 2/50, 0/50, 4/50)	Large intestine: adenoma (0/50, 0/50, 3/50, 7/50) Mammary gland: fibroadenoma, multiple (22/50, 24/50, 43/50, 38/50)	Liver: hepatocellular adenoma (27/50, 40/50, 40/50, 31/50); hepatocellular carcinoma (19/50, 25/50, 36/50, 45/50); hepatocellular adenoma or carcinoma (34/50, 44/50, 49/50, 49/50); hepatoblastoma (4/50, 11/50, 28/50, 34/50)	Liver: hepatocellular adenoma (27/50, 48/50, 44/50, 46/50); hepatocellular carcinoma (14/50, 23/50, 26/50, 20/50); hepatocellular adenoma or carcinoma (31/50, 49/50, 46/50, 46/50)
Equivocal findings	Pancreatic islets: adenoma (3/50, 4/50, 9/50, 3/50) Liver: hepatocellular adenoma (2/50, 0/50, 3/50, 4/50)	Liver: hepatocellular adenoma (0/50, 0/50, 0/50, 3/50)	None	None
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Positive in TA100 with and without S9; negative in TA98 with or without S9; negative in *Escherichia coli* strain WP2 *uvrA*/pKM101 with or without S9
Micronucleated erythrocytes Mouse peripheral blood *in vivo:*	Negative

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on bromochloroacetic acid on February 27, 2008, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 27, 2008, the draft Technical Report on the toxicology and carcinogenesis studies of bromochloroacetic acid received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. R.L. Melnick, NIEHS, described the occurrence of bromochloroacetic acid as a drinking water disinfection by-product and the results of the NTP studies. The proposed conclusions were *clear evidence of carcinogenic activity* of bromochloroacetic acid in male and female rats and mice.

Dr. Soper, the first principal reviewer, felt the study was well done and agreed with the proposed conclusions. He explored the occurrence of the hepatocellular neoplasms in male and female rats and inquired about the rationale for classifying them as equivocal, rather than some evidence.

Dr. Bunton, the second principal reviewer, also felt the study was well done. She also inquired about the intent of the language for the proposed conclusions for the hepatocellular lesions.

Dr. Novak, the third principal reviewer, inquired if there were any other clinical pathology measurements to assess the metabolic state of the animals.

Dr. Melnick explained that the phrase "may have been related" to chemical exposure was equivalent to *equivocal evidence* at that particular site in a study where a higher level of evidence was seen in other tissues. He noted that while the hepatocellular adenomas were indeed uncommon tumors, occurring at a background rate of about 1%, the occurrence of just a few such tumors best fit the interpretive category of equivocal evidence. He noted that the clinical pathology measurements were performed in the 3-month studies but not the 2-year studies.

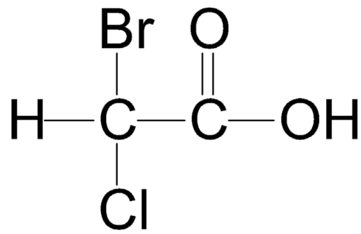
Dr. P.C. Howard, NCTR, inquired about the rationale for classifying the malignant mesotheliomas and large intestine tumors in male rats as clear evidence with statistical significance by either the trend test or pairwise comparison, but not both, at each site. Dr. Melnick explained that large intestine neoplasms are extremely rare in control rats, and the occurrence of several such tumors in exposed animals was a strong indication of a chemical-related effect. Moreover, for the intestinal tumors there was supporting evidence from an even stronger response in the female rats. Dr. Melnick also noted that the incidence of mesotheliomas exceeded the historical control range in all exposed groups and that the incidences of mesotheliomas were increased in a previous NTP study of a related chemical, dibromoacetic acid.

Dr. Crump asked if it was standard practice to combine the incidences of mesotheliomas from all sites. Dr. Melnick said that all the mesotheliomas that occur in the peritoneum are combined. Dr. Crump also suggested that the marginally increased incidences of skin fibroma and fibrosarcoma be mentioned in the text.

Dr. Cattley noted that the proposed conclusion for female rats was based largely on intestinal adenomas, and he suggested that more explanation of the knowledge about the possible progression of these tumors to malignancy from other studies be included in the discussion.

Dr. Soper moved, and Dr. Mirsalis seconded, that the conclusions be accepted as written. The motion was carried unanimously with eight votes.

INTRODUCTION



BROMOCHLOROACETIC ACID

CAS No. 5589-96-8

Chemical Formula: $C_2H_2BrClO_2$ Molecular Weight: 173.40

Synonyms: Acetic acid; bromochloro (9CI); bromochloroacetate; bromochloroethanoic acid

Chemical and Physical Properties

Bromochloroacetic acid is a crystalline compound (melting point, 27.5° C; boiling point, 215° C at 760 mm Hg) (Weast, 1983). It is a moderately strong acid; the pKa for dihaloacetic acids, including bromochloroacetic acid, dichloroacetic acid, and dibromoacetic acid, is approximately 1.3 (Schultz *et al.*, 1999; Urbansky, 2000). In dilute solutions at pH greater than 6, more than 99.99% of the chemical exists as the dissociated carboxylate anion, bromochloroacetate. Thus, under most conditions of exposure and in biological tissues, this chemical exists as the carboxylate anion. In contrast to dichloroacetate and dibromoacetate, bromochloroacetate contains an asymmetric carbon atom and, therefore, can exist in two nonsuperimposable forms, the (+)- and (–)-bromochloroacetate stereoisomers. Although bromochloroacetic acid was the test article for this Technical Report, in the animal, it is described as bromo-chloroacetate 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 hypobromous 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 compounds, 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), these processes are very slow in ambient water, and 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 class of 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. Controlled laboratory studies (Huang *et al.*, 2004) also showed that dibromoacetic acid was produced by ozonation of organic fractions of source water containing high ambient bromide concentrations; bromochloroacetic acid was not measured in this study. Treatment of natural waters with chloramine or chlorine dioxide produced haloacetic acids, including bromochloroacetic acid; however, the levels of these by-products were substantially less than those formed by free chlorine (Hua and Reckhow, 2007). Preozonation increased the concentration of dihaloacetic acids formed by free chlorine and reduced the concentration formed by chloramination.

Levels of haloacetic acids in drinking water are regulated by the United States Environmental Protection Agency (USEPA) (40 CFR, § 141.64). Under the disinfection by-products rule, the sum of the concentrations 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. However, bromochloroacetic acid is not included in the five haloacetic acids regulated by the USEPA under the current disinfection by-products rule. 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 bromochloroacetic acid ranged up to 19 µg/L in finished water samples and in the distribution systems.

Absorption, Distribution, Metabolism, and Excretion

Dihaloacetates are rapidly absorbed from the gastrointestinal tract after oral exposure (James *et al.*, 1998; Schultz *et al.*, 1999). The maximum blood concentration of bromochloroacetate in F344/N rats was reached 1.5 hours after administration by gavage (Schultz *et al.*, 1999).

Dihaloacetates exhibit low binding to rat plasma proteins; in plasma obtained from dosed F344 rats, 93% of the measured bromochloroacetate was in the unbound fraction (Schultz *et al.*, 1999). Forty-eight hours after oral administration of ^{14}C-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. No data are available on placental transfer of bromochloroacetate.

The oral bioavailability of bromochloroacetate was reported to be 47% in male F344/N rats (Schultz *et al.*, 1999). The lower bioavailability of bromochloroacetate compared to dichloroacetate is due to greater first-pass metabolism of bromochloroacetate 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). Glyoxylate is the initial stable metabolite of dichloroacetate formed by purified GST-ζ (Tong *et al.*, 1998b). The major metabolites identified in the urine or blood of rats or mice administered dichloroacetate are glyoxylate, glycolate, and oxalate (Lin *et al.*, 1993; James *et al.*, 1998; Narayanan *et al.*, 1999).

In addition to these metabolites, approximately 30% of radioactivity from orally administered ^{14}C-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 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. Glyoxylate may induce toxicity by reacting covalently with proteins, e.g., N-terminal amino groups or lysine ε-amino groups (Anderson *et al.*, 2004).

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 bromochloroacetate, the plasma half-life after intravenous injection is approximately 45 minutes (Schultz *et al.*, 1999). Elimination of dihaloacetates is primarily by metabolism; approximately 2% of an intravenous dose (500 µmol bromochloroacetate/kg body weight; 86.7 mg/kg) is excreted as the parent compound in urine, and less than 0.1% is excreted in feces. Bromine substitution increases the rate of clearance of dihaloacetates.

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. Elimination of dichloroacetate in rats was dramatically reduced even after a single dose of 50 mg/kg (James *et al.*, 1998).

Gonzalez-Leon *et al.* (1999) reported that 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. 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 reductions 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) was 0.21 day (Anderson *et al.*, 1999). Treatment of male F344/N rats with 0.2 g dichloroacetate/L drinking water for 7 days reduced liver GST-ζ activity by 90%; this reduction markedly decreased the total body clearance of dichloroacetate and increased its oral bioavailability (Saghir and Schultz, 2002). Guo *et al.* (2006) reported that exposures as low as 2.5 µg dichloroacetate/kg per day for 8 weeks also significantly decreased GST-ζ activity in Sprague-Dawley rats; this exposure level is similar to what humans may be exposed from consumption of chlorinated drinking water.

Bromochloroacetate and dibromoacetate are also suicide substrates for GST-ζ; 12 hours after a single injection of these dihaloacetates (0.30 mmol/kg), GST-ζ activity in the rat liver was reduced to 19% and 17%, respectively, of that in controls (Anderson *et al.*, 1999). Hydrolysis

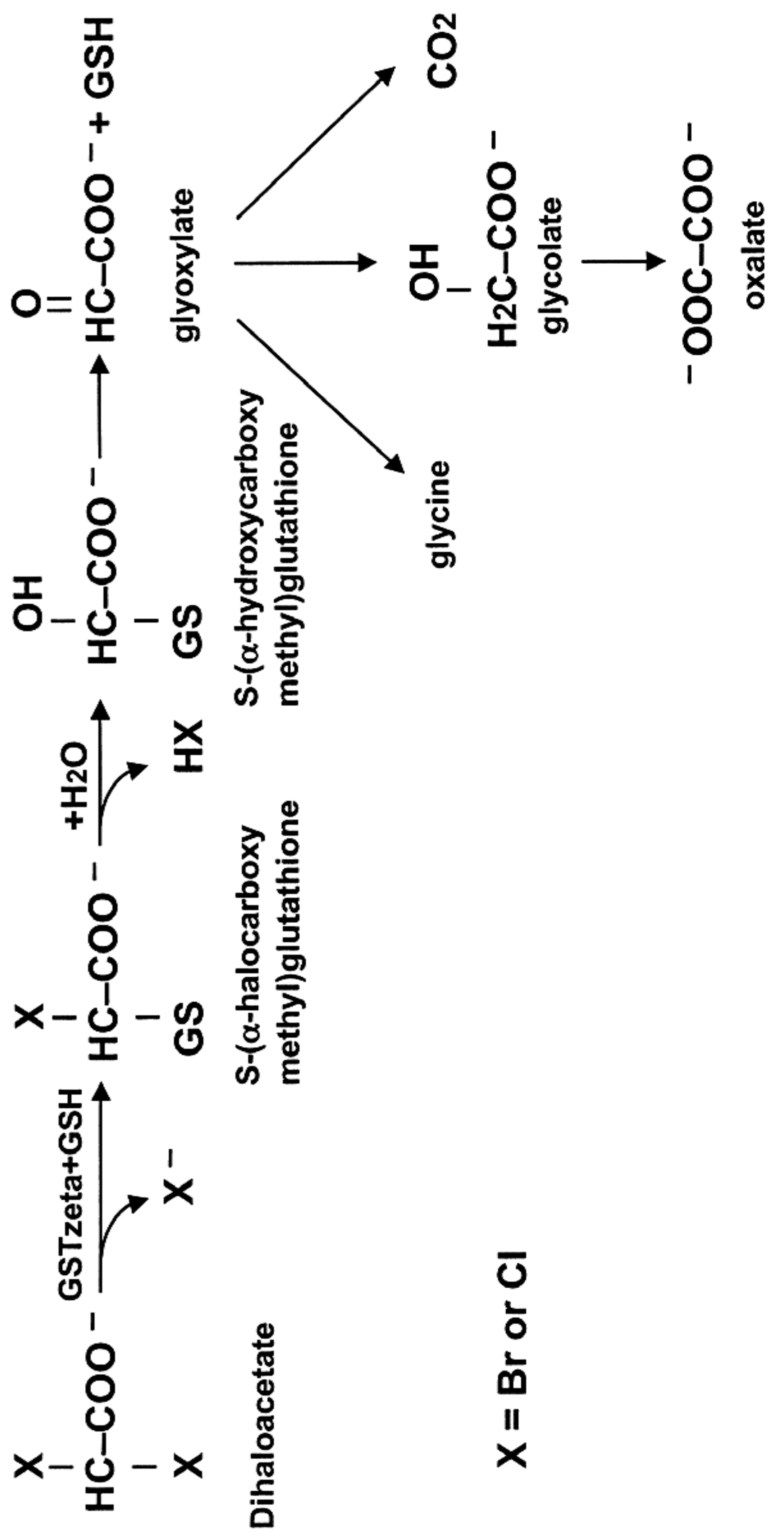


FIGURE 1
Scheme for the Biotransformation of Dihaloacetates (Tong *et al.*, 1998b)

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 (Anderson *et al.*, 1999; Wempe *et al.*, 1999). Thus, hydrolysis of this intermediate and inactivation of GST-ζ are competing reactions. Recovery of GST-ζ activity occurs via *de novo* synthesis of the protein. GST-ζ activity was decreased 95% in Sprague-Dawley rats after exposure to 50 mg/kg dichloroacetate in drinking water for 1 week; after cessation of exposure, GST-ζ activity returned to 60% of control levels by 1 week and fully recovered by 8 weeks postexposure (Guo *et al.*, 2006). Because GST-ζ is identical to maleylacetoacetate isomerase, the enzyme that catalyzes the penultimate step in the tyrosine degradation pathway, loss of this enzyme by exposure to dihaloacetates causes accumulation of maleylacetoacetate and maleylacetone. The latter compound may cause tissue damage by reacting with cellular nucleophiles. In GST-ζ knockout mice, [^{13}C]di-chloroacetate was not metabolized to glyoxylate or monochloroacetate to any significant extent, and very high levels of dichloroacetate were excreted in the urine (Ammini *et al.*, 2003). Thus, GST-ζ is the only enzyme that significantly catalyzes the metabolic clearance of dichloroacetate.

The elimination half-life of (–)-bromochloroacetate in naive male F344 rats is approximately fivefold smaller than that of (+)-bromochloroacetate, indicating that the rate of GST-ζ-catalyzed metabolism of bromochloroacetate is much faster for the (–) stereoisomer (Schultz and Sylvester, 2001). In rats treated with dichloroacetate to deplete GST-ζ activity, the elimination half-life of (–)-bromochloroacetate was increased to a value similar to that of (+)-bromochloroacetate. Because the metabolism of the bromochloroacetate stereoisomers in naive and GST-ζ-depleted cytosol of rats is dependent on the presence of glutathione, 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

Much more health effects data are available on dichloroacetate and dibromoacetate than on bromo-chloroacetate. Results of toxicity studies on dichloro-acetate and dibromoacetate included below are informative of the potential health effects of bromochloroacetate because of qualitative similarities in the metabolism of the chlorinated/brominated dihaloacetates.

Experimental Animals

The LD_{50} 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 administered 250 mg dibromoacetate/kg per day by gavage, 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. Bull *et al.* (1990) reported that 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. 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, bromochloroacetate and dibromoacetate caused glycogen accumulation in the liver of B6C3F1 mice (Kato-Weinstein *et al.*, 2001). However, dibromoacetate, but not bromochloroacetate, produced transient increases (2 to 4 weeks) in liver cell replication rates and in peroxisomal acyl-CoA oxidase activity. Dibromoacetate and dichloroacetate also induced peroxisomal palmitoyl-CoA oxidase activity in primary rat hepatocyte cultures (Walgren *et al.*, 2004). Hepatocellular vacuolization was observed in F344/N rats (increased incidence) and B6C3F1 mice (increased severity) administered dibromoacetic acid in drinking water at concentrations ranging from 125 to 2,000 mg/L for 3 months (Melnick *et al.*, 2007; NTP, 2007a).

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 tri-chloroacetate (Plewa *et al*., 2002).

Humans

No studies on the toxicity of bromochloroacetate 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 dihaloacetates. Initial studies evaluated the effects of dibromoacetate and dichloroacetate, while later studies also examined the potential male reproductive toxicity of bromochloroacetate.

Administration of 72 or 216 mg/kg bromochloroacetate to male Sprague-Dawley rats for 14 days 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). Similar effects were observed with dichloroacetate in Sprague-Dawley rats (Linder *et al.*, 1997a) and with dibromoacetate in Sprague-Dawley rats (Linder *et al.*, 1994a,b, 1997b) and in F344/N rats and B6C3F1 mice (Melnick *et al.*, 2007; NTP, 2007a). The formation of atypical residual bodies was suggested to be a result of impairment of degradative processes of Sertoli cells (Linder *et al.*, 1994b, 1997b). In spite of the changes in sperm quality caused by dibromoacetate at daily doses up to approximately 150 mg/kg, the germinal epithelium appeared intact and there were no obvious changes in sperm production in exposed rats. At higher doses of dibromoacetate, germinal epithelial atrophy was induced in exposed rats (Linder *et al.*, 1997b; Melnick *et al.*, 2007).

Juvenile and adult C57BL/6 male mice were exposed daily to 0, 8, 24, 72, or 216 mg/kg bromochloroacetate for 14 days and evaluated for reproductive performance in a 40-day continuous breeding assay (Tully *et al.*, 2005). Juvenile mice, exposed from postnatal day 8 through postnatal day 21, were allowed to mature to 14 weeks of age and then entered into the 40-day mating study. Effects on fertility were observed only during the first 10 days of mating in mice exposed as adults (10 weeks old). In the two highest exposure groups, the mean number of litters per male, the percentage of litters per female bred, and the total number of fetuses per male were reduced. In addition, histopathologic evaluations of testes after the final dosing with bromochloroacetate revealed spermatids with abnormal head morphology and atypical residual bodies. Thus, diminished male fertility was attributed to disruption of spermatid differentiation by bromochloroacetate. Treatment with bromochloroacetate did not affect relative testis, epididymal, or seminal vesicle weights.

The fertility of cauda epididymal sperm evaluated by *in utero* insemination was reduced in male rats exposed to 8, 24, or 72 mg bromochloroacetate/kg for 14 days (Klinefelter *et al.*, 2002). In male Sprague-Dawley rats gavaged daily with up to 50 mg dibromoacetate/kg body weight for up to 79 days, fertility was not altered; however, male fertility was compromised in rats treated for 15 days or longer 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. In fertility assessments by intrauterine insemination, the ED_{50} for decreased male fertility of cauda epididymal sperm collected from Sprague-Dawley rats receiving 2.7 mg/kg (15.6 µmol/kg) bromochloroacetate by gavage for 14 days was similar to the ED_{50} for dibromoacetate (3.5 mg/kg, 16.1 µmol/kg) (Kaydos *et al.*, 2004).

In a short-term reproductive toxicity screen, male and female rats were exposed to 0, 60, 200, or 600 ppm (mg/L) of bromochloroacetic acid in the drinking water during cohabitation and separated after mating (NTP, 1998). Treatment of dams continued until gestation day 20, at which time they were examined for fertility and pregnancy status. Treatment of males continued until 30 days after mating, at which time they were necropsied and examined for sperm counts, motility, and morphology, organ weights, and histopathologic changes in reproductive organs. In a concomitant developmental toxicity screen, pregnant rats were exposed to the same concentrations of bromochloroacetic acid in drinking water from gestation day 6 until onset of birth. At postnatal day 5, dams were necropsied and uteri were examined; also at that time, pups were examined for external malformations and soft tissue abnormalities. Treatment related findings in these studies included a significant decrease in total implants per litter and a significant decrease in the number of live fetuses per litter at 600 ppm.

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). 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 or F_1 generation female rats. In parental and F_1 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 or 650 ppm exposure groups. Delays in preputial separation and vaginal opening were observed in 650 ppm groups of F_1 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.

Exposure of mouse whole embryo cultures to haloacetates for up to 26 hours caused defects in neural tube closure, craniofacial abnormalities, proencephalic and pharyngeal arch hypoplasia, deficient caudal growth, and eye and heart defects (Hunter *et al.*, 1996, 2006a). Bromochloroacetate and dibromoacetate were more potent than dichloroacetate at disrupting embryogenesis and altering morphogenesis. Bromochloroacetate was also more potent than dibromochloroacetate and bromodichloroacetate at inducing neural tube defects (Hunter *et al.*, 2006b). Bromochloroacetate, dibromoacetate, and dichloroacetate also caused developmental abnormalities (mainly delayed caudal development) in rat embryonic cultures, with dichloroacetate being the least potent of these three dihaloacetates (Andrews *et al.*, 2004). The developmental effects of these haloacetates appeared to be additive in mixture studies in whole embryo cultures.

Bromochloroacetate, but not dibromoacetate, caused an increase in the incidence of malformations (axial deformities and gut malformations) in the *Xenopus* frog embryo teratogenesis assay (Weber *et al.*, 2004); however, effects were seen only at exposure concentrations between 10,000 and 12,000 ppm.

Humans

No human studies have been reported on reproductive or developmental effects of bromochloroacetate *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). Although associations between stillbirth risk and exposure to disinfection by-products have been demonstrated, no association was observed with haloacetic acids after controlling for exposures to trihalomethanes (King *et al.*, 2005). Associations between exposure to the five dihaloacetic acids regulated by the USEPA and impaired fetal growth have been reported (Hinckley *et al.*, 2005).

Carcinogenicity

Experimental Animals

No studies have been reported on the carcinogenicity of bromochloroacetate 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 or 7 of 22, respectively) were less than those induced with dichloroacetate (25 of 26 or 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 liver tumors were 3/20 (15%) in controls and 18/24 (75%) in dichloroacetate-exposed mice (Daniel *et al.*, 1992). In a subsequent drinking water study, B6C3F1 mice were exposed to 0, 0.05, 0.5, 1.0, 2.0, or 3.5 g/L dichloroacetic acid for 100 weeks (DeAngelo *et al.*, 1999). Mean daily doses were 0, 8, 84, 168, 315, or 429 mg dichloroacetic acid/kg, and the incidences of hepatocellular carcinoma were 26%, 33%, 48%, 71%, 95%, or 100%, respectively. The liver tumor response was significantly increased at exposures of 0.5 g/L or greater.

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 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 basophilic and/or clear cell foci that do not respond to normal homeostatic control mechanisms (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.

Dibromoacetic acid was administered for 2 years to male and female F344 rats and B6C3F1 mice in drinking water at concentrations of 0, 50, 500, or 1,000 mg/L (Melnick *et al.*, 2007; NTP, 2007a). Exposure-related neoplasms were increased at multiple sites; these included mononuclear cell leukemia and abdominal cavity mesotheliomas in rats and liver and lung neoplasms in mice. The increased incidences of hepatocellular neoplasms in male mice were significant even in the 50 mg/L group, an exposure equivalent to an average daily dose of approximately 4 mg/kg.

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 ≈ 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 or 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 increased mRNA expression of the c-*myc* and insulin-like growth factor II genes (Tao *et al.*, 2004). Dibromoacetate and dichloroacetate also caused hypomethylation of DNA in kidneys of male B6C3F1 mice and male F344 rats exposed to these agents via the drinking water (Tao *et al.*, 2005). Treatment with dibromoacetate also caused glycogen accumulation and peroxisome proliferation in the mouse and rat liver. Thus, dibromoacetate and dichloroacetate appear to induce similar biochemical and molecular effects.

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 dichloro-acetate-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 bromochloroacetate 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 USEPA (2003). Based on sufficient evidence of carcinogenicity in experimental animals, dichloroacetate is listed by the International Agency for Research on Cancer (IARC, 2004) as possibly carcinogenic to humans (Group 2B).

Genetic Toxicity

Although several studies have demonstrated genotoxicity of dihaloacetates, little information is available on bromochloroacetate. 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 standard Ames tests using *S. typhimurium* strains TA98 and/or TA100, with and without metabolic activation (DeMarini *et al.*, 1994; Kargalioglu *et al.*, 2002; NTP, 2007a,b); dibromoacetate was the more potent mutagen in these two strains (Kargalioglu *et al.*, 2002). 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 bromochloroacetate or dibromo-acetate 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). Glyoxylate, an intermediate of dihaloacetate metabolism, 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 dichloro-acetate/L drinking water for 60 weeks (Leavitt *et al.*, 1997); consistent with the bacterial data, mutations in mice 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 (Fuscoe *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. Similar studies with dichloroacetate in male and female Tg.AC, p53$^{(+/-)}$, and B6C3F1 mice (3 months of exposure, highest concentration of 2 g/L) resulted in no increase in micronucleated erythrocytes (NTP, 2007b); in these latter studies, both the duration of treatment and the concentration levels of dichloroacetate were lower than in the studies conducted by Fuscoe *et al.* (1996), and this may have been a factor in the different outcomes of these two studies. Significant increases in micronucleated normochromatic erythrocytes were observed, however, in peripheral blood of male B6C3F1 mice treated with dibromoacetate for 3 months in drinking water; no increases in micronucleated erythrocytes were noted in female mice in this study (NTP, 2007a). 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

Bromochloroacetic acid and dibromoacetic acid were nominated to the NTP by the USEPA for toxicity and carcinogenicity studies in rats and mice because of widespread human exposure to these water disinfection by-products and because a related dihaloacetic acid, dichloroacetic acid, 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 these chemicals. Results of the NTP studies on dibromoacetic acid were reported previously (Melnick *et al.,* 2007; NTP, 2007a)

MATERIALS AND METHODS

Procurement and Characterization of Bromochloroacetic Acid

Bromochloroacetic acid was obtained from Carbolabs, Inc. (Woodbridge, CT), in two lots (II-37A and 11388A). Lot II-37A was used in the 2-week and 3-month studies, and lot 11388A was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH); identity and purity analyses were also conducted by the study laboratory, Southern Research Institute (Birmingham, AL) (Appendix I). Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the bromochloroacetic acid studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a waxy, off-white solid, was identified as bromochloroacetic acid by infrared and nuclear magnetic resonance spectroscopy. The water content of lots II-37A and 11388A was determined by Karl Fischer titration. The purity of both lots was determined by functional group titration and high-performance liquid chromatography (HPLC). Purity was also determined using ion chromatography for lot II-37A and differential scanning calorimetry (DSC) for lot 11388A.

For lot II-37A, Karl Fischer titration indicated 180 ppm water. Functional group titration indicated a purity of 100.3% ± 0.6%. Ion chromatography indicated one major peak and three impurities with a combined area of 1.7%. HPLC indicated an area percent purity of 95.7%. The overall purity of lot II-37A was determined to be greater than 95%.

For lot 11388A, Karl Fischer titration indicated 0.65% water. Functional group titration indicated a purity of 98.7% ± 0.8% and DSC indicated a purity of 96.7%. HPLC indicated an area percent purity of 98.1% The overall purity of lot 11388A was determined to be greater than 96%.

To identify and quantitate the largest impurity, gas chromatography/mass spectrometry, HPLC, and standard addition were used. The impurity was identified as dibromoacetic acid at concentrations of 2.35% for lot II-37A and 0.83% for lot 11388A.

To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles sealed with Teflon®-lined lids. Stability was monitored during the 2-week, 3-month, and 2-year studies using HPLC; no degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice during the 2-week studies and approximately every 4 weeks during the 3-month and 2-year studies by mixing bromochloroacetic acid with tap water (Table I1). The level of bromochloroacetic acid measured in tap water used for these formulations was never greater than 6.5 µg/L. Stability studies of a 62.5 mg/L formulation were performed by the analytical chemistry laboratory using HPLC. Stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass or Nalgene® containers at 5° C and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of bromochloroacetic acid were conducted by the study laboratory using HPLC. During the 2-week studies, the dose formulations were analyzed twice; all six of the dose formulations for rats and mice were within 10% of the target concentrations (Table I2). During the 3-month studies, the dose formulations were analyzed at the beginning, midpoint, and end of the studies; all 15 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 15 samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks. All 78 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I4). Animal room samples of these dose formulations were also analyzed; all 12 samples for rats and all 12 for mice 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 to 5 weeks old. Animals were quarantined for 12 (rats) or 14 (mice) 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, 62.5, 125, 250, 500, or 1,000 mg bromochloroacetic acid/L 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, and water consumption was recorded twice weekly by cage on a 3-day/4-day schedule. 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 rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on the colon, small intestine (duodenum, jejunum, ileum), kidney, liver, lung, and stomach (forestomach and glandular) in all control rats and mice and 1,000 mg/L mice. Table 1 lists the tissues and organs examined.

3-Month Studies

The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to bromochloroacetic 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 to 5 weeks old. Rats were quarantined for 13 (males) or 14 (females) days, and mice were quarantined for 11 (females) or 12 (males) days; rats and mice were 5 to 7 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, 62.5, 125, 250, 500, or 1,000 mg bromochloroacetic acid/L in the drinking water for 14 weeks; groups of 10 male and 10 female clinical pathology rats 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 clinical pathology rats on days 3 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 CO_2/O_2 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).

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice exposed to 0, 250, 500, and 1,000mg/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 epididymal spermatozoal motility and concentration. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on 0 and 1,000 mg/L rats and mice. Organs with possible treatment-related effects were examined to a no-effect level in the remaining exposure groups. Table 1 lists the tissues and organs examined. The pathology findings underwent a quality assessment and any discrepancies were resolved by the NTP Pathology Working Group (Maronpot and Boorman, 1982; Boorman *et al.,* 1985).

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed to 0, 250, 500, or 1,000 mg bromochloroacetic acid/L in the drinking water for up to 105 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 6 to 7 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program using three to five male and female sentinel rats and mice at 6, 12, and 18 months and five male and five female 1,000 mg/L rats and mice at the end of the studies (Appendix L).

Animal Maintenance

Male rats were housed 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 every 2 weeks. 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. Body weights were recorded initially, and water consumption and body weights were recorded weekly for the first 13 weeks and at 4-week intervals thereafter.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. 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, adrenal medulla, thyroid gland, and pancreatic islets for male and female rats; the epididymis, seminal vesicle, mesentery, pancreas, testis, prostate gland, colon, and rectum in male rats; the mammary gland, lung, spleen, mesenteric lymph nodes, skin, and kidney of female rats; the liver and pancreatic islets of male and female mice; the kidney, cecum, preputial gland, bone marrow, mandibular and mesenteric lymph nodes, and spleen of male mice; and the mesentery, forestomach, and skin of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, and/or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups 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 Bromochloroacetic 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 B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 12 days Mice: 14 days	Rats: 13 (males) or 14 (females) days Mice: 11 (females) or 12 (males) days	14 days
Average Age When Studies Began		
6 weeks	5 to 7 weeks	6 to 7 weeks
Date of First Exposure		
Rats: July 31, 2000 Mice: August 2, 2000	Rats: October 24 (males) or 25 (females), 2000 Mice: October 22 (females) or 23 (males), 2000	Rats: September 26, 2001 Mice: October 10, 2001
Duration of Exposure		
15 days	14 weeks	105 weeks
Date of Last Exposure		
Rats: August 14, 2000 Mice: August 16, 2000	Rats: January 24 (males) or 25 (females), 2001 Mice: January 22 (females) or 23 (males), 2001	Rats: September 26 (males) or October 1, 2003 (females) Mice: October 13 (males) or 16 (females), 2003
Necropsy Dates		
Rats: August 14, 2000 Mice: August 16, 2000	Rats: January 24 (males) or 25 (females), 2001 Mice: January 22 (females) or 23 (males), 2001	Rats: September 24-26 (males) or October 1 (females), 2003 Mice: October 8-13 (males) or 16 (females), 2003
Average Age at Necropsy		
7 to 9 weeks	18 to 20 weeks	110 to 112 weeks
Size of Study Groups		
5 males and 5 females	Core studies: 10 males and 10 females Clinical pathology: 10 male and 10 female rats	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

Table 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Bromochloroacetic Acid

2-Week Studies	3-Month Studies	2-Year 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
Diet		
Irradiated NTP-2000 wafer rodent feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	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 *ad libitum*, 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		
Irradiated hardwood chips (P.J. Murphy Forest Products, Inc., Montville, NJ), changed once (male mice) or twice weekly	Same as 2-week studies	Same as 2-week studies
Rack 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)	Same as 2-week studies; changed every 2 weeks	Same as 3-month studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations		
0, 62.5, 125, 250, 500, or 1,000 mg/L in drinking water	0, 62.5, 125, 250, 500, or 1,000 mg/L in drinking water	0, 250, 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 twice weekly.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings and water consumption were recorded weekly.	Observed twice daily; animals were weighed initially, weekly for 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 Bromochloroacetic Acid

2-Week Studies	3-Month Studies	2-Year Studies
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology rats on days 3 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, platelet, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials. ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology		
Microscopic examinations were performed on 0 and 1,000 mg/L animals. In addition to gross lesions and tissue masses, the following tissues were examined: colon, small intestine (duodenum, jejunum, ileum), kidney, liver, lung, and stomach.	Complete histopathologic examinations were performed on core study animals exposed to 0 or 1,000 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, 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.	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 (female 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, urinary bladder, and uterus.

Table 1
Experimental Design and Materials and Methods in the Drinking Water Studies of Bromochloroacetic Acid

2-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study male animals in the 0, 250, 500, and 1,000 mg/L groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, 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 females exposed to 0, 250, 500, or 1,000 mg/L for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None

Statistical Methods

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site divided by the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., 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 A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power. This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each 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, liver enzyme, 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. 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. Proportions of regularly cycling females in each exposure group were compared to the control group using the Fisher exact test (Gart *et al.,* 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

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

Quality Assurance Methods

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assessment 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 bromochloroacetic acid was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli* and increases in the frequency of micronucleated erythrocytes in peripheral blood of exposed mice. Micronuclei (literally "small nuclei" or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acentric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle *et al.*, 1983). The protocols for these studies and the results are given in Appendix E.

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

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (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 and body weight gains of exposed male and female rats were similar to those of the controls. Water consumption by exposed and control groups was similar. Drinking water concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 9, 18, 35, 75, and 140 mg bromochloroacetic acid/kg body weight to males and 8, 17, 35, 70, and 130 mg/kg to females. There were no clinical findings related to bromo-chloroacetic acid exposure.

The relative liver weights of 500 mg/L males and 1,000 mg/L males and females were significantly greater than those of the controls (Table G1). Absolute and relative right kidney weights of males exposed to 1,000 mg/L were significantly increased. Absolute and

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

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption[c] Week 1	Week 2
Male							
0	5/5	89 ± 2	150 ± 4	61 ± 3		13.0	14.3
62.5	5/5	85 ± 3	143 ± 4	58 ± 2	95	13.0	14.0
125	5/5	87 ± 2	152 ± 5	66 ± 4	101	13.8	14.3
250	5/5	90 ± 3	156 ± 4	66 ± 2	104	15.0	13.6
500	5/5	86 ± 3	155 ± 2	69 ± 3	103	14.7	15.7
1,000	5/5	85 ± 3	144 ± 4	59 ± 2	96	13.4	14.6
Female							
0	5/5	81 ± 3	123 ± 3	42 ± 1		12.2	12.6
62.5	5/5	81 ± 3	120 ± 2	39 ± 3	98	11.5	12.1
125	5/5	84 ± 1	121 ± 2	37 ± 3	98	12.8	12.5
250	5/5	85 ± 2	124 ± 2	39 ± 1	100	13.3	12.9
500	5/5	85 ± 2	126 ± 3	42 ± 1	103	12.8	12.8
1,000	5/5	85 ± 2	121 ± 3	37 ± 2	98	12.0	11.7

[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Dunnett's test.
[c] Water consumption is expressed as grams per animal per day.

relative lung weights of 62.5 and 125 mg/L males and relative lung weight of 250 mg/L males were significantly decreased.

No exposure-related gross or histopathologic lesions were observed in male or female rats.

Exposure Concentration Selection Rationale: Based on the lack of mortality, clinical signs of toxicity, water consumption changes, and body weight changes, bromochloroacetic acid exposure concentrations selected for the 3-month drinking water study in rats were 62.5, 125, 250, 500, and 1,000 mg/L.

3-Month Study

All rats survived to the end of the study (Table 3). Final mean body weights and body weight gains of exposed male and female rats were similar to those of the controls. Water consumption by exposed and control groups was similar. Drinking water concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 5, 10, 20, 40, and 75 mg bromochloroacetic acid/kg body weight to males and 5, 10, 20, 40, and 85 mg/kg to females. There were no clinical findings related to bromochloroacetic acid exposure. Absolute and relative liver weights of male and female rats exposed to 500 or 1,000 mg/L were significantly increased (Table G2). The relative liver weights of the 125 and 250 mg/L males were also significantly increased.

The absolute and relative right kidney weights of males exposed to 1,000 mg/L, relative right kidney weights of 1,000 mg/L females, and absolute kidney weights of 500 mg/L males were also significantly increased.

The hematology and clinical chemistry data for rats in the 3-month toxicity study of bromochloroacetic acid are listed in Table F1. Except for decreases in serum activities of alanine aminotransferase and sorbitol

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

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption[c] Week 1	Week 13
Male							
0	10/10	112 ± 4	326 ± 4	214 ± 3		15.9	15.2
62.5	10/10	111 ± 4	303 ± 8	192 ± 7	93	16.2	13.3
125	10/10	112 ± 3	323 ± 11	211 ± 9	99	17.1	14.8
250	10/10	111 ± 4	333 ± 5	222 ± 3	102	17.0	14.5
500	10/10	113 ± 3	341 ± 8	228 ± 7	104	17.2	15.3
1,000	10/10	112 ± 3	333 ± 7	221 ± 5	102	15.6	14.9
Female							
0	10/10	94 ± 2	188 ± 3	94 ± 4		13.3	10.7
62.5	10/10	94 ± 2	193 ± 4	99 ± 4	102	13.6	11.0
125	10/10	93 ± 2	194 ± 2	101 ± 3	103	13.7	11.1
250	10/10	92 ± 3	190 ± 5	98 ± 3	101	13.9	10.6
500	10/10	94 ± 2	192 ± 3	98 ± 4	102	13.9	11.1
1,000	10/10	94 ± 2	186 ± 3	93 ± 3	99	13.0	11.1

[a] Number of animals surviving at 14 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Differences from the control group are not significant by Williams' or Dunnett's test.
[c] Water consumption is expressed as grams per animal per day.

dehydrogenase, no changes occurred in hematology or clinical chemistry variables that were considered related to bromochloroacetic acid administration. Alanine aminotransferase activities were significantly decreased in 500 and 1,000 mg/L males and females on day 21, in 250 mg/L or greater males at week 14, and in 1,000 mg/L females at week 14. Sorbitol dehydrogenase activities were significantly decreased in 250 mg/L or greater males and 500 and 1,000 mg/L females at week 14. The amount of the decreases varied between 10% and 48% depending on the enzyme, sex, exposure group, and sampling time. While there did not appear to be an exposure concentration relationship, the decreases were larger at week 14 than on day 21. The significance of the enzyme activity decreases was unknown, but may suggest an effect related to altered enzyme synthesis, release, catabolism, or inhibition (Schmidt and Schmidt, 1987, 1989; Pappas, 1989).

There were no significant changes seen in the sperm parameters or estrous cyclicity of male or female rats (Tables H1 and H2).

In the liver, there were significantly increased incidences of diffuse cytoplasmic vacuolization in males and females exposed to 1,000 mg/L (male: 0 mg/L, 0/10; 62.5 mg/L, 0/0; 125 mg/L, 0/10; 250 mg/L, 0/10; 500 mg/L, 0/10; 1,000 mg/L, 10/10; female: 0/10, 0/1, 0/3, 0/10, 0/10, 6/10).

Exposure Concentration Selection Rationale: Based on the lack of mortality, water consumption changes, and body weight changes, bromochloroacetic acid exposure concentrations selected for the 2-year drinking water study in rats were 250, 500, and 1,000 mg/L. Higher exposures were excluded because of the extent of liver enlargement observed in this study (33% increase in 1,000 mg/L males). The concentrations of bromochloroacetic acid selected for the 2-year study are similar to exposure levels used to evaluate the chronic toxicity and carcinogenicity of dichloroacetic acid and dibromoacetic acid in rats.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Exposure to bromochloroacetic acid had no effect on survival of male or female rats.

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 500 mg/L males were 8% less than the control group after week 81, and those of 1,000 mg/L males were 10% less than the control group after week 69 (Figure 3; Table 5). Mean body weights of 1,000 mg/L females were 10% less than the control group after week 85 (Figure 3; Table 6). Water consumption by exposed males and females was similar to that by controls during the study (Tables J1 and J2). Drinking water concentrations of 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 10, 20, and 40 mg/kg to males and 13, 25, and 50 mg/kg to females. No chemical-related clinical findings, other than increased incidences of thinness in exposed males (0 mg/L, 6%; 250 mg/L, 9%; 500 mg/L, 15%; 1,000 mg/L, 17%) were observed.

Table 4
Survival of Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Animals initially in study	50	50	50	50
Moribund	15	17	17	17
Natural deaths	4	7	8	4
Animals surviving to study termination	31	26	25	29
Percent probability of survival at end of study[a]	62	52	50	58
Mean survival (days)[b]	686	683	666	674
Survival analysis[c]	P=0.826	P=0.434	P=0.277	P=0.774
Female				
Animals initially in study	50	50	50	50
Moribund	12	14	9	13
Natural deaths	4	5	4	2
Animals surviving to study termination	34	31	37[d]	35
Percent probability of survival at end of study	68	62	74	70
Mean survival (days)	687	685	694	696
Survival analysis	P=0.572N	P=0.694	P=0.613N	P=0.901N

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

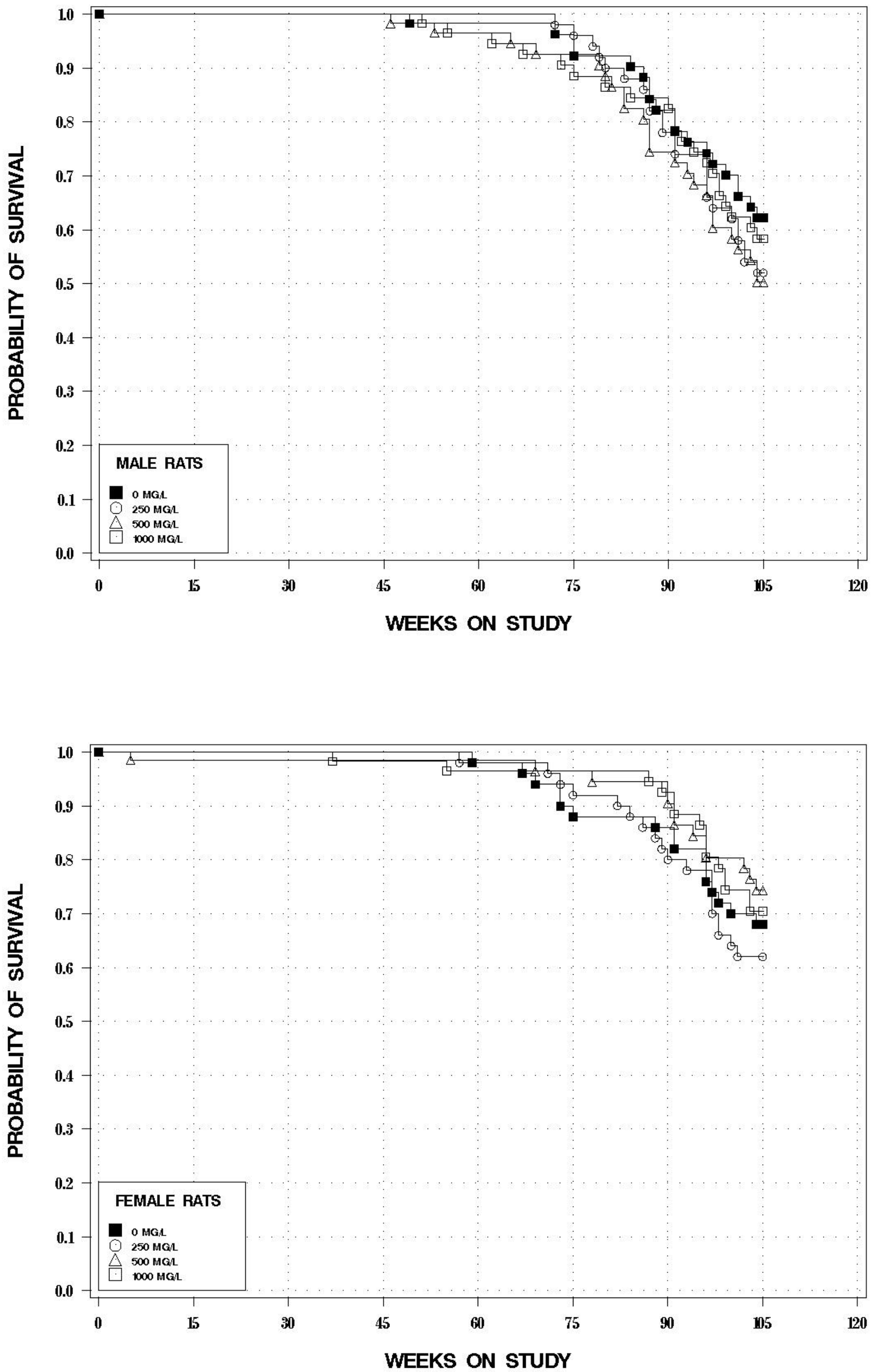
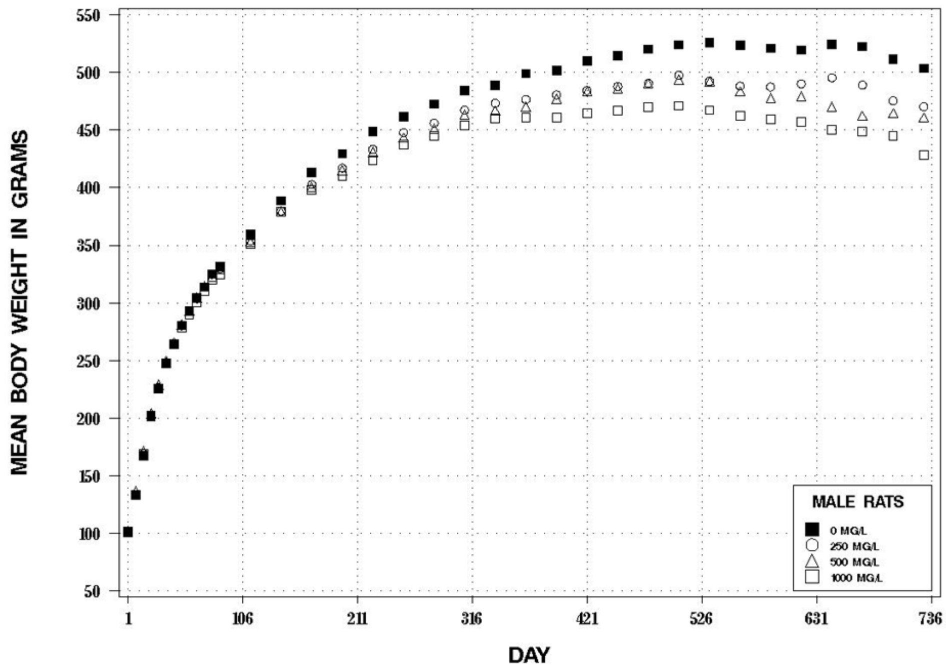


FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Bromochloroacetic Acid in Drinking Water for 2 Years



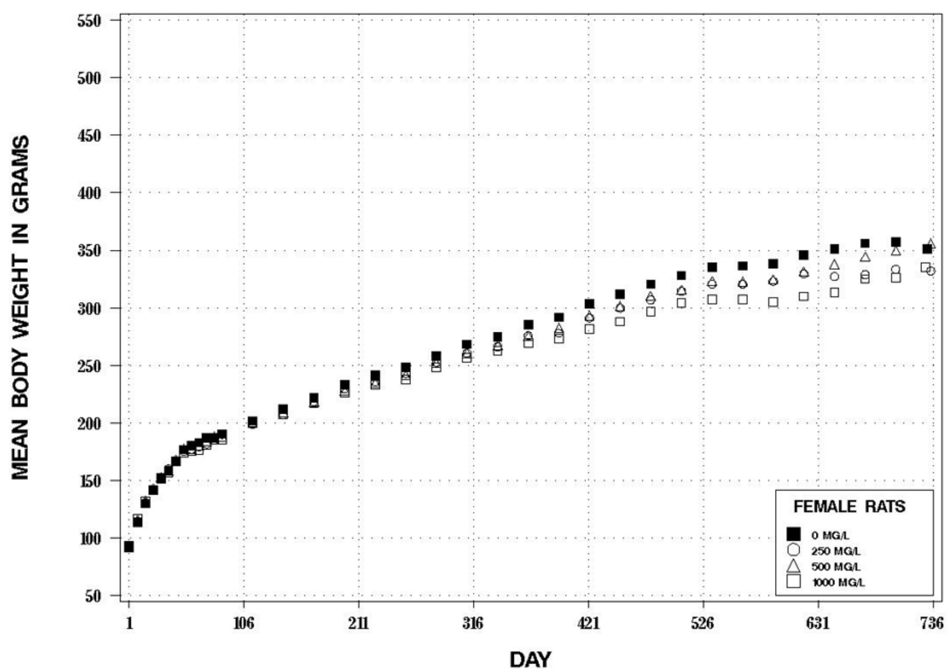


FIGURE 3
Growth Curves for Male and Female Rats Exposed to Bromochloroacetic Acid in Drinking Water for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

Days on Study	0 mg/L Av. Wt. (g)	0 mg/L No. of Survivors	250 mg/L Av. Wt. (g)	250 mg/L Wt. (% of controls)	250 mg/L No. of Survivors	500 mg/L Av. Wt. (g)	500 mg/L Wt. (% of controls)	500 mg/L No. of Survivors	1,000 mg/L Av. Wt. (g)	1,000 mg/L Wt. (% of controls)	1,000 mg/L No. of Survivors
1	101	50	101	99	50	102	101	50	101	100	50
8	133	50	133	101	50	136	103	50	134	101	50
15	166	50	169	101	50	171	103	50	170	102	50
22	200	50	201	100	50	204	102	50	202	101	50
29	225	50	226	100	50	229	102	50	226	101	50
36	246	50	247	101	50	250	102	50	247	101	50
43	263	50	263	100	50	267	101	50	264	100	50
50	279	50	279	100	50	282	101	50	278	100	50
57	292	50	291	100	50	294	101	50	289	99	50
64	302	50	303	100	50	305	101	50	300	99	50
71	312	50	313	100	50	315	101	50	310	99	50
78	323	50	323	100	50	324	100	50	320	99	50
85	330	50	328	100	50	331	100	50	324	98	50
113	357	50	353	99	50	355	99	50	350	98	50
141	387	50	379	98	50	381	99	50	378	98	50
169	411	50	402	98	50	403	98	50	397	97	50
197	429	50	416	97	50	417	97	50	409	96	50
225	448	50	432	97	50	432	97	50	422	94	50
253	461	50	447	97	50	445	97	50	437	95	50
281	472	50	455	97	50	453	96	50	445	94	50
309	484	50	466	96	50	464	96	50	454	94	50
337	493	49	472	96	50	468	95	49	459	93	50
365	499	49	475	95	50	471	95	49	460	92	49
393	502	49	480	96	50	477	95	48	461	92	48
421	510	49	484	95	50	483	95	48	465	91	48
449	515	49	488	95	50	486	95	48	467	91	47
477	520	49	491	94	50	490	94	47	470	90	46
505	524	48	497	95	49	494	94	46	471	90	46
533	526	46	492	94	48	492	94	46	467	89	44
561	524	46	488	93	45	484	92	43	463	88	43
589	521	45	487	94	44	478	92	41	459	88	42
617	519	41	490	94	41	479	92	37	457	88	42
645	524	39	495	95	37	470	90	36	450	86	38
673	522	37	489	94	33	462	89	33	449	86	36
701	512	34	475	93	31	465	91	29	445	87	31
Mean for weeks											
1-13	244		244	100		247	101		243	100	
14-52	438		425	97		424	97		417	95	
53-101	517		487	94		479	93		460	89	

Table 6
Mean Body Weights and Survival of Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

Days on Study	0 mg/L		250 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	93	50	94	101	50	93	100	50	94	102	50
9	114	50	116	102	50	117	103	50	118	103	50
16	130	50	132	102	50	133	102	50	132	102	50
23	142	50	143	101	50	144	101	50	142	100	50
30	152	50	154	101	50	154	101	49	152	99	50
37	159	50	162	102	50	160	101	49	157	99	50
44	167	50	168	101	50	169	101	49	167	100	50
51	177	50	177	100	50	179	101	49	175	99	50
58	180	50	179	99	50	178	99	49	176	98	50
65	183	50	181	99	50	182	100	49	176	97	50
72	187	50	184	98	50	186	99	49	181	97	50
79	187	50	187	100	50	190	102	49	185	99	50
86	191	50	192	101	50	190	100	49	185	97	50
114	201	50	201	100	50	202	100	49	200	99	50
142	211	50	209	99	50	211	100	49	208	98	50
170	222	50	221	100	50	221	100	49	217	98	50
198	234	50	231	99	50	229	98	49	226	97	50
226	242	50	237	98	50	237	98	49	233	96	50
254	249	50	244	98	50	244	98	49	238	96	50
282	259	50	254	98	50	254	98	49	248	96	49
310	269	50	263	98	50	263	98	49	257	96	49
338	276	50	267	97	50	270	98	49	263	96	49
366	286	50	277	97	50	278	97	49	270	95	49
394	292	50	278	95	50	283	97	49	274	94	48
422	303	49	291	96	49	294	97	49	282	93	48
450	312	49	300	96	49	302	97	49	288	92	48
478	321	48	307	96	49	311	97	48	297	93	48
506	328	45	314	96	48	316	96	48	304	93	48
534	335	44	321	96	46	323	97	48	308	92	48
562	337	44	321	95	46	323	96	47	307	91	48
590	339	44	323	95	44	325	96	47	305	90	48
618	346	43	327	94	41	332	96	47	311	90	46
646	351	41	327	93	39	338	96	43	314	89	44
674	356	38	329	92	38	345	97	40	325	91	40
702	357	35	334	93	32	350	98	40	326	91	37
Mean for weeks											
1-13	159		159	101		160	101		157	99	
14-52	240		236	99		237	99		232	97	
53-101	328		311	95		317	97		301	92	

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant mesothelioma and neoplasms and/or nonneoplastic lesions of the large intestine, mammary gland, pancreatic islets, liver, and lung. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the 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 was significantly increased in the 500 mg/L group of male rats (Tables 7 and A2). The incidences in all exposed groups of males exceeded the historical range in drinking water controls (Tables 7 and A3a). Since the distinction between benign and malignant mesothelioma is not clear (Hall, 1990), all mesotheliomas were classified as malignant. Malignant mesothelioma did not occur in female rats. Malignant mesotheliomas were found in multiple locations within and on peritoneal surfaces of the mesentery, peritoneum, testes, epididymis, prostate gland, seminal vesicles, pancreas, adrenal gland, spleen, kidney, urinary bladder, gastrointestinal tract, and skeletal muscle. The frequency of occurrence by anatomic site in the current study appeared to be similar to that described for other water disinfection by-products (potassium bromate and dibromoacetic acid) that induced mesotheliomas in F344/N rats in 2-year bioassays (Wolf *et al.*, 1998; NTP, 2007a).

Grossly, mesotheliomas appeared as multiple, small nodules (1 to 2 mm or less) on peritoneal surfaces. When extensive involvement occurred, there was often an increased quantity of reddish-brown fluid in the abdominal cavity. Microscopically, mesothelioma consisted of a proliferation of mesothelial cells with an increased nuclear to cytoplasmic ratio on a fibrovascular stroma (Plates 1 and 2). The amount of stromal component varied considerably, and at times, the pleomorphic mesothelial cells took on a tubular appearance or occurred in packets that were densely packed. At other times, the fibrous component appeared to be dominant.

Table 7
Incidences of Malignant Mesothelioma in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Malignant Mesothelioma[a]				
Overall rate[b]	1/50 (2%)	5/50 (10%)	10/50 (20%)	6/50 (12%)
Adjusted rate[c]	2.3%	11.7%	23.7%	14.0%
Terminal rate[d]	0/31 (0%)	2/26 (8%)	5/25 (20%)	2/29 (7%)
First incidence (days)	608	555	479	556
Poly-3 test[e]	P=0.062	P=0.097	P=0.003	P=0.052

[a] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 9/300 (3.0% ± 2.8%), range 0%-6%
[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.

Large Intestine: Increased incidences of adenomas of the colon or rectum occurred with positive trends in male and female rats, and the incidence in 1,000 mg/L females was significantly increased compared to controls (Tables 8, A1, A2, B1, and B2). The incidences in 250 mg/L males, 500 mg/L females, and 1,000 mg/L males and females exceeded the historical ranges for controls in drinking water studies and all routes (Tables 8, A3b, and B3a). A total of 18 adenomas occurred in the large intestines of 16 exposed rats (six males and 10 females). One female had an adenoma in the colon and an adenoma in the rectum, and one female had two adenomas in the colon. Two cases of mucosal hyperplasia were seen, one in the colon of a female rat exposed to 1,000 mg/L, and another in the rectum of a female rat exposed to 500 mg/L (Tables 8 and B4).

Microscopically, the adenomas were polypoid masses extending into the lumina of the large intestine from fibrovascular stalks attached to the intestinal walls (Plate 3). The polypoid masses consisted of moderately well-differentiated cuboidal to columnar epithelial cells forming coiled tubular gland-like structures. The epithelial cells were basophilic with prominent nucleoli and occasional mitotic figures. Hyperplasia was characterized by an increase in the crypt length (thickness of the mucosa) and an increase in the density of the crypts.

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Large Intestine in Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Colon[a]	50	50	50	50
Adenoma[b]	0	1	0	3
Rectum	50	50	50	50
Adenoma	0	1	0	1
Colon or Rectum: Adenoma[c]				
Overall rate[d]	0/50 (0%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate[e]	0.0%	4.8%	0.0%	9.5%
Terminal rate[f]	0/31 (0%)	2/26 (8%)	0/25 (0%)	3/29 (10%)
First incidence (days)	—[h]	729 (T)	—	468
Poly-3 test[g]	P=0.031	P=0.231	—[i]	P=0.057

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Large Intestine in Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female				
Colon	50	50	50	50
Hyperplasia				1 (2.0)[j]
Adenoma, Multiple	0	0	0	1
Adenoma (includes multiple)	0	0	2	6**
Rectum	50	50	50	50
Hyperplasia			1 (4.0)	
Adenoma	0	0	1	2
Colon or Rectum: Adenoma[k]				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	7/50 (14%)
Adjusted rate	0.0%	0.0%	6.6%	15.5%
Terminal rate	0/34 (0%)	0/31 (0%)	3/37 (8%)	6/35 (17%)
First incidence (days)	—	—	729 (T)	692
Poly-3 test	P<0.001	—	P=0.127	P=0.009

** Significantly different (P≤0.01) from the control group by the Poly-3 test
(T) Terminal sacrifice
a Number necropsied
b Number of animals with neoplasm
c Historical incidence for 2-year drinking water studies (mean ± standard deviation): 0/300; all routes: 2/1,199 (0.2% ± 0.6%), range 0%-2%
d Number of neoplasm-bearing animals/number of animals necropsied
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.
h Not applicable; no neoplasms in animal group
i Value of statistic cannot be computed.
j Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
k Historical incidence for drinking water studies: 0/250; all routes: 2/1,100 (0.2% ± 0.6%), range 0%-2%

Mammary Gland: The incidences of fibroadenoma in all groups of female rats exceeded or were at the high end of the historical ranges for controls in drinking water studies and all routes of exposure (Tables 9 and B3b). There were increased incidences of multiple fibro-adenomas with concomitant decreased incidences of single mass fibroadenoma in the 500 and 1,000 mg/L females (Tables 9 and B1). The number of fibro-adenomas per fibroadenoma-bearing rat was also significantly increased in the 500 and 1,000 mg/L groups compared to controls. Fibroadenomas were composed of a proliferation of glandular epithelium (ducts, ductules, and alveoli) and prominent, mature connective tissue. Almost all of the fibroadenomas were visible grossly at the time of necropsy.

Pancreatic Islets: The incidence of adenoma in males exposed to 500 mg/L was significantly increased compared to controls (0 mg/L, 3/50; 250 mg/L, 4/50; 500 mg/L, 9/50; 1,000 mg/L, 3/50; Tables A1 and A2) and exceeded the historical range in drinking

Table 9
Incidences of Mammary Gland Fibroadenoma in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Number Necropsied	50	50	50	50
Fibroadenoma, Single	21	19	4**	8**
Fibroadenoma, Multiple	22	24	43**	38**
Number of fibroadenomas/ fibroadenoma-bearing rat	1.84	2.05	3.53▲▲	3.98▲▲
Fibroadenoma (includes multiple)[a]				
Overall rate[b]	43/50 (86%)	43/50 (86%)	47/50 (94%)	46/50 (92%)
Adjusted rate[c]	92.0%	90.0%	96.6%	96.9%
Terminal rate[d]	32/34 (94%)	27/31 (87%)	36/37 (97%)	35/35 (100%)
First incidence (days)	505	525	478	618
Poly-3 test[e]	P=0.107	P=0.504N	P=0.274	P=0.248

** Significantly different (P≤0.01) from the control group by the Poly-3 test
▲▲ Significantly different (P≤0.01) from the control group by the Kruskal-Wallis analysis of variance
[a] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 176/250 (70.4% ± 9.8%), range 62%-86%; all routes: 574/1,100 (52.2 ± 14.7), range 24%-86%
[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**.

water controls [23/296 (8% ± 2%), range 6%-10%; Table A3c]. There were no significant increases in the incidences of carcinoma, adenoma or carcinoma (combined), or hyperplasia in male rats (Tables A1, A2, and A4). No increased incidences of pancreatic islet neoplasms or nonneoplastic lesions were observed in female rats (Tables B1, B2, and B4).

Microscopically, the islet cell adenomas were characterized by an expansive mass of islet cells usually larger than 500 µm in diameter that were compressing adjacent acini (Plate 4). The expanding islet cell adenomas often had entrapped acinar cells.

Liver: The incidences of hepatocellular adenoma occurred with a positive trend in female rats (Tables 10 and B2). The incidences in 500 mg/L males and 1,000 mg/L males and females, while not significantly increased over controls, exceeded the historical ranges in drinking water controls (Tables 10, A3d, and B3c). No hepatocellular carcinomas were observed in male or female rats.

Microscopically, the hepatocellular adenomas were characterized by a proliferation of hepatocytes that resulted in compression of adjacent parenchyma and by loss of the normal lobular architecture of the liver due to irregularity of hepatic cords.

The incidences of eosinophilic focus in 500 mg/L females and 1,000 mg/L males and females and mixed cell focus in 1,000 mg/L females were significantly greater than those of the control groups (Tables 10, A4, and B4). The foci of cellular alteration observed (classified according to the tinctorial characteristics of the cytoplasm) were usually circumscribed lesions with little or no compression of the surrounding hepatic parenchyma.

Table 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus[a]	2	5	4	8*
Mixed Cell Focus	4	3	4	2
Hepatocellular Adenoma, Multiple	0	0	0	1
Hepatocellular Adenoma (includes multiple)[b]				
Overall rate[c]	2/50 (4%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate[d]	4.6%	0.0%	7.5%	9.5%
Terminal rate[e]	2/31 (7%)	0/26 (0%)	3/25 (12%)	1/29 (3%)
First incidence (days)	729 (T)	—[g]	729 (T)	682
Poly-3 test[f]	P=0.107	P=0.242N	P=0.468	P=0.324
Female				
Number Examined Microscopically	50	50	50	50
Eosinophilic Focus	1	6	9*	15**
Mixed Cell Focus	1	4	6	10**
Hepatocellular Adenoma[h]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.6%
Terminal rate	0/34 (0%)	0/31 (0%)	0/37 (0%)	3/35 (9%)
First incidence (days)	—	—	—	729 (T)
Poly-3 test	P=0.012	—[i]	—	P=0.125

* Significantly different ($P \leq 0.05$) from the control group by the Poly-3 test
** $P \leq 0.01$
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 4/300 (1.3% ± 1.6%), range 0%-4%
[c] Number of neoplasm-bearing animals/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] Not applicable; no neoplasm in animal group
[h] Historical incidence for drinking water studies: 3/250 (1.2% ± 1.8%), range 0%-4%
[i] Value of statistic cannot be computed.

Lung: The incidence of alveolar epithelium hyperplasia was significantly increased in 1,000 mg/L females (0 mg/L, 5/50; 250 mg/L, 7/50; 500 mg/L, 8/50; 1,000 mg/L, 18/50; Table B4); however, there was no corresponding increase in severity. The focal hyperplasia was characterized by increased cellularity and thickness of alveolar walls, with the underlying architecture being intact and alveolar lumina being visible. Epithelial cells had cuboidal appearance, and macrophages in alveolar lumina contributed to the overall hypercellular appearance, with little evidence of inflammation. The incidences of histiocytic cellular infiltration were significantly increased in all exposed groups of females (36/50, 44/50, 44/50, 45/50; Table B4).

Skin: Increased incidences of fibroma and fibroma or fibrosarcoma (combined) were seen in 1,000 mg/L female rats [fibroma: 1/50, 1/50, 2/50, 4/50; fibroma or fibrosarcoma (combined): 1/50, 1/50, 2/50, 6/50; Table B4]. The incidence of fibroma in 1,000 mg/L females was within the historical control range for drinking water studies, and the incidence of fibrosarcoma in this group was slightly above the historical control range for drinking water studies (Table B3d). Because the increased incidences were not significantly greater compared to the controls and the incidence of fibroma was within the NTP historical control range, these increases are not considered related to exposure to bromo-chloroacetic acid.

Mice

2-Week Study

All mice survived to the end of the study (Table 11). The final mean body weight and body weight gain of male mice exposed to 250 mg/L was significantly greater than those of the controls, but the mean body weights of all other exposed groups of males and all groups of exposed females were similar to those of the controls. Water consumption by exposed and control groups was similar. Drinking water concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 10, 20, 40, 80, and 170 mg bromochloroacetic acid/kg body weight to males and 9, 17, 40, 75, and 155 mg/kg to females. There were no clinical findings related to bromochloroacetic acid exposure.

There were no chemical-related differences in organ weights between exposed and control mice (Table G3).

No gross or histopathologic lesions related to exposure to bromochloroacetic acid were observed in male or female mice.

Exposure Concentration Selection Rationale: Based on the lack of mortality, clinical signs of toxicity, water consumption changes, and body weight changes, bromochloroacetic acid exposure concentrations selected for the 3-month drinking water study in mice were 62.5, 125, 250, 500, and 1,000 mg/L.

Table 11
Survival, Body Weights, and Water Consumption of Mice in the 2-Week Drinking Water Study of Bromochloroacetic Acid

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption[c] Week 1	Week 2
Male							
0	5/5	21.5 ± 0.3	24.3 ± 0.2	2.8 ± 0.5		3.6	3.6
62.5	5/5	21.4 ± 0.5	24.7 ± 0.4	3.3 ± 0.5	102	3.4	3.8
125	5/5	21.8 ± 0.3	24.9 ± 0.4	3.1 ± 0.2	102	3.5	3.0
250	5/5	21.0 ± 0.4	26.0 ± 0.4*	5.0 ± 0.4**	107	3.3	3.7
500	5/5	20.9 ± 0.4	25.3 ± 0.4	4.4 ± 0.7	104	3.8	3.4
1,000	5/5	20.8 ± 0.7	24.5 ± 0.4	3.7 ± 0.4	101	3.8	3.5
Female							
0	5/5	18.8 ± 0.2	20.7 ± 0.3	2.0 ± 0.4		3.1	2.9
62.5	5/5	18.4 ± 0.3	20.5 ± 0.5	2.1 ± 0.3	99	2.8	2.9
125	5/5	18.9 ± 0.2	21.6 ± 0.3	2.7 ± 0.1	104	2.7	2.4
250	5/5	18.8 ± 0.2	21.4 ± 0.4	2.6 ± 0.3	103	2.9	2.9
500	5/5	18.9 ± 0.2	21.2 ± 0.2	2.3 ± 0.2	102	2.9	2.8
1,000	5/5	18.7 ± 0.1	21.2 ± 0.3	2.5 ± 0.3	103	2.2	3.7

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
** $P \leq 0.01$
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.

3-Month Study

All mice survived to the end of the study (Table 12). Final mean body weights of exposed male and female mice were similar to those of the controls; however, body weight gains of females exposed to 250 mg/L or greater were significantly decreased. Water consumption by exposed and control groups was similar. Drinking water concentrations of 62.5, 125, 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 8, 16, 32, 65, and 125 mg bromo-chloroacetic acid/kg body weight to males and 8, 17, 35, 70, and 140 mg/kg to females. There were no clinical findings related to bromochloroacetic acid exposure.

Absolute and relative liver weights of 1,000 mg/L males and all exposed groups of females were significantly increased (Table G4). The relative liver weight of 500 mg/L males was also significantly increased.

There were no hematologic effects in the mice exposed to bromochloroacetic acid (Table F2).

There were no significant changes seen in the reproductive histopathology or estrous cyclicity of the female mice at any exposure concentration (Table H4). There were significant (24%) increases in the absolute and relative numbers of testicular spermatids in 250 mg/L male mice; however, in the absence of other effects and because this increase in testicular spermatids was not exposure concentration related, the effect is not considered biologically significant (Table H3).

Table 12
Survival, Body Weights, and Water Consumption of Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Water Consumption[c] Week 1	Week 13
Male							
0	10/10	21.7 ± 0.3	38.5 ± 1.0	16.8 ± 0.8		4.3	3.6
62.5	10/10	22.0 ± 0.6	38.9 ± 1.4	16.9 ± 0.9	101	4.0	3.2
125	10/10	22.2 ± 0.5	41.0 ± 1.4	18.8 ± 1.2	107	4.5	3.3
250	10/10	21.8 ± 0.3	40.0 ± 1.1	18.3 ± 1.1	104	5.5	3.5
500	10/10	21.9 ± 0.3	38.5 ± 0.8	16.6 ± 0.8	100	4.2	3.7
1,000	10/10	22.4 ± 0.3	38.5 ± 0.9	16.1 ± 0.8	100	4.8	3.5
Female							
0	10/10	18.1 ± 0.4	31.8 ± 1.0	13.7 ± 1.0		4.2	2.9
62.5	10/10	18.5 ± 0.3	32.5 ± 0.9	14.0 ± 1.0	102	3.1	3.1
125	10/10	17.8 ± 0.3	29.7 ± 1.0	11.9 ± 0.9	93	3.1	3.2
250	10/10	18.4 ± 0.2	28.4 ± 1.0	10.0 ± 0.9*	89	3.1	3.9
500	10/10	18.4 ± 0.3	30.2 ± 1.1	11.8 ± 0.9*	95	2.7	3.1
1,000	10/10	18.5 ± 0.2	29.1 ± 0.8	10.6 ± 0.8*	92	3.1	3.0

* Significantly different ($P \leq 0.05$) from the control group by Williams' test
[a] Number of animals surviving at 14 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.

In the liver, there were significantly increased incidences of periportal cytoplasmic vacuolization in males and females exposed to 500 and 1,000 mg/L (Table 13). In the spleen, there were increased incidences of hematopoietic cell proliferation in 62.5, 125, and 250 mg/L males and 125 and 1,000 mg/L females.

Exposure Concentration Selection Rationale: Based on the lack of mortality, water consumption changes, and body weight changes, bromochloroacetic acid exposure concentrations selected for the 2-year drinking water study in mice were 250, 500, and 1,000 mg/L. Higher exposures were excluded because of the extent of liver enlargement observed in this study (32% increase in 1,000 mg/L females). The concentrations of bromochloroacetic acid selected for the 2-year study are similar to exposure levels used to evaluate the chronic toxicity and carcinogenicity of dichloroacetic acid and dibromoacetic acid in mice.

Table 13
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Liver[a]	10	10	10	10	10	10
Periportal, Vacuolization Cytoplasmic[b]	0	0	1 (1.0)[c]	1 (2.0)	10** (1.6)	10** (2.0)
Spleen	10	6	5	6	10	10
Hematopoietic Cell Proliferation	2 (1.0)	6** (1.0)	5** (1.0)	6** (1.0)	3 (1.0)	3 (1.0)
Female						
Liver	10	10	10	10	10	10
Periportal, Vacuolization Cytoplasmic	0	0	0	1 (1.0)	10** (1.6)	10** (1.9)
Spleen	10	9	5	9	10	10
Hematopoietic Cell Proliferation	2 (1.0)	6 (1.2)	5** (1.0)	4 (1.3)	5 (1.2)	9** (1.7)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked

2-Year Study

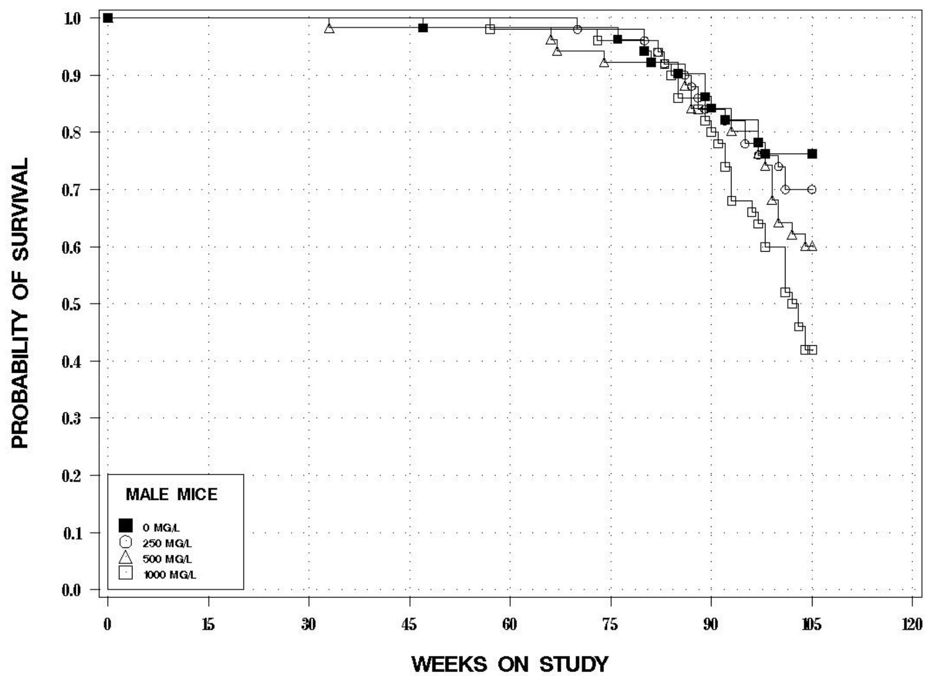
Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 14 and in the Kaplan-Meier survival curves (Figure 4). Survival of 1,000 mg/L males was significantly less than that of the control group largely due to an increased incidence of malignant liver neoplasms.

Table 14
Survival of Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Animals initially in study	50	50	50	50
Moribund	6	5	9	15
Natural deaths	6	10	11	14
Animals surviving to study termination	38	35	30	21
Percent probability of survival at end of study[a]	76	70	60	42
Mean survival (days)[b]	696	698	684	680
Survival analysis[c]	P<0.001	P=0.677	P=0.175	P=0.003
Female				
Animals initially in study	50	50	50	50
Moribund	4	3	3	2
Natural deaths	10	5	15	8
Animals surviving to study termination	36	42	32	40[d]
Percent probability of survival at end of study	72	84	64	80
Mean survival (days)	689	713	703	708
Survival analysis	P=0.727N	P=0.222N	P=0.550	P=0.444N

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



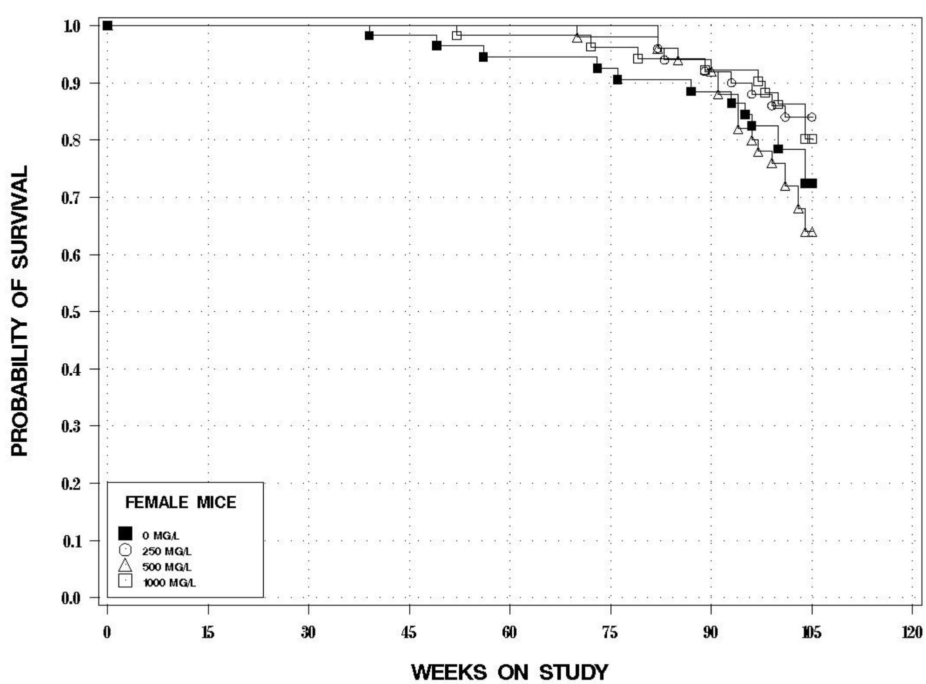


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Bromochloroacetic Acid in Drinking Water for 2 Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 1,000 mg/L males were 12% less than the control group after week 97, and those of 1,000 mg/L females were 8% less than the control group after week 21 (Tables 15 and 16; Figure 5). Water consumption by exposed males and females was similar to that by controls during the study (Tables J3 and J4). Drinking water concentrations of 250, 500, and 1,000 mg/L resulted in average daily doses of approximately 25, 50, and 90 mg/kg to males and 15, 30, and 60 mg/kg to females. No clinical findings related to chemical exposure were observed in females; the numbers of male mice with masses were increased in all exposed groups.

Table 15
Mean Body Weights and Survival of Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

Days on Study	0 mg/L		250 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	21.1	50	21.6	102	50	21.3	101	50	21.4	101	50
9	23.1	50	23.9	104	50	23.5	102	50	23.4	102	50
16	24.6	50	25.0	102	50	24.7	101	50	24.8	101	50
23	26.3	50	26.4	100	50	26.5	101	50	26.3	100	50
30	28.0	50	28.4	101	50	28.4	102	50	27.9	100	50
37	29.6	50	30.1	102	50	29.9	101	50	29.4	99	50
44	30.6	50	31.7	104	50	31.0	102	50	30.8	101	50
51	32.3	50	33.4	103	50	32.9	102	50	32.8	102	50
58	34.2	50	34.9	102	50	34.2	100	50	34.4	101	50
65	35.4	50	36.0	102	50	35.4	100	50	35.3	100	50
72	36.9	50	37.4	102	50	36.8	100	50	36.7	100	50
79	38.1	50	38.5	101	50	37.8	99	50	37.7	99	50
86	39.5	50	39.8	101	50	39.0	99	50	39.0	99	50
114	44.3	50	44.4	100	50	43.6	99	50	43.6	98	50
142	46.7	50	46.8	100	50	46.5	100	50	46.5	100	50
170	48.4	50	48.7	101	50	48.0	99	50	48.4	100	50
198	49.3	50	49.7	101	50	49.2	100	50	49.4	100	50
226	50.0	50	50.4	101	50	50.5	101	49	50.3	101	50
254	50.8	50	51.7	102	50	51.6	102	49	51.2	101	50
282	51.9	50	52.5	101	50	52.2	101	49	51.9	100	50
310	52.5	50	53.2	101	50	53.0	101	49	52.4	100	50
338	53.0	49	53.5	101	50	53.3	101	49	52.6	99	50
366	53.3	49	53.7	101	50	53.4	100	49	52.9	99	50
394	53.9	49	54.3	101	50	54.1	100	49	53.6	99	50
422	54.0	49	54.5	101	50	54.4	101	49	53.7	99	49
450	54.1	49	54.1	100	50	54.2	100	49	53.4	99	49
478	53.6	49	54.0	101	50	54.5	102	47	53.2	99	49
506	53.0	49	54.0	102	49	54.6	103	47	53.0	100	48
534	53.9	48	54.5	101	49	55.2	103	46	53.1	99	48
562	52.7	47	52.7	100	48	54.2	103	46	50.7	96	48
590	52.0	46	51.6	99	46	52.5	101	45	50.1	96	45
618	50.2	44	50.3	100	42	50.9	101	42	48.1	96	42
646	49.2	41	48.3	98	41	48.3	98	41	45.7	93	37
674	48.0	39	46.0	96	38	44.9	94	38	42.4	88	33
702	46.8	38	42.7	91	37	43.8	94	32	40.6	87	28
Mean for weeks											
1-13	30.7		31.3	102		30.9	101		30.8	100	
14-52	49.7		50.1	101		49.8	100		49.6	100	
53-101	51.9		51.6	99		51.9	100		50.0	96	

Table 16
Mean Body Weights and Survival of Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

Days on Study	0 mg/L		250 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	18.4	100	50	18.5	101	50
8	18.6	50	18.9	102	50	18.9	102	50	19.0	102	50
15	20.3	50	20.3	100	50	20.6	102	50	20.3	100	50
22	21.5	50	21.4	100	50	21.7	101	50	21.7	101	50
29	23.0	50	22.8	99	50	22.7	99	50	22.9	100	50
36	23.3	50	23.2	100	50	23.2	100	50	23.5	101	50
43	24.3	50	24.3	100	50	24.1	99	50	24.6	102	50
50	25.4	50	25.3	100	50	25.3	100	50	25.5	101	50
57	26.9	50	27.0	100	50	26.6	99	50	26.7	99	50
64	27.8	50	28.1	101	50	27.7	99	50	27.3	98	50
71	29.7	50	29.7	100	50	28.9	97	50	29.3	99	50
78	30.6	50	31.1	102	50	30.5	100	50	30.3	99	50
85	32.1	50	32.4	101	50	32.0	100	50	31.2	97	50
113	37.4	50	37.4	100	50	36.5	98	50	35.2	94	50
141	42.5	50	42.4	100	50	40.2	95	50	39.2	92	50
169	46.3	50	47.1	102	50	44.9	97	50	43.2	93	50
197	49.8	50	50.2	101	50	48.0	96	50	45.5	91	50
225	52.9	50	53.6	101	50	51.6	98	50	48.1	91	50
253	55.0	50	56.4	103	50	53.6	97	50	50.5	92	50
281	56.6	49	57.7	102	50	55.9	99	50	51.9	92	50
309	58.2	49	59.0	101	50	57.7	99	50	54.3	93	50
337	58.3	49	58.5	100	50	57.8	99	50	54.0	93	50
365	59.1	48	59.1	100	50	58.5	99	50	55.7	94	49
393	59.3	47	59.2	100	50	59.3	100	50	56.8	96	49
421	61.4	47	60.2	98	50	60.6	99	50	58.0	95	49
449	62.1	47	60.6	98	50	61.1	99	50	58.7	95	49
477	62.0	47	60.7	98	50	61.3	99	50	58.7	95	49
505	61.4	47	60.1	98	50	60.9	99	49	58.4	95	48
533	63.0	45	61.8	98	50	62.3	99	49	59.6	95	48
561	62.3	45	61.1	98	50	61.2	98	49	59.7	96	47
589	62.0	45	60.1	97	47	60.5	98	48	59.2	96	47
617	61.8	44	59.0	95	47	59.1	96	47	58.2	94	47
645	62.2	43	59.2	95	46	57.9	93	44	58.0	93	46
673	60.8	41	57.7	95	44	55.6	92	39	55.0	91	45
701	61.0	39	57.0	93	43	55.1	90	38	54.7	90	43
Mean for weeks											
1-13	24.8		24.9	100		24.7	100		24.7	100	
14-52	50.8		51.4	101		49.6	98		46.9	92	
53-101	61.4		59.7	97		59.5	97		57.7	94	

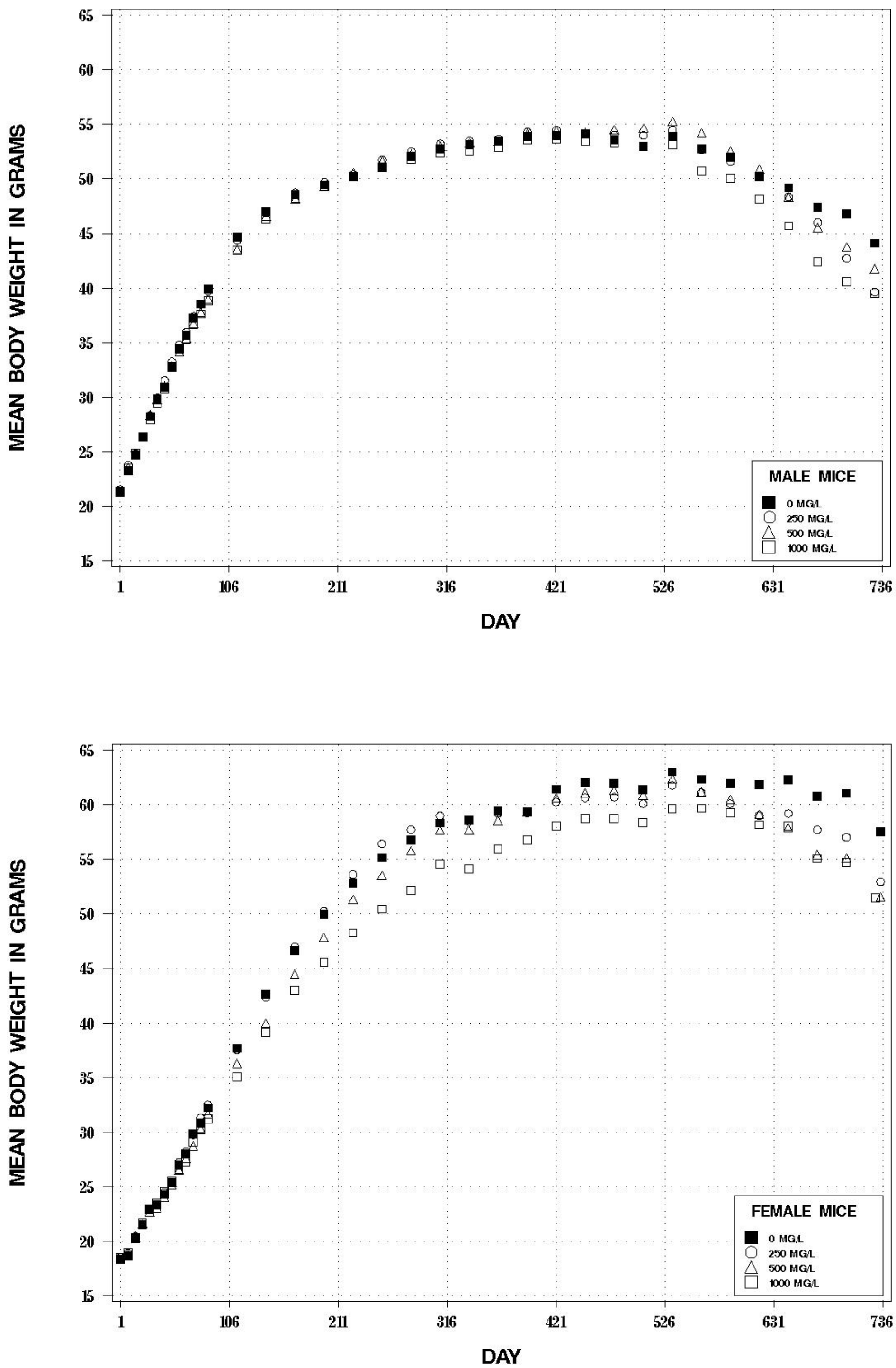


FIGURE 5
Growth Curves for Male and Female Mice Exposed to Bromochloroacetic Acid in Drinking Water for 2 Years

Pathology and Statistical Analyses

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

Liver: There were significantly increased incidences of hepatocellular adenoma in 250 and 500 mg/L male mice and in all exposed groups of female mice, hepatocellular carcinoma in 500 and 1,000 mg/L males and 500 mg/L females, hepatocellular adenoma or carcinoma (combined) in all exposed groups of males and females, and hepatoblastoma in all exposed groups of males (Tables 17, C2, and D2). In addition, the incidences of multiple hepatocellular adenoma and multiple hepatocellular carcinoma in exposed males and females and multiple hepatoblastoma in exposed males were significantly increased (Tables 17, C1, and D1). The incidences of hepatocellular adenoma in 250 and 500 mg/L males and all exposed groups of females exceeded the historical ranges in drinking water controls (Tables C3, and D3a). The incidences of hepatocellular carcinoma and of hepatocellular adenoma or carcinoma (combined) in all exposed groups of male and female mice exceeded the historical ranges in drinking water controls. In 500 and 1,000 mg/L males, the incidences of hepatoblastoma exceeded the historical ranges in drinking water controls.

The hepatocellular adenomas were composed of well-differentiated hepatocytes that formed discrete, compressive masses lacking normal lobular architecture (Plate 5). Microscopically, hepatocellular carcinomas were characterized by irregular masses or hepatocytes lacking normal lobular architecture. Additionally, variable numbers of atypical hepatocytes and mitotic figures and necrosis were present. Neoplastic hepatocytes formed variable numbers of trabeculae that were three or more cells thick (Plate 6). Some of the carcinomas metastasized to other organs, such as mesentery and lungs. Hepatoblastomas, more commonly seen among the male exposed groups, frequently arose within hepatocellular adenomas and hepatocellular carcinomas or within the parenchyma of the liver. These neoplasms were composed of cells with hyperchromatic irregular nuclei and scant basophilic cytoplasm that resembled hepatoblasts of a developing fetal liver (Plate 7). Some of the hepatoblastomas metastasized to other organs, including the mesentery, pancreas, and lungs. Hepatoblastomas frequently contained large blood-filled spaces, hemorrhage, and necrosis.

There were increased incidences of eosinophilic foci in all exposed groups of female mice. Microscopically, eosinophilic foci were generally composed of 10 or more enlarged hepatocytes with cytoplasm with a distinct granular pink or ground glass appearance. Slight compression of the adjacent parenchyma was occasionally present, but compression was not present at all margins (Plate 8).

There were also exposure concentration-related increased incidences of cytoplasmic vacuolization in all exposed groups of male and female mice. The lesion typically consisted of the presence of clear, approximately 10 to 20 micron diameter vacuoles filling the hepatocellular cytoplasm. Although special stains were not used to identify the material in the vacuoles, it was suggestive of lipid droplets. Virtually the entire liver parenchyma was involved in the more severely affected livers, but there was relative sparing of the midzonal and centrilobular regions in the less severely affected cases. There were increased incidences of centrilobular necrosis in males exposed to 500 or 1,000 mg/L that were considered secondary to the space-occupying or hemorrhage associated with hepatic neoplasms.

Table 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Vacuolization Cytoplasmic[a]	3 (3.3)[b]	12** (2.6)	17** (3.2)	19** (3.2)
Centrilobular, Necrosis	1 (1.0)	2 (4.0)	4 (2.5)	8* (2.4)
Hepatocellular Adenoma, Multiple	13	27**	25**	19
Hepatocellular Adenoma (includes multiple)[c]				
Overall rate[d]	27/50 (54%)	40/50 (80%)	40/50 (80%)	31/50 (62%)
Adjusted rate[e]	58.7%	83.6%	83.7%	67.4%
Terminal rate[f]	23/38 (61%)	30/35 (86%)	25/30 (83%)	17/21 (81%)
First incidence (days)	617	555	462	397
Poly-3 test[g]	P=0.402	P=0.005	P=0.005	P=0.252
Hepatocellular Carcinoma, Multiple	9	9	20**	32**
Hepatocellular Carcinoma (includes multiple)[h]				
Overall rate	19/50 (38%)	25/50 (50%)	36/50 (72%)	45/50 (90%)
Adjusted rate	39.6%	52.5%	76.9%	92.7%
Terminal rate	12/38 (32%)	18/35 (51%)	22/30 (73%)	20/21 (95%)
First incidence (days)	328	486	469	397
Poly-3 test	P<0.001	P=0.143	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma[i]				
Overall rate	34/50 (68%)	44/50 (88%)	49/50 (98%)	49/50 (98%)
Adjusted rate	70.6%	89.7%	99.9%	98.6%
Terminal rate	26/38 (68%)	32/35 (91%)	30/30 (100%)	21/21 (100%)
First incidence (days)	328	486	462	397
Poly-3 test	P<0.001	P=0.013	P<0.001	P<0.001
Hepatoblastoma, Multiple	0	2	12**	14**
Hepatoblastoma[j]				
Overall rate	4/50 (8%)	11/50 (22%)	28/50 (56%)	34/50 (68%)
Adjusted rate	8.8%	23.8%	61.3%	73.7%
Terminal rate	3/38 (8%)	7/35 (20%)	17/30 (57%)	17/21 (81%)
First incidence (days)	621	609	602	505
Poly-3 test	P<0.001	P=0.047	P<0.001	P<0.001
Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	35/50 (70%)	45/50 (90%)	49/50 (98%)	50/50 (100%)
Adjusted rate	72.7%	91.0%	99.9%	100.0%
Terminal rate	27/38 (71%)	32/35 (91%)	30/30 (100%)	21/21 (100%)
First incidence (days)	328	486	462	397
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001

Table 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female				
Number Examined Microscopically	50	50	50	50
Hepatocyte, Vacuolization Cytoplasmic	3 (3.0)	11* (2.5)	27** (2.8)	42** (3.3)
Eosinophilic Focus	13	22	31**	24*
Hepatocellular Adenoma, Multiple	16	37**	34**	43**
Hepatocellular Adenoma (includes multiple)[k]				
Overall rate	27/50 (54%)	48/50 (96%)	44/50 (88%)	46/50 (92%)
Adjusted rate	59.4%	96.0%	90.9%	95.2%
Terminal rate	22/36 (61%)	40/42 (95%)	30/32 (94%)	39/40 (98%)
First incidence (days)	645	570	568	551
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Hepatocellular Carcinoma, Multiple	1	8*	12**	5
Hepatocellular Carcinoma (includes multiple)[l]				
Overall rate	14/50 (28%)	23/50 (46%)	26/50 (52%)	20/50 (40%)
Adjusted rate	31.1%	48.3%	56.1%	42.3%
Terminal rate	11/36 (31%)	21/42 (50%)	20/32 (63%)	17/40 (43%)
First incidence (days)	609	667	657	618
Poly-3 test	P=0.249	P=0.067	P=0.011	P=0.185
Hepatocellular Adenoma or Carcinoma[m]				
Overall rate	31/50 (62%)	49/50 (98%)	46/50 (92%)	46/50 (92%)
Adjusted rate	67.6%	98.0%	94.6%	95.2%
Terminal rate	24/36 (67%)	41/42 (98%)	31/32 (97%)	39/40 (98%)
First incidence (days)	609	570	568	551
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001

* Significantly different (P ≤ 0.05) from the control group by the Poly-3 test
** P ≤ 0.01
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 140/247 (56.7% ± 13.0%), range 37%-72%
[d] Number of neoplasm-bearing animals/number of animals with liver 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.
[h] Historical incidence for drinking water studies: 91/247 (36.9% ± 8.6%), range 28%-48%
[i] Historical incidence for drinking water studies: 182/247 (73.7% ± 11.7%), range 57%-85%
[j] Historical incidence for drinking water studies: 28/247 (11.3% ± 13.6%), range 0%-34%
[k] Historical incidence for drinking water studies: 133/297 (44.8% ± 11.9%), range 29%-61%
[l] Historical incidence for drinking water studies: 51/297 (17.1% ± 9.5%), range 6%-28%
[m] Historical incidence for drinking water studies: 158/297 (53.1% ± 11.3%), range 35%-63%

Harderian Gland: There were significantly increased incidences of Harderian gland adenoma in 250 and 1,000 mg/L females (0 mg/L, 1/50; 250 mg/L, 7/50; 500 mg/L, 1/50; 1,000 mg/L, 7/50; Table D2). This increase was probably due to the low incidence in the controls. The historical incidence of Harderian gland adenoma in 2-year drinking water studies is 34/300 (11% ± 8%, range 2%-22%; Table D3b). The incidence of focal hyperplasia was also significantly increased in the 250 mg/L female group (0/50, 5/50, 4/50, 1/50; Table D4). The main characteristics of hyperplasia were proliferating epithelium that was one layer thick, projection of the epithelium into the acinar lumen, a lack of compression of the adjacent parenchyma, and maintenance of the architecture of the gland. Adenoma was well demarcated and compressed the surrounding gland, and the cells were cuboidal to columnar, similar to those found in a normal gland.

Other Organs: The incidence of bone marrow hyperplasia was significantly increased in 1,000 mg/L males (0 mg/L, 29/50; 250 mg/L, 21/48; 500 mg/L, 31/47; 1,000 mg/L, 40/48; Table C4), and the incidences of hematopoietic cell proliferation in the spleen were increased in 500 and 1,000 mg/L males (20/50, 23/50, 39/50, 40/50; Table C4)

Genetic Toxicology

In the first of two independent bacterial mutation assays, bromochloroacetic acid (33 to 3,333 µg/plate) was mutagenic in *Salmonella typhimurium* strain TA100, with and without rat or hamster liver activation enzymes (S9); no mutagenicity was detected in strain TA98 in tests conducted with and without hamster and rat S9 (Table E1). In the second assay, lower concentrations of bromochloroacetic acid (10 to 500 µg/plate) were tested in TA100 without S9, and no mutagenicity was detected (Table E1). However, mutagenicity was observed in TA100 (1,000 to 10,000 µg/plate) in the presence of rat S9; no significant increases in mutant colonies were seen in *Escherichia coli* WP2 *uvrA*/pKM101 with or without S9. No significant increases in the frequency of micronucleated normochromatic erythrocytes were observed in blood samples of male or female B6C3F1 mice exposed to bromochloroacetic acid (62.5 to 1,000 mg/L) for 3 months in drinking water, indicating no induction of chromosomal damage in proerythrocytes by bromochloroacetic acid under these conditions in these mice. The percentage of immature erythrocytes (polychromatic erythrocytes) among total erythrocytes in blood of male and female mice was not significantly altered, indicating a lack of chemical-induced changes in erythropoiesis.

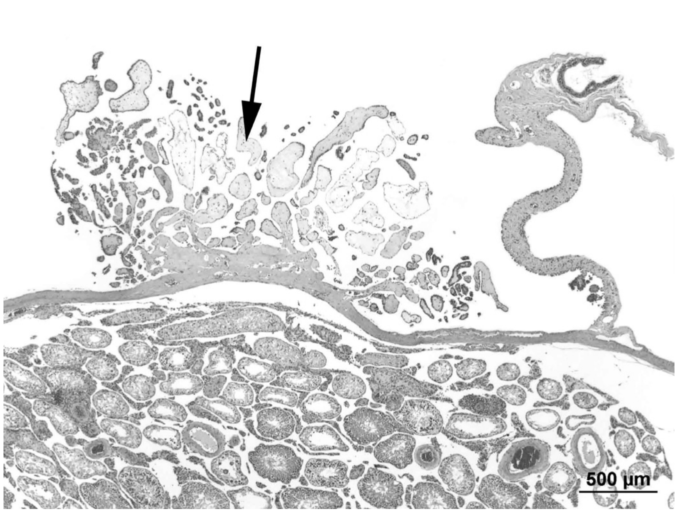


Plate 1
Malignant mesothelioma (arrow) growing on the serosal surface of the testis in a male F344/N rat exposed to 1,000 mg/L bromochloroacetic acid in drinking water for 2 years. H&E

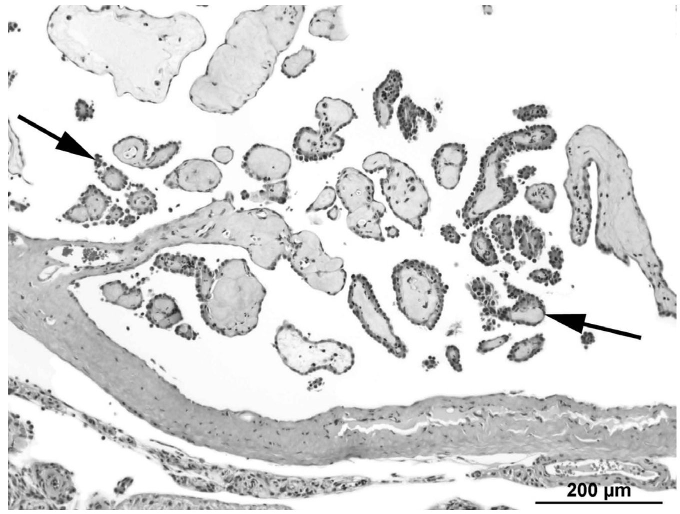


Plate 2
Higher magnification of Plate 1 showing the structure of papillary exophytic projection (arrows) in the malignant mesothelioma. H&E

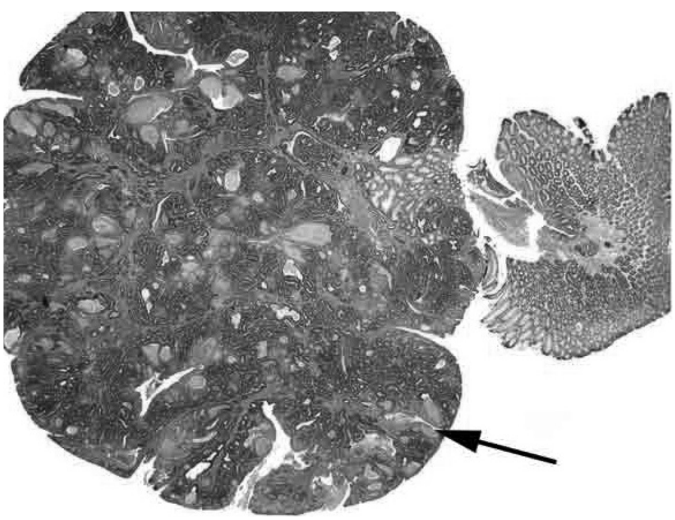


Plate 3
Adenoma (arrow) in the large intestine of a male F344/N rat exposed to 1,000 mg/L bromochloroacetic acid in drinking water for 2 years. The neoplasm is a polypoid mass extending into the lumen of the colon. H&E

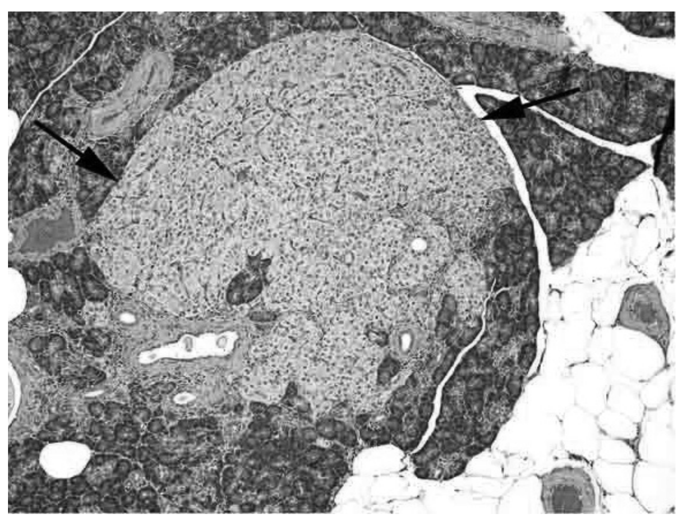


Plate 4
Benign pancreatic islet adenoma (arrows) in a male F344/N rat exposed to 500 mg/L bromochloroacetic acid in drinking water for 2 years. The neoplasm is characterized by an expansive mass of islet cells, compression of adjacent acini, and entrapment of acinar cells. H&E

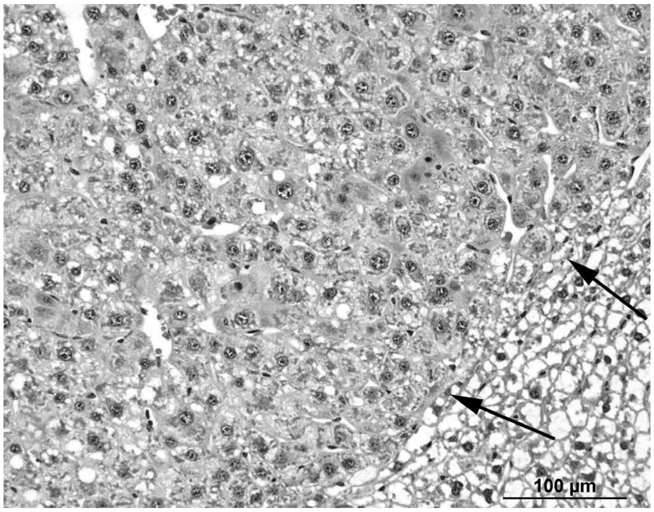


Plate 5
Hepatocellular adenoma in the liver of a female B6C3F1 mouse exposed to 1,000 mg/L bromochloroacetic acid in drinking water for 2 years. The neoplasm is composed of well-differentiated hepatocytes that form discrete compressive masses (arrows) lacking normal lobular architecture. The masses impinge perpendicularly or obliquely on the cords of the adjacent liver. H&E

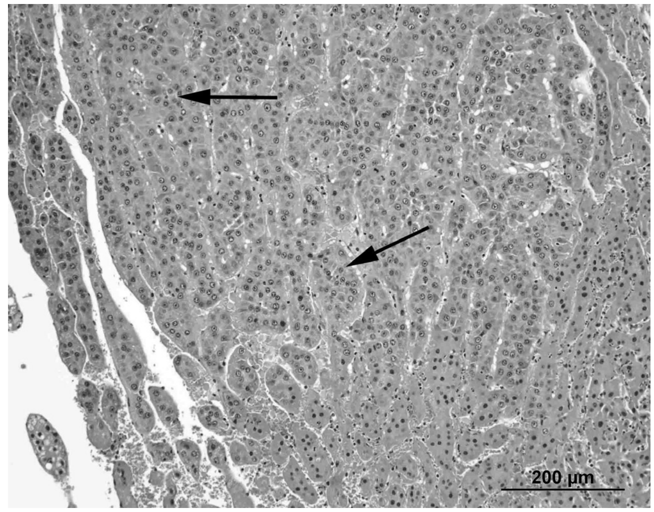


Plate 6
Hepatocellular carcinoma in the liver of a female B6C3F1 mouse exposed to 1,000 mg/L bromochloroacetic acid in drinking water for 2 years. The neoplastic hepatocytes are forming trabeculae (arrows) that are three or more cells thick. H&E

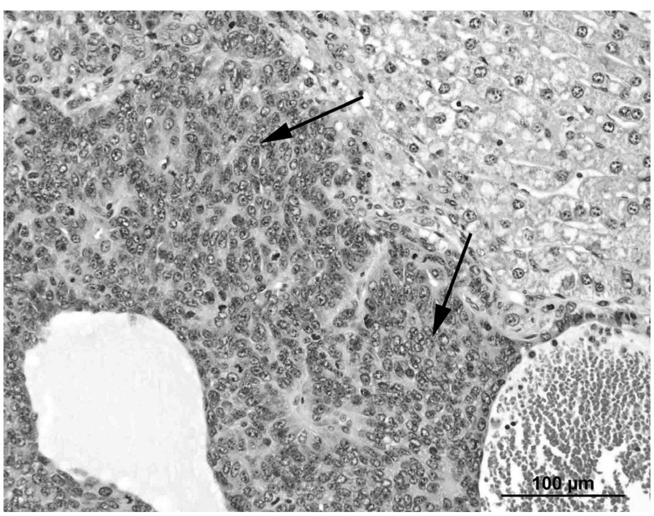


Plate 7
Hepatoblastoma in the liver of a male B6C3F1 mouse exposed to 1,000 mg/L bromochloroacetic acid in drinking water for 2 years. The neoplasm is composed of cells with hyperchromatic irregular nuclei and scant basophilic cytoplasm (arrows) that resemble hepatoblasts of a developing fetal liver. H&E

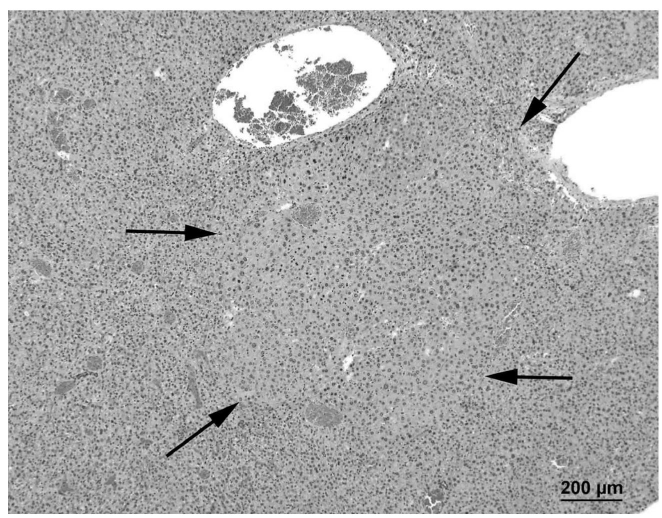


Plate 8
Eosinophilic focus in the liver of a female B6C3F1 mouse exposed to 500 mg/L bromochloroacetic acid in drinking water for 2 years. Note that slight compression of the adjacent parenchyma is present at the margins (arrows). H&E

DISCUSSION AND CONCLUSIONS

Bromochloroacetic acid is a drinking water disinfection by-product formed by the reaction of chlorine-containing oxidizing agents with natural organic matter in source water containing bromide. While the United States Environmental Protection Agency (USEPA) regulates drinking water levels of monochloroacetic acid, dichloroacetic acid, trichloroacetic acid, monobromoacetic acid, and dibromoacetic acid at a total limit of 60 µg/L under the disinfection by-product rule (USEPA, 1998), bromochloroacetic acid is not included in that rule, and no drinking water standard has been established for exposure to this disinfection by-product in the United States. Toxicity and carcinogenicity studies of bromochloroacetic acid and of dibromoacetic acid administered in drinking water to rats and mice were nominated to the National Toxicology Program (NTP) by the USEPA because of widespread human exposure to these water disinfection by-products 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, 1999; Daniel *et al.*, 1992) and rats (DeAngelo *et al.*, 1996). Results of the NTP studies on dibromoacetic acid were reported previously (Melnick *et al.*, 2007; NTP, 2007a).

Drinking water concentrations of bromochloroacetic acid ranged from 62.5 to 1,000 mg/L in the 2-week and 3-month studies in rats and mice. The higher exposure concentrations of bromochloroacetic acid are similar to those that induced tumors in rats or mice exposed to dichloroacetic acid (DeAngelo *et al.*, 1991, 1996, 1999; Daniel *et al.*, 1992; NTP, 2007b) or dibromoacetic acid (NTP, 2007a). Higher exposure concentrations of bromochloroacetic acid were not used because it was anticipated that brominated haloacetic acids would be more active than dichloroacetic acid. For example, in short-term studies, bromochloroacetic acid was more potent than dichloroacetic acid in causing oxidative damage (lipid peroxidation) and forming 8-hydroxydeoxyguanosine adducts in mouse liver nuclear DNA (Austin *et al.*, 1996; Parrish *et al.*, 1996). The main findings among the 2-week and 3-month studies were increased kidney weights in male rats, increased liver weights (absolute and/or relative) in male and female rats and mice, increased incidences of hepatocellular cytoplasmic vacuolization in both sexes of rats and mice, and increased incidences of hematopoietic cell proliferation in the spleen of male and female mice. Because liver weights in the 1,000 mg/L groups of both species were increased considerably compared to controls in the 3-month studies (33% in male rats, 21% in female rats, 18% in male mice, and 32% in female mice), higher exposure concentrations were not selected for the 2-year studies in rats and mice.

Based on SMVCE results, the reproductive organ weights, and histopathology of the reproductive organs, there was no evidence of toxicity to the reproductive system in the current 3-month studies in rats and mice. In contrast to these findings, previous studies have established bromochloroacetic acid as a reproductive toxicant. Klinefelter *et al.* (2002) observed numerous changes in male reproductive parameters (decreased epididymal sperm counts, decreased number of motile sperm, increased number of epididymal sperm with misshapen heads or tail defects, increased number of atypical residual bodies in seminiferous tubules, and increased number of Step 19 spermatids retained in Stages X and XI of the spermatogenic cycle) in male Sprague-Dawley rats administered 72 mg/kg bromo-chloroacetic acid for 14 days. In addition, the fertility of cauda epididymal sperm evaluated by *in utero* insemination was reduced in male rats exposed to 1.6 mg bromochloroacetic acid/kg body weight or greater doses for 14 days (Klinefelter *et al.*, 2002; Kaydos *et al.*, 2004), and drinking water exposure to bromochloro-acetic acid caused a decrease in total implants per litter and a decrease in the number of live fetuses per litter in a short-term toxicity screen in Sprague-Dawley rats (NTP, 1998).

A particularly noteworthy finding in the 2-year study in rats was the increased incidences of large intestine (colon or rectum) adenoma in males and females. Although the incidence was significantly increased only in 1,000 mg/L females, the incidences in 250 and 1,000 mg/L males and in 500 and 1,000 mg/L females exceeded the historical control ranges for these rarely occurring neoplasms; in control male and female rats, large intestine adenomas occur at a rate of less than 0.2%. Based on the rarity of these neoplasms, the incidences in male and female rats were considered to be clearly related to exposure to bromochloroacetic acid. The determination of *clear evidence* of carcinogenicity of bromochloroacetic acid in male and female rats is supported by numerous studies indicating that adenoma of the large intestine can progress to carcinoma (Deschner, 1983; Chang, 1984; Nigro, 1985). In addition, among the 12 studies in the NTP database in which large intestine neoplasms were induced in rats, 11 included the observation of large intestine carcinomas. Because of the recognized ability of benign intestinal neoplasms to progress to malignant neoplasms, the NTP combines incidences of benign and malignant intestinal neoplasms when evaluating evidence of a carcinogenic effect in the large intestine (McConnell *et al.,* 1986). Increased colorectal cancer risk has been associated with human exposure to drinking water disinfection by-products (Morris *et al.*, 1992). Gavage administration of 50 or 100 mg/kg bromodichloromethane in rats induced high incidences of large intestine neoplasms (Dunnick *et al*., 1987; NTP, 1987); however, no large intestine neoplasms were observed in male rats administered bromodichloromethane in drinking water for 2 years at exposures up to 700 mg/L (NTP, 2006). Thus, bromo-chloroacetic acid is the first water disinfection by-product shown to produce large intestine neoplasms in laboratory animals after drinking water exposure.

In male rats, a significantly increased incidence of abdominal mesothelioma was observed in the 500 mg/L group, and the incidences of mesotheliomas in all exposed groups of male rats exceeded the historical range in drinking water controls. Similar to the induction of mesotheliomas in male rats exposed to dibromoacetic acid, this malignant lesion was observed at multiple sites throughout the abdominal cavity (peritoneum). 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 (NTP, 2007c). Both mutagenic and nonmutagenic chemicals induced these neoplasms. Phenoxybenzamine hydrochloride was the only chemical that induced neoplasms in the abdominal cavity mesothelium of both sexes of rats and mice (NCI, 1978). Another disinfection by-product, potassium bromate, also induced mesotheliomas of the peritoneum in male F344 rats (Kurokawa *et al.*, 1983). Based on the above studies, there is no apparent relationship between chemical structure and this neoplastic response.

In a 2-year study of potassium bromate administered to male F344 rats in drinking water at concentrations ranging from 0.02 to 0.4 g/L, mesothelioma was detected on the tunica vaginalis in one animal euthanized after 52 weeks of treatment (0.2 g/L); while after only 78 weeks of treatment, mesotheliomas were present on multiple abdominal organs (Wolf *et al.*, 1998). Based on these findings, Wolf *et al.* (1998) suggested that the tunica vaginalis might be the site of origin of bromate-induced mesotheliomas. However, the time-dependent incidence of preneoplastic lesions and mesotheliomas in male rats exposed to potassium bromate was consistent with either the tunica vaginalis or the spleen as the site of origin of these neoplasms (Crosby *et al.*, 2000a). In the current study of bromochloroacetic acid, the earliest death of a male rat with mesothelioma occurred after 68 weeks of exposure. Neoplasms were observed on multiple organs throughout the abdominal cavity in most of the early death animals bearing mesotheliomas in the current study. Thus, it is not possible to draw a definitive conclusion on the site of origin of the peritoneal mesotheliomas.

Mesotheliomas were collected from F344/N rats treated with bromochloroacetic acid in the current study and examined for gene expression profiles by microarray analyses of isolated RNA (Kim *et al.*, 2006). Gene expressions were altered in several cancer-related pathways, including growth and proliferation, cell cycle progression, apoptosis, invasion, and metastasis, and exhibited changes similar to those in human pleural mesotheliomas.

The incidence of mammary gland fibroadenoma in female rats was not increased in the current study; however, the incidences of multiple fibroadenomas were increased in 500 and 1,000 mg/L females. Because of the high incidence of fibroadenomas in the control group (86%), which is the highest incidence of these neoplasms observed in control female F344/N rats by any route of exposure, an alternative means of evaluating a response is to examine the multiplicity of these lesions in relation to exposure to bromochloroacetic acid. In addition to increases in the incidences of multiple fibroadenomas, the numbers of fibroadenomas per fibroadenoma-bearing female rat in the 500 and 1,000 mg/L groups were significantly greater than the control group. This finding provides compelling evidence of an exposure-related effect by bromo-chloro-acetic acid on mammary gland fibroadenomas in female rats.

The incidences of hepatocellular adenomas in 500 and 1,000 mg/L male rats and in 1,000 mg/L female rats were not significantly increased compared to the controls, but they exceeded the historical range for these neoplasms in drinking water controls. Because these neoplasms are uncommon in rats and because there were also increased incidences of altered hepatic foci (eosinophilic foci in male and female rats and mixed cell foci in female rats), the increased incidences of hepatocellular adenoma in both sexes of rats may have been related to bromochloroacetic acid exposure. The 3-month study in rats also showed that the liver is a target organ of bromochloroacetic acid administered in drinking water.

The significantly increased incidence of pancreatic islet neoplasms in 500 mg/L male rats, which exceeded the historical range in drinking water controls but was not increased in any other exposed group, may have been related to exposure to bromochloroacetic acid. The lack of an effect in 1,000 mg/L male rats or in female rats adds to the uncertainty to the response in 500 mg/L males.

In the 2-year study in mice, survival was significantly less in 1,000 mg/L males compared to controls. The increase in natural deaths and moribund sacrifices in males was associated with increased liver neoplasms. Exposure of mice to bromochloroacetic acid produced significant chemical-related increased incidences of hepatocellular adenoma or carcinoma (combined) in males and females and hepatoblastoma in males. These responses are similar to those observed with dibromoacetic acid in male and female mice (Melnick *et al.*, 2007; NTP, 2007a). The liver neoplasm response was associated with exposure concentration-related increased incidences of hepatocyte cytoplasmic vacuolization in males and females, a small increased incidence in centrilobular necrosis in 1,000 mg/L males, and increased incidences of eosinophilic foci in females that were significant in the 500 and 1,000 mg/L groups. A species difference in susceptibility to bromochloroacetic acid-induced liver neoplasia is noted by comparing the nearly 100% responses in mice with the marginal responses in rats exposed to equivalent concentrations.

In addition to alterations in the liver, increased incidences of alveolar epithelial hyperplasia of the lung in rats and hematopoietic cell proliferation of the spleen and bone marrow hyperplasia in male mice indicate that other organs are potential targets of bromochloroacetate toxicity with chronic exposure. The latter responses were not predicted from the 3-month study that found no hematological effects of mice exposed to bromochloroacetic acid.

The current 2-year studies demonstrate that bromochloroacetic acid is a multiple-organ carcinogen in laboratory animals; the primary sites of neoplasm induction identified were the large intestine of male and female rats, the abdominal cavity mesothelium of male rats, the mammary gland of female rats, and the liver of mice. Bromochloroacetic acid induced neoplasms at several organ sites in common with dibromoacetic acid and/or dichloroacetic acid (Table 18; NTP, 2007a,b). The mechanism(s) of neoplasm induction by bromochloro-acetic acid or the related compounds, dichloroacetic acid and dibromoacetic acid, are not known. Reduction of GST-ζ (also known as maleylacetoacetate isomerase) activity by dihaloacetic acids may be involved in the carcinogenicity of these chemicals by causing accumulation of toxic intermediates of the tyrosine degradation pathway (Ammini *et al.,* 2003). The mouse liver was a common site of neoplasm induction by each of these haloacetic acids. For dichloroacetic acid, Carter *et al.* (2003) suggested that the induction of liver neoplasms in mice is due to selective growth of focal lesions of a cell type that does not respond to mitoinhibitory homeostatic control mechanisms. Neither hepatocellular necrosis nor regenerative hyperplasia accounted for the development of preneoplastic lesions or liver neoplasms in mice treated with any of these three dihaloacetic acids. 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). DNA damage due to oxidative stress in the livers of mice exposed to halogenated acetic acids, including bromochloroacetic acid (Austin *et al.*, 1996), may also contribute to the hepatocarcinogenicity of these chemicals. A study of gene expression in immortalized rat peritoneal mesothelial cells incubated with potassium bromate detected increases in oxidative stress responsive genes, as well as changes in genes that regulate DNA repair and cell cycling (Crosby *et al.*, 2000b). The carcinogenicity of bromochloroacetic acid

Table 18
Tumor Induction by Dihaloacetic Acids in F344/N Rats and B6C3F1 Mice

Dihaloacetic Acid	Exposure Concentrations	Rats	Mice
Dichloroacetic Acid (DeAngelo *et al.,* 1991, 1996, 1999)	500 to 5,000 mg/L (3.9 to 39 mmol/L)	Hepatocellular adenoma or carcinoma (males)	Hepatocellular adenoma or carcinoma (males)
Dibromoacetic Acid (NTP, 2007a)	50 to 1,000 mg/L (0.23 to 4.6 mmol/L)	Peritoneal mesothelioma (males) Mononuclear cell leukemia (females, equivocal in males)	Hepatocellular adenoma or carcinoma (males and females) Hepatoblastoma (males) Alveolar/bronchiolar adenoma or carcinoma (males, equivocal in females)
Bromochloroacetic Acid	250 to 1,000 mg/L (1.4 to 5.8 mmol/L)	Peritoneal mesothelioma (males) Large intestine adenoma (males and females) Mammary gland fibroadenoma (females) Pancreatic islet adenoma (equivocal in males) Hepatocellular adenoma (equivocal in males and females)	Hepatocellular adenoma or carcinoma (males and females) Hepatoblastoma (males)

may also involve a genotoxic mechanism since this chemical induces mutations in *Salmonella typhimurium* strain TA100 with and without S9 metabolic activation (Appendix E). In addition, glyoxylate, a metabolite of dihaloacetate biotransformation, is mutagenic in strains TA100, TA102, and TA104 (Sayato *et al.*, 1987). Thus, it is possible that oxidative stress, DNA damage, and selective growth of preneoplastic cells are involved in the carcinogenesis of bromo-chloroacetic acid.

The 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 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 urine and plasma-time course data for bromochloroacetate and glyoxylate in rats and mice (Appendix M), as well as published physiological and biochemical parameters, a physiologically based pharmacokinetic model (Appendix N) was created to characterize the absorption, metabolism, and elimination of this chemical in rats and mice and to estimate blood and liver concentrations of bromochloroacetate and glyoxylate and liver levels of GST-ζ activity in rats and mice exposed to the same drinking water concentrations of bromochloroacetic acid that were used in the 2-year studies. Several model-based predictions relate to the tissue dosimetry of bromochloroacetate and its metabolite, glyoxylate, after 60 days of drinking water exposure:

1) GST-ζ activity in livers of rats and mice decreases dramatically with increasing exposure concentrations of bromochloroacetic acid. At equivalent exposure concentrations, GST-ζ is inactivated to a greater extent in rats than in mice even though the daily dose in mice is nearly twice that of rats. At the 1,000 mg/L concentration, less than 10% of the control GST-ζ activity remains in rats and mice.
2) The concentrations of bromochloroacetate or glyoxylate in blood are similar to those in the liver of rats and mice; this is because the liver-to-blood partition coefficients for these compounds are close to 1.0.
3) At equivalent exposure concentrations, tissue levels of glyoxylate are approximately 10 to 15 times higher in mice than in rats; this apparent species difference is due in part to differences in daily uptake between rats and mice and the greater sensitivity of rats to GST-ζ inactivation. The latter factor also contributes to the much higher tissue concentrations of bromochloroacetate in rats than in mice.
4) Tissue concentrations of bromochloroacetate increase disproportionately with increasing concentrations of bromochloroacetic acid in the drinking water. For example, average daily blood concentrations (areas under the blood concentration curve) increased three- to fourfold at the 500 mg/L concentration compared to the 250 mg/L concentration, and increased an additional 2.5- to 3.5-fold at the 1,000 mg/L concentration. The nonlinear relationship between exposure and tissue concentration of bromochloroacetate reflects the consequence of suicidal inactivation of GST-ζ.

Because several assumptions in the dihaloacetate disposition model are being tested (e.g., a secondary GST-ζ-independent pathway metabolizes bromochloroacetate, all of the metabolic product from (+)-bromochloroacetate binds covalently to GST-ζ, and age does not affect the activity of metabolic enzymes), no tumor dose-response analyses have been performed yet with various model-predicted dose metrics (e.g., time- and age-dependent tissue concentrations of bromochloroacetate and/or glyoxylate).

Conclusions

Under the conditions of these 2-year studies, there was *clear evidence of carcinogenic activity** of bromochloroacetic acid in male F344/N rats based on increased incidences of malignant mesotheliomas and adenomas of the large intestine. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in female F344/N rats based on increased incidences of adenomas of the large intestine; increased incidences of multiple fibroadenomas of the mammary gland in female rats were also considered to be exposure related. Increased incidences of pancreatic islet adenomas in male rats and of hepatocellular adenomas in male and female rats may have been related to bromochloroacetic acid exposure. There was *clear evidence of carcinogenic activity* of bromochloroacetic acid in male and female B6C3F1 mice based on increased incidences of hepatocellular neoplasms and hepatoblastoma (males only).

Exposure to bromochloroacetic acid for 2 years resulted in increased incidences of nonneoplastic lesions in the liver of male rats, liver and lung of female rats, and liver of male and female mice.

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

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

Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	17	17	17
Natural deaths	4	7	8	4
Survivors				
Terminal sacrifice	31	26	25	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(48)	(48)	(47)	(49)
Intestine large, colon	(50)	(49)	(50)	(48)
Adenoma		1 (2%)		3 (6%)
Intestine large, rectum	(50)	(50)	(50)	(49)
Adenoma		1 (2%)		1 (2%)
Intestine small, duodenum	(50)	(49)	(50)	(49)
Intestine small, ileum	(49)	(46)	(47)	(49)
Intestine small, jejunum	(47)	(46)	(46)	(48)
Liver	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, spleen		1 (2%)		
Hepatocellular adenoma	2 (4%)		3 (6%)	3 (6%)
Hepatocellular adenoma, multiple				1 (2%)
Mesentery	(6)	(16)	(15)	(15)
Fibrosarcoma		2 (13%)		
Fibrosarcoma, metastatic, spleen		1 (6%)		
Oral mucosa	(0)	(0)	(1)	(1)
Squamous cell papilloma				1 (100%)
Pharyngeal, squamous cell papilloma			1 (100%)	
Pancreas	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery		2 (4%)		
Fibrosarcoma, metastatic, spleen		1 (2%)		
Acinus, adenoma	2 (4%)		1 (2%)	1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(0)	(1)	(0)	(1)
Squamous cell papilloma		1 (100%)		
Tooth	(0)	(0)	(0)	(2)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Schwannoma benign				1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	8 (16%)	13 (26%)	7 (14%)	11 (22%)
Pheochromocytoma malignant	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Bilateral, pheochromocytoma benign	1 (2%)		1 (2%)	1 (2%)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Endocrine System (continued)				
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	4 (8%)	9 (18%)	3 (6%)
Carcinoma	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Pituitary gland	(48)	(50)	(50)	(50)
Pars distalis, adenoma	23 (48%)	30 (60%)	23 (46%)	21 (42%)
Pars distalis, carcinoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	3 (6%)	7 (14%)	7 (14%)	8 (16%)
C-cell, carcinoma	1 (2%)	1 (2%)		
Follicular cell, adenoma	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Follicular cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
Peritoneum	(0)	(4)	(2)	(3)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, mesentery		1 (2%)		
Preputial gland	(50)	(49)	(50)	(49)
Adenoma	4 (8%)	7 (14%)	7 (14%)	8 (16%)
Carcinoma	2 (4%)	1 (2%)		
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, spleen		1 (2%)		
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	38 (76%)	39 (78%)	39 (78%)	43 (86%)
Interstitial cell, adenoma	8 (16%)	7 (14%)	5 (10%)	3 (6%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(14)	(31)	(19)	(25)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung				1 (4%)
Lymph node, mandibular	(1)	(3)	(0)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, spleen		1 (2%)		
Spleen	(50)	(50)	(50)	(50)
Fibrosarcoma		3 (6%)		
Hemangiosarcoma				1 (2%)
Thymus	(49)	(50)	(50)	(48)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Thymoma malignant				1 (2%)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Fibroadenoma	3 (6%)	4 (8%)	3 (6%)	4 (8%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)	2 (4%)	1 (2%)
Keratoacanthoma	1 (2%)		2 (4%)	1 (2%)
Squamous cell papilloma	3 (6%)	4 (8%)	4 (8%)	2 (4%)
Squamous cell papilloma, multiple		1 (2%)		
Trichoepithelioma	3 (6%)			
Sebaceous gland, adenoma				1 (2%)
Subcutaneous tissue, fibroma	7 (14%)	7 (14%)	2 (4%)	4 (8%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	3 (6%)		
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, liposarcoma	1 (2%)			
Subcutaneous tissue, neural crest tumor	1 (2%)		1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			1 (2%)	
Thymoma malignant, metastatic, thymus				1 (2%)
Skeletal muscle	(1)	(4)	(0)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (100%)
Fibrosarcoma, metastatic, mesentery		2 (50%)		
Fibrosarcoma, metastatic, spleen		1 (25%)		
Hemangioma		1 (25%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant			1 (2%)	
Oligodendroglioma malignant			1 (2%)	1 (2%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)		1 (2%)
Alveolar/bronchiolar carcinoma	1 (2%)			2 (4%)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Thymoma malignant, metastatic, thymus				1 (2%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(1)	(1)	(1)	(0)
Adenoma			1 (100%)	
Carcinoma	1 (100%)	1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(49)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	21 (42%)	23 (46%)	19 (38%)	21 (42%)
Lymphoma malignant				1 (2%)
Mesothelioma malignant	1 (2%)	5 (10%)	10 (20%)	6 (12%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	50	50	50
Total primary neoplasms	147	175	156	162
Total animals with benign neoplasms	50	50	49	48
Total benign neoplasms	113	132	119	126
Total animals with malignant neoplasms	27	33	33	31
Total malignant neoplasms	33	43	36	35
Total animals with metastatic neoplasms	1	3		2
Total metastatic neoplasms	1	11		6
Total animals with uncertain neoplasms-benign or malignant	1		1	1
Total uncertain neoplasms	1		1	1

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

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	9/50 (18%)	13/50 (26%)	8/50 (16%)	12/50 (24%)
Adjusted rate[b]	20.5%	30.4%	19.1%	28.8%
Terminal rate[c]	8/31 (26%)	10/26 (39%)	3/25 (12%)	10/29 (35%)
First incidence (days)	504	668	555	717
Poly-3 test[d]	P=0.327	P=0.206	P=0.544N	P=0.260
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	10/50 (20%)	14/50 (28%)	8/50 (16%)	13/50 (26%)
Adjusted rate	22.8%	32.5%	19.1%	30.7%
Terminal rate	9/31 (29%)	10/26 (39%)	3/25 (12%)	10/29 (35%)
First incidence (days)	504	618	555	521
Poly-3 test	P=0.353	P=0.218	P=0.440N	P=0.277
Large Intestine (Colon or Rectum): Adenoma				
Overall rate	0/50 (0%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	4.8%	0.0%	9.5%
Terminal rate	0/31 (0%)	2/26 (8%)	0/25 (0%)	3/29 (10%)
First incidence (days)	—[e]	729 (T)	—	468
Poly-3 test	P=0.031	P=0.231	—[f]	P=0.057
Liver: Hepatocellular Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.6%	0.0%	7.5%	9.5%
Terminal rate	2/31 (7%)	0/26 (0%)	3/25 (12%)	1/29 (3%)
First incidence (days)	729 (T)	—	729 (T)	682
Poly-3 test	P=0.107	P=0.242N	P=0.468	P=0.324
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	3/50 (6%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	7.0%	2.4%	0.0%	7.1%
Terminal rate	3/31 (10%)	1/26 (4%)	0/25 (0%)	1/29 (3%)
First incidence (days)	729 (T)	729 (T)	—	380
Poly-3 test	P=0.522	P=0.314N	P=0.131N	P=0.656
Mammary Gland: Fibroadenoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted rate	6.9%	9.4%	7.3%	9.6%
Terminal rate	2/31 (7%)	2/26 (8%)	2/25 (8%)	4/29 (14%)
First incidence (days)	668	668	549	729 (T)
Poly-3 test	P=0.436	P=0.488	P=0.635	P=0.477
Pancreatic Islets: Adenoma				
Overall rate	3/50 (6%)	4/50 (8%)	9/50 (18%)	3/50 (6%)
Adjusted rate	7.0%	9.4%	21.6%	7.1%
Terminal rate	3/31 (10%)	2/26 (8%)	5/25 (20%)	2/29 (7%)
First incidence (days)	729 (T)	679	549	434
Poly-3 test	P=0.502	P=0.491	P=0.049	P=0.654
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	6/50 (12%)	10/50 (20%)	4/50 (8%)
Adjusted rate	11.6%	14.1%	24.0%	9.4%
Terminal rate	5/31 (16%)	3/26 (12%)	6/25 (24%)	3/29 (10%)
First incidence (days)	729 (T)	679	549	434
Poly-3 test	P=0.472N	P=0.488	P=0.110	P=0.512N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	23/48 (48%)	30/50 (60%)	23/50 (46%)	21/50 (42%)
Adjusted rate	52.0%	64.7%	52.4%	48.0%
Terminal rate	16/31 (52%)	17/26 (65%)	13/25 (52%)	14/29 (48%)
First incidence (days)	337	504	575	510
Poly-3 test	P=0.226N	P=0.149	P=0.570	P=0.434N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	23/48 (48%)	30/50 (60%)	24/50 (48%)	21/50 (42%)
Adjusted rate	52.0%	64.7%	54.7%	48.0%
Terminal rate	16/31 (52%)	17/26 (65%)	14/25 (56%)	14/29 (48%)
First incidence (days)	337	504	575	510
Poly-3 test	P=0.235N	P=0.149	P=0.484	P=0.434N
Preputial Gland: Adenoma				
Overall rate	4/50 (8%)	7/49 (14%)	7/50 (14%)	8/49 (16%)
Adjusted rate	9.3%	16.4%	17.0%	19.3%
Terminal rate	4/31 (13%)	4/25 (16%)	5/25 (20%)	4/29 (14%)
First incidence (days)	729 (T)	504	561	631
Poly-3 test	P=0.153	P=0.252	P=0.233	P=0.156
Preputial Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	8/49 (16%)	7/50 (14%)	8/49 (16%)
Adjusted rate	11.4%	18.7%	17.0%	19.3%
Terminal rate	4/31 (13%)	4/25 (16%)	5/25 (20%)	4/29 (14%)
First incidence (days)	504	504	561	631
Poly-3 test	P=0.248	P=0.256	P=0.335	P=0.239
Skin: Squamous Cell Papilloma				
Overall rate	3/50 (6%)	5/50 (10%)	4/50 (8%)	2/50 (4%)
Adjusted rate	6.9%	11.7%	9.8%	4.8%
Terminal rate	2/31 (7%)	3/26 (12%)	3/25 (12%)	2/29 (7%)
First incidence (days)	701	549	561	729 (T)
Poly-3 test	P=0.345N	P=0.351	P=0.468	P=0.518N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	4/50 (8%)	5/50 (10%)	6/50 (12%)	2/50 (4%)
Adjusted rate	9.2%	11.7%	14.5%	4.8%
Terminal rate	3/31 (10%)	3/26 (12%)	3/25 (12%)	2/29 (7%)
First incidence (days)	701	549	561	729 (T)
Poly-3 test	P=0.285N	P=0.495	P=0.339	P=0.355N
Skin: Trichoepithelioma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.9%	0.0%	0.0%	0.0%
Terminal rate	2/31 (7%)	0/26 (0%)	0/25 (0%)	0/29 (0%)
First incidence (days)	701	—	—	—
Poly-3 test	P=0.045N	P=0.123N	P=0.132N	P=0.126N
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	2/50 (4%)	1/50 (2%)
Adjusted rate	6.9%	2.4%	4.9%	2.4%
Terminal rate	2/31 (7%)	1/26 (4%)	0/25 (0%)	1/29 (3%)
First incidence (days)	701	729 (T)	674	729 (T)
Poly-3 test	P=0.289N	P=0.315N	P=0.529N	P=0.319N

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma				
Overall rate	6/50 (12%)	6/50 (12%)	8/50 (16%)	3/50 (6%)
Adjusted rate	13.9%	14.0%	19.2%	7.2%
Terminal rate	5/31 (16%)	4/26 (15%)	3/25 (12%)	3/29 (10%)
First incidence (days)	701	549	561	729 (T)
Poly-3 test	P=0.240N	P=0.616	P=0.355	P=0.261N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	7/50 (14%)	7/50 (14%)	2/50 (4%)	4/50 (8%)
Adjusted rate	16.1%	16.3%	4.9%	9.6%
Terminal rate	6/31 (19%)	3/26 (12%)	1/25 (4%)	3/29 (10%)
First incidence (days)	637	596	631	682
Poly-3 test	P=0.145N	P=0.603	P=0.095N	P=0.283N
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.3%	7.1%	0.0%	0.0%
Terminal rate	0/31 (0%)	1/26 (4%)	0/25 (0%)	0/29 (0%)
First incidence (days)	601	702	—	—
Poly-3 test	P=0.163N	P=0.294	P=0.516N	P=0.509N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	8/50 (16%)	9/50 (18%)	2/50 (4%)	4/50 (8%)
Adjusted rate	18.2%	20.9%	4.9%	9.6%
Terminal rate	6/31 (19%)	4/26 (15%)	1/25 (4%)	3/29 (10%)
First incidence (days)	601	596	631	682
Poly-3 test	P=0.071N	P=0.480	P=0.058N	P=0.200N
Spleen: Fibrosarcoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	0.0%	7.0%	0.0%	0.0%
Terminal rate	0/31 (0%)	2/26 (8%)	0/25 (0%)	0/29 (0%)
First incidence (days)	—	525	—	—
Poly-3 test	P=0.324N	P=0.116	—	—
Testes: Adenoma				
Overall rate	46/50 (92%)	46/50 (92%)	44/50 (88%)	46/50 (92%)
Adjusted rate	96.6%	96.1%	93.2%	96.0%
Terminal rate	31/31 (100%)	26/26 (100%)	25/25 (100%)	28/29 (97%)
First incidence (days)	525	525	366	434
Poly-3 test	P=0.519N	P=0.707N	P=0.352N	P=0.682N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	3/50 (6%)	7/50 (14%)	7/50 (14%)	8/50 (16%)
Adjusted rate	7.0%	16.4%	17.3%	19.1%
Terminal rate	3/31 (10%)	4/26 (15%)	5/25 (20%)	5/29 (17%)
First incidence (days)	729 (T)	609	652	682
Poly-3 test	P=0.099	P=0.150	P=0.131	P=0.087
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	8/50 (16%)	7/50 (14%)	8/50 (16%)
Adjusted rate	9.3%	18.6%	17.3%	19.1%
Terminal rate	4/31 (13%)	4/26 (15%)	5/25 (20%)	5/29 (17%)
First incidence (days)	729 (T)	609	652	682
Poly-3 test	P=0.184	P=0.173	P=0.224	P=0.160

Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	7.0%	4.7%	5.0%	4.8%
Terminal rate	3/31 (10%)	1/26 (4%)	2/25 (8%)	2/29 (7%)
First incidence (days)	729 (T)	713	729 (T)	729 (T)
Poly-3 test	P=0.446N	P=0.511N	P=0.532N	P=0.517N
All Organs: Mononuclear Leukemia				
Overall rate	21/50 (42%)	23/50 (46%)	19/50 (38%)	21/50 (42%)
Adjusted rate	44.9%	50.7%	42.7%	47.3%
Terminal rate	9/31 (29%)	11/26 (42%)	6/25 (24%)	11/29 (38%)
First incidence (days)	525	549	366	434
Poly-3 test	P=0.530	P=0.364	P=0.500N	P=0.494
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	5/50 (10%)	10/50 (20%)	6/50 (12%)
Adjusted rate	2.3%	11.7%	23.7%	14.0%
Terminal rate	0/31 (0%)	2/26 (8%)	5/25 (20%)	2/29 (7%)
First incidence (days)	608	555	479	556
Poly-3 test	P=0.062	P=0.097	P=0.003	P=0.052
All Organs: Benign Tumors				
Overall rate	50/50 (100%)	50/50 (100%)	49/50 (98%)	48/50 (96%)
Adjusted rate	100.0%	100.0%	99.8%	99.5%
Terminal rate	31/31 (100%)	26/26 (100%)	25/25 (100%)	29/29 (100%)
First incidence (days)	337	504	366	434
Poly-3 test	P=0.892N	—	P=1.000N	P=1.000N
All Organs: Malignant Tumors				
Overall rate	27/50 (54%)	33/50 (66%)	33/50 (66%)	31/50 (62%)
Adjusted rate	56.9%	69.0%	68.5%	63.5%
Terminal rate	14/31 (45%)	14/26 (54%)	13/25 (52%)	13/29 (45%)
First incidence (days)	504	525	321	357
Poly-3 test	P=0.381	P=0.153	P=0.164	P=0.326
All Organs: Benign or Malignant Tumors				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	31/31 (100%)	26/26 (100%)	25/25 (100%)	29/29 (100%)
First incidence (days)	337	504	321	357
Poly-3 test	—	—	—	—

(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, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

Table A3a
Historical Incidence of Malignant Mesothelioma in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	1/50
Bromodichloromethane	3/50
Dibromoacetic acid	3/50
Dibromoacetonitrile	0/50
Sodium chlorate	0/50
Sodium dichromate dihydrate (VI)	2/50
Overall Historical Incidence: Drinking Water Studies	
Total (%)	9/300 (3.0%)
Mean ± standard deviation	3.0% ± 2.8%
Range	0%-6%
Overall Historical Incidence: All Routes	
Total (%)	34/1,199 (2.8%)
Mean ± standard deviation	2.8% ± 2.3%
Range	0%-6%

[a] Data as of October 4, 2007

Table A3b
Historical Incidence of Adenoma of the Large Intestine in Untreated Male F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	0/50
Bromodichloromethane	0/50
Dibromoacetic acid	0/50
Dibromoacetonitrile	0/50
Sodium chlorate	0/50
Sodium dichromate dihydrate (VI)	0/50
Overall Historical Incidence: Drinking Water Studies	
Total (%)	0/300 (0.0%)
Overall Historical Incidence: All Routes	
Total (%)	2/1,199 (0.2%)
Mean ± standard deviation	0.2% ± 0.6%
Range	0%-2%

[a] Data as of October 4, 2007

Table A3c
Historical Incidence of Neoplasms of the Pancreatic Islets in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	3/50	2/50	5/50
Bromodichloromethane	3/49	0/49	3/49
Dibromoacetic acid	5/48	0/48	5/48
Dibromoacetonitrile	5/49	1/49	6/49
Sodium chlorate	3/50	2/50	5/50
Sodium dichromate dihydrate (VI)	4/50	0/50	4/50
Overall Historical Incidence: Drinking Water Studies			
Total (%)	23/296 (7.8%)	5/296 (1.7%)	28/296 (9.5%)
Mean ± standard deviation	7.8% ± 2.1%	1.7% ± 2.0%	9.5% ± 2.1%
Range	6%-10%	0%-4%	6%-12%
Overall Historical Incidence: All Routes			
Total (%)	73/1,194 (6.1%)	27/1,194 (2.3%)	100/1,194 (8.4%)
Mean ± standard deviation	6.1% ± 3.2%	2.3% ± 2.6%	8.4% ± 4.1%
Range	0%-12%	0%-10%	0%-18%

[a] Data as of October 4, 2007

Table A3d
Historical Incidence of Neoplasms of the Liver in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	2/50	0/50	2/50
Bromodichloromethane	1/50	0/50	1/50
Dibromoacetic acid	1/50	0/50	1/50
Dibromoacetonitrile	0/50	0/50	0/50
Sodium chlorate	0/50	0/50	0/50
Sodium dichromate dihydrate (VI)	0/50	0/50	0/50
Overall Historical Incidence: Drinking Water Studies			
Total (%)	4/300 (1.3%)	0/300 (0.0%)	4/300 (1.3%)
Mean ± standard deviation	1.3% ± 1.6%		1.3% ± 1.6%
Range	0%-4%		0%-4%
Overall Historical Incidence: All Routes			
Total (%)	10/1,199 (0.8%)	2/1,199 (0.2%)	12/1,199 (1.0%)
Mean ± standard deviation	0.8% ± 1.3%	0.2% ± 0.6%	1.0% ± 1.3%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of October 4, 2007

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	15	17	17	17
Natural deaths	4	7	8	4
Survivors				
Terminal sacrifice	31	26	25	29
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(48)	(48)	(47)	(49)
Edema	5 (10%)	1 (2%)	1 (2%)	6 (12%)
Inflammation	1 (2%)			1 (2%)
Intestine large, colon	(50)	(49)	(50)	(48)
Inflammation		1 (2%)		
Intestine large, rectum	(50)	(50)	(50)	(49)
Intestine small, duodenum	(50)	(49)	(50)	(49)
Intestine small, ileum	(49)	(46)	(47)	(49)
Inflammation				1 (2%)
Intestine small, jejunum	(47)	(46)	(46)	(48)
Inflammation	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Amyloid deposition		1 (2%)		
Angiectasis	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Basophilic focus	6 (12%)	5 (10%)	4 (8%)	3 (6%)
Clear cell focus	22 (44%)	21 (42%)	23 (46%)	20 (40%)
Degeneration, cystic	13 (26%)	13 (26%)	12 (24%)	18 (36%)
Eosinophilic focus	2 (4%)	5 (10%)	4 (8%)	8 (16%)
Hepatodiaphragmatic nodule	11 (22%)	13 (26%)	7 (14%)	4 (8%)
Inflammation, chronic	10 (20%)	12 (24%)	12 (24%)	8 (16%)
Malformation				1 (2%)
Mixed cell focus	4 (8%)	3 (6%)	4 (8%)	2 (4%)
Necrosis, focal		2 (4%)	2 (4%)	
Bile duct, hyperplasia	45 (90%)	49 (98%)	46 (92%)	44 (88%)
Centrilobular, necrosis	4 (8%)	7 (14%)	8 (16%)	8 (16%)
Hepatocyte, vacuolization cytoplasmic	3 (6%)	6 (12%)	3 (6%)	2 (4%)
Mesentery	(6)	(16)	(15)	(15)
Accessory spleen			1 (7%)	1 (7%)
Angiectasis			1 (7%)	
Degeneration				1 (7%)
Fat, necrosis	6 (100%)	8 (50%)	9 (60%)	9 (60%)
Oral mucosa	(0)	(0)	(1)	(1)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	29 (58%)	28 (56%)	26 (52%)	29 (58%)
Cyst				1 (2%)
Acinus, hyperplasia, focal	7 (14%)	3 (6%)	2 (4%)	7 (14%)
Salivary glands	(50)	(50)	(50)	(50)
Inflammation				1 (2%)

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

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	4 (8%)	4 (8%)	4 (8%)	6 (12%)
Erosion			1 (2%)	1 (2%)
Inflammation, chronic active		1 (2%)		
Ulcer	3 (6%)	3 (6%)	2 (4%)	5 (10%)
Epithelium, hyperplasia	6 (12%)	5 (10%)	5 (10%)	8 (16%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema	4 (8%)	3 (6%)	1 (2%)	6 (12%)
Erosion	7 (14%)	5 (10%)	7 (14%)	8 (16%)
Hyperplasia	1 (2%)			
Ulcer	2 (4%)	3 (6%)	2 (4%)	
Tongue	(0)	(1)	(0)	(1)
Hyperplasia				1 (100%)
Tooth	(0)	(0)	(0)	(2)
Malformation				2 (100%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	46 (92%)	48 (96%)	48 (96%)
Thrombosis	4 (8%)	3 (6%)	4 (8%)	6 (12%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	23 (46%)	27 (54%)	28 (56%)	19 (38%)
Hyperplasia, focal	11 (22%)	13 (26%)	12 (24%)	9 (18%)
Hyperplasia, diffuse		1 (2%)		
Hypertrophy, focal	5 (10%)	3 (6%)	3 (6%)	4 (8%)
Necrosis	1 (2%)			
Bilateral, atrophy			1 (2%)	
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	19 (38%)	22 (44%)	20 (40%)	28 (56%)
Necrosis				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		1 (2%)
Pituitary gland	(48)	(50)	(50)	(50)
Pars distalis, angiectasis	23 (48%)	27 (54%)	23 (46%)	20 (40%)
Pars distalis, cyst	5 (10%)	1 (2%)	4 (8%)	4 (8%)
Pars distalis, hemorrhage	1 (2%)			
Pars distalis, hyperplasia				1 (2%)
Pars distalis, hyperplasia, focal	10 (21%)	8 (16%)	9 (18%)	9 (18%)
Pars intermedia, cyst			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst	2 (4%)	3 (6%)	3 (6%)	1 (2%)
C-cell, hyperplasia	21 (42%)	15 (30%)	15 (30%)	24 (48%)
Follicle, cyst	11 (22%)	11 (22%)	5 (10%)	9 (18%)
General Body System				
Peritoneum	(0)	(4)	(2)	(3)

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Hyperplasia, mesothelium		1 (2%)		
Preputial gland	(50)	(49)	(50)	(49)
Cyst	4 (8%)	4 (8%)	3 (6%)	7 (14%)
Inflammation, chronic	3 (6%)	2 (4%)	8 (16%)	6 (12%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	36 (72%)	32 (64%)	35 (70%)	34 (68%)
Epithelium, hyperplasia	7 (14%)	4 (8%)	7 (14%)	5 (10%)
Seminal vesicle	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	13 (26%)	12 (24%)	7 (14%)	10 (20%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	7 (14%)	8 (16%)	3 (6%)
Myelofibrosis		1 (2%)		2 (4%)
Lymph node	(14)	(31)	(19)	(25)
Deep cervical, hemorrhage		1 (3%)		
Mediastinal, congestion	1 (7%)			
Mediastinal, hemorrhage		6 (19%)	1 (5%)	4 (16%)
Mediastinal, hyperplasia, lymphoid	6 (43%)	8 (26%)	9 (47%)	7 (28%)
Pancreatic, amyloid deposition		1 (3%)		
Pancreatic, hyperplasia, lymphoid		4 (13%)	4 (21%)	
Lymph node, mandibular	(1)	(3)	(0)	(0)
Hyperplasia, lymphoid		1 (33%)		
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid		9 (18%)	4 (8%)	
Spleen	(50)	(50)	(50)	(50)
Accessory spleen				1 (2%)
Amyloid deposition		1 (2%)		
Fibrosis	1 (2%)	2 (4%)	2 (4%)	3 (6%)
Hematopoietic cell proliferation	5 (10%)	7 (14%)	6 (12%)	3 (6%)
Necrosis		1 (2%)		
Pigmentation		1 (2%)	2 (4%)	1 (2%)
Lymphoid follicle, hyperplasia		1 (2%)		
Thymus	(49)	(50)	(50)	(48)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	21 (42%)	14 (28%)	16 (32%)	18 (36%)
Hyperplasia	2 (4%)			1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hyperplasia	2 (4%)			2 (4%)
Inflammation, chronic	1 (2%)			
Epidermis, hyperplasia			1 (2%)	

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis		4 (8%)		2 (4%)
Femur, osteopetrosis	1 (2%)			1 (2%)
Skeletal muscle	(1)	(4)	(0)	(1)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	5 (10%)	8 (16%)	11 (22%)	5 (10%)
Hemorrhage	2 (4%)	3 (6%)	4 (8%)	1 (2%)
Necrosis	1 (2%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)	2 (4%)		3 (6%)
Infiltration cellular, histiocyte	24 (48%)	23 (46%)	27 (54%)	25 (50%)
Inflammation, chronic	5 (10%)	3 (6%)	7 (14%)	6 (12%)
Alveolar epithelium, hyperplasia	7 (14%)	14 (28%)	14 (28%)	10 (20%)
Nose	(50)	(50)	(50)	(50)
Foreign body	14 (28%)	9 (18%)	14 (28%)	18 (36%)
Inflammation, chronic	18 (36%)	9 (18%)	16 (32%)	21 (42%)
Nasolacrimal duct, cyst	1 (2%)	1 (2%)		
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	2 (4%)	3 (6%)	3 (6%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)	1 (2%)		3 (6%)
Zymbal's gland	(1)	(1)	(1)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	1 (2%)			1 (2%)
Infarct	1 (2%)	2 (4%)		2 (4%)
Nephropathy	47 (94%)	47 (94%)	43 (86%)	45 (90%)
Pelvis, inflammation				1 (2%)
Renal tubule, pigmentation			1 (2%)	
Transitional epithelium, hyperplasia	1 (2%)			2 (4%)
Urinary bladder	(50)	(50)	(50)	(49)
Hemorrhage				1 (2%)
Inflammation				1 (2%)

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

Table B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	14	9	13
Natural deaths	4	5	4	2
Survivors				
Died last week of study			1	
Terminal sacrifice	34	31	36	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(47)	(48)	(49)
Leiomyosarcoma				1 (2%)
Intestine large, colon	(50)	(49)	(50)	(50)
Adenoma			2 (4%)	5 (10%)
Adenoma, multiple				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Adenoma			1 (2%)	2 (4%)
Intestine small, duodenum	(49)	(48)	(49)	(49)
Intestine small, ileum	(47)	(47)	(48)	(48)
Intestine small, jejunum	(47)	(47)	(48)	(48)
Leiomyosarcoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma				3 (6%)
Leiomyosarcoma, metastatic, uncertain primary site				1 (2%)
Mesentery	(9)	(9)	(9)	(6)
Leiomyosarcoma				1 (17%)
Oral mucosa	(2)	(0)	(0)	(1)
Carcinoma, metastatic, thyroid gland	1 (50%)			
Squamous cell carcinoma				1 (100%)
Pharyngeal, squamous cell papilloma	1 (50%)			
Pancreas	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Leiomyosarcoma				1 (2%)
Tongue	(1)	(0)	(1)	(0)
Carcinoma, metastatic, thyroid gland	1 (100%)			
Squamous cell papilloma			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	1 (2%)			

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Adrenal medulla	(49)	(47)	(50)	(50)
Pheochromocytoma benign	2 (4%)	1 (2%)	4 (8%)	4 (8%)
Pheochromocytoma complex				1 (2%)
Pheochromocytoma malignant	2 (4%)	1 (2%)		
Bilateral, pheochromocytoma benign				1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Carcinoma				1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, adenoma	28 (56%)	33 (66%)	30 (60%)	26 (52%)
Pars distalis, carcinoma			1 (2%)	
Thyroid gland	(49)	(50)	(50)	(50)
Bilateral, C-cell, adenoma		1 (2%)	2 (4%)	1 (2%)
C-cell, adenoma	6 (12%)	6 (12%)	8 (16%)	7 (14%)
C-cell, carcinoma	1 (2%)			
Follicular cell, adenoma		1 (2%)	2 (4%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(47)
Adenoma	16 (32%)	10 (20%)	8 (16%)	9 (19%)
Carcinoma		2 (4%)		1 (2%)
Bilateral, adenoma	1 (2%)	1 (2%)		3 (6%)
Ovary	(50)	(50)	(50)	(50)
Bilateral, carcinoma, metastatic, thyroid gland	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Adenocarcinoma		1 (2%)		
Adenoma		1 (2%)		
Polyp stromal	9 (18%)	6 (12%)	13 (26%)	11 (22%)
Vagina	(0)	(1)	(0)	(1)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(15)	(20)	(11)	(16)
Deep cervical, carcinoma, metastatic, thyroid gland	1 (7%)			
Mediastinal, carcinoma, metastatic, thyroid gland	1 (7%)			
Lymph node, mandibular	(0)	(2)	(1)	(1)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Thymus	(50)	(50)	(50)	(50)
Thymoma benign				1 (2%)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)	1 (2%)		3 (6%)
Fibroadenoma	21 (42%)	19 (38%)	4 (8%)	8 (16%)
Fibroadenoma, multiple	22 (44%)	24 (48%)	43 (86%)	38 (76%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Keratoacanthoma		1 (2%)	1 (2%)	
Trichoepithelioma		1 (2%)		
Pinna, neural crest tumor			1 (2%)	
Sebaceous gland, carcinoma				1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Subcutaneous tissue, fibroma, multiple			1 (2%)	
Subcutaneous tissue, fibrosarcoma				2 (4%)
Subcutaneous tissue, schwannoma NOS		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma		1 (2%)		
Mandible, squamous cell carcinoma, metastatic, oral mucosa				1 (2%)
Vertebra, osteosarcoma	1 (2%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland			1 (2%)	
Granular cell tumor benign			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		2 (4%)	2 (4%)
Alveolar/bronchiolar carcinoma		1 (2%)		
Carcinoma, metastatic, mammary gland				1 (2%)
Carcinoma, metastatic, skin				1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Osteosarcoma, metastatic, bone		1 (2%)		
Squamous cell carcinoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal’s gland	(0)	(2)	(0)	(0)
Adenoma		1 (50%)		
Carcinoma		1 (50%)		

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Lipoma		1 (2%)		
Renal tubule, adenoma	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)			
Leukemia mononuclear	15 (30%)	12 (24%)	12 (24%)	12 (24%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	49	49	50
Total primary neoplasms	135	131	139	157
Total animals with benign neoplasms	49	48	48	47
Total benign neoplasms	113	110	125	130
Total animals with malignant neoplasms	18	18	13	23
Total malignant neoplasms	22	20	13	27
Total animals with metastatic neoplasms	1	1	1	4
Total metastatic neoplasms	8	1	1	4
Total animals with malignant neoplasms of uncertain primary site				1
Total animals with uncertain neoplasms-benign or malignant		1	1	
Total uncertain neoplasms		1	1	

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

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	2/49 (4%)	1/47 (2%)	4/50 (8%)	5/50 (10%)
Adjusted rate[b]	4.6%	2.5%	8.8%	11.0%
Terminal rate[c]	2/34 (6%)	0/29 (0%)	3/37 (8%)	3/35 (9%)
First incidence (days)	729 (T)	699	709	692
Poly-3 test[d]	P=0.097	P=0.520N	P=0.362	P=0.236
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	4/49 (8%)	2/47 (4%)	4/50 (8%)	6/50 (12%)
Adjusted rate	9.3%	4.9%	8.8%	13.2%
Terminal rate	4/34 (12%)	0/29 (0%)	3/37 (8%)	4/35 (11%)
First incidence (days)	729 (T)	610	709	692
Poly-3 test	P=0.221	P=0.359N	P=0.614N	P=0.401
Clitoral Gland: Adenoma				
Overall rate	17/50 (34%)	11/49 (22%)	8/50 (16%)	12/47 (26%)
Adjusted rate	38.4%	25.8%	17.5%	27.9%
Terminal rate	15/34 (44%)	8/30 (27%)	7/37 (19%)	9/33 (27%)
First incidence (days)	505	618	668	618
Poly-3 test	P=0.189N	P=0.150N	P=0.022N	P=0.205N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	17/50 (34%)	13/49 (27%)	8/50 (16%)	13/47 (28%)
Adjusted rate	38.4%	30.4%	17.5%	30.0%
Terminal rate	15/34 (44%)	9/30 (30%)	7/37 (19%)	9/33 (27%)
First incidence (days)	505	618	668	618
Poly-3 test	P=0.217N	P=0.286N	P=0.022N	P=0.273N
Large Intestine (Colon or Rectum): Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	7/50 (14%)
Adjusted rate	0.0%	0.0%	6.6%	15.5%
Terminal rate	0/34 (0%)	0/31 (0%)	3/37 (8%)	6/35 (17%)
First incidence (days)	—[e]	—	729 (T)	692
Poly-3 test	P<0.001	—[f]	P=0.127	P=0.009
Liver: Hepatocellular Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	0.0%	6.6%
Terminal rate	0/34 (0%)	0/31 (0%)	0/37 (0%)	3/35 (9%)
First incidence (days)	—	—	—	729 (T)
Poly-3 test	P=0.012	—	—	P=0.125
Mammary Gland: Fibroadenoma				
Overall rate	43/50 (86%)	43/50 (86%)	47/50 (94%)	46/50 (92%)
Adjusted rate	92.0%	90.0%	96.6%	96.9%
Terminal rate	32/34 (94%)	27/31 (87%)	36/37 (97%)	35/35 (100%)
First incidence (days)	505	525	478	618
Poly-3 test	P=0.107	P=0.504N	P=0.274	P=0.248
Mammary Gland: Carcinoma				
Overall rate	1/50 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	2.3%	2.3%	0.0%	6.5%
Terminal rate	1/34 (3%)	1/31 (3%)	0/37 (0%)	1/35 (3%)
First incidence (days)	729 (T)	729 (T)	—	381
Poly-3 test	P=0.171	P=0.758	P=0.491N	P=0.329

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	43/50 (86%)	43/50 (86%)	47/50 (94%)	47/50 (94%)
Adjusted rate	92.0%	90.0%	96.6%	97.2%
Terminal rate	32/34 (94%)	27/31 (87%)	36/37 (97%)	35/35 (100%)
First incidence (days)	505	525	478	381
Poly-3 test	P=0.090	P=0.504N	P=0.274	P=0.217
Pancreatic Islets: Adenoma				
Overall rate	3/49 (6%)	1/50 (2%)	1/50 (2%)	2/50 (4%)
Adjusted rate	7.0%	2.3%	2.2%	4.4%
Terminal rate	3/34 (9%)	1/31 (3%)	0/37 (0%)	2/35 (6%)
First incidence (days)	729 (T)	729 (T)	668	729 (T)
Poly-3 test	P=0.454N	P=0.304N	P=0.283N	P=0.476N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	3/49 (6%)	1/50 (2%)	1/50 (2%)	3/50 (6%)
Adjusted rate	7.0%	2.3%	2.2%	6.6%
Terminal rate	3/34 (9%)	1/31 (3%)	0/37 (0%)	3/35 (9%)
First incidence (days)	729 (T)	729 (T)	668	729 (T)
Poly-3 test	P=0.524	P=0.304N	P=0.283N	P=0.638N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	28/50 (56%)	33/50 (66%)	30/50 (60%)	26/50 (52%)
Adjusted rate	60.0%	70.5%	64.3%	55.3%
Terminal rate	19/34 (56%)	22/31 (71%)	23/37 (62%)	18/35 (51%)
First incidence (days)	468	493	631	604
Poly-3 test	P=0.226N	P=0.191	P=0.414	P=0.400N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	28/50 (56%)	33/50 (66%)	31/50 (62%)	26/50 (52%)
Adjusted rate	60.0%	70.5%	66.4%	55.3%
Terminal rate	19/34 (56%)	22/31 (71%)	24/37 (65%)	18/35 (51%)
First incidence (days)	468	493	631	604
Poly-3 test	P=0.233N	P=0.191	P=0.331	P=0.400N
Skin: Keratoacanthoma, Trichoepithelioma, or Basal Cell Adenoma				
Overall rate	0/50 (0%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	0.0%	6.9%	2.2%	0.0%
Terminal rate	0/34 (0%)	1/31 (3%)	1/37 (3%)	0/35 (0%)
First incidence (days)	—	626	729 (T)	—
Poly-3 test	P=0.350N	P=0.118	P=0.509	—
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	4/50 (8%)
Adjusted rate	2.3%	2.3%	4.4%	8.8%
Terminal rate	1/34 (3%)	1/31 (3%)	2/37 (5%)	3/35 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	682
Poly-3 test	P=0.080	P=0.758	P=0.515	P=0.191
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	1/50 (2%)	2/50 (4%)	6/50 (12%)
Adjusted rate	2.3%	2.3%	4.4%	13.1%
Terminal rate	1/34 (3%)	1/31 (3%)	2/37 (5%)	3/35 (9%)
First incidence (days)	729 (T)	729 (T)	729 (T)	618
Poly-3 test	P=0.012	P=0.758	P=0.515	P=0.064

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/49 (12%)	7/50 (14%)	10/50 (20%)	8/50 (16%)
Adjusted rate	13.9%	16.2%	21.8%	17.6%
Terminal rate	4/34 (12%)	5/31 (16%)	8/37 (22%)	5/35 (14%)
First incidence (days)	637	674	631	668
Poly-3 test	P=0.363	P=0.502	P=0.246	P=0.429
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	7/49 (14%)	7/50 (14%)	10/50 (20%)	8/50 (16%)
Adjusted rate	16.2%	16.2%	21.8%	17.6%
Terminal rate	5/34 (15%)	5/31 (16%)	8/37 (22%)	5/35 (14%)
First incidence (days)	637	674	631	668
Poly-3 test	P=0.455	P=0.613N	P=0.347	P=0.546
Uterus: Stromal Polyp				
Overall rate	9/50 (18%)	6/50 (12%)	13/50 (26%)	11/50 (22%)
Adjusted rate	20.5%	13.6%	28.0%	24.2%
Terminal rate	8/34 (24%)	3/31 (10%)	11/37 (30%)	9/35 (26%)
First incidence (days)	634	395	478	636
Poly-3 test	P=0.237	P=0.277N	P=0.282	P=0.438
All Organs: Mononuclear Leukemia				
Overall rate	15/50 (30%)	12/50 (24%)	12/50 (24%)	12/50 (24%)
Adjusted rate	33.4%	26.5%	25.5%	26.0%
Terminal rate	8/34 (24%)	5/31 (16%)	4/37 (11%)	8/35 (23%)
First incidence (days)	612	395	627	618
Poly-3 test	P=0.287N	P=0.315N	P=0.274N	P=0.294N
All Organs: Benign Tumors				
Overall rate	49/50 (98%)	48/50 (96%)	48/50 (96%)	47/50 (94%)
Adjusted rate	99.6%	97.3%	98.7%	98.1%
Terminal rate	34/34 (100%)	30/31 (97%)	37/37 (100%)	35/35 (100%)
First incidence (days)	468	395	478	604
Poly-3 test	P=0.500N	P=0.442N	P=0.791N	P=0.619N
All Organs: Malignant Tumors				
Overall rate	18/50 (36%)	18/50 (36%)	13/50 (26%)	23/50 (46%)
Adjusted rate	40.0%	39.5%	27.6%	46.6%
Terminal rate	11/34 (32%)	9/31 (29%)	5/37 (14%)	12/35 (34%)
First incidence (days)	612	395	627	258
Poly-3 test	P=0.285	P=0.565N	P=0.147N	P=0.332

Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
All Organs: Benign or Malignant Tumors				
Overall rate	49/50 (98%)	49/50 (98%)	49/50 (98%)	50/50 (100%)
Adjusted rate	99.6%	99.3%	100.0%	100.0%
Terminal rate	34/34 (100%)	31/31 (100%)	37/37 (100%)	35/35 (100%)
First incidence (days)	468	395	478	258
Poly-3 test	P=0.781	P=0.986N	P=1.000	P=1.000

(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, liver, 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 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 B3a
Historical Incidence of Adenoma of the Large Intestine in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	0/50
Dibromoacetic acid	0/50
Dibromoacetonitrile	0/50
Sodium chlorate	0/50
Sodium dichromate dihydrate (VI)	0/50
Overall Historical Incidence: Drinking Water Studies	
Total (%)	0/250 (0.0%)
Overall Historical Incidence: All Routes	
Total (%)	2/1,100 (0.2%)
Mean ± standard deviation	0.2% ± 0.6%
Range	0%-2%

[a] Data as of October 4, 2007

Table B3b
Historical Incidence of Fibroadenoma of the Mammary Gland in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	43/50
Dibromoacetic acid	32/50
Dibromoacetonitrile	31/50
Sodium chlorate	33/50
Sodium dichromate dihydrate (VI)	37/50
Overall Historical Incidence: Drinking Water Studies	
Total (%)	176/250 (70.4%)
Mean ± standard deviation	70.4% ± 9.8%
Range	62%-86%
Overall Historical Incidence: All Routes	
Total (%)	574/1,100 (52.2%)
Mean ± standard deviation	52.2% ± 14.7%
Range	24%-86%

[a] Data as of October 4, 2007

Table B3c
Historical Incidence of Hepatocellular Adenoma of the Liver in Untreated Female F344/N Rats[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	0/50
Dibromoacetic acid	2/50
Dibromoacetonitrile	0/50
Sodium chlorate	0/50
Sodium dichromate dihydrate (VI)	1/50
Overall Historical Incidence: Drinking Water Studies	
Total (%)	3/250 (1.2%)
Mean ± standard deviation	1.2% ± 1.8%
Range	0%-4%
Overall Historical Incidence: All Routes	
Total (%)	14/1,099 (1.3%)
Mean ± standard deviation	1.3% ± 2.8%
Range	0%-12%

[a] Data as of October 4, 2007

Table B3d
Historical Incidence of Subcutaneous Skin Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls	
	Fibroma	Fibrosarcoma
Historical Incidence: Drinking Water Studies		
Bromochloroacetic acid	1/50	0/50
Dibromoacetic acid	1/50	0/50
Dibromoacetonitrile	0/50	1/50
Sodium chlorate	4/50	0/50
Sodium dichromate dihydrate (VI)	1/50	0/50
Overall Historical Incidence: Drinking Water Studies		
Total (%)	7/250 (2.8%)	1/250 (0.4%)
Mean ± standard deviation	2.8% ± 3.0%	0.4% ± 0.9%
Range	0%-8%	0%-2%
Overall Historical Incidence: All Routes		
Total (%)	19/1,100 (1.7%)	5/1,100 (0.5%)
Mean ± standard deviation	1.7% ± 2.1%	0.5% ± 0.9%
Range	0%-8%	0%-2%

[a] Data as of October 4, 2007

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	14	9	13
Natural deaths	4	5	4	2
Survivors				
Died last week of study			1	
Terminal sacrifice	34	31	36	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(47)	(48)	(49)
Intestine large, colon	(50)	(49)	(50)	(50)
Hyperplasia				1 (2%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Hyperplasia			1 (2%)	
Inflammation				1 (2%)
Intestine small, duodenum	(49)	(48)	(49)	(49)
Ectopic tissue		1 (2%)		
Intestine small, ileum	(47)	(47)	(48)	(48)
Intestine small, jejunum	(47)	(47)	(48)	(48)
Liver	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)	2 (4%)	
Basophilic focus	39 (78%)	37 (74%)	37 (74%)	31 (62%)
Clear cell focus	14 (28%)	11 (22%)	16 (32%)	17 (34%)
Degeneration, cystic	1 (2%)			
Eosinophilic focus	1 (2%)	6 (12%)	9 (18%)	15 (30%)
Hemorrhage	1 (2%)			
Hepatodiaphragmatic nodule	11 (22%)	10 (20%)	5 (10%)	8 (16%)
Inflammation, chronic	14 (28%)	17 (34%)	19 (38%)	10 (20%)
Mixed cell focus	1 (2%)	4 (8%)	6 (12%)	10 (20%)
Necrosis, focal			1 (2%)	2 (4%)
Bile duct, hyperplasia	19 (38%)	19 (38%)	21 (42%)	16 (32%)
Hepatocyte, vacuolization cytoplasmic	6 (12%)	4 (8%)	3 (6%)	3 (6%)
Mesentery	(9)	(9)	(9)	(6)
Accessory spleen	1 (11%)		1 (11%)	
Hemorrhage				1 (17%)
Fat, necrosis	7 (78%)	9 (100%)	8 (89%)	4 (67%)
Oral mucosa	(2)	(0)	(0)	(1)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	21 (42%)	20 (40%)	22 (44%)	22 (44%)
Cyst	1 (2%)			
Acinus, hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema		2 (4%)	2 (4%)	1 (2%)
Erosion			1 (2%)	
Perforation				1 (2%)
Ulcer	1 (2%)	2 (4%)		1 (2%)
Epithelium, hyperplasia	6 (12%)	2 (4%)	3 (6%)	6 (12%)

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

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Edema		1 (2%)	3 (6%)	2 (4%)
Erosion	2 (4%)		5 (10%)	2 (4%)
Ulcer		2 (4%)		1 (2%)
Tongue	(1)	(0)	(1)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	44 (88%)	43 (86%)	48 (96%)	44 (88%)
Thrombosis		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	17 (34%)	11 (22%)	12 (24%)	13 (26%)
Hyperplasia, focal	17 (34%)	17 (34%)	18 (36%)	25 (50%)
Hypertrophy, focal	5 (10%)	2 (4%)	3 (6%)	6 (12%)
Adrenal medulla	(49)	(47)	(50)	(50)
Hemorrhage				1 (2%)
Hyperplasia	5 (10%)	6 (13%)	4 (8%)	7 (14%)
Necrosis				1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	
Pituitary gland	(50)	(50)	(50)	(50)
Pars distalis, angiectasis	32 (64%)	32 (64%)	33 (66%)	30 (60%)
Pars distalis, cyst	28 (56%)	27 (54%)	31 (62%)	29 (58%)
Pars distalis, hyperplasia				1 (2%)
Pars distalis, hyperplasia, focal	14 (28%)	11 (22%)	6 (12%)	17 (34%)
Pars intermedia, cyst			1 (2%)	
Thyroid gland	(49)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)	1 (2%)	2 (4%)	1 (2%)
C-cell, hyperplasia	25 (51%)	28 (56%)	23 (46%)	23 (46%)
Follicle, cyst	5 (10%)	3 (6%)	2 (4%)	4 (8%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(49)	(50)	(47)
Cyst	4 (8%)	6 (12%)	7 (14%)	7 (15%)
Hyperplasia	1 (2%)			1 (2%)
Inflammation, chronic	2 (4%)	2 (4%)	3 (6%)	2 (4%)
Inflammation, chronic active				1 (2%)
Ovary	(50)	(50)	(50)	(50)
Cyst	7 (14%)	10 (20%)	6 (12%)	11 (22%)

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Genital System (continued)				
Uterus	(50)	(50)	(50)	(50)
Cyst			1 (2%)	1 (2%)
Hemorrhage		1 (2%)		1 (2%)
Hydrometra	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Inflammation, chronic		1 (2%)		
Cervix, cyst			1 (2%)	1 (2%)
Cervix, hypertrophy				1 (2%)
Endometrium, cyst				1 (2%)
Endometrium, hyperplasia	1 (2%)			
Vagina	(0)	(1)	(0)	(1)
Cyst		1 (100%)		
Inflammation, acute				1 (100%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	7 (14%)	5 (10%)	6 (12%)
Myelofibrosis	1 (2%)			
Lymph node	(15)	(20)	(11)	(16)
Deep cervical, hyperplasia, lymphoid	1 (7%)			
Mediastinal, hemorrhage	2 (13%)	1 (5%)		2 (13%)
Mediastinal, hyperplasia, lymphoid	6 (40%)	14 (70%)	8 (73%)	11 (69%)
Pancreatic, hyperplasia, lymphoid	1 (7%)		1 (9%)	1 (6%)
Lymph node, mandibular	(0)	(2)	(1)	(1)
Hyperplasia, lymphoid				1 (100%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Edema				1 (2%)
Hyperplasia, lymphoid	2 (4%)	3 (6%)	3 (6%)	8 (16%)
Spleen	(50)	(50)	(50)	(50)
Fibrosis			1 (2%)	2 (4%)
Hematopoietic cell proliferation	3 (6%)	11 (22%)	10 (20%)	8 (16%)
Necrosis	1 (2%)			
Pigmentation	3 (6%)	6 (12%)	4 (8%)	2 (4%)
Thymus	(50)	(50)	(50)	(50)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	47 (94%)	47 (94%)	46 (92%)	45 (90%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Edema		1 (2%)		
Hyperkeratosis	1 (2%)			
Inflammation, chronic	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis		1 (2%)		
Femur, osteopetrosis	1 (2%)	1 (2%)		3 (6%)

Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	10 (20%)	15 (30%)	9 (18%)	10 (20%)
Hemorrhage			3 (6%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion		2 (4%)		
Hemorrhage	1 (2%)			2 (4%)
Infiltration cellular, histiocyte	36 (72%)	44 (88%)	44 (88%)	45 (90%)
Inflammation, chronic	8 (16%)	10 (20%)	6 (12%)	7 (14%)
Metaplasia, osseous			1 (2%)	
Alveolar epithelium, hyperplasia	5 (10%)	7 (14%)	8 (16%)	18 (36%)
Pleura, fibrosis	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	1 (2%)	3 (6%)	2 (4%)
Inflammation, chronic	3 (6%)	1 (2%)	3 (6%)	6 (12%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Harderian gland	(50)	(50)	(50)	(50)
Inflammation, chronic	3 (6%)	5 (10%)	4 (8%)	
Zymbal's gland	(0)	(2)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Infarct		2 (4%)	2 (4%)	7 (14%)
Nephropathy	36 (72%)	42 (84%)	43 (86%)	40 (80%)
Cortex, inflammation, chronic				1 (2%)
Transitional epithelium, hyperplasia, diffuse		3 (6%)	2 (4%)	6 (12%)
Transitional epithelium, inflammation, chronic active		3 (6%)	3 (6%)	6 (12%)
Urinary bladder	(50)	(50)	(50)	(50)

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

Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	5	9	15
Natural deaths	6	10	11	14
Survivors				
Terminal sacrifice	38	35	30	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Intestine large, cecum	(49)	(49)	(49)	(49)
Carcinoma	1 (2%)	1 (2%)		2 (4%)
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(48)	(47)	(48)	(47)
Adenoma	3 (6%)			1 (2%)
Carcinoma	1 (2%)	2 (4%)		
Intestine small, jejunum	(47)	(47)	(46)	(43)
Carcinoma	1 (2%)		1 (2%)	
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Hemangiosarcoma, multiple			2 (4%)	
Hepatoblastoma	4 (8%)	9 (18%)	16 (32%)	20 (40%)
Hepatoblastoma, multiple		2 (4%)	12 (24%)	14 (28%)
Hepatocellular adenoma	14 (28%)	13 (26%)	15 (30%)	12 (24%)
Hepatocellular adenoma, multiple	13 (26%)	27 (54%)	25 (50%)	19 (38%)
Hepatocellular carcinoma	10 (20%)	16 (32%)	16 (32%)	13 (26%)
Hepatocellular carcinoma, multiple	9 (18%)	9 (18%)	20 (40%)	32 (64%)
Mast cell tumor malignant		1 (2%)		
Mesentery	(15)	(12)	(8)	(8)
Hemangiosarcoma		1 (8%)		
Hepatoblastoma, metastatic, liver		1 (8%)	1 (13%)	3 (38%)
Hepatocellular carcinoma, metastatic, liver				1 (13%)
Pancreas	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(49)	(50)
Hemangioma		1 (2%)		
Squamous cell carcinoma	1 (2%)	1 (2%)	1 (2%)	
Squamous cell papilloma	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Squamous cell papilloma, multiple		1 (2%)		
Stomach, glandular	(50)	(50)	(49)	(50)
Squamous cell carcinoma, metastatic, stomach, forestomach			1 (2%)	
Tooth	(2)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(2)	(2)	(1)	(4)
Heart	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Capsule, adenoma	7 (14%)		5 (10%)	4 (8%)
Capsule, adenoma, multiple	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(47)
Islets, pancreatic	(50)	(50)	(48)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Parathyroid gland	(32)	(40)	(37)	(38)
Pituitary gland	(50)	(50)	(48)	(49)
Pars distalis, adenoma	1 (2%)	1 (2%)		
Pars intermedia, adenoma	1 (2%)			
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, carcinoma				1 (2%)
Follicular cell, adenoma	2 (4%)		3 (6%)	1 (2%)
Follicular cell, adenoma, multiple	1 (2%)		1 (2%)	
Follicular cell, carcinoma				1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (100%)
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(48)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Hepatoblastoma, metastatic, liver				1 (2%)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)			
Hematopoietic System				
Bone marrow	(50)	(48)	(47)	(48)
Hemangiosarcoma	1 (2%)			1 (2%)
Mast cell tumor malignant		1 (2%)		
Lymph node	(5)	(6)	(5)	(3)
Lymph node, mandibular	(50)	(45)	(41)	(45)
Lymph node, mesenteric	(45)	(45)	(44)	(45)
Carcinoma, metastatic, intestine large, cecum	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Hemangioma	1 (2%)			
Hemangiosarcoma	1 (2%)		3 (6%)	1 (2%)
Mast cell tumor malignant		1 (2%)		
Thymus	(46)	(40)	(36)	(40)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Mast cell tumor benign			1 (2%)	
Mast cell tumor malignant		1 (2%)		
Squamous cell papilloma	1 (2%)		1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)			
Subcutaneous tissue, hemangiosarcoma			1 (2%)	

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(2)	(3)	(2)	(6)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (50%)			1 (17%)
Granular cell tumor benign			1 (50%)	1 (17%)
Hemangiosarcoma				1 (17%)
Hepatoblastoma, metastatic, liver			1 (50%)	
Hepatocellular carcinoma, metastatic, liver				1 (17%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(1)	(2)	(2)	(5)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	5 (10%)	6 (12%)	3 (6%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	9 (18%)	10 (20%)	4 (8%)	6 (12%)
Alveolar/bronchiolar carcinoma, multiple	3 (6%)	1 (2%)		5 (10%)
Hemangiosarcoma			1 (2%)	
Hepatoblastoma, metastatic, liver		1 (2%)	7 (14%)	8 (16%)
Hepatocellular carcinoma, metastatic, liver	4 (8%)	4 (8%)	10 (20%)	11 (22%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(49)	(50)
Adenoma	5 (10%)	9 (18%)	9 (18%)	7 (14%)
Adenoma, multiple				1 (2%)
Carcinoma		1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Mast cell tumor malignant		1 (2%)		
Renal tubule, adenoma	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Renal tubule, carcinoma			1 (2%)	1 (2%)
Urethra	(1)	(0)	(0)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Hemangiosarcoma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	2 (4%)	
Lymphoma malignant	2 (4%)	4 (8%)	1 (2%)	2 (4%)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	48	49	50
Total primary neoplasms	109	126	158	158
Total animals with benign neoplasms	41	44	42	38
Total benign neoplasms	64	62	71	55
Total animals with malignant neoplasms	36	40	46	49
Total malignant neoplasms	45	64	87	103
Total animals with metastatic neoplasms	6	6	19	23
Total metastatic neoplasms	6	7	20	27

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

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Adrenal Cortex: Adenoma				
Overall rate[a]	9/50 (18%)	0/50 (0%)	5/50 (10%)	4/50 (8%)
Adjusted rate[b]	19.8%	0.0%	11.5%	9.5%
Terminal rate[c]	7/38 (18%)	0/35 (0%)	4/30 (13%)	3/21 (14%)
First incidence (days)	617	—[e]	645	616
Poly-3 test[d]	P=0.247N	P=0.002N	P=0.217N	P=0.146N
Harderian Gland: Adenoma				
Overall rate	5/50 (10%)	9/50 (18%)	9/50 (18%)	8/50 (16%)
Adjusted rate	11.1%	20.0%	20.7%	18.5%
Terminal rate	5/38 (13%)	8/35 (23%)	7/30 (23%)	3/21 (14%)
First incidence (days)	729 (T)	610	674	576
Poly-3 test	P=0.272	P=0.194	P=0.174	P=0.251
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	10/50 (20%)	10/50 (20%)	8/50 (16%)
Adjusted rate	11.1%	22.1%	22.7%	18.5%
Terminal rate	5/38 (13%)	8/35 (23%)	7/30 (23%)	3/21 (14%)
First incidence (days)	729 (T)	610	607	576
Poly-3 test	P=0.295	P=0.132	P=0.118	P=0.251
Small Intestine (Duodenum): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate	6.7%	0.0%	0.0%	2.4%
Terminal rate	3/38 (8%)	0/35 (0%)	0/30 (0%)	1/21 (5%)
First incidence (days)	729 (T)	—	—	729 (T)
Poly-3 test	P=0.247N	P=0.119N	P=0.126N	P=0.332N
Small Intestine (Duodenum or Jejunum): Adenoma or Carcinoma				
Overall rate	5/50 (10%)	2/50 (4%)	1/50 (2%)	1/50 (2%)
Adjusted rate	11.0%	4.5%	2.3%	2.4%
Terminal rate	4/38 (11%)	2/35 (6%)	1/30 (3%)	1/21 (5%)
First incidence (days)	555	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.066N	P=0.223N	P=0.113N	P=0.122N
Kidney (Renal Tubule): Adenoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)[f]	3/50 (6%)[f]
Adjusted rate	8.9%	4.5%	4.6%	7.2%
Terminal rate	4/38 (11%)	2/35 (6%)	2/30 (7%)	3/21 (14%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.508N	P=0.339N	P=0.355N	P=0.541N
Liver: Hemangiosarcoma				
Overall rate	2/50 (4%)	1/50 (2%)	5/50 (10%)	3/50 (6%)
Adjusted rate	4.4%	2.2%	11.2%	7.2%
Terminal rate	1/38 (3%)	0/35 (0%)	1/30 (3%)	1/21 (5%)
First incidence (days)	630	703	590	701
Poly-3 test	P=0.240	P=0.503N	P=0.210	P=0.465
Liver: Hepatocellular Adenoma				
Overall rate	27/50 (54%)	40/50 (80%)	40/50 (80%)	31/50 (62%)
Adjusted rate	58.7%	83.6%	83.7%	67.4%
Terminal rate	23/38 (61%)	30/35 (86%)	25/30 (83%)	17/21 (81%)
First incidence (days)	617	555	462	397
Poly-3 test	P=0.402	P=0.005	P=0.005	P=0.252

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Liver: Hepatocellular Carcinoma				
Overall rate	19/50 (38%)	25/50 (50%)	36/50 (72%)	45/50 (90%)
Adjusted rate	39.6%	52.5%	76.9%	92.7%
Terminal rate	12/38 (32%)	18/35 (51%)	22/30 (73%)	20/21 (95%)
First incidence (days)	328	486	469	397
Poly-3 test	P<0.001	P=0.143	P<0.001	P<0.001
Liver: Hepatocellular Adenoma or Hepatocellular Carcinoma				
Overall rate	34/50 (68%)	44/50 (88%)	49/50 (98%)	49/50 (98%)
Adjusted rate	70.6%	89.7%	99.9%	98.6%
Terminal rate	26/38 (68%)	32/35 (91%)	30/30 (100%)	21/21 (100%)
First incidence (days)	328	486	462	397
Poly-3 test	P<0.001	P=0.013	P<0.001	P<0.001
Liver: Hepatoblastoma				
Overall rate	4/50 (8%)	11/50 (22%)	28/50 (56%)	34/50 (68%)
Adjusted rate	8.8%	23.8%	61.3%	73.7%
Terminal rate	3/38 (8%)	7/35 (20%)	17/30 (57%)	17/21 (81%)
First incidence (days)	621	609	602	505
Poly-3 test	P<0.001	P=0.047	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	21/50 (42%)	32/50 (64%)	43/50 (86%)	49/50 (98%)
Adjusted rate	43.8%	66.3%	90.7%	98.0%
Terminal rate	14/38 (37%)	23/35 (66%)	27/30 (90%)	20/21 (95%)
First incidence (days)	328	486	469	397
Poly-3 test	P<0.001	P=0.019	P<0.001	P<0.001
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	35/50 (70%)	45/50 (90%)	49/50 (98%)	50/50 (100%)
Adjusted rate	72.7%	91.0%	99.9%	100.0%
Terminal rate	27/38 (71%)	32/35 (91%)	30/30 (100%)	21/21 (100%)
First incidence (days)	328	486	462	397
Poly-3 test	P<0.001	P=0.014	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	4/50 (8%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	8.8%	11.2%	13.6%	7.1%
Terminal rate	3/38 (8%)	4/35 (11%)	2/30 (7%)	1/21 (5%)
First incidence (days)	621	703	602	633
Poly-3 test	P=0.456N	P=0.493	P=0.354	P=0.540N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	12/50 (24%)	11/50 (22%)	4/50 (8%)	11/50 (22%)
Adjusted rate	25.9%	24.3%	9.3%	25.8%
Terminal rate	8/38 (21%)	8/35 (23%)	4/30 (13%)	5/21 (24%)
First incidence (days)	526	640	729 (T)	576
Poly-3 test	P=0.446N	P=0.527N	P=0.036N	P=0.590N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	15/50 (30%)	12/50 (24%)	9/50 (18%)	14/50 (28%)
Adjusted rate	32.1%	26.5%	20.4%	32.5%
Terminal rate	10/38 (26%)	9/35 (26%)	5/30 (17%)	6/21 (29%)
First incidence (days)	526	640	602	576
Poly-3 test	P=0.526	P=0.360N	P=0.150N	P=0.573

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Spleen: Hemangiosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.2%	0.0%	6.9%	2.4%
Terminal rate	1/38 (3%)	0/35 (0%)	2/30 (7%)	0/21 (0%)
First incidence (days)	729 (T)	—	607	570
Poly-3 test	P=0.437	P=0.501N	P=0.295	P=0.747
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	2/50 (4%)
Adjusted rate	4.5%	6.7%	2.3%	4.7%
Terminal rate	2/38 (5%)	2/35 (6%)	0/30 (0%)	1/21 (5%)
First incidence (days)	729 (T)	640	692	576
Poly-3 test	P=0.524N	P=0.501	P=0.512N	P=0.672
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	2/50 (4%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	4.5%	8.9%	4.6%	4.7%
Terminal rate	2/38 (5%)	3/35 (9%)	0/30 (0%)	1/21 (5%)
First incidence (days)	729 (T)	640	607	576
Poly-3 test	P=0.487N	P=0.338	P=0.685	P=0.672
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rate	6.7%	0.0%	9.3%	2.4%
Terminal rate	3/38 (8%)	0/35 (0%)	4/30 (13%)	1/21 (5%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P=0.438N	P=0.119N	P=0.479	P=0.332N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	3/50 (6%)	0/50 (0%)	4/50 (8%)	2/50 (4%)
Adjusted rate	6.7%	0.0%	9.3%	4.8%
Terminal rate	3/38 (8%)	0/35 (0%)	4/30 (13%)	1/21 (5%)
First incidence (days)	729 (T)	—	729 (T)	712
Poly-3 test	P=0.529	P=0.119N	P=0.479	P=0.533N
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	9/50 (18%)	5/50 (10%)
Adjusted rate	6.6%	2.2%	20.0%	11.7%
Terminal rate	2/38 (5%)	0/35 (0%)	4/30 (13%)	1/21 (5%)
First incidence (days)	630	703	590	570
Poly-3 test	P=0.108	P=0.309N	P=0.057	P=0.325
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	9/50 (18%)	5/50 (10%)
Adjusted rate	8.8%	4.5%	20.0%	11.7%
Terminal rate	2/38 (5%)	1/35 (3%)	4/30 (13%)	1/21 (5%)
First incidence (days)	630	703	590	570
Poly-3 test	P=0.217	P=0.344N	P=0.111	P=0.461
All Organs: Malignant Lymphoma				
Overall rate	2/50 (4%)	4/50 (8%)	1/50 (2%)	2/50 (4%)
Adjusted rate	4.5%	8.7%	2.3%	4.8%
Terminal rate	2/38 (5%)	2/35 (6%)	1/30 (3%)	1/21 (5%)
First incidence (days)	729 (T)	555	729 (T)	616
Poly-3 test	P=0.460N	P=0.346	P=0.514N	P=0.671

Table C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
All Organs: Benign Tumors				
Overall rate	41/50 (82%)	44/50 (88%)	42/50 (84%)	38/50 (76%)
Adjusted rate	88.1%	91.0%	87.3%	80.3%
Terminal rate	35/38 (92%)	32/35 (91%)	26/30 (87%)	19/21 (91%)
First incidence (days)	617	555	462	397
Poly-3 test	P=0.094N	P=0.443	P=0.585N	P=0.206N
All Organs: Malignant Tumors				
Overall rate	36/50 (72%)	40/50 (80%)	46/50 (92%)	49/50 (98%)
Adjusted rate	72.3%	80.7%	95.3%	98.0%
Terminal rate	25/38 (66%)	26/35 (74%)	28/30 (93%)	20/21 (95%)
First incidence (days)	328	486	469	397
Poly-3 test	P<0.001	P=0.226	P=0.002	P<0.001
All Organs: Benign or Malignant Tumors				
Overall rate	47/50 (94%)	48/50 (96%)	49/50 (98%)	50/50 (100%)
Adjusted rate	94.0%	96.9%	99.9%	100.0%
Terminal rate	35/38 (92%)	34/35 (97%)	30/30 (100%)	21/21 (100%)
First incidence (days)	328	486	462	397
Poly-3 test	P=0.037	P=0.419	P=0.126	P=0.119

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, kidney, liver, lung, spleen, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the 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] One renal tubule carcinoma occurred in an animal that also had renal tubule adenoma.

Table C3
Historical Incidence of Neoplasms of the Liver in Untreated Male B6C3F1 Mice[a]

Study	Incidence in Controls			
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma	Hepatoblastoma
Historical Incidence: Drinking Water Studies				
Bromochloroacetic acid	27/50	19/50	34/50	4/50
Dibromoacetic acid	18/49	14/49	28/49	0/49
Dibromoacetonitrile	29/50	24/50	37/50	1/50
Sodium chlorate	30/48	20/48	41/48	6/48
Sodium dichromate dihydrate (VI)	36/50	14/50	42/50	17/50
Overall Historical Incidence: Drinking Water Studies				
Total (%)	140/247 (56.7%)	91/247 (36.8%)	182/247 (73.7%)	28/247 (11.3%)
Mean ± standard deviation	56.7% ± 13.0%	36.9% ± 8.6%	73.7% ± 11.7%	11.3% ± 13.6%
Range	37%-72%	28%-48%	57%-85%	0%-34%
Overall Historical Incidence: All Routes				
Total (%)	544/1,146 (47.5%)	317/1,146 (27.7%)	729/1,146 (63.6%)	43/1,146 (3.8%)
Mean ± standard deviation	47.5% ± 14.9%	27.7% ± 9.2%	63.6% ± 15.6%	3.8% ± 7.4%
Range	14%-72%	8%-48%	20%-85%	0%-34%

[a] Data as of October 4, 2007

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	6	5	9	15
Natural deaths	6	10	11	14
Survivors				
Terminal sacrifice	38	35	30	21
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Ulcer	1 (2%)			
Intestine large, cecum	(49)	(49)	(49)	(49)
Developmental malformation				1 (2%)
Edema	1 (2%)	1 (2%)		1 (2%)
Inflammation				1 (2%)
Intestine large, colon	(50)	(50)	(50)	(50)
Ulcer			1 (2%)	
Intestine small, duodenum	(48)	(47)	(48)	(47)
Intestine small, jejunum	(47)	(47)	(46)	(43)
Developmental malformation				1 (2%)
Hyperplasia, lymphoid			1 (2%)	
Epithelium, hyperplasia				1 (2%)
Peyer's patch, inflammation, suppurative			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Amyloid deposition	1 (2%)			
Angiectasis	2 (4%)			
Basophilic focus	5 (10%)	11 (22%)	8 (16%)	4 (8%)
Clear cell focus	15 (30%)	9 (18%)	4 (8%)	1 (2%)
Eosinophilic focus	20 (40%)	25 (50%)	14 (28%)	19 (38%)
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Hemorrhage	1 (2%)		1 (2%)	
Infarct	1 (2%)			
Infiltration cellular, mixed cell	4 (8%)	1 (2%)	2 (4%)	2 (4%)
Mixed cell focus	5 (10%)	9 (18%)	6 (12%)	4 (8%)
Necrosis, focal	5 (10%)	5 (10%)	7 (14%)	7 (14%)
Regeneration	1 (2%)			
Bile duct, cyst multilocular	1 (2%)			
Centrilobular, necrosis	1 (2%)	2 (4%)	4 (8%)	8 (16%)
Hepatocyte, fatty change			1 (2%)	
Hepatocyte, vacuolization cytoplasmic	3 (6%)	12 (24%)	17 (34%)	19 (38%)
Mesentery	(15)	(12)	(8)	(8)
Angiectasis		1 (8%)		
Fat, necrosis	15 (100%)	9 (75%)	6 (75%)	3 (38%)
Pancreas	(50)	(50)	(50)	(50)
Necrosis				1 (2%)
Acinus, cytoplasmic alteration	2 (4%)	5 (10%)	5 (10%)	8 (16%)
Salivary glands	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid	2 (4%)	4 (8%)	3 (6%)	5 (10%)
Necrosis		1 (2%)		

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

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Alimentary System (continued)				
Stomach, forestomach	(50)	(50)	(49)	(50)
Diverticulum				2 (4%)
Dysplasia			1 (2%)	
Inflammation, chronic		1 (2%)		3 (6%)
Ulcer	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Epithelium, hyperplasia, squamous	6 (12%)	6 (12%)	5 (10%)	9 (18%)
Stomach, glandular	(50)	(50)	(49)	(50)
Erosion	3 (6%)	2 (4%)	4 (8%)	3 (6%)
Metaplasia, atypical				1 (2%)
Ulcer	2 (4%)		1 (2%)	
Glands, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Tooth	(2)	(0)	(0)	(0)
Malformation	1 (50%)			
Cardiovascular System				
Blood vessel	(2)	(2)	(1)	(4)
Hypertrophy		1 (50%)		1 (25%)
Inflammation, chronic		1 (50%)		1 (25%)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Hemorrhage		1 (2%)		
Inflammation, chronic	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Mineralization	1 (2%)	1 (2%)	2 (4%)	5 (10%)
Thrombosis		1 (2%)	1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	2 (4%)	7 (14%)	2 (4%)	2 (4%)
Angiectasis				1 (2%)
Degeneration, fatty	1 (2%)			
Hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Hypertrophy, focal	5 (10%)	8 (16%)	3 (6%)	2 (4%)
Capsule, hyperplasia	8 (16%)	10 (20%)	2 (4%)	2 (4%)
Adrenal medulla	(50)	(50)	(50)	(47)
Hyperplasia	1 (2%)	2 (4%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(48)	(50)
Hyperplasia	35 (70%)	29 (58%)	21 (44%)	10 (20%)
Parathyroid gland	(32)	(40)	(37)	(38)
Cyst		2 (5%)	1 (3%)	1 (3%)
Pituitary gland	(50)	(50)	(48)	(49)
Pars distalis, cyst	5 (10%)	6 (12%)	3 (6%)	3 (6%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Follicle, degeneration, focal	9 (18%)	18 (36%)	10 (20%)	8 (16%)
Follicular cell, hyperplasia	18 (36%)	14 (28%)	14 (28%)	5 (10%)
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Inflammation, chronic	1 (2%)	1 (2%)		
Necrosis				1 (2%)
Spermatocele		1 (2%)		
Preputial gland	(48)	(50)	(50)	(50)
Foreign body				1 (2%)
Granuloma				1 (2%)
Hyperplasia	1 (2%)			
Inflammation, chronic	19 (40%)	27 (54%)	21 (42%)	22 (44%)
Duct, ectasia	41 (85%)	41 (82%)	41 (82%)	47 (94%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	2 (4%)	3 (6%)	2 (4%)	1 (2%)
Seminal vesicle	(50)	(50)	(50)	(50)
Degeneration	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic	1 (2%)			
Testes	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Germinal epithelium, atrophy	4 (8%)	3 (6%)	3 (6%)	8 (16%)
Hematopoietic System				
Bone marrow	(50)	(48)	(47)	(48)
Depletion cellular		1 (2%)		
Hyperplasia	29 (58%)	21 (44%)	31 (66%)	40 (83%)
Lymph node	(5)	(6)	(5)	(3)
Iliac, hematopoietic cell proliferation		1 (17%)	1 (20%)	
Iliac, hyperplasia, lymphoid	1 (20%)	1 (17%)		
Iliac, pigmentation	2 (40%)		2 (40%)	
Inguinal, pigmentation		1 (17%)		
Mediastinal, hemorrhage		1 (17%)		3 (100%)
Mediastinal, hyperplasia, lymphoid	1 (20%)		1 (20%)	
Mediastinal, pigmentation		1 (17%)	1 (20%)	3 (100%)
Lymph node, mandibular	(50)	(45)	(41)	(45)
Atrophy	1 (2%)	6 (13%)	3 (7%)	18 (40%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	
Hyperplasia, lymphoid	9 (18%)	10 (22%)	4 (10%)	
Pigmentation		1 (2%)	1 (2%)	2 (4%)
Lymph node, mesenteric	(45)	(45)	(44)	(45)
Angiectasis				1 (2%)
Atrophy	5 (11%)	5 (11%)	2 (5%)	15 (33%)
Ectasia			1 (2%)	
Hematopoietic cell proliferation	1 (2%)	2 (4%)	2 (5%)	3 (7%)
Hemorrhage	5 (11%)	3 (7%)	4 (9%)	2 (4%)
Hyperplasia, lymphoid	7 (16%)	3 (7%)	2 (5%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)		
Angiectasis				1 (2%)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	20 (40%)	23 (46%)	39 (78%)	40 (80%)
Lymphoid follicle, atrophy	3 (6%)	5 (10%)	6 (12%)	6 (12%)
Lymphoid follicle, hyperplasia	3 (6%)	2 (4%)	2 (4%)	1 (2%)

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Hematopoietic System (continued)				
Thymus	(46)	(40)	(36)	(40)
Atrophy	14 (30%)	22 (55%)	18 (50%)	24 (60%)
Cyst	8 (17%)	4 (10%)	3 (8%)	3 (8%)
Hyperplasia, lymphoid				1 (3%)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		
Edema		1 (2%)	5 (10%)	1 (2%)
Hemorrhage		1 (2%)		
Ulcer	2 (4%)	4 (8%)	5 (10%)	4 (8%)
Epidermis, hyperplasia	2 (4%)	3 (6%)	5 (10%)	4 (8%)
Sebaceous gland, hyperplasia			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Necrosis, focal				1 (2%)
Cranium, osteopetrosis		1 (2%)	1 (2%)	
Femur, fibrous osteodystrophy				1 (2%)
Skeletal muscle	(2)	(3)	(2)	(6)
Atrophy	1 (50%)			1 (17%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Gliosis		1 (2%)		
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Peripheral nerve	(1)	(2)	(2)	(5)
Atrophy	1 (100%)	1 (50%)	1 (50%)	2 (40%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Edema		1 (2%)		1 (2%)
Foreign body	1 (2%)			1 (2%)
Hemorrhage	1 (2%)	3 (6%)	1 (2%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)		1 (2%)
Infiltration cellular, histiocyte	10 (20%)	14 (28%)	5 (10%)	7 (14%)
Inflammation, suppurative	1 (2%)			2 (4%)
Thrombosis				2 (4%)
Alveolar epithelium, hyperplasia	3 (6%)	6 (12%)	4 (8%)	3 (6%)
Nose	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			1 (2%)
Inflammation, chronic	2 (4%)			1 (2%)

Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hemorrhage		1 (2%)		
Inflammation, chronic		1 (2%)	1 (2%)	
Cornea, hyperplasia		1 (2%)	1 (2%)	
Harderian gland	(50)	(50)	(49)	(50)
Hyperplasia, focal	4 (8%)	1 (2%)	2 (4%)	2 (4%)
Inflammation, chronic			1 (2%)	
Acinus, atrophy	1 (2%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	10 (20%)	10 (20%)	16 (32%)	2 (4%)
Glomerulosclerosis			1 (2%)	1 (2%)
Hydronephrosis	1 (2%)	1 (2%)		2 (4%)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	
Infarct	11 (22%)	2 (4%)	6 (12%)	5 (10%)
Inflammation, suppurative		1 (2%)		1 (2%)
Inflammation, chronic	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Metaplasia, osseous	6 (12%)	6 (12%)	3 (6%)	1 (2%)
Nephropathy	40 (80%)	39 (78%)	31 (62%)	29 (58%)
Renal tubule, accumulation, hyaline droplet		1 (2%)	2 (4%)	
Renal tubule, dilatation, diffuse	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Renal tubule, hyperplasia	1 (2%)	1 (2%)		
Renal tubule, necrosis	1 (2%)	2 (4%)	1 (2%)	3 (6%)
Renal tubule, pigmentation	1 (2%)	4 (8%)	21 (42%)	24 (48%)
Urethra	(1)	(0)	(0)	(0)
Angiectasis	1 (100%)			
Inflammation, acute	1 (100%)			
Urinary bladder	(50)	(50)	(49)	(50)
Edema		2 (4%)	2 (4%)	1 (2%)
Inflammation, chronic		1 (2%)	1 (2%)	
Transitional epithelium, hyperplasia			1 (2%)	

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

Table D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	3	3	2
Natural deaths	10	5	15	8
Survivors				
Died last week of study				1
Terminal sacrifice	36	42	32	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(42)	(49)	(41)	(41)
Intestine large, cecum	(48)	(50)	(46)	(47)
Sarcoma, metastatic, mesentery			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(49)
Intestine small, duodenum	(48)	(45)	(45)	(46)
Adenoma	1 (2%)		1 (2%)	1 (2%)
Sarcoma, metastatic, mesentery			1 (2%)	
Intestine small, ileum	(47)	(50)	(47)	(46)
Sarcoma, metastatic, mesentery			1 (2%)	
Intestine small, jejunum	(48)	(48)	(46)	(44)
Carcinoma	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	1 (2%)			
Hepatoblastoma		1 (2%)		1 (2%)
Hepatocellular adenoma	11 (22%)	11 (22%)	10 (20%)	3 (6%)
Hepatocellular adenoma, multiple	16 (32%)	37 (74%)	34 (68%)	43 (86%)
Hepatocellular carcinoma	13 (26%)	15 (30%)	14 (28%)	15 (30%)
Hepatocellular carcinoma, multiple	1 (2%)	8 (16%)	12 (24%)	5 (10%)
Sarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Sarcoma, metastatic, skin				1 (2%)
Mesentery	(14)	(16)	(15)	(7)
Carcinoma, metastatic, pancreas	1 (7%)			
Hemangioma				1 (14%)
Sarcoma	1 (7%)	1 (6%)	2 (13%)	
Sarcoma, metastatic, skin				1 (14%)
Sarcoma, metastatic, uncertain primary site	1 (7%)		1 (7%)	
Pancreas	(49)	(50)	(49)	(49)
Sarcoma, metastatic, mesentery		1 (2%)	1 (2%)	
Sarcoma, metastatic, skin				1 (2%)
Acinus, carcinoma	1 (2%)			
Salivary glands	(50)	(50)	(48)	(48)
Stomach, forestomach	(50)	(50)	(50)	(50)
Sarcoma, metastatic, mesentery			2 (4%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)	
Squamous cell papilloma		1 (2%)	2 (4%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	1 (2%)			
Sarcoma, metastatic, mesentery			1 (2%)	

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas	1 (2%)			
Hemangiosarcoma		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Carcinoma, metastatic, thyroid gland	1 (2%)			
Sarcoma, metastatic, skin				1 (2%)
Adrenal medulla	(50)	(50)	(49)	(48)
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma malignant	1 (2%)			
Islets, pancreatic	(49)	(50)	(48)	(49)
Adenoma	1 (2%)	2 (4%)	4 (8%)	2 (4%)
Adenoma, multiple		1 (2%)		
Parathyroid gland	(36)	(47)	(44)	(47)
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, adenoma	4 (8%)	3 (6%)	4 (8%)	6 (12%)
Pars distalis, carcinoma				1 (2%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(49)	(49)
C-cell, adenoma				1 (2%)
C-cell, carcinoma	1 (2%)			
Follicular cell, adenoma	2 (4%)	1 (2%)	3 (6%)	
Follicular cell, adenoma, multiple	1 (2%)			
General Body System				
Tissue NOS	(1)	(0)	(2)	(0)
Hemangiosarcoma			1 (50%)	
Sarcoma, metastatic, uncertain primary site	1 (100%)		1 (50%)	
Genital System				
Clitoral gland	(43)	(50)	(49)	(46)
Ovary	(47)	(50)	(44)	(48)
Choriocarcinoma				1 (2%)
Cystadenoma	2 (4%)	3 (6%)	1 (2%)	3 (6%)
Granulosa cell tumor benign				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Polyp stromal	2 (4%)			1 (2%)
Sarcoma stromal			1 (2%)	
Vagina	(1)	(1)	(0)	(0)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Lymph node	(11)	(11)	(4)	(5)
Sarcoma, metastatic, skin		1 (9%)		
Inguinal, osteosarcoma, metastatic, bone				1 (20%)
Mediastinal, carcinoma, metastatic, pancreas	1 (9%)			
Mediastinal, carcinoma, metastatic, thyroid gland	1 (9%)			
Lymph node, mandibular	(49)	(49)	(46)	(42)
Lymph node, mesenteric	(50)	(48)	(47)	(47)
Spleen	(49)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)		1 (2%)	1 (2%)
Thymus	(49)	(47)	(49)	(48)
Sarcoma, metastatic, mesentery			1 (2%)	
Sarcoma, metastatic, uncertain primary site			1 (2%)	
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Carcinoma	2 (4%)			1 (2%)
Fibroadenoma			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Subcutaneous tissue, fibrous histiocytoma	1 (2%)		1 (2%)	
Subcutaneous tissue, hemangioma	2 (4%)			1 (2%)
Subcutaneous tissue, hemangiosarcoma		1 (2%)	1 (2%)	1 (2%)
Subcutaneous tissue, lipoma		1 (2%)		
Subcutaneous tissue, sarcoma	1 (2%)	2 (4%)	6 (12%)	3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma				1 (2%)
Skeletal muscle	(2)	(2)	(4)	(2)
Carcinoma, metastatic, pancreas	1 (50%)			
Hemangiosarcoma		1 (50%)		
Rhabdomyosarcoma				1 (50%)
Sarcoma, metastatic, mesentery			2 (50%)	
Sarcoma, metastatic, skin			1 (25%)	1 (50%)
Sarcoma, metastatic, uncertain primary site			1 (25%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)			
Peripheral nerve	(1)	(2)	(1)	(1)

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

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)	2 (4%)	5 (10%)
Alveolar/bronchiolar carcinoma	6 (12%)	2 (4%)	1 (2%)	1 (2%)
Alveolar/bronchiolar carcinoma, multiple				2 (4%)
Carcinoma, metastatic, pancreas	1 (2%)			
Carcinoma, metastatic, thyroid gland	1 (2%)			
Choriocarcinoma, metastatic, ovary				1 (2%)
Hepatocellular carcinoma, metastatic, liver	1 (2%)	1 (2%)	5 (10%)	2 (4%)
Osteosarcoma, metastatic, bone				1 (2%)
Sarcoma, metastatic, mesentery			1 (2%)	
Sarcoma, metastatic, skin		1 (2%)	1 (2%)	
Sarcoma, metastatic, uncertain primary site	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(48)
Carcinoma, metastatic, Harderian gland	1 (2%)			
Harderian gland	(49)	(50)	(50)	(47)
Adenoma		7 (14%)	1 (2%)	7 (15%)
Adenoma, multiple	1 (2%)			
Carcinoma	2 (4%)	2 (4%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)		1 (2%)	1 (2%)
Leukemia granulocytic			1 (2%)	
Lymphoma malignant	18 (36%)	19 (38%)	15 (30%)	7 (14%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	42	49	50	50
Total primary neoplasms	98	122	122	122
Total animals with benign neoplasms	32	49	46	47
Total benign neoplasms	45	69	64	79
Total animals with malignant neoplasms	37	36	39	33
Total malignant neoplasms	53	53	58	43
Total animals with metastatic neoplasms	5	4	10	5
Total metastatic neoplasms	15	5	24	10
Total animals with malignant neoplasms of uncertain primary site	1		1	

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

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Harderian Gland: Adenoma				
Overall rate[a]	1/50 (2%)	7/50 (14%)	1/50 (2%)	7/50 (14%)
Adjusted rate[b]	2.2%	14.5%	2.2%	14.7%
Terminal rate[c]	1/36 (3%)	4/42 (10%)	0/32 (0%)	6/40 (15%)
First incidence (days)	729 (T)	570	489	500
Poly-3 test[d]	P=0.091	P=0.040	P=0.752N	P=0.038
Harderian Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	9/50 (18%)	2/50 (4%)	7/50 (14%)
Adjusted rate	6.7%	18.6%	4.3%	14.7%
Terminal rate	1/36 (3%)	6/42 (14%)	1/32 (3%)	6/40 (15%)
First incidence (days)	696	570	489	500
Poly-3 test	P=0.342	P=0.079	P=0.485N	P=0.184
Liver: Hepatocellular Adenoma				
Overall rate	27/50 (54%)	48/50 (96%)	44/50 (88%)	46/50 (92%)
Adjusted rate	59.4%	96.0%	90.9%	95.2%
Terminal rate	22/36 (61%)	40/42 (95%)	30/32 (94%)	39/40 (98%)
First incidence (days)	645	570	568	551
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Liver: Hepatocellular Carcinoma				
Overall rate	14/50 (28%)	23/50 (46%)[e]	26/50 (52%)	20/50 (40%)[e]
Adjusted rate	31.1%	48.3%	56.1%	42.3%
Terminal rate	11/36 (31%)	21/42 (50%)	20/32 (63%)	17/40 (43%)
First incidence (days)	609	667	657	618
Poly-3 test	P=0.249	P=0.067	P=0.011	P=0.185
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	31/50 (62%)	49/50 (98%)[e]	46/50 (92%)	46/50 (92%)[e]
Adjusted rate	67.6%	98.0%	94.6%	95.2%
Terminal rate	24/36 (67%)	41/42 (98%)	31/32 (97%)	39/40 (98%)
First incidence (days)	609	570	568	551
Poly-3 test	P<0.001	P<0.001	P<0.001	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	2/50 (4%)	1/50 (2%)	2/50 (4%)	5/50 (10%)
Adjusted rate	4.5%	2.1%	4.4%	10.7%
Terminal rate	2/36 (6%)	0/42 (0%)	2/32 (6%)	5/40 (13%)
First incidence (days)	729 (T)	707	729 (T)	729 (T)
Poly-3 test	P=0.080	P=0.479N	P=0.686N	P=0.238
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate	13.3%	4.2%	2.2%	6.4%
Terminal rate	5/36 (14%)	2/42 (5%)	1/32 (3%)	3/40 (8%)
First incidence (days)	609	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.210N	P=0.117N	P=0.054N	P=0.222N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	8/50 (16%)	3/50 (6%)	3/50 (6%)	8/50 (16%)
Adjusted rate	17.8%	6.3%	6.6%	17.1%
Terminal rate	7/36 (19%)	2/42 (5%)	3/32 (9%)	8/40 (20%)
First incidence (days)	609	707	729 (T)	729 (T)
Poly-3 test	P=0.413	P=0.083N	P=0.094N	P=0.572N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Ovary: Cystadenoma				
Overall rate	2/47 (4%)	3/50 (6%)	1/44 (2%)	3/48 (6%)
Adjusted rate	4.8%	6.3%	2.5%	6.6%
Terminal rate	2/34 (6%)	3/42 (7%)	1/29 (3%)	2/39 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	673
Poly-3 test	P=0.500	P=0.558	P=0.513N	P=0.543
Pancreatic Islets: Adenoma				
Overall rate	1/49 (2%)	3/50 (6%)	4/48 (8%)	2/49 (4%)
Adjusted rate	2.3%	6.3%	9.0%	4.3%
Terminal rate	1/36 (3%)	2/42 (5%)	3/32 (9%)	2/40 (5%)
First incidence (days)	729 (T)	693	595	729 (T)
Poly-3 test	P=0.481	P=0.331	P=0.180	P=0.518
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	4/48 (8%)	3/49 (6%)	4/48 (8%)	6/49 (12%)
Adjusted rate	9.4%	6.5%	9.1%	13.1%
Terminal rate	3/35 (9%)	3/41 (7%)	3/30 (10%)	6/39 (15%)
First incidence (days)	728	729 (T)	624	729 (T)
Poly-3 test	P=0.254	P=0.457N	P=0.630N	P=0.415
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	4/48 (8%)	3/49 (6%)	4/48 (8%)	7/49 (14%)
Adjusted rate	9.4%	6.5%	9.1%	15.2%
Terminal rate	3/35 (9%)	3/41 (7%)	3/30 (10%)	7/39 (18%)
First incidence (days)	728	729 (T)	624	729 (T)
Poly-3 test	P=0.153	P=0.457N	P=0.630N	P=0.303
Skin (Subcutaneous Tissue): Sarcoma				
Overall rate	1/50 (2%)	2/50 (4%)	6/50 (12%)	3/50 (6%)
Adjusted rate	2.2%	4.2%	13.0%	6.4%
Terminal rate	1/36 (3%)	1/42 (2%)	3/32 (9%)	3/40 (8%)
First incidence (days)	729 (T)	619	653	729 (T)
Poly-3 test	P=0.228	P=0.524	P=0.061	P=0.324
Skin (Subcutaneous Tissue): Fibrous Histiocytoma or Sarcoma				
Overall rate	2/50 (4%)	2/50 (4%)	7/50 (14%)	3/50 (6%)
Adjusted rate	4.5%	4.2%	15.2%	6.4%
Terminal rate	2/36 (6%)	1/42 (2%)	4/32 (13%)	3/40 (8%)
First incidence (days)	729 (T)	619	653	729 (T)
Poly-3 test	P=0.341	P=0.669N	P=0.087	P=0.523
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	4.4%	6.4%
Terminal rate	0/36 (0%)	1/42 (2%)	2/32 (6%)	2/40 (5%)
First incidence (days)	—[f]	729 (T)	729 (T)	725
Poly-3 test	P=0.067	P=0.512	P=0.242	P=0.128
Thyroid Gland: Follicular Cell Adenoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/49 (6%)	0/49 (0%)
Adjusted rate	6.7%	2.1%	6.7%	0.0%
Terminal rate	3/36 (8%)	1/42 (2%)	2/32 (6%)	0/40 (0%)
First incidence (days)	729 (T)	729 (T)	725	—
Poly-3 test	P=0.134N	P=0.284N	P=0.662N	P=0.113N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
All Organs: Hemangioma				
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Adjusted rate	4.5%	0.0%	0.0%	6.4%
Terminal rate	2/36 (6%)	0/42 (0%)	0/32 (0%)	3/40 (8%)
First incidence (days)	729 (T)	—	—	729 (T)
Poly-3 test	P=0.241	P=0.224N	P=0.232N	P=0.523
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	2/50 (4%)	3/50 (6%)
Adjusted rate	2.2%	6.3%	4.4%	6.4%
Terminal rate	1/36 (3%)	3/42 (7%)	2/32 (6%)	3/40 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.323	P=0.327	P=0.507	P=0.324
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	3/50 (6%)	3/50 (6%)	2/50 (4%)	6/50 (12%)
Adjusted rate	6.7%	6.3%	4.4%	12.8%
Terminal rate	3/36 (8%)	3/42 (7%)	2/32 (6%)	6/40 (15%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.160	P=0.634N	P=0.490N	P=0.268
All Organs: Malignant Lymphoma				
Overall rate	18/50 (36%)	19/50 (38%)	15/50 (30%)	7/50 (14%)
Adjusted rate	39.3%	39.9%	32.8%	14.9%
Terminal rate	14/36 (39%)	18/42 (43%)	14/32 (44%)	6/40 (15%)
First incidence (days)	340	646	670	700
Poly-3 test	P=0.003N	P=0.559	P=0.334N	P=0.006N
All Organs: Benign Tumors				
Overall rate	32/50 (64%)	49/50 (98%)	46/50 (92%)	47/50 (94%)
Adjusted rate	70.4%	98.0%	93.7%	95.9%
Terminal rate	26/36 (72%)	41/42 (98%)	31/32 (97%)	39/40 (98%)
First incidence (days)	645	570	489	500
Poly-3 test	P<0.001	P<0.001	P<0.002	P<0.001
All Organs: Malignant Tumors				
Overall rate	38/50 (76%)	36/50 (72%)	40/50 (80%)	33/50 (66%)
Adjusted rate	79.9%	74.5%	84.4%	68.0%
Terminal rate	27/36 (75%)	32/42 (76%)	29/32 (91%)	27/40 (68%)
First incidence (days)	340	619	635	359
Poly-3 test	P=0.131N	P=0.351N	P=0.375	P=0.135N

Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
All Organs: Benign or Malignant Tumors				
Overall rate	43/50 (86%)	49/50 (98%)	50/50 (100%)	50/50 (100%)
Adjusted rate	90.4%	98.0%	100.0%	100.0%
Terminal rate	32/36 (89%)	41/42 (98%)	32/32 (100%)	40/40 (100%)
First incidence (days)	340	570	489	359
Poly-3 test	P=0.009	P=0.108	P=0.030	P=0.030

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, 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 group incidence are the P values corresponding to pairwise comparisons between the chamber 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 hepatocellular carcinoma.
[f] Not applicable; no neoplasms in animal group

Table D3a
Historical Incidence of Neoplasms of the Liver in Untreated Female B6C3F1 Mice[a]

Study	Incidence in Controls: Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	27/50	14/50	31/50
Bromodichloromethane	24/50	13/50	30/50
Dibromoacetic acid	19/49	3/49	22/49
Dibromoacetonitrile	19/50	10/50	27/50
Sodium chlorate	30/49	3/49	31/49
Sodium dichromate dihydrate (VI)	14/49	8/49	17/49
Overall Historical Incidence: Drinking Water Studies			
Total (%)	133/297 (44.8%)	51/297 (17.2%)	158/297 (53.2%)
Mean ± standard deviation	44.8% ± 11.9%	17.1% ± 9.5%	53.1% ± 11.3%
Range	29%-61%	6%-28%	35%-63%
Overall Historical Incidence: All Routes			
Total (%)	345/1,245 (27.7%)	131/1,245 (10.5%)	419/1,245 (33.7%)
Mean ± standard deviation	27.8% ± 17.0%	10.5% ± 7.7%	33.7% ± 19.1%
Range	6%-62%	0%-28%	8%-64%

[a] Data as of October 4, 2007

TABLE D3b
Historical Incidence of Neoplasms of the Harderian Gland in Untreated Female B6C3F1 Mice[a]

Study	Incidence in Controls		
	Adenoma	**Carcinoma**	**Adenoma or Carcinoma**
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	1/50	2/50	3/50
Bromodichloromethane	3/50	1/50	4/50
Dibromoacetic acid	9/50	1/50	10/50
Dibromoacetonitrile	6/50	2/50	8/50
Sodium chlorate	11/50	1/50	12/50
Sodium dichromate dihydrate (VI)	4/50	1/50	5/50
Overall Historical Incidence: Drinking Water Studies			
Total (%)	34/300 (11.3%)	8/300 (2.7%)	42/300 (14.0%)
Mean ± standard deviation	11.3% ± 7.6%	2.7% ± 1.0%	14.0% ± 7.2%
Range	2%-22%	2%-4%	6%-24%
Overall Historical Incidence: All Routes			
Total (%)	128/1,249 (10.3%)	33/1,249 (2.6%)	160/1,249 (12.8%)
Mean ± standard deviation	10.2% ± 4.9%	2.6% ± 2.4%	12.8% ± 5.6%
Range	2%-22%	0%-10%	6%-24%

[a] Data as of October 4, 2007

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	4	3	3	2
Natural deaths	10	5	15	8
Survivors				
Died last week of study				1
Terminal sacrifice	36	42	32	39
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(42)	(49)	(41)	(41)
Intestine large, cecum	(48)	(50)	(46)	(47)
Edema		1 (2%)		1 (2%)
Ulcer		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(49)
Diverticulum		1 (2%)		
Intestine small, duodenum	(48)	(45)	(45)	(46)
Epithelium, hyperplasia		1 (2%)		1 (2%)
Intestine small, ileum	(47)	(50)	(47)	(46)
Epithelium, hyperplasia		2 (4%)		
Intestine small, jejunum	(48)	(48)	(46)	(44)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Epithelium, hyperplasia		1 (2%)	1 (2%)	
Peyer's patch, inflammation, suppurative				1 (2%)
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		2 (4%)	
Autolysis				1 (2%)
Basophilic focus	1 (2%)	3 (6%)	6 (12%)	5 (10%)
Clear cell focus	4 (8%)	4 (8%)	2 (4%)	
Eosinophilic focus	13 (26%)	22 (44%)	31 (62%)	24 (48%)
Hematopoietic cell proliferation	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Hemorrhage	2 (4%)		1 (2%)	
Infiltration cellular, mixed cell	4 (8%)	4 (8%)	5 (10%)	
Mixed cell focus	5 (10%)	10 (20%)	6 (12%)	1 (2%)
Necrosis, focal	3 (6%)	2 (4%)	2 (4%)	2 (4%)
Tension lipidosis		1 (2%)		1 (2%)
Bile duct, cyst multilocular		1 (2%)		1 (2%)
Centrilobular, necrosis				2 (4%)
Hepatocyte, fatty change		1 (2%)	1 (2%)	
Hepatocyte, fatty change, focal	1 (2%)			
Hepatocyte, hyperplasia, nodular	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	3 (6%)	11 (22%)	27 (54%)	42 (84%)
Kupffer cell, hyperplasia	1 (2%)			
Mesentery	(14)	(16)	(15)	(7)
Inflammation, suppurative			1 (7%)	
Proliferation connective tissue			1 (7%)	
Fat, necrosis	8 (57%)	15 (94%)	9 (60%)	5 (71%)
Pancreas	(49)	(50)	(49)	(49)
Atrophy		1 (2%)		
Cyst	1 (2%)	1 (2%)		
Acinus, cytoplasmic alteration	3 (6%)	4 (8%)	2 (4%)	3 (6%)

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

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Alimentary System (continued)				
Salivary glands	(50)	(50)	(48)	(48)
Atrophy				1 (2%)
Hyperplasia, lymphoid	17 (34%)	19 (38%)	12 (25%)	12 (25%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			1 (2%)
Edema		1 (2%)		
Erosion	1 (2%)			
Inflammation, chronic	2 (4%)		1 (2%)	
Ulcer		2 (4%)	1 (2%)	
Epithelium, hyperplasia, squamous	3 (6%)	2 (4%)	1 (2%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	2 (4%)		1 (2%)	2 (4%)
Ulcer				2 (4%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy		1 (2%)	2 (4%)	
Inflammation, chronic			1 (2%)	
Mineralization		2 (4%)	2 (4%)	
Thrombosis	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(49)	(50)
Accessory adrenal cortical nodule	10 (20%)	8 (16%)	6 (12%)	10 (20%)
Hyperplasia, focal	2 (4%)			
Hyperplasia, diffuse			1 (2%)	
Capsule, hyperplasia				2 (4%)
Zona reticularis, vacuolization cytoplasmic		1 (2%)		2 (4%)
Adrenal medulla	(50)	(50)	(49)	(48)
Hyperplasia		1 (2%)		1 (2%)
Islets, pancreatic	(49)	(50)	(48)	(49)
Hyperplasia	3 (6%)	3 (6%)		
Parathyroid gland	(36)	(47)	(44)	(47)
Cyst	1 (3%)	3 (6%)		4 (9%)
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, angiectasis	2 (4%)			
Pars distalis, cyst	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Pars distalis, hyperplasia, focal	10 (21%)	7 (14%)	4 (8%)	6 (12%)
Pars intermedia, hyperplasia				1 (2%)
Thyroid gland	(50)	(50)	(49)	(49)
Inflammation, granulomatous		2 (4%)		
C-cell, hyperplasia				1 (2%)
Follicle, cyst	1 (2%)	4 (8%)	4 (8%)	3 (6%)
Follicle, degeneration, focal	19 (38%)	22 (44%)	20 (41%)	13 (27%)
Follicular cell, hyperplasia	15 (30%)	18 (36%)	11 (22%)	9 (18%)
General Body System				
Tissue NOS	(1)	(0)	(2)	(0)

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Genital System				
Clitoral gland	(43)	(50)	(49)	(46)
Cyst	1 (2%)			
Ovary	(47)	(50)	(44)	(48)
Amyloid deposition	1 (2%)			
Angiectasis	6 (13%)	6 (12%)	4 (9%)	6 (13%)
Cyst	20 (43%)	15 (30%)	12 (27%)	10 (21%)
Hyperplasia, tubular		1 (2%)		
Thrombosis			1 (2%)	
Corpus luteum, hyperplasia				3 (6%)
Thecal cell, hyperplasia	1 (2%)	1 (2%)		
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Hemorrhage				1 (2%)
Endometrium, hyperplasia, cystic	39 (78%)	46 (92%)	44 (88%)	44 (88%)
Vagina	(1)	(1)	(0)	(0)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Depletion cellular		1 (2%)		
Hyperplasia	14 (28%)	10 (20%)	22 (44%)	14 (28%)
Myelofibrosis		1 (2%)		
Lymph node	(11)	(11)	(4)	(5)
Iliac, ectasia	2 (18%)			
Iliac, hematopoietic cell proliferation				1 (20%)
Iliac, hyperplasia, lymphoid	1 (9%)	1 (9%)		
Inguinal, hyperplasia, lymphoid	1 (9%)			
Mediastinal, hyperplasia, lymphoid		2 (18%)	1 (25%)	
Renal, hyperplasia, lymphoid	2 (18%)			
Lymph node, mandibular	(49)	(49)	(46)	(42)
Atrophy	1 (2%)	1 (2%)	2 (4%)	
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	2 (4%)	6 (12%)	4 (9%)	7 (17%)
Pigmentation	7 (14%)	9 (18%)	3 (7%)	6 (14%)
Lymph node, mesenteric	(50)	(48)	(47)	(47)
Atrophy	2 (4%)	2 (4%)	2 (4%)	
Ectasia	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	2 (4%)	2 (4%)		
Hemorrhage	4 (8%)			
Hyperplasia, lymphoid	4 (8%)	5 (10%)	1 (2%)	2 (4%)
Spleen	(49)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Hematopoietic cell proliferation	33 (67%)	40 (80%)	39 (78%)	27 (54%)
Pigmentation		4 (8%)	3 (6%)	2 (4%)
Lymphoid follicle, atrophy	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Lymphoid follicle, hyperplasia	8 (16%)	13 (26%)	8 (16%)	9 (18%)
Thymus	(49)	(47)	(49)	(48)
Atrophy	2 (4%)	5 (11%)	11 (22%)	5 (10%)
Cyst			2 (4%)	1 (2%)
Hyperplasia, lymphoid	6 (12%)	7 (15%)	2 (4%)	5 (10%)

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	2 (4%)	8 (16%)	6 (12%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Edema			1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosis	1 (2%)	6 (12%)	4 (8%)	4 (8%)
Cranium, osteopetrosis	1 (2%)	1 (2%)		
Skeletal muscle	(2)	(2)	(4)	(2)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	2 (4%)		2 (4%)	1 (2%)
Hemorrhage	1 (2%)			
Peripheral nerve	(1)	(2)	(1)	(1)
Atrophy		2 (100%)	1 (100%)	1 (100%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body				2 (4%)
Hemorrhage	2 (4%)	5 (10%)	5 (10%)	6 (12%)
Hyperplasia, lymphoid	1 (2%)	3 (6%)		
Infiltration cellular, histiocyte	5 (10%)	2 (4%)	4 (8%)	6 (12%)
Metaplasia, osseous	1 (2%)			
Alveolar epithelium, hyperplasia	2 (4%)	4 (8%)	1 (2%)	1 (2%)
Nose	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)			1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(48)
Atrophy	1 (2%)	1 (2%)		2 (4%)
Cataract		1 (2%)		1 (2%)
Inflammation, chronic		1 (2%)		
Harderian gland	(49)	(50)	(50)	(47)
Hyperplasia, focal		5 (10%)	4 (8%)	1 (2%)
Inflammation, chronic	1 (2%)			

Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Drinking Water Study of Bromochloroacetic Acid

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Casts granular	2 (4%)	1 (2%)		1 (2%)
Hydronephrosis			1 (2%)	
Infarct	8 (16%)	4 (8%)	2 (4%)	3 (6%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic	4 (8%)		3 (6%)	3 (6%)
Metaplasia, osseous	1 (2%)	2 (4%)	3 (6%)	2 (4%)
Nephropathy	5 (10%)	5 (10%)	8 (16%)	6 (12%)
Renal tubule, accumulation, hyaline droplet	1 (2%)			1 (2%)
Renal tubule, necrosis	1 (2%)		1 (2%)	
Renal tubule, pigmentation			1 (2%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Edema			1 (2%)	
Inflammation, chronic	7 (14%)	4 (8%)	3 (6%)	6 (12%)
Transitional epithelium, hyperplasia			1 (2%)	

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Two independent mutagenicity assays were conducted with bromochloroacetic acid. Testing was performed in *Salmonella typhimurium* strains TA98 and TA100 for the first assay as reported by Zeiger *et al*. (1992). The second assay, conducted with the same lot of bromochloroacetic acid tested in the 2-year studies, used a slightly modified protocol (activation only with 10% rat liver S9) and also employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *S. typhimurium* strain TA100. Bromochloroacetic acid was sent to the testing laboratory as a coded aliquot. It was incubated with the bacterial tester strains (TA98, TA100, *E.coli* WP2) 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 bromochloroacetic acid. In both tests, the highest concentration tested was limited by toxicity in the absence of S9; with S9, concentrations in the second trial (conducted at SITEK Research Laboratories) achieved the limit concentration of 10,000 µg/plate, but substantial toxicity was seen at this concentration level. 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.

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 1,000 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. 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 judgment of the overall evidence for activity of the chemical in an assay.

Results

In the first of two independent bacterial mutation assays, bromochloroacetic acid (33 to 3,333 µg/plate) was mutagenic in *S. typhimurium* strain TA100, with and without rat or hamster liver activation enzymes (S9); no mutagenicity was detected in strain TA98 in tests conducted with and without hamster and rat S9 (Table E1). In the second assay, lower concentrations of bromochloroacetic acid (10 to 500 µg/plate) were tested in TA100 without S9, and no mutagenicity was detected (Table E1). However, mutagenicity was observed in TA100 (1,000 to 10,000 µg/plate) in the presence of rat S9; no significant increases in mutant colonies were seen in *E. coli* WP2 *uvrA*/pKM101 with or without S9. No significant increases in the frequency of micronucleated NCEs were observed in blood samples of male or female B6C3F1 mice exposed to bromochloroacetic acid (62.5 to 1,000 mg/L) for 3 months in drinking water, indicating no induction of chromosomal damage in proerythrocytes by bromochloroacetic acid under these conditions in these mice. The percentage of immature erythrocytes (polychromatic erythrocytes) among total erythrocytes in blood of male and female mice was not significantly altered, indicating a lack of chemical-induced changes in erythropoiesis.

Table E1
Mutagenicity of Bromochloroacetic Acid in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+30% hamster S9 Trial 1	+30% hamster S9 Trial 2	+30% rat S9 Trial 1	+30% rat S9 Trial 2
Study performed at SRI International							
TA100	0	125 ± 10.0	118 ± 7.0	131 ± 14.0	143 ± 12.0	140 ± 6.0	132 ± 3.0
	33	101 ± 10.0	117 ± 5.0	113 ± 13.0	145 ± 4.0	136 ± 3.0	119 ± 11.0
	100	168 ± 8.0	150 ± 9.0	146 ± 6.0	173 ± 7.0	176 ± 7.0	147 ± 5.0
	333	253 ± 17.0	170 ± 4.0	236 ± 11.0	225 ± 7.0	291 ± 12.0	340 ± 12.0
	666		373 ± 4.0		408 ± 16.0		426 ± 9.0
	1,000	540 ± 24.0	482 ± 3.0	589 ± 8.0	500 ± 28.0	681 ± 20.0	532 ± 27.0
	3,333	1,242 ± 56.0		1,178 ± 59.0		1,159 ± 38.0	
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[c]		1,187 ± 16.0	1,352 ± 27.0	1,250 ± 63.0	763 ± 25.0	838 ± 37.0	673 ± 38.0
TA98	0	18 ± 2.0		25 ± 2.0		20 ± 2.0	
	33	16 ± 1.0		24 ± 5.0		21 ± 0.0	
	100	16 ± 2.0		22 ± 4.0		26 ± 2.0	
	333	16 ± 2.0		23 ± 3.0		21 ± 2.0	
	1,000	18 ± 4.0		17 ± 4.0		21 ± 4.0	
	3,333	15 ± 3.0		25 ± 1.0		22 ± 3.0	
Trial summary		Negative		Negative		Negative	
Positive control		422 ± 9.0		904 ± 34.0		513 ± 32.0	

Table E1
Mutagenicity of Bromochloroacetic Acid in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
Study performed at SITEK Research Laboratories					
TA100	0	165 ± 6.0	168 ± 2.0	149 ± 13.0	146 ± 25.0
	10	174 ± 6.0	154 ± 1.0		
	50	193 ± 5.0	193 ± 13.0		
	100	174 ± 14.0	182 ± 5.0		
	250	156 ± 8.0	152 ± 15.0		
	500	54 ± 6.0	58 ± 5.0		
	1,000			216 ± 4.0	236 ± 8.0
	2,500			318 ± 18.0	318 ± 12.0
	5,000			331 ± 8.0	330 ± 11.0
	7,500			205 ± 4.0	234 ± 7.0
	10,000			15 ± 4.0	5 ± 3.0
Trial summary		Negative	Negative	Positive	Positive
Positive control		621 ± 18.0	558 ± 7.0	449 ± 30.0	367 ± 8.0
***Escherichia coli* WP2 *uvrA*/pKM101**					
	0	293 ± 6.0	278 ± 6.0	265 ± 9.0	278 ± 14.0
	10	312 ± 11.0	308 ± 7.0		
	50	284 ± 4.0	286 ± 8.0		
	100	281 ± 2.0	277 ± 7.0		
	250	315 ± 5.0	312 ± 3.0		
	500	76 ± 5.0	75 ± 5.0		
	1,000			304 ± 5.0	291 ± 14.0
	2,500			355 ± 13.0	315 ± 3.0
	5,000			305 ± 11.0	358 ± 13.0
	7,500			304 ± 6.0	281 ± 41.0
	10,000			34 ± 11.0	2 ± 1.0
Trial summary		Negative	Negative	Negative	Negative
Positive control		869 ± 11.0	873 ± 2.0	612 ± 20.0	554 ± 17.0

[a] The detailed protocol for the study performed by SRI International 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), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA*/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.

Table E2
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Administration of Bromochloroacetic 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					
Water	0	10	4.80 ± 0.57		4.4 ± 0.3
Bromochloroacetic acid	62.5	10	5.10 ± 0.59	0.3812	4.6 ± 0.1
	125	10	3.90 ± 0.67	0.8332	4.3 ± 0.2
	250	10	5.30 ± 0.56	0.3090	4.5 ± 0.2
	500	8	4.75 ± 0.59	0.5193	4.3 ± 0.3
	1,000	10	4.50 ± 0.58	0.6224	4.6 ± 0.2
			P=0.603[d]		
Female					
Water	0	10	2.60 ± 0.43		4.0 ± 0.3
Bromochloroacetic acid	62.5	10	3.30 ± 0.45	0.1807	4.5 ± 0.3
	125	10	4.20 ± 0.53	0.0260	4.3 ± 0.3
	250	10	4.60 ± 0.54	0.0091	4.5 ± 0.2
	500	10	3.80 ± 0.68	0.0665	4.6 ± 0.2
	1,000	10	3.30 ± 0.47	0.1807	4.5 ± 0.2
			P=0.467		

[a] Study was performed at ILS, Inc. The detailed protocol is 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 Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
Hematology						
n						
Day 3	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Hematocrit (auto) (%)						
Day 3	44.9 ± 0.8	42.8 ± 0.5	43.4 ± 0.7	43.5 ± 0.5	44.7 ± 0.6	43.1 ± 0.7
Day 21	49.9 ± 0.9	49.1 ± 0.7	49.9 ± 0.5	51.0 ± 0.9	52.9 ± 1.0	49.0 ± 0.6
Week 14	44.6 ± 0.5	44.2 ± 0.4	45.6 ± 0.5	44.5 ± 0.4	44.7 ± 0.3	44.6 ± 0.4
Hematocrit (spun) (%)						
Day 3	46.0 ± 0.8	43.3 ± 0.6*	43.9 ± 0.8	44.0 ± 0.5	45.3 ± 0.6	43.3 ± 0.9
Day 21	49.7 ± 0.9	48.8 ± 0.7	50.0 ± 0.5	50.2 ± 0.8	52.2 ± 0.8	48.9 ± 0.6
Week 14	45.2 ± 0.5	44.5 ± 0.5	45.9 ± 0.5	45.2 ± 0.5	44.7 ± 0.4	45.1 ± 0.5
Hemoglobin (g/dL)						
Day 3	15.2 ± 0.4	14.4 ± 0.2	14.5 ± 0.3	14.6 ± 0.2	14.9 ± 0.2	14.3 ± 0.3
Day 21	15.8 ± 0.3	15.3 ± 0.2	15.4 ± 0.1	16.0 ± 0.3	16.5 ± 0.3	15.2 ± 0.2
Week 14	14.5 ± 0.2	14.2 ± 0.1	14.8 ± 0.2	14.4 ± 0.1	14.5 ± 0.1	14.4 ± 0.1
Erythrocytes ($10^6/\mu L$)						
Day 3	7.74 ± 0.18	7.27 ± 0.07	7.40 ± 0.12	7.45 ± 0.06	7.60 ± 0.10	7.32 ± 0.11
Day 21	8.58 ± 0.15	8.28 ± 0.12	8.40 ± 0.08	8.63 ± 0.16	8.93 ± 0.23	8.27 ± 0.13
Week 14	8.72 ± 0.09	8.49 ± 0.07	8.80 ± 0.06	8.62 ± 0.07	8.72 ± 0.07	8.68 ± 0.06
Reticulocytes ($10^5/\mu L$)						
Day 3	7.19 ± 0.11	7.47 ± 0.27	6.61 ± 0.25	6.62 ± 0.23	7.14 ± 0.20	6.76 ± 0.25
Day 21	3.39 ± 0.22	4.20 ± 0.14	4.55 ± 0.17**	4.48 ± 0.14**	3.65 ± 0.20	4.54 ± 0.12**
Week 14	2.50 ± 0.06	2.84 ± 0.07*	2.81 ± 0.09	2.67 ± 0.08	2.86 ± 0.03**	2.83 ± 0.13
Nucleated erythrocytes (/100 leukocytes)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10	0.30 ± 0.15
Mean cell volume (fL)						
Day 3	58.0 ± 0.4	58.8 ± 0.3	58.6 ± 0.2	58.4 ± 0.3	58.8 ± 0.2	58.9 ± 0.3
Day 21	58.2 ± 0.4	59.3 ± 0.1	59.4 ± 0.4	59.2 ± 0.4	59.3 ± 0.6	59.4 ± 0.4
Week 14	51.2 ± 0.2	52.1 ± 0.2	51.8 ± 0.2	51.6 ± 0.2	51.3 ± 0.2	51.4 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	19.6 ± 0.1	19.7 ± 0.2	19.6 ± 0.1	19.6 ± 0.1	19.6 ± 0.1	19.5 ± 0.1
Day 21	18.4 ± 0.2	18.5 ± 0.2	18.4 ± 0.1	18.5 ± 0.2	18.5 ± 0.2	18.5 ± 0.2
Week 14	16.6 ± 0.1	16.7 ± 0.1	16.8 ± 0.2	16.7 ± 0.1	16.6 ± 0.1	16.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	33.6 ± 0.2	33.5 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.4 ± 0.2	33.1 ± 0.1
Day 21	31.7 ± 0.4	31.2 ± 0.3	31.0 ± 0.1	31.3 ± 0.3	31.3 ± 0.2	31.0 ± 0.2
Week 14	32.5 ± 0.2	32.1 ± 0.2	32.4 ± 0.2	32.3 ± 0.1	32.4 ± 0.2	32.4 ± 0.2
Platelets ($10^3/\mu L$)						
Day 3	847.5 ± 19.1	875.3 ± 19.8	838.2 ± 35.7	831.2 ± 31.5	792.9 ± 70.7	849.8 ± 32.1
Day 21	729.7 ± 29.7	764.2 ± 21.6	773.5 ± 23.1	787.8 ± 23.9	729.8 ± 14.4	766.4 ± 16.6
Week 14	577.6 ± 16.1	570.2 ± 9.7	591.3 ± 18.7	569.1 ± 14.8	581.1 ± 9.8	578.6 ± 13.7
Leukocytes ($10^3/\mu L$)						
Day 3	7.79 ± 0.76	7.70 ± 0.38	6.91 ± 0.40	6.76 ± 0.53	6.43 ± 0.51	7.83 ± 0.76
Day 21	10.08 ± 0.51	11.10 ± 0.63	11.94 ± 0.52	10.16 ± 0.33	11.57 ± 0.67	11.20 ± 0.45
Week 14	6.81 ± 0.30	8.54 ± 0.39*	7.22 ± 0.32	7.50 ± 0.47	7.11 ± 0.50	7.12 ± 0.34
Segmented neutrophils ($10^3/\mu L$)						
Day 3	0.83 ± 0.09	0.84 ± 0.04	0.72 ± 0.04	0.75 ± 0.07	0.80 ± 0.07	0.83 ± 0.05
Day 21	0.89 ± 0.03	0.87 ± 0.04	0.98 ± 0.05	0.93 ± 0.04	0.91 ± 0.03	0.95 ± 0.06
Week 14	1.10 ± 0.06	1.20 ± 0.05	1.02 ± 0.04	1.12 ± 0.05	1.08 ± 0.07	1.01 ± 0.05

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

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 14	9	10	10	10	10	10
Lymphocytes ($10^3/\mu L$)						
Day 3	6.44 ± 0.61	6.39 ± 0.35	5.79 ± 0.35	5.55 ± 0.45	5.24 ± 0.43	6.53 ± 0.68
Day 21	8.67 ± 0.44	9.70 ± 0.58	10.40 ± 0.45	8.67 ± 0.25	10.09 ± 0.61	9.74 ± 0.40
Week 14	4.99 ± 0.28	6.48 ± 0.37	5.48 ± 0.27	5.41 ± 0.40	5.13 ± 0.42	5.19 ± 0.32
Monocytes ($10^3/\mu L$)						
Day 3	0.15 ± 0.02	0.16 ± 0.01	0.13 ± 0.01	0.13 ± 0.02	0.14 ± 0.02	0.16 ± 0.02
Day 21	0.13 ± 0.02	0.13 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	0.17 ± 0.02	0.14 ± 0.01
Week 14	0.09 ± 0.02	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Basophils ($10^3/\mu L$)						
Day 3	0.036 ± 0.006	0.027 ± 0.006	0.031 ± 0.006	0.024 ± 0.003	0.022 ± 0.005	0.031 ± 0.006
Day 21	0.043 ± 0.004	0.054 ± 0.007	0.048 ± 0.004	0.043 ± 0.004	0.049 ± 0.005	0.049 ± 0.004
Week 14	0.048 ± 0.009	0.064 ± 0.008	0.052 ± 0.010	0.075 ± 0.008	0.069 ± 0.011	0.059 ± 0.009
Eosinophils ($10^3/\mu L$)						
Day 3	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.06 ± 0.04	0.03 ± 0.01	0.02 ± 0.00
Day 21	0.05 ± 0.02	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
Week 14	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.06 ± 0.01
Large unstained cells ($10^3/\mu L$)						
Day 3	0.31 ± 0.05	0.27 ± 0.03	0.21 ± 0.02	0.24 ± 0.03	0.21 ± 0.03	0.27 ± 0.03
Day 21	0.31 ± 0.04	0.32 ± 0.03	0.32 ± 0.02	0.34 ± 0.03	0.31 ± 0.03	0.30 ± 0.03
Week 14	0.52 ± 0.03	0.65 ± 0.03	0.52 ± 0.05	0.74 ± 0.06*	0.70 ± 0.09	0.72 ± 0.06
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	15.8 ± 0.5[b]	13.7 ± 0.4*	13.5 ± 0.4*	14.0 ± 0.4	14.0 ± 0.6	13.6 ± 0.5*
Day 21	19.5 ± 0.7	19.5 ± 1.6	21.5 ± 0.4	17.3 ± 0.8	19.9 ± 1.1	20.3 ± 0.9
Week 14	13.6 ± 0.8	13.3 ± 0.5	14.2 ± 0.6	12.0 ± 0.5	14.5 ± 0.7	13.7 ± 0.5
Creatinine (mg/dL)						
Day 3	0.49 ± 0.01[b]	0.48 ± 0.01	0.50 ± 0.00	0.51 ± 0.02[b]	0.49 ± 0.01	0.49 ± 0.01
Day 21	0.57 ± 0.02	0.54 ± 0.02	0.53 ± 0.02	0.57 ± 0.02	0.52 ± 0.01	0.51 ± 0.01*
Week 14	0.62 ± 0.01	0.62 ± 0.02	0.65 ± 0.03	0.62 ± 0.01	0.61 ± 0.02	0.63 ± 0.02
Total protein (g/dL)						
Day 3	5.7 ± 0.1[b]	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.7 ± 0.1	5.5 ± 0.0
Day 21	6.4 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.9 ± 0.1	7.1 ± 0.2*	6.2 ± 0.1
Week 14	6.7 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.6 ± 0.0	6.8 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 3	3.9 ± 0.1[b]	3.8 ± 0.1	3.8 ± 0.1	3.9 ± 0.1	3.8 ± 0.1	3.7 ± 0.0
Day 21	4.4 ± 0.0	4.4 ± 0.1	4.5 ± 0.1	4.6 ± 0.0	4.8 ± 0.1*	4.4 ± 0.0
Week 14	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0	4.3 ± 0.0	4.3 ± 0.0	4.4 ± 0.0
Alanine aminotransferase (IU/L)						
Day 3	67 ± 2	69 ± 4	65 ± 2	69 ± 2	70 ± 3	70 ± 2
Day 21	53 ± 2	51 ± 2	50 ± 1	52 ± 2	37 ± 2**	42 ± 2**
Week 14	82 ± 11	75 ± 9	59 ± 6	46 ± 3**	48 ± 7**	43 ± 5**

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

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Alkaline phosphatase (IU/L)						
Day 3	622 ± 36	770 ± 24**	725 ± 20	744 ± 20	756 ± 30	728 ± 15
Day 21	360 ± 32	425 ± 23	410 ± 9	487 ± 12**	374 ± 9	390 ± 9
Week 14	183 ± 5	192 ± 5	178 ± 2	186 ± 7	165 ± 10	192 ± 9
Creatine kinase (IU/L)						
Day 3	349 ± 33	445 ± 107	279 ± 23	390 ± 104	382 ± 51	331 ± 30
Day 21	317 ± 53	329 ± 55	283 ± 29	286 ± 25	296 ± 26	249 ± 16
Week 14	258 ± 28	175 ± 19	235 ± 29	255 ± 38	327 ± 36	302 ± 47
Sorbitol dehydrogenase (IU/L)						
Day 3	20 ± 1	17 ± 1[b]	19 ± 1	20 ± 1	21 ± 2	19 ± 0
Day 21	21 ± 1	20 ± 1	21 ± 1	24 ± 1	23 ± 1	20 ± 1
Week 14	33 ± 2	33 ± 2	31 ± 3	25 ± 1**	28 ± 3*	23 ± 1**
Bile acids (µmol/L)						
Day 3	33.3 ± 4.5	23.3 ± 1.4[b]	35.3 ± 4.0	29.2 ± 2.9	32.2 ± 3.7	31.2 ± 2.0
Day 21	31.8 ± 4.3	26.9 ± 1.6	34.3 ± 2.0	27.7 ± 2.3	21.1 ± 1.0	29.2 ± 1.8
Week 14	25.3 ± 2.2	18.7 ± 1.4	39.1 ± 16.1	22.0 ± 1.4	34.9 ± 8.2	24.0 ± 2.4
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 3	44.5 ± 1.0	44.7 ± 0.9	45.7 ± 0.9	45.0 ± 0.8	43.9 ± 0.9	46.2 ± 0.9
Day 21	47.5 ± 0.9	48.5 ± 0.6	47.3 ± 0.6	47.3 ± 0.5	46.7 ± 0.5	45.7 ± 0.6
Week 14	44.6 ± 0.3	43.9 ± 0.5	44.2 ± 0.3	43.3 ± 0.2*	44.0 ± 0.4	43.9 ± 0.3
Hematocrit (spun) (%)						
Day 3	43.7 ± 1.0	43.8 ± 0.8	45.2 ± 0.8	44.4 ± 0.8	43.2 ± 0.8	45.6 ± 0.9
Day 21	47.5 ± 0.8	48.2 ± 0.6	47.3 ± 0.7	47.5 ± 0.5	46.6 ± 0.5	45.9 ± 0.5
Week 14	45.2 ± 0.4	44.4 ± 0.5	44.6 ± 0.4	43.9 ± 0.3	44.4 ± 0.4	44.5 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.9 ± 0.3	14.9 ± 0.3	15.2 ± 0.3	15.1 ± 0.2	14.7 ± 0.3	15.4 ± 0.3
Day 21	14.7 ± 0.3	15.0 ± 0.2	14.8 ± 0.2	14.8 ± 0.1	14.5 ± 0.1	14.3 ± 0.2
Week 14	14.3 ± 0.1	14.1 ± 0.2	14.2 ± 0.1	14.1 ± 0.1	14.1 ± 0.1	14.3 ± 0.1
Erythrocytes (10^6/µL)						
Day 3	7.46 ± 0.15	7.52 ± 0.15	7.66 ± 0.14	7.54 ± 0.13	7.41 ± 0.11	7.74 ± 0.16
Day 21	8.11 ± 0.15	8.27 ± 0.13	8.14 ± 0.08	8.09 ± 0.07	7.98 ± 0.08	7.91 ± 0.11
Week 14	8.33 ± 0.06	8.22 ± 0.10	8.28 ± 0.06	8.08 ± 0.04*	8.15 ± 0.08	8.28 ± 0.06
Reticulocytes (10^5/µL)						
Day 3	5.57 ± 0.28	5.53 ± 0.24	5.51 ± 0.26	5.56 ± 0.30	5.61 ± 0.26	5.79 ± 0.16
Day 21	2.83 ± 0.14	2.83 ± 0.09	2.76 ± 0.11	2.68 ± 0.10	2.74 ± 0.08	2.49 ± 0.09
Week 14	2.17 ± 0.08	2.40 ± 0.10	2.42 ± 0.10	2.26 ± 0.10	2.25 ± 0.07	2.33 ± 0.09
Nucleated erythrocytes (/100 leukocytes)						
Day 3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Day 21	0.30 ± 0.21	0.20 ± 0.20	0.33 ± 0.17	0.20 ± 0.13	0.30 ± 0.15	0.50 ± 0.27
Week 14	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.20 ± 0.20	0.10 ± 0.10	0.00 ± 0.00

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

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Mean cell volume (fL)						
Day 3	59.7 ± 0.3	59.5 ± 0.3	59.6 ± 0.3	59.7 ± 0.3	59.3 ± 0.4	59.6 ± 0.3
Day 21	58.6 ± 0.3	58.7 ± 0.4	58.1 ± 0.3	58.5 ± 0.3	58.5 ± 0.3	57.8 ± 0.4
Week 14	53.5 ± 0.1	53.4 ± 0.1	53.5 ± 0.1	53.6 ± 0.2	53.9 ± 0.1	53.0 ± 0.1
Mean cell hemoglobin (pg)						
Day 3	20.0 ± 0.1	19.8 ± 0.1	19.9 ± 0.1	20.0 ± 0.1	19.9 ± 0.1	19.8 ± 0.1
Day 21	18.1 ± 0.1	18.2 ± 0.2	18.1 ± 0.1	18.3 ± 0.2	18.1 ± 0.1	18.1 ± 0.1
Week 14	17.2 ± 0.1	17.1 ± 0.1	17.2 ± 0.1	17.4 ± 0.1	17.3 ± 0.1	17.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	33.5 ± 0.2	33.3 ± 0.2	33.4 ± 0.2	33.5 ± 0.2	33.6 ± 0.1	33.3 ± 0.2
Day 21	31.0 ± 0.1	31.0 ± 0.1	31.2 ± 0.1	31.3 ± 0.3	31.1 ± 0.1	31.3 ± 0.2
Week 14	32.1 ± 0.1	32.0 ± 0.1	32.1 ± 0.2	32.5 ± 0.2	32.1 ± 0.1	32.5 ± 0.2
Platelets ($10^3/\mu L$)						
Day 3	795.1 ± 28.1	878.0 ± 41.0	874.0 ± 17.6	830.9 ± 19.2	814.0 ± 27.9	801.0 ± 27.8
Day 21	666.5 ± 18.3	661.0 ± 16.5	662.2 ± 34.2	646.9 ± 16.4	674.2 ± 24.7	651.9 ± 18.7
Week 14	623.9 ± 12.5	596.1 ± 15.5	632.7 ± 11.0	627.1 ± 25.6	620.4 ± 9.1	619.5 ± 14.7
Leukocytes ($10^3/\mu L$)						
Day 3	7.88 ± 0.55	7.91 ± 0.60	8.55 ± 0.56	7.25 ± 0.60	6.68 ± 0.61	8.25 ± 0.55
Day 21	10.15 ± 0.46	10.65 ± 0.25	10.01 ± 0.25	9.94 ± 0.37	9.86 ± 0.39	9.57 ± 0.56
Week 14	7.03 ± 0.34	7.00 ± 0.43	7.58 ± 0.22	6.75 ± 0.39	7.46 ± 0.22	6.66 ± 0.37
Segmented neutrophils ($10^3/\mu L$)						
Day 3	0.64 ± 0.05	0.63 ± 0.06	0.77 ± 0.09	0.60 ± 0.05	0.59 ± 0.05	0.72 ± 0.06
Day 21	0.77 ± 0.03	0.86 ± 0.03	0.74 ± 0.06	0.91 ± 0.05*	1.01 ± 0.08**	0.93 ± 0.08*
Week 14	1.08 ± 0.07	1.15 ± 0.08	1.12 ± 0.06	1.07 ± 0.05	1.16 ± 0.06	1.22 ± 0.11
Lymphocytes ($10^3/\mu L$)						
Day 3	6.76 ± 0.46	6.86 ± 0.50	7.30 ± 0.44	6.23 ± 0.51	5.75 ± 0.53	7.07 ± 0.46
Day 21	8.83 ± 0.42	9.25 ± 0.22	8.75 ± 0.20	8.43 ± 0.35	8.26 ± 0.32	7.96 ± 0.43
Week 14	5.02 ± 0.29	4.89 ± 0.35	5.56 ± 0.22	4.77 ± 0.33	5.42 ± 0.17	4.46 ± 0.23
Monocytes ($10^3/\mu L$)						
Day 3	0.12 ± 0.01	0.12 ± 0.01	0.14 ± 0.02	0.12 ± 0.01	0.10 ± 0.01	0.14 ± 0.02
Day 21	0.17 ± 0.02	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.17 ± 0.01
Week 14	0.10 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
Basophils ($10^3/\mu L$)						
Day 3	0.032 ± 0.006	0.028 ± 0.004	0.037 ± 0.005	0.029 ± 0.005	0.030 ± 0.006	0.038 ± 0.006
Day 21	0.041 ± 0.004	0.037 ± 0.002	0.050 ± 0.005	0.050 ± 0.008	0.043 ± 0.005	0.043 ± 0.007
Week 14	0.056 ± 0.008	0.052 ± 0.006	0.060 ± 0.009	0.049 ± 0.005	0.051 ± 0.006	0.051 ± 0.009
Eosinophils ($10^3/\mu L$)						
Day 3	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.03 ± 0.00
Day 21	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.00
Week 14	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.06 ± 0.00	0.06 ± 0.00	0.07 ± 0.01
Large unstained cells ($10^3/\mu L$)						
Day 3	0.29 ± 0.04	0.25 ± 0.03	0.28 ± 0.03	0.23 ± 0.03	0.19 ± 0.03	0.26 ± 0.03
Day 21	0.31 ± 0.03	0.32 ± 0.02	0.31 ± 0.02	0.37 ± 0.05	0.33 ± 0.02	0.43 ± 0.06
Week 14	0.72 ± 0.06	0.75 ± 0.09	0.71 ± 0.04	0.71 ± 0.04	0.68 ± 0.03	0.67 ± 0.08

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

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Female (continued)						
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	9	10	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	14.9 ± 0.8	13.3 ± 0.6	13.1 ± 0.3	13.8 ± 0.8	13.7 ± 0.4	12.8 ± 0.6
Day 21	14.4 ± 0.4	15.6 ± 0.4	15.1 ± 0.5	14.8 ± 0.5	14.4 ± 0.3	13.8 ± 0.3
Week 14	13.6 ± 0.5	14.3 ± 0.5	13.8 ± 0.3	14.3 ± 0.4	13.0 ± 0.3	12.7 ± 0.2
Creatinine (mg/dL)						
Day 3	0.44 ± 0.02	0.46 ± 0.02	0.43 ± 0.02	0.44 ± 0.02	0.46 ± 0.02	0.41 ± 0.01
Day 21	0.54 ± 0.02	0.54 ± 0.02	0.51 ± 0.02	0.54 ± 0.02	0.52 ± 0.01	0.51 ± 0.01
Week 14	0.63 ± 0.02	0.59 ± 0.01	0.58 ± 0.01*	0.57 ± 0.02*	0.56 ± 0.02**	0.55 ± 0.02**
Total protein (g/dL)						
Day 3	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.8 ± 0.1	5.7 ± 0.1	5.5 ± 0.1[b]
Day 21	6.2 ± 0.1	6.3 ± 0.1	6.4 ± 0.1	6.2 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
Week 14	6.9 ± 0.2	6.6 ± 0.2	6.8 ± 0.1	6.8 ± 0.1	6.9 ± 0.1	7.2 ± 0.1
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.0 ± 0.0	3.9 ± 0.1	3.9 ± 0.1
Day 21	4.4 ± 0.1	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.6 ± 0.0*	4.6 ± 0.0**
Week 14	4.7 ± 0.1	4.5 ± 0.1	4.7 ± 0.1	4.8 ± 0.1	4.7 ± 0.1	5.0 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	56 ± 2	52 ± 3	57 ± 2	55 ± 2	56 ± 1	58 ± 3
Day 21	41 ± 1	40 ± 1	40 ± 1	38 ± 2	36 ± 1**	37 ± 1**
Week 14	53 ± 7	68 ± 8	55 ± 6	45 ± 4	33 ± 4	31 ± 2*
Alkaline phosphatase (IU/L)						
Day 3	550 ± 24	555 ± 20	552 ± 12	552 ± 17	592 ± 18	584 ± 14
Day 21	363 ± 8	365 ± 18	359 ± 10	355 ± 13	357 ± 12	334 ± 12
Week 14	140 ± 5	147 ± 5	146 ± 7	143 ± 2	139 ± 3	135 ± 2
Creatine kinase (IU/L)						
Day 3	352 ± 89	317 ± 36	485 ± 107	432 ± 73	281 ± 33	433 ± 69
Day 21	242 ± 24	203 ± 15	299 ± 29	278 ± 29	262 ± 27	241 ± 28
Week 14	277 ± 31	267 ± 34	248 ± 29	284 ± 34	263 ± 30	291 ± 33
Sorbitol dehydrogenase (IU/L)						
Day 3	23 ± 1	20 ± 1	24 ± 2	22 ± 2	20 ± 1	20 ± 1
Day 21	23 ± 1	23 ± 1	21 ± 1	24 ± 2	21 ± 1	22 ± 1
Week 14	30 ± 2	36 ± 4	27 ± 2	24 ± 1	23 ± 2*	18 ± 1**
Bile acids (µmol/L)						
Day 3	21.6 ± 2.2	18.4 ± 1.1	22.7 ± 2.9	19.1 ± 2.2	23.4 ± 2.1	23.5 ± 1.8
Day 21	19.1 ± 1.8	17.4 ± 1.1	18.2 ± 1.9	21.5 ± 1.5	20.6 ± 2.1	19.5 ± 2.4
Week 14	24.6 ± 2.3	30.2 ± 3.8	30.9 ± 3.5	22.1 ± 2.6	28.0 ± 1.9	24.0 ± 2.1

* 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

Table F2
Hematology Data for Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Male						
n	10	10	10	10	9	10
Hematocrit (auto) (%)	52.0 ± 0.5	51.6 ± 0.4	51.8 ± 0.6	51.6 ± 0.6	52.1 ± 0.5	51.4 ± 0.5
Hematocrit (spun) (%)	51.1 ± 0.4	50.8 ± 0.5	51.0 ± 0.6	51.2 ± 0.7	51.3 ± 0.6	50.9 ± 0.6
Hemoglobin (g/dL)	17.6 ± 0.3	17.1 ± 0.1	17.1 ± 0.2	17.3 ± 0.2	17.5 ± 0.3	17.2 ± 0.2
Erythrocytes ($10^6/\mu L$)	11.13 ± 0.12	11.09 ± 0.10	10.98 ± 0.14	11.04 ± 0.12	11.15 ± 0.14	11.01 ± 0.10
Reticulocytes ($10^5/\mu L$)	4.15 ± 0.07	4.07 ± 0.10	4.32 ± 0.11	4.09 ± 0.13	4.26 ± 0.08	4.03 ± 0.11
Nucleated erythrocytes (/100 leukocytes)	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.15	0.00 ± 0.00
Mean cell volume (fL)	46.8 ± 0.2	46.6 ± 0.1	47.2 ± 0.2	46.8 ± 0.2	46.7 ± 0.2	46.6 ± 0.1
Mean cell hemoglobin (pg)	15.8 ± 0.2	15.4 ± 0.1	15.6 ± 0.1	15.7 ± 0.1	15.7 ± 0.2	15.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.5	33.1 ± 0.2	33.1 ± 0.2	33.5 ± 0.2	33.6 ± 0.3	33.5 ± 0.1
Platelets ($10^3/\mu L$)	828.7 ± 49.5	828.4 ± 50.4	825.6 ± 49.5	826.3 ± 54.9	788.1 ± 54.7	784.8 ± 38.2
Leukocytes ($10^3/\mu L$)	5.38 ± 0.23	5.23 ± 0.23	4.96 ± 0.31	4.79 ± 0.29	5.11 ± 0.52	5.07 ± 0.19
Segmented neutrophils ($10^3/\mu L$)	0.55 ± 0.05	0.50 ± 0.04	0.48 ± 0.03	0.47 ± 0.02	0.47 ± 0.05	0.49 ± 0.03
Lymphocytes ($10^3/\mu L$)	4.50 ± 0.19	4.45 ± 0.21	4.18 ± 0.28	3.95 ± 0.26	4.31 ± 0.45	4.27 ± 0.16
Monocytes ($10^3/\mu L$)	0.06 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
Basophils ($10^3/\mu L$)	0.018 ± 0.002	0.013 ± 0.003	0.015 ± 0.002	0.012 ± 0.002	0.016 ± 0.006	0.017 ± 0.003
Eosinophils ($10^3/\mu L$)	0.08 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.14 ± 0.06	0.09 ± 0.02	0.09 ± 0.01
Large unstained cells ($10^3/\mu L$)	0.16 ± 0.02	0.14 ± 0.01	0.16 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.16 ± 0.01
Female						
n	9	10	10	10	10	10
Hematocrit (auto) (%)	50.0 ± 1.0	48.9 ± 0.7	48.1 ± 0.6	48.1 ± 0.6	50.5 ± 1.4	48.3 ± 0.7
Hematocrit (spun) (%)	49.8 ± 0.7[b]	48.9 ± 0.8	48.9 ± 0.6	48.7 ± 0.7	50.7 ± 1.5	47.9 ± 0.9
Hemoglobin (g/dL)	17.0 ± 0.4	16.3 ± 0.2	16.3 ± 0.2	16.3 ± 0.2	16.9 ± 0.4	16.3 ± 0.3
Erythrocytes ($10^6/\mu L$)	10.96 ± 0.30	10.48 ± 0.13	10.38 ± 0.12	10.40 ± 0.13	10.86 ± 0.25	10.49 ± 0.17
Reticulocytes ($10^5/\mu L$)	4.33 ± 0.14[c]	4.15 ± 0.18	4.08 ± 0.26	4.21 ± 0.22	4.06 ± 0.16	4.10 ± 0.15
Nucleated erythrocytes (/100 leukocytes)	0.10 ± 0.10[b]	0.20 ± 0.13	0.10 ± 0.10	0.10 ± 0.10	0.10 ± 0.10	0.00 ± 0.00
Mean cell volume (fL)	45.7 ± 0.3	46.6 ± 0.3	46.4 ± 0.2	46.3 ± 0.2	46.4 ± 0.2	46.0 ± 0.1
Mean cell hemoglobin (pg)	15.6 ± 0.1	15.6 ± 0.1	15.7 ± 0.1	15.7 ± 0.1	15.6 ± 0.1	15.5 ± 0.1
Mean cell hemoglobin concentration (g/dL)	34.0 ± 0.2	33.4 ± 0.2	33.9 ± 0.2	33.9 ± 0.1	33.5 ± 0.3	33.7 ± 0.2
Platelets ($10^3/\mu L$)	838.3 ± 41.8	863.5 ± 50.7	847.8 ± 44.1	786.6 ± 52.6	722.9 ± 68.7	788.9 ± 68.5
Leukocytes ($10^3/\mu L$)	4.55 ± 0.34	4.83 ± 0.49	4.39 ± 0.31	4.90 ± 0.41	3.96 ± 0.33	4.57 ± 0.46
Segmented neutrophils ($10^3/\mu L$)	0.51 ± 0.07	0.47 ± 0.06	0.43 ± 0.04	0.60 ± 0.10	0.36 ± 0.04	0.61 ± 0.06
Lymphocytes ($10^3/\mu L$)	3.73 ± 0.29	4.01 ± 0.40	3.66 ± 0.27	4.00 ± 0.31	3.36 ± 0.29	3.68 ± 0.40
Monocytes ($10^3/\mu L$)	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
Basophils ($10^3/\mu L$)	0.017 ± 0.003	0.017 ± 0.003	0.014 ± 0.003	0.013 ± 0.003	0.017 ± 0.004	0.009 ± 0.001
Eosinophils ($10^3/\mu L$)	0.09 ± 0.01	0.08 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01**	0.07 ± 0.01
Large unstained cells ($10^3/\mu L$)	0.15 ± 0.02	0.19 ± 0.03	0.17 ± 0.01	0.16 ± 0.02	0.13 ± 0.01	0.16 ± 0.01

** Significantly different ($P \leq 0.01$) from the control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=8

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 Study of Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	5	5	5	5	5	5
Male						
Necropsy body wt	150 ± 4	143 ± 4	152 ± 5	156 ± 4	155 ± 2	144 ± 4
Heart						
Absolute	0.566 ± 0.016	0.532 ± 0.017	0.552 ± 0.022	0.564 ± 0.013	0.558 ± 0.007	0.546 ± 0.013
Relative	3.768 ± 0.063	3.719 ± 0.084	3.624 ± 0.062	3.625 ± 0.101	3.597 ± 0.056	3.798 ± 0.052
R. Kidney						
Absolute	0.662 ± 0.014	0.626 ± 0.019	0.670 ± 0.024	0.698 ± 0.034	0.698 ± 0.012	0.728 ± 0.010*
Relative	4.411 ± 0.079	4.376 ± 0.076	4.399 ± 0.044	4.471 ± 0.123	4.496 ± 0.033	5.070 ± 0.102**
Liver						
Absolute	7.638 ± 0.318	7.118 ± 0.195	7.510 ± 0.364	8.028 ± 0.324	8.488 ± 0.246	7.914 ± 0.136
Relative	50.785 ± 1.162	49.758 ± 0.589	49.237 ± 0.969	51.432 ± 0.774	54.635 ± 0.836*	55.172 ± 1.704**
Lung						
Absolute	1.314 ± 0.153	0.933 ± 0.035*[b]	0.950 ± 0.054*[b]	0.988 ± 0.041	1.064 ± 0.078	1.024 ± 0.082
Relative	8.734 ± 0.980	6.487 ± 0.147*[b]	6.182 ± 0.310*[b]	6.343 ± 0.228*	6.853 ± 0.485	7.076 ± 0.372
R. Testis						
Absolute	0.703 ± 0.073	0.634 ± 0.069	0.621 ± 0.100	0.720 ± 0.101	0.817 ± 0.054	0.596 ± 0.028
Relative	4.646 ± 0.417	4.397 ± 0.353	4.023 ± 0.510	4.568 ± 0.542	5.253 ± 0.283	4.145 ± 0.160
Thymus						
Absolute	0.431 ± 0.024	0.425 ± 0.008	0.435 ± 0.029	0.391 ± 0.025	0.439 ± 0.010	0.411 ± 0.017
Relative	2.873 ± 0.155	2.986 ± 0.127	2.856 ± 0.169	2.528 ± 0.222	2.830 ± 0.084	2.861 ± 0.098
Female						
Necropsy body wt	123 ± 3	120 ± 2	121 ± 2	124 ± 2	126 ± 3	121 ± 3
Heart						
Absolute	0.456 ± 0.010	0.432 ± 0.008	0.470 ± 0.011	0.446 ± 0.008	0.470 ± 0.012	0.460 ± 0.010
Relative	3.708 ± 0.060	3.601 ± 0.094	3.899 ± 0.106	3.610 ± 0.047	3.719 ± 0.024	3.801 ± 0.048
R. Kidney						
Absolute	0.550 ± 0.028	0.538 ± 0.015	0.560 ± 0.011	0.542 ± 0.010	0.622 ± 0.029	0.602 ± 0.039
Relative	4.459 ± 0.126	4.483 ± 0.130	4.647 ± 0.121	4.386 ± 0.038	4.912 ± 0.112	4.965 ± 0.264
Liver						
Absolute	5.462 ± 0.190	5.304 ± 0.191	5.288 ± 0.194	5.362 ± 0.201	6.048 ± 0.200	5.954 ± 0.230
Relative	44.350 ± 0.730	44.168 ± 1.481	43.827 ± 1.398	43.361 ± 1.203	47.840 ± 0.839	49.144 ± 1.226**
Lung						
Absolute	0.876 ± 0.051	0.822 ± 0.043	0.820 ± 0.056	0.786 ± 0.039	0.852 ± 0.032	0.834 ± 0.053
Relative	7.104 ± 0.329	6.857 ± 0.401	6.779 ± 0.356	6.359 ± 0.280	6.754 ± 0.271	6.894 ± 0.433
Thymus						
Absolute	0.368 ± 0.005	0.345 ± 0.010	0.333 ± 0.009	0.331 ± 0.016	0.335 ± 0.012	0.328 ± 0.011
Relative	2.995 ± 0.062	2.877 ± 0.100	2.761 ± 0.068	2.687 ± 0.154	2.652 ± 0.092	2.713 ± 0.115

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** Significantly different (P≤0.01) from the control group by Williams' test
a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
b n=4

Table G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	334 ± 4	311 ± 9	333 ± 12	341 ± 5	349 ± 8	342 ± 7
Heart						
Absolute	0.856 ± 0.015	0.793 ± 0.024	0.814 ± 0.030	0.852 ± 0.017	0.896 ± 0.021	0.878 ± 0.022
Relative	2.562 ± 0.033	2.549 ± 0.028	2.446 ± 0.026*	2.500 ± 0.035	2.566 ± 0.020	2.565 ± 0.028
R. Kidney						
Absolute	0.933 ± 0.015	0.881 ± 0.029	0.925 ± 0.029	0.961 ± 0.018	1.025 ± 0.036*	1.095 ± 0.025**
Relative	2.794 ± 0.041	2.828 ± 0.036	2.790 ± 0.068	2.819 ± 0.031	2.933 ± 0.061	3.200 ± 0.038**
Liver						
Absolute	9.644 ± 0.203	9.101 ± 0.397	10.300 ± 0.468	10.447 ± 0.179	11.766 ± 0.382**	12.845 ± 0.417**
Relative	28.857 ± 0.444	29.128 ± 0.522	30.876 ± 0.462*	30.654 ± 0.277*	33.664 ± 0.638**	37.473 ± 0.616**
Lung						
Absolute	1.462 ± 0.070	1.377 ± 0.068	1.360 ± 0.041	1.392 ± 0.047	1.436 ± 0.047	1.484 ± 0.044
Relative	4.366 ± 0.179	4.409 ± 0.130	4.120 ± 0.164	4.087 ± 0.132	4.113 ± 0.100	4.342 ± 0.126
R. Testis						
Absolute	1.388 ± 0.030	1.164 ± 0.116*	1.374 ± 0.025	1.443 ± 0.029	1.454 ± 0.030	1.419 ± 0.043
Relative	4.152 ± 0.069	3.673 ± 0.317	4.157 ± 0.107	4.236 ± 0.082	4.171 ± 0.067	4.144 ± 0.087
Thymus						
Absolute	0.260 ± 0.008	0.263 ± 0.012	0.266 ± 0.013	0.249 ± 0.008	0.252 ± 0.010	0.249 ± 0.010
Relative	0.780 ± 0.025	0.845 ± 0.035	0.805 ± 0.040	0.731 ± 0.023	0.725 ± 0.030	0.726 ± 0.020
Female						
Necropsy body wt	191 ± 3	197 ± 4	198 ± 2	193 ± 5	194 ± 3	191 ± 3
Heart						
Absolute	0.566 ± 0.011	0.573 ± 0.013	0.565 ± 0.010	0.569 ± 0.013	0.567 ± 0.011	0.552 ± 0.012
Relative	2.963 ± 0.039	2.909 ± 0.052	2.852 ± 0.039	2.957 ± 0.047	2.922 ± 0.053	2.892 ± 0.029
R. Kidney						
Absolute	0.620 ± 0.012	0.628 ± 0.017	0.625 ± 0.007	0.620 ± 0.017	0.639 ± 0.014	0.658 ± 0.012
Relative	3.247 ± 0.046	3.184 ± 0.047	3.159 ± 0.059	3.217 ± 0.038	3.290 ± 0.037	3.452 ± 0.058**
Liver						
Absolute	5.679 ± 0.114	5.832 ± 0.232	5.852 ± 0.143	6.084 ± 0.179	6.429 ± 0.147**	6.896 ± 0.184**
Relative	29.722 ± 0.302	29.530 ± 0.817	29.594 ± 0.903	31.576 ± 0.520	33.133 ± 0.677**	36.110 ± 0.598**
Lung						
Absolute	1.047 ± 0.029	1.063 ± 0.055	0.983 ± 0.024	1.016 ± 0.034	1.038 ± 0.041	0.924 ± 0.034
Relative	5.487 ± 0.153	5.387 ± 0.252	4.970 ± 0.147	5.272 ± 0.121	5.340 ± 0.175	4.834 ± 0.126*
Thymus						
Absolute	0.216 ± 0.009	0.216 ± 0.007	0.212 ± 0.008	0.200 ± 0.010	0.211 ± 0.010	0.199 ± 0.010
Relative	1.130 ± 0.036	1.094 ± 0.028	1.070 ± 0.035	1.038 ± 0.049	1.090 ± 0.055	1.043 ± 0.044

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

Table G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	5	5	5	5	5	5
Male						
Necropsy body wt	24.3 ± 0.2	24.7 ± 0.4	24.9 ± 0.4	26.0 ± 0.4*	25.3 ± 0.4	24.5 ± 0.4
Heart						
Absolute	0.130 ± 0.003	0.124 ± 0.008	0.120 ± 0.003	0.130 ± 0.004	0.120 ± 0.003	0.124 ± 0.005
Relative	5.343 ± 0.137	5.005 ± 0.276	4.833 ± 0.189	5.006 ± 0.220	4.741 ± 0.127	5.062 ± 0.199
R. Kidney						
Absolute	0.228 ± 0.015	0.244 ± 0.013	0.240 ± 0.007	0.268 ± 0.011	0.248 ± 0.009	0.252 ± 0.008
Relative	9.350 ± 0.536	9.856 ± 0.444	9.649 ± 0.261	10.294 ± 0.351	9.789 ± 0.285	10.279 ± 0.229
Liver						
Absolute	1.310 ± 0.033	1.316 ± 0.059	1.368 ± 0.037	1.400 ± 0.034	1.416 ± 0.043	1.406 ± 0.045
Relative	53.800 ± 1.009	53.157 ± 1.669	54.967 ± 1.025	53.779 ± 0.673	55.888 ± 1.119	57.332 ± 1.089
Lung						
Absolute	0.164 ± 0.007	0.154 ± 0.007	0.162 ± 0.004	0.164 ± 0.007	0.158 ± 0.002	0.158 ± 0.005
Relative	6.734 ± 0.242	6.228 ± 0.280	6.524 ± 0.231	6.299 ± 0.220	6.244 ± 0.102	6.445 ± 0.144
R. Testis						
Absolute	0.099 ± 0.003	0.099 ± 0.004	0.098 ± 0.001	0.101 ± 0.003	0.098 ± 0.004	0.099 ± 0.001
Relative	4.077 ± 0.110	4.016 ± 0.203	3.959 ± 0.072	3.895 ± 0.091	3.877 ± 0.196	4.034 ± 0.038
Thymus						
Absolute	0.052 ± 0.002	0.053 ± 0.002	0.046 ± 0.005	0.053 ± 0.002	0.056 ± 0.003	0.051 ± 0.002
Relative	2.147 ± 0.091	2.135 ± 0.076	1.867 ± 0.219	2.022 ± 0.068	2.201 ± 0.081	2.098 ± 0.056
Female						
Necropsy body wt	20.7 ± 0.3	20.5 ± 0.6	21.6 ± 0.3	21.4 ± 0.4	21.2 ± 0.2	21.2 ± 0.3
Heart						
Absolute	0.110 ± 0.004	0.108 ± 0.007	0.112 ± 0.005	0.110 ± 0.000	0.116 ± 0.004	0.112 ± 0.006
Relative	5.321 ± 0.272	5.254 ± 0.279	5.172 ± 0.195	5.147 ± 0.098	5.472 ± 0.139	5.264 ± 0.219
R. Kidney						
Absolute	0.156 ± 0.010	0.158 ± 0.004	0.166 ± 0.004	0.162 ± 0.002	0.170 ± 0.004	0.170 ± 0.009
Relative	7.538 ± 0.510	7.703 ± 0.182	7.674 ± 0.191	7.579 ± 0.153	8.023 ± 0.143	7.990 ± 0.338
Liver						
Absolute	1.006 ± 0.027	0.970 ± 0.038	1.048 ± 0.047	1.032 ± 0.027	1.032 ± 0.027	1.060 ± 0.027
Relative	48.535 ± 0.864	47.181 ± 0.951	48.371 ± 1.743	48.222 ± 0.888	48.700 ± 0.864	49.949 ± 1.436
Lung						
Absolute	0.144 ± 0.004	0.196 ± 0.030	0.172 ± 0.017	0.152 ± 0.005	0.152 ± 0.004	0.142 ± 0.006
Relative	6.964 ± 0.276	9.521 ± 1.398*	7.934 ± 0.729	7.102 ± 0.179	7.177 ± 0.163	6.680 ± 0.210
Thymus						
Absolute	0.061 ± 0.005	0.068 ± 0.002	0.070 ± 0.003	0.070 ± 0.004	0.065 ± 0.003	0.068 ± 0.002
Relative	2.960 ± 0.209	3.325 ± 0.119	3.214 ± 0.116	3.265 ± 0.219	3.049 ± 0.105	3.211 ± 0.160

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

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

Table G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	62.5 mg/L	125 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body wt	40.0 ± 1.0	40.1 ± 1.3	42.4 ± 1.3	41.2 ± 1.1	39.6 ± 0.8	39.3 ± 1.0
Heart						
Absolute	0.150 ± 0.004	0.152 ± 0.003	0.153 ± 0.003	0.151 ± 0.004	0.152 ± 0.003	0.145 ± 0.004
Relative	3.757 ± 0.072	3.812 ± 0.084	3.627 ± 0.068	3.664 ± 0.038	3.841 ± 0.076	3.686 ± 0.066
R. Kidney						
Absolute	0.295 ± 0.008	0.308 ± 0.008	0.334 ± 0.011*	0.312 ± 0.005	0.305 ± 0.009	0.291 ± 0.009
Relative	7.395 ± 0.188	7.713 ± 0.166	7.893 ± 0.126	7.603 ± 0.160	7.699 ± 0.204	7.389 ± 0.089
Liver						
Absolute	1.524 ± 0.064	1.590 ± 0.069	1.743 ± 0.097	1.698 ± 0.107	1.688 ± 0.054	1.802 ± 0.081*
Relative	38.059 ± 0.966	39.646 ± 0.937	40.946 ± 1.320	40.904 ± 1.445	42.538 ± 0.812**	45.632 ± 1.002**
Lung						
Absolute	0.280 ± 0.011	0.296 ± 0.010	0.278 ± 0.016	0.280 ± 0.013	0.285 ± 0.010	0.290 ± 0.011
Relative	7.004 ± 0.226	7.472 ± 0.361	6.624 ± 0.420	6.813 ± 0.319	7.226 ± 0.306	7.386 ± 0.275
R. Testis						
Absolute	0.119 ± 0.002	0.118 ± 0.003	0.119 ± 0.003	0.118 ± 0.001	0.115 ± 0.003	0.122 ± 0.002
Relative	2.992 ± 0.089	2.959 ± 0.070	2.822 ± 0.075	2.887 ± 0.071	2.901 ± 0.060	3.098 ± 0.052
Thymus						
Absolute	0.036 ± 0.001	0.040 ± 0.002	0.040 ± 0.002	0.039 ± 0.002	0.036 ± 0.002	0.037 ± 0.001
Relative	0.899 ± 0.034	0.984 ± 0.033	0.943 ± 0.035	0.930 ± 0.036	0.906 ± 0.045	0.938 ± 0.017
Female						
Necropsy body wt	29.9 ± 1.1	32.7 ± 1.0	29.7 ± 1.0	29.0 ± 0.9	29.9 ± 1.2	29.0 ± 0.8
Heart						
Absolute	0.113 ± 0.004	0.127 ± 0.003*	0.121 ± 0.003	0.121 ± 0.004	0.123 ± 0.003	0.126 ± 0.003
Relative	3.796 ± 0.088	3.911 ± 0.146	4.099 ± 0.123	4.175 ± 0.119	4.176 ± 0.198	4.359 ± 0.122**
R. Kidney						
Absolute	0.166 ± 0.006	0.187 ± 0.004*	0.179 ± 0.005	0.176 ± 0.005	0.169 ± 0.004	0.181 ± 0.004
Relative	5.595 ± 0.177	5.751 ± 0.168	6.060 ± 0.199	6.082 ± 0.156	5.734 ± 0.278	6.269 ± 0.186
Liver						
Absolute	1.042 ± 0.055	1.269 ± 0.043**	1.180 ± 0.039**	1.176 ± 0.029**	1.323 ± 0.038**	1.371 ± 0.035**
Relative	34.835 ± 1.103	38.899 ± 1.224**	39.805 ± 0.713**	40.596 ± 0.590**	44.574 ± 1.423**	47.317 ± 0.632**
Lung						
Absolute	0.188 ± 0.005	0.224 ± 0.013	0.222 ± 0.016	0.224 ± 0.014	0.259 ± 0.019**	0.217 ± 0.015
Relative	6.358 ± 0.236	6.915 ± 0.481	7.563 ± 0.632	7.811 ± 0.600	8.812 ± 0.740*	7.545 ± 0.569
Thymus						
Absolute	0.043 ± 0.002	0.049 ± 0.003	0.041 ± 0.003	0.040 ± 0.001	0.045 ± 0.002	0.043 ± 0.003
Relative	1.439 ± 0.059	1.508 ± 0.069	1.390 ± 0.080	1.389 ± 0.058	1.514 ± 0.043	1.482 ± 0.086

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

APPENDIX H
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

Table H1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	334 ± 4	341 ± 5	349 ± 8	342 ± 7
L. Cauda epididymis	0.1796 ± 0.0050	0.1847 ± 0.0070	0.1868 ± 0.0067	0.1768 ± 0.0042
L. Epididymis	0.4568 ± 0.0074	0.4914 ± 0.0362	0.5610 ± 0.0951	0.4559 ± 0.0113
L. Testis	1.4806 ± 0.0308	1.5474 ± 0.0266	1.5561 ± 0.0300	1.5315 ± 0.0376
Spermatid measurements				
Spermatid heads (10^6/g testis)	137.1 ± 4.5	129.9 ± 3.1	128.4 ± 4.8	134.5 ± 4.4
Spermatid heads (10^6/testis)	187.5 ± 7.8	185.4 ± 3.6	183.8 ± 7.2	188.4 ± 7.6
Epididymal spermatozoal measurements				
Motility (%)	73.76 ± 1.46	72.11 ± 1.95	73.41 ± 1.46	68.21 ± 1.93
Sperm (10^6/g cauda epididymis)	663.1 ± 25.4	670.9 ± 27.8	618.7 ± 42.9	530.9 ± 55.3
Sperm (10^6/cauda epididymis)	119 ± 6	124 ± 7	115 ± 8	108 ± 7

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

Table H2
Estrous Cycle Characterization for Female Rats in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	191 ± 3	193 ± 5	194 ± 3	191 ± 3
Proportion regular cycling females[b]	10/10	10/10	10/10	9/10
Estrous cycle length (days)	4.95 ± 0.05	5.15 ± 0.17	5.15 ± 0.11	5.25 ± 0.11
Estrous stages (% of cycle)				
Diestrus	40.0	36.7	35.0	38.3
Proestrus	12.5	17.5	17.5	16.7
Estrus	30.0	26.7	29.2	25.0
Metestrus	17.5	19.2	18.3	20.0

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight), Dunn's test (estrous cycle length), or Fisher's exact test (proportion of regular cycling females). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

Table H3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	40.0 ± 1.0	41.2 ± 1.1	39.6 ± 0.8	39.3 ± 1.0
L. Cauda epididymis	0.0228 ± 0.0021	0.0189 ± 0.0020	0.0216 ± 0.0017	0.0230 ± 0.0008
L. Epididymis	0.0537 ± 0.0020	0.0549 ± 0.0029	0.0525 ± 0.0014	0.0549 ± 0.0021
L. Testis	0.1196 ± 0.0015	0.1222 ± 0.0021	0.1145 ± 0.0037	0.1201 ± 0.0015
Spermatid measurements				
Spermatid heads (10^6/g testis)	161.9 ± 10.2	202.1 ± 6.2*	176.7 ± 7.5	186.5 ± 14.0
Spermatid heads (10^6/testis)	17.30 ± 1.04	21.50 ± 0.94*	17.94 ± 1.09	19.35 ± 1.30
Epididymal spermatozoal measurements				
Motility (%)	75.76 ± 1.40	72.74 ± 1.46	74.61 ± 2.02	71.43 ± 1.82
Sperm heads (10^6/g cauda epididymis)	1,102 ± 116	1,449 ± 256	1,186 ± 115	1,072 ± 71
Sperm heads (10^6/cauda epididymis)	23 ± 1	23 ± 2	24 ± 1	25 ± 2

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body and tissue weights) or Dunn's test (epididymal spermatozoal measurements).

Table H4
Estrous Cycle Characterization for Female Mice in the 3-Month Drinking Water Study of Bromochloroacetic Acid[a]

	0 mg/L	250 mg/L	500 mg/L	1,000 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	29.9 ± 1.1	29.0 ± 0.9	29.9 ± 1.2	29.0 ± 0.8
Proportion regular cycling females[b]	6/10	9/10	7/10	9/10
Estrous cycle length (days)	4.95 ± 0.42	4.17 ± 0.17[c]	4.00 ± 0.20	4.30 ± 0.13
Estrous stages (% of cycle)				
Diestrus	31.7	36.7	30.8	31.7
Proestrus	17.5	13.3	17.5	15.8
Estrus	30.8	27.5	31.7	30.0
Metestrus	20.0	22.5	20.0	22.5

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weight), Dunn's test (estrous cycle length) or Fisher's exact test (proportion of regular cycling females). By multivariate analysis of variance, exposed females do not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or under in one of 10 animals.

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF BROMOCHLOROACETIC ACID

Bromochloroacetic acid was obtained from Carbolabs, Inc. (Woodbridge, CT), in two lots (II-37A and 11388A). Lot II-37A was used in the 2-week and 3-month studies and lot 11388A was used in the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Columbus Operations (Columbus, OH); identity and purity analyses were also conducted by the study laboratory, Southern Research Institute (Birmingham, AL). Karl Fischer titration was performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the bromochloroacetic acid studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a waxy, off-white solid, were identified as bromochloroacetic acid by the analytical chemistry laboratory and the study laboratory using infrared and proton nuclear magnetic resonance (NMR) spectroscopy; the analytical chemistry laboratory also used carbon-13 NMR spectroscopy. All spectra were consistent with the literature spectra (*Aldrich,* 1997) of other haloacetic acids, computer generated spectra, and the structure of bromochloroacetic acid. Representative infrared, proton NMR, and carbon-13 NMR spectra are presented in Figures I1, I2, and I3, respectively.

The water content of both lots was determined by Karl Fischer titration. The purity of lots II-37A and 11388A was determined by the analytical chemistry laboratory using functional group titration and high-performance liquid chromatography (HPLC) by system A. Purity was also determined using ion chromatography (IC) for lot II-37A and differential scanning calorimetry (DSC) for lot 11388A. The study laboratory determined the purity of lots II-37A and 11388A using HPLC by system B. IC (Dionex, Sunnyvale, CA) was performed using an Ionpac® AS11 column (250 mm × 4 mm), a mobile phase of A) 3 mM sodium hydroxide and B) 50 mM sodium hydroxide, and suppressed conductivity detection. The mobile phase gradient was held at 100% A for 9 minutes, linearly changed to 100% B in 15 minutes, held for 31.9 minutes, then linearly changed to 100% A in 0.1 minutes and held for 24 minutes; the flow rate was 1.5 mL/minute. DSC was performed using a Perkin-Elmer DSC-7 instrument (Perkin Elmer, Waltham, MA) scanning from approximately –20° C to 31° C at a rate of 1° C per minute under a nitrogen atmosphere.

A) HPLC (Waters Corporation, Milford, MA) system included a Prodigy™ ODS-3, 150 mm × 4.6 mm, 5-µm column (Phenomenex, Torrance, CA) with a mobile phase of A) 15 mM phosphoric acid and B) 30 mM phosphoric acid:acetonitrile (1:1) a linear gradient from 100% A to 100% B in 20 minutes, held for 15 minutes, linear to 100% A in 5 minutes, held for 15 minutes, a flow rate of 1 mL/minute, and ultraviolet light detection at 220 nm.

B) HPLC system included a Phenomenex Aqua® C18 150 mm × 4.6 mm, 3-µm column (Phenomenex) with a mobile phase of A) 80:20 0.1 M phosphoric acid:acetonitrile and B) 10:90 0.1 M phosphoric acid:acetonitrile, beginning with 100% A held for 2 minutes, then linear gradients to 90% A:10% B in 3 minutes, to 80% A:20% B in 4 minutes, and to 100% B in 6 minutes, held for 15 minutes, a linear gradient to 100% A in 20 minutes, held for 25 minutes, a flow rate of 1 mL/minute, and ultraviolet light detection at 220 nm.

For lot II-37A, Karl Fischer titration indicated 180 ppm water. Functional group titration indicated a purity of 100.3 ± 0.6%. IC indicated one major peak and three impurities with a combined area of 1.7% (0.4%, 1.1%, and 0.2%). HPLC indicated an area percent purity of 95.7%. The overall purity of lot II-37A was determined to be greater than 95%.

For lot 11388A, Karl Fischer titration indicated 0.65% water. Functional group titration indicated a purity of 98.7% ± 0.8%, and DSC indicated a purity of 96.7%. HPLC indicated an area percent purity of 98.1%. The overall purity of lot 11388A was determined to be greater than 96%.

To identify and quantitate the largest impurity, the analytical chemistry laboratory used gas chromatography/mass spectrometry (GC/MS) on a methylated sample, HPLC by system A, and standard addition. The GC/MS system included a Carlo Erba (Fisons Ltd., Valencia, CA) or Hewlett-Packard (Palo Alto, CA) gas chromatograph, a VOCOL™ 30 m × 0.25 mm, 1.5-µm film column (Sigma-Aldrich, St. Louis, MO), an oven temperature program of 45° C for 1 minute, increased to 60° C at 1° C per minute, held for 1 minute, increased to 200° C at 10° C per minute, held for 2 minutes, helium carrier gas at 15 psi, and mass spectrometry (Micromass Co., UK or Hewlett-Packard) detection. The impurity was identified as dibromoacetic acid at concentrations of 2.35% for lot II-37A and 0.83% for lot 11388A.

To ensure stability, the bulk chemical was stored at room temperature in amber glass bottles sealed with Teflon®-lined lids. Periodic purity analyses of the bulk chemical were performed by the study laboratory using HPLC by system B at the beginning and end of the 2-week, 3-month, and 2-year studies, and approximately every 6 months during the 2-year studies; no degradation of the bulk chemical occurred.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice during the 2-week studies and approximately every 4 weeks during the 3-month and 2-year studies. The dose formulations were prepared by mixing bromochloroacetic acid with tap water (Table I1). The level of bromochloroacetic acid measured in tap water used for these formulations was never greater than 6.5 µg/L. Formulations were adjusted to pH 5 with 1 N sodium hydroxide or 1 N hydrochloric acid and stored protected from light in sealed Nalgene® containers at 5° C for up to 42 days.

Stability studies of a 62.5 mg/L formulation were performed by the analytical chemistry laboratory using HPLC by a system similar to system B. Stability was confirmed for at least 42 days for dose formulations stored in sealed amber glass or Nalgene® containers at 5° C and for at least 7 days under simulated animal room conditions.

Periodic analyses of the dose formulations of bromochloroacetic acid were conducted by the study laboratory using HPLC by system B. During the 2-week studies, the dose formulations were analyzed twice; all six of the dose formulations for rats and mice were within 10% of the target concentrations (Table I2). Animal room samples of these dose formulations were also analyzed; all five of the animal room samples for rats and all five for mice 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 15 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table I3). Animal room samples of these dose formulations were also analyzed; all 15 samples for rats and all 15 for mice were within 10% of the target concentrations. During the 2-year studies, the dose formulations were analyzed approximately every 8 to 12 weeks (Table I4). All 78 dose formulations analyzed for rats and mice were within 10% of the target concentrations. Animal room samples of these dose formulations were also analyzed; all 12 samples for rats and all 12 for mice were within 10% of the target concentrations.

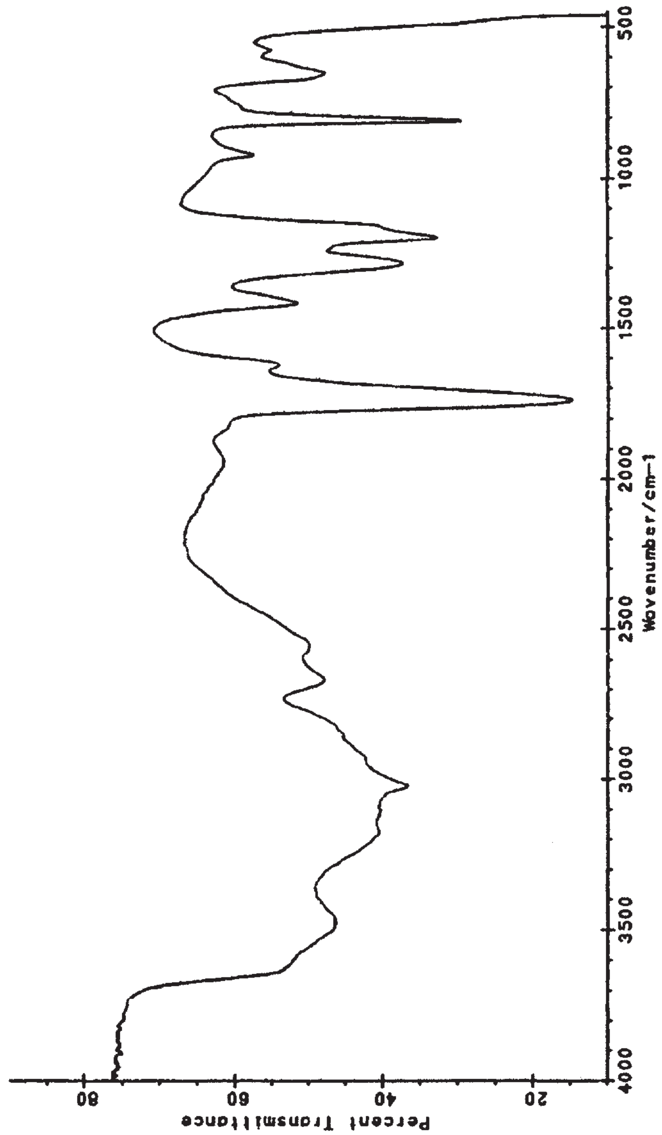

Figure I1
Infrared Absorption Spectrum of Bromochloroacetic Acid

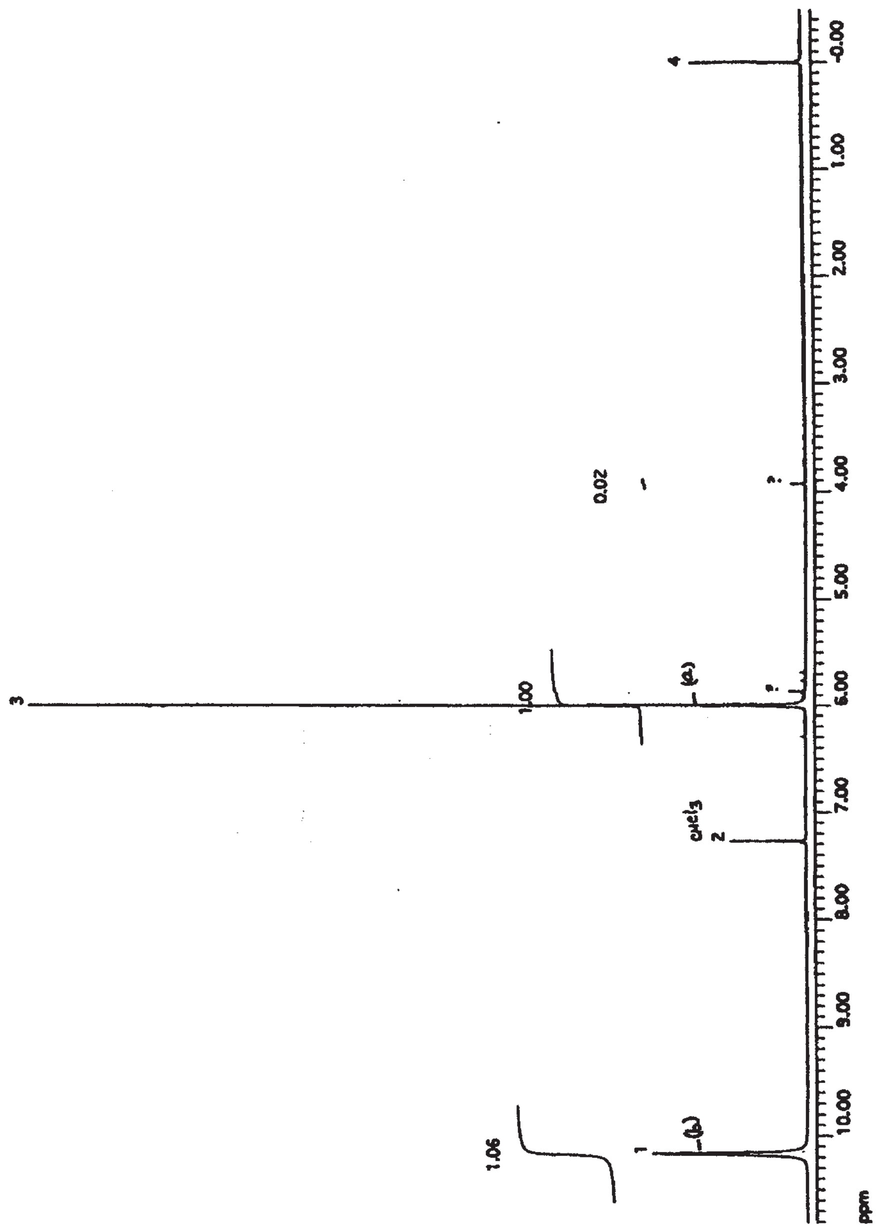

Figure I2
Proton Nuclear Magnetic Resonance Spectrum of Bromochloroacetic Acid

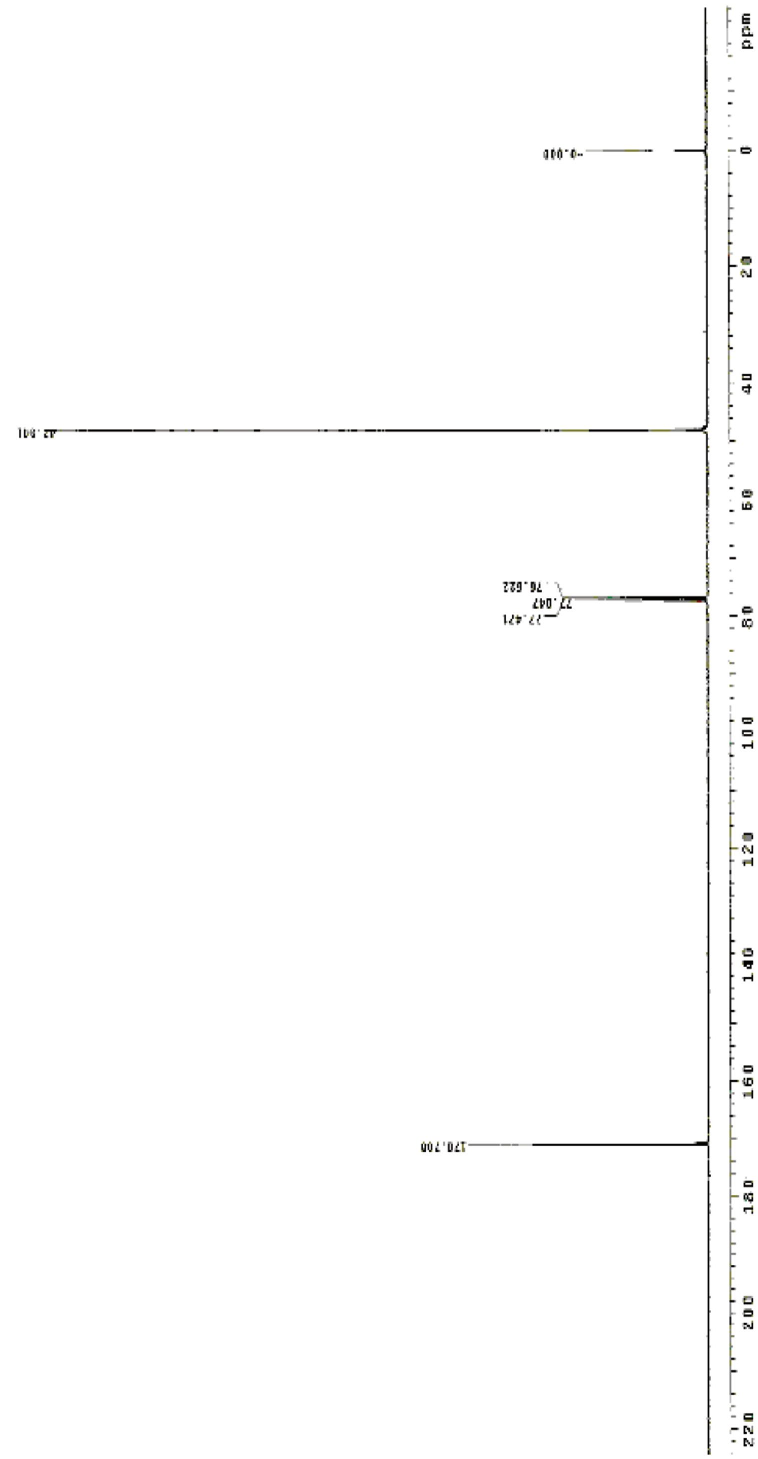

Figure I3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Bromochloroacetic Acid

Table I1
Preparation and Storage of Dose Formulations in the Drinking Water Studies of Bromochloroacetic Acid

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
A premix solution was prepared by adding the appropriate amount of bromochloroacetic acid to tap water in a volumetric flask or beaker and mixing with a magnetic stir bar until in solution. The premix was transferred to a mixing tank partially filled with tap water; rinsate was added from the premix container five times with continual mixing for approximately 2 minutes, then filled to final volume and mixed for up to 5 minutes; the pH was adjusted to 5 by the addition of 1 N sodium hydroxide or 1 N hydrochloric acid, then mixed an additional 10 minutes. The dose formulations were prepared twice.	Same as 2-week studies. The dose formulations were prepared approximately every 4 weeks.	Same as 2-week studies. The dose formulations were prepared approximately every 4 weeks.
Chemical Lot Numbers		
II-37A	II-37A	11388A
Maximum Storage Time		
28 days	42 days	42 days
Storage Conditions		
Stored in sealed Nalgene® containers protected from light at 5° C	Same as 2-week studies	Same as 2-week studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	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 Bromochloroacetic Acid

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
July 21, 2000	July 24, 2000	62.5	61.1	–2
		125	123	–2
		250	250	0
		500	494	–1
		1,000	1,015	+2
August 10, 2000	August 10, 2000	250	252	+1
Animal Room Samples				
Rats				
July 21, 2000	August 17, 2000	62.5	61.7	–1
		125	124	–1
		500	495	–1
		1,000	997	0
August 10, 2000	August 17, 2000	250	251	0
Mice				
July 21, 2000	August 17, 2000	62.5	61.9	–1
		125	124	–1
		500	500	0
		1,000	1,007	+1
August 10, 2000	August 17, 2000	250	247	–1

[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 Bromochloroacetic Acid

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
October 13, 2000	October 16, 2000	62.5	66.4	+6
		125	133	+6
		250	261	+4
		500	540	+8
		1,000	1,065	+7
November 10, 2000	November 13, 2000	62.5	61.5	–2
		125	123	–2
		250	251	0
		500	501	0
		1,000	1,009	+1
January 4, 2001	January 8, 2001	62.5	67.2	+8
		125	134	+7
		250	257	+3
		500	534	+7
		1,000	992	–1
Animal Room Samples				
Rats				
October 13, 2000	November 20, 2000	62.5	61.0	–2
		125	125	0
		250	250	0
		500	499	0
		1,000	993	0
November 10, 2000	December 18, 2000	62.5	63.2	+1
		125	125	0
		250	253	+1
		500	504	+1
		1,000	1,021	+2
January 4, 2001	January 25, 2001	62.5	67.3	+8
		125	131	+5
		250	256	+2
		500	510	+2
		1,000	964	–4

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

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Animals Room Samples (continued)				
Mice				
October 13, 2000	November 21, 2000	62.5	61.8	–1
		125	124	–1
		250	249	0
		500	497	–1
		1,000	995	–1
November 10, 2000	December 20, 2000	62.5	62.8	+1
		125	123	–1
		250	255	+2
		500	505	+1
		1,000	995	–1
January 4, 2001	January 30, 2001	62.5	67.1	+7
		125	133	+6
		250	249	0
		500	530	+6
		1,000	964	–4

[a] Results of duplicate analyses

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

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
September 13, 2001	September 14, 2001	250	250	0
		250	252	+1
		500	505	+1
		500	508	+2
		1,000	1,000	0
		1,000	1,032	+3
November 8, 2001	November 9, 2001	250	252	+1
		250	250	0
		500	508	+2
		500	509	+2
		1,000	997	0
		1,000	1,002	0
January 30, 2002	February 1, 2002	250	250	0
		250	251	0
		500	485	–3
		500	504	+1
		1,000	996	0
		1,000	1,001	0
March 27, 2002	March 28, 2002	250	255	+2
		250	254	+2
		500	518	+4
		500	511	+2
		1,000	1,015	+2
		1,000	1,011	+1
June 19, 2002	June 20, 2002	250	252	+1
		250	251	0
		500	508	+2
		500	511	+2
		1,000	1,014	+1
		1,000	1,012	+1
August 15, 2002	August 16, 2002	250	250	0
		250	251	0
		500	500	0
		500	500	0
		1,000	993	–1
		1,000	1,020	+2
November 7, 2002	November 8, 2002	250	249	0
		250	249	0
		500	498	0
		500	495	–1
		1,000	989	–1
		1,000	998	0

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

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Rats and Mice (continued)				
December 5, 2002	December 18, 2002	250	253	+1
		250	252	+1
		500	505	+1
		500	500	0
		1,000	1,005	+1
		1,000	999	0
January 2, 2003	January 6, 2003	250	249	0
		250	247	–1
		500	502	0
		500	500	0
		1,000	989	–1
		1,000	993	–1
March 27, 2003	March 28, 2003	250	247	–1
		250	251	0
		500	504	+1
		500	510	+2
		1,000	987	–1
		1,000	997	0
May 21, 2003	May 22, 2003	250	250	0
		250	249	0
		500	499	0
		500	500	0
		1,000	985	–2
		1,000	1,013	+1
July 17, 2003	July 21, 2003	250	250	0
		250	249	0
		500	502	0
		500	501	0
		1,000	989	–1
		1,000	1,010	+1
August 14, 2003	August 18, 2003	250	249	0
		250	251	0
		500	494	–1
		500	508	+2
		1,000	1,013	+1
		1,000	1,008	+1

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

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Animal Room Samples				
Rats				
September 13, 2001	October 23, 2001	250	249	0
		500	503	+1
		1,000	999	0
March 27, 2002	May 7, 2002	250	252	+1
		500	507	+1
		1,000	1,005	+1
December 5, 2002	January 14, 2003	250	253	+1
		500	503	+1
		1,000	998	0
July 17, 2003	August 26, 2003	250	256	+2
		500	505	+1
		1,000	1,001	0
Mice				
September 13, 2001	October 23, 2001	250	249	0
		500	498	0
		1,000	992	–1
March 27, 2002	May 7, 2002	250	252	+1
		500	503	+1
		1,000	1,004	0
December 5, 2002	January 14, 2003	250	248	–1
		500	499	0
		1,000	1,002	0
July 17, 2003	August 26, 2003	250	252	+1
		500	510	+2
		1,000	1,008	+1

[a] Results of duplicate analyses

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

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

	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
Week	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	16.2	102	16.2	101	40	16.4	103	80	16.4	101	163
2	17.2	133	17.5	134	33	17.5	137	64	17.6	134	132
3	18.9	168	18.8	169	28	18.8	172	55	18.7	169	110
4	19.3	201	19.1	202	24	19.5	204	48	19.1	202	95
5	19.2	226	18.7	227	21	18.7	229	41	18.7	226	83
6	19.2	248	18.5	248	19	18.8	250	38	18.4	248	74
7	19.6	265	19.0	264	18	19.1	266	36	18.8	264	71
8	19.3	281	18.8	280	17	18.6	281	33	18.6	279	67
9	19.0	293	18.5	292	16	18.5	293	32	18.4	290	64
10	16.2	304	17.1	304	14	16.4	305	27	16.1	300	54
11	17.2	314	17.1	314	14	16.8	314	27	16.6	310	54
12	17.3	325	16.9	325	13	17.1	323	27	17.0	320	53
13	17.1	331	17.1	330	13	16.9	330	26	17.2	325	53
17	16.7	359	16.9	354	12	16.8	353	24	16.2	351	46
21	17.2	389	17.1	380	11	17.0	379	22	16.4	379	43
25	16.5	413	16.4	403	10	16.1	401	20	15.6	398	39
29	15.7	429	15.5	417	9	15.4	415	19	14.7	410	36
33	17.1	449	16.7	433	10	16.5	431	19	15.9	424	38
37	17.5	462	16.7	448	9	16.6	444	19	15.7	437	36
41	17.5	472	17.0	456	9	17.0	451	19	15.9	445	36
45	16.6	484	16.8	467	9	16.0	463	17	15.4	454	34
49	16.5	489	16.1	473	9	16.0	467	17	14.8	460	32
53	16.7	499	17.3	476	9	16.7	470	18	15.2	461	33
57	16.8	502	16.8	480	9	16.3	477	17	15.3	461	33
61	16.3	510	16.3	484	8	16.2	483	17	15.0	465	32
65	16.5	515	16.3	488	8	16.0	486	17	15.1	467	32
69	16.8	520	16.1	491	8	16.2	490	17	15.3	470	33
73	16.3	524	15.9	497	8	16.2	494	16	14.7	471	31
77	17.3	526	16.4	492	8	17.2	492	18	15.7	467	34
81	17.8	524	17.7	488	9	18.3	483	19	16.9	463	37
85	18.5	521	17.6	487	9	18.1	478	19	17.3	459	38
89	18.2	519	18.4	490	9	18.1	479	19	16.4	457	36
93	17.9	524	17.2	495	9	17.0	470	18	16.2	450	36
97	17.3	522	17.0	489	9	16.3	462	18	16.4	449	37
101	18.3	512	18.0	475	10	17.5	465	19	17.9	445	40
Mean for weeks											
1-13	18.1	245	17.9	245	21	17.9	247	41	17.8	244	83
14-52	16.8	438	16.6	426	10	16.4	423	20	15.6	418	38
53-101	17.3	517	17.0	487	9	16.9	479	18	16.0	460	35

a Grams of water consumed per animal per day
b Milligrams of bromochloroacetic 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 Bromochloroacetic Acid

	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
Week	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	13.5	93	13.7	93	37	14.2	92	77	13.8	93	148
2	14.2	114	14.4	115	31	14.5	116	62	14.1	117	120
3	14.5	130	14.4	131	28	14.1	132	53	13.8	132	105
4	14.1	142	14.2	142	25	13.9	143	49	13.6	142	96
5	14.4	153	13.9	153	23	13.5	153	44	13.4	151	89
6	14.6	159	13.7	160	21	13.8	159	43	13.3	157	85
7	14.9	167	14.0	167	21	14.1	168	42	13.7	167	82
8	14.3	177	13.5	175	19	13.2	178	37	13.2	175	76
9	13.5	180	12.9	177	18	12.8	178	36	12.6	176	72
10	12.4	183	11.4	179	16	11.7	181	32	10.5	177	59
11	12.3	187	11.6	182	16	11.5	184	31	11.1	181	61
12	12.0	187	11.7	185	16	11.6	188	31	11.6	185	63
13	12.4	191	11.7	190	15	11.6	188	31	11.9	185	64
17	12.4	202	11.7	199	15	11.5	200	29	11.6	201	58
21	11.8	212	11.6	207	14	11.6	209	28	11.1	208	54
25	11.9	222	11.3	218	13	11.0	219	25	11.1	217	51
29	11.5	233	11.0	230	12	10.7	228	24	10.5	226	46
33	12.2	242	12.1	236	13	11.4	235	24	11.1	233	48
37	12.4	248	12.1	243	12	11.7	242	24	11.3	238	48
41	12.2	258	11.8	253	12	11.6	252	23	11.5	248	46
45	11.2	268	11.9	261	11	11.2	261	22	11.0	256	43
49	11.4	275	11.5	266	11	10.9	268	20	11.0	263	42
53	12.1	285	11.9	276	11	11.5	276	21	11.2	270	42
57	12.5	292	12.2	278	11	11.9	283	21	11.6	274	42
61	12.3	303	12.5	291	11	11.8	294	20	11.6	282	41
65	12.0	312	11.8	300	10	11.6	302	19	11.5	288	40
69	12.3	321	12.8	307	10	11.8	310	19	12.1	297	41
73	12.1	328	12.6	314	10	11.9	316	19	12.3	304	40
77	12.4	335	13.3	321	10	12.8	323	20	12.4	308	40
81	13.7	337	14.3	321	11	13.4	323	21	13.2	307	43
85	15.1	339	14.4	323	11	13.9	325	21	14.3	305	47
89	14.7	346	14.7	330	11	14.1	332	21	13.9	310	45
93	14.1	351	14.2	327	11	13.8	338	20	13.3	314	42
97	14.3	356	14.3	329	11	13.9	345	20	14.2	325	44
101	15.5	357	14.5	334	11	14.1	350	20	14.2	326	44
Mean for weeks											
1-13	13.6	159	13.2	158	22	13.1	158	44	12.8	157	86
14-52	11.9	240	11.7	235	13	11.3	235	24	11.1	232	48
53-101	13.3	328	13.3	312	11	12.8	317	20	12.8	301	42

[a] Grams of water consumed per animal per day
[b] Milligrams of bromochloroacetic 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 Bromochloroacetic Acid

	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
Week	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	4.2	21.3	4.0	21.5	46	3.8	21.4	89	3.8	21.4	178
2	4.3	23.3	4.4	23.8	46	4.0	23.6	85	3.7	23.5	157
3	4.1	24.7	4.1	24.8	41	3.9	24.7	79	4.0	24.8	161
4	4.2	26.4	3.9	26.4	37	3.9	26.4	74	4.0	26.3	152
5	4.3	28.2	4.1	28.3	36	4.3	28.4	76	3.9	28.0	139
6	4.4	29.8	4.0	29.9	33	4.1	29.8	69	3.9	29.5	132
7	4.5	30.9	4.1	31.5	33	4.0	31.0	65	3.9	30.7	127
8	4.6	32.7	4.3	33.2	32	4.1	32.9	62	4.0	32.8	122
9	4.2	34.4	3.7	34.8	27	3.7	34.2	54	3.5	34.4	102
10	4.2	35.7	3.8	35.9	26	3.8	35.4	54	3.6	35.3	102
11	4.1	37.3	3.8	37.4	25	3.6	36.7	49	3.5	36.6	96
12	4.0	38.5	3.9	38.5	25	3.7	37.8	49	3.6	37.6	96
13	3.9	39.9	3.7	39.7	23	3.8	39.0	49	3.6	38.9	93
17	3.7	44.6	3.4	44.4	19	3.4	43.6	39	3.2	43.4	74
21	3.9	47.0	3.6	46.9	19	3.4	46.6	37	3.3	46.3	71
25	4.0	48.6	3.9	48.7	20	3.5	48.2	36	3.1	48.2	64
29	4.1	49.5	4.0	49.7	20	3.7	49.3	38	3.5	49.3	71
33	4.3	50.2	4.1	50.4	20	3.9	50.5	39	3.5	50.2	70
37	4.5	51.0	4.3	51.7	21	3.9	51.7	38	3.6	51.1	71
41	4.6	52.1	4.4	52.5	21	4.4	52.3	42	3.9	51.8	75
45	4.5	52.8	4.2	53.2	20	4.0	53.0	38	3.6	52.4	69
49	4.7	53.2	4.7	53.4	22	4.5	53.3	42	3.9	52.5	74
53	4.6	53.5	4.7	53.6	22	4.5	53.4	42	4.1	52.9	78
57	4.6	53.9	4.7	54.3	22	4.5	54.1	42	3.8	53.6	71
61	4.9	54.0	5.1	54.5	23	4.8	54.4	44	4.0	53.7	75
65	5.1	54.1	5.3	54.1	25	4.6	54.2	42	4.0	53.4	75
69	5.2	53.6	5.4	54.0	25	4.6	54.5	42	4.2	53.2	79
73	5.2	53.0	5.7	54.0	26	5.3	54.6	49	4.8	53.0	91
77	5.3	53.9	5.2	54.5	24	5.0	55.2	45	4.6	53.1	87
81	5.1	52.7	5.3	52.7	25	5.7	54.2	53	4.9	50.7	97
85	5.0	52.0	5.2	51.6	25	5.7	52.5	54	5.2	50.1	104
89	5.1	50.2	5.3	50.3	26	5.9	50.9	58	5.0	48.1	104
93	4.9	49.2	5.3	48.3	27	6.3	48.3	65	5.2	45.7	114
97	5.2	47.4	5.3	46.0	29	7.0	45.5	77	5.7	42.4	135
101	5.5	46.8	5.6	42.7	33	7.6	43.8	87	6.3	40.6	155
Mean for weeks											
1-13	4.2	31.0	4.0	31.2	33	3.9	30.9	66	3.8	30.8	127
14-52	4.3	49.9	4.1	50.1	20	3.9	49.8	39	3.5	49.5	71
53-101	5.1	51.9	5.2	51.6	26	5.5	52.0	54	4.8	50.0	97

a Grams of water consumed per animal per day
b Milligrams of bromochloroacetic 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 Bromochloroacetic Acid

	0 mg/L		250 mg/L			500 mg/L			1,000 mg/L		
Week	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	3.0	18.4	3.1	18.5	42	3.1	18.4	85	2.8	18.5	151
2	3.0	18.6	3.2	18.9	42	3.1	18.8	82	3.0	18.9	158
3	3.1	20.3	3.3	20.4	40	3.3	20.5	80	3.2	20.3	158
4	3.2	21.5	3.3	21.5	38	3.2	21.6	74	3.3	21.7	152
5	3.8	23.0	3.5	22.9	38	3.4	22.7	75	3.3	22.9	144
6	3.3	23.3	3.7	23.3	40	3.4	23.0	74	3.4	23.5	145
7	3.7	24.3	3.6	24.5	37	3.6	24.1	75	3.2	24.6	130
8	3.8	25.4	3.4	25.4	34	3.4	25.2	68	3.3	25.6	129
9	3.7	27.0	3.4	27.2	31	3.4	26.5	64	3.5	26.6	132
10	3.5	28.0	3.3	28.2	29	3.5	27.6	63	3.1	27.3	114
11	3.2	29.8	3.2	29.8	27	3.3	28.8	57	3.0	29.1	103
12	3.2	30.8	3.2	31.3	26	3.3	30.3	54	3.2	30.2	106
13	3.7	32.2	3.2	32.5	25	3.4	31.7	54	3.0	31.2	96
17	2.9	37.7	2.8	37.5	19	2.9	36.3	40	2.6	35.1	74
21	2.7	42.6	2.8	42.3	17	2.7	40.0	34	2.5	39.1	64
25	2.4	46.6	2.4	47.0	13	2.4	44.5	27	2.4	43.0	56
29	2.4	50.0	2.4	50.2	12	2.4	47.9	25	2.4	45.6	53
33	2.5	52.8	2.5	53.6	12	2.5	51.3	24	2.2	48.2	46
37	2.6	55.1	2.7	56.4	12	2.6	53.5	24	2.3	50.4	46
41	2.8	56.8	2.7	57.7	12	2.6	55.8	23	2.7	52.2	52
45	2.6	58.3	2.5	59.0	11	2.4	57.7	21	2.3	54.6	42
49	2.8	58.5	2.7	58.4	12	2.5	57.7	22	2.7	54.1	50
53	3.1	59.4	2.8	59.2	12	2.7	58.5	23	2.5	55.9	45
57	2.6	59.3	2.8	59.2	12	2.6	59.3	22	2.3	56.8	41
61	2.8	61.4	2.8	60.2	12	2.6	60.6	21	2.7	58.0	47
65	2.9	62.1	2.8	60.6	12	2.7	61.1	22	2.4	58.7	41
69	3.0	62.0	2.7	60.7	11	3.0	61.3	25	2.5	58.7	43
73	2.8	61.4	2.9	60.1	12	2.7	60.9	22	2.8	58.4	48
77	3.1	63.0	3.1	61.8	13	2.9	62.3	23	2.6	59.6	44
81	3.4	62.3	3.1	61.1	13	2.8	61.2	23	2.6	59.7	44
85	3.5	62.0	3.2	60.1	13	3.1	60.5	26	2.8	59.2	47
89	3.4	61.8	3.5	59.0	15	3.6	59.1	31	3.2	58.2	55
93	3.3	62.2	3.4	59.2	14	3.6	57.9	31	2.7	58.0	47
97	3.9	60.8	4.1	57.7	18	4.1	55.4	37	3.4	55.1	62
101	4.0	61.0	4.9	57.0	22	4.8	55.1	44	3.6	54.7	66
Mean for weeks											
1-13	3.4	24.8	3.3	25.0	35	3.3	24.6	70	3.2	24.6	132
14-52	2.6	50.9	2.6	51.3	13	2.6	49.4	27	2.5	46.9	54
53-101	3.2	61.4	3.2	59.7	14	3.2	59.5	27	2.8	57.8	48

[a] Grams of water consumed per animal per day
[b] Milligrams of bromochloroacetic 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

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	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

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

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.8 ± 0.59	13.3 – 15.7	25
Crude fat (% by weight)	8.1 ± 0.27	7.6 – 8.6	25
Crude fiber (% by weight)	9.0 ± 0.44	8.0 – 9.9	25
Ash (% by weight)	5.2 ± 0.26	4.7 – 5.8	25
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,957 ± 116	3,060 – 8,900	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	7.8 ± 1.31	5.9 – 11.4	25
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	1.001 ± 0.052	0.873 – 1.110	25
Phosphorus (%)	0.605 ± 0.034	0.555 – 0.701	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

Table K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.37 ± 0.152	0.18 – 0.50	25
Cadmium (ppm)	0.05 ± 0.017	0.04 – 0.09	25
Lead (ppm)	0.07 ± 0.028	0.05 – 0.17	25
Mercury (ppm)	< 0.02		25
Selenium (ppm)	0.21 ± 0.054	0.14 – 0.36	25
Aflatoxins (ppb)	< 5.00		25
Nitrate nitrogen (ppm)[c]	14.8 ± 3.61	7.88 – 23.2	25
Nitrite nitrogen (ppm)[c]	< 0.61		25
BHA (ppm)[d]	< 1.0		25
BHT (ppm)[d]	< 1.0		25
Aerobic plate count (CFU/gm)	28 ± 71	10 – 360	25
Coliform (MPN/gm)	3.0 ± 3.0	3.0 – 3.0	25
Escherichia coli (MPN/gm)	< 10		25
Salmonella (MPN/gm)	Negative		25
Total nitrosoamines (ppb)[e]	4.2 ± 1.54	2.3 – 8.4	25
N-Nitrosodimethylamine (ppb)[e]	2.7 ± 1.42	1.2 – 6.9	25
N-Nitrosopyrrolidine (ppb)[e]	1.5 ± 0.58	0.9 – 3.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.103 ± 0.063	0.020 – 0.259	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.317 ± 0.471	0.020 – 1.850	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfane sulfate	<0.03		25

[a] All samples were irradiated. CFU=colony-forming units; MPN=most probable number; BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] All values were corrected for percent recovery.

APPENDIX L
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

During the 3-month studies, serum samples were collected by the study laboratory from five male and five female sentinel rats and mice at the end of the study.

During the 2-year studies, serum samples were collected from three to five male and female sentinel rats and mice at 6, 12, and 18 months and from five 1,000 mg/L male and female rats and mice at the end of the study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titers. At 18 months, fecal samples were obtained for PCR analysis. 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	
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	
Mycoplasma arthritidis	6 months and study termination
Mycoplasma pulmonis	6 months and 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
PVM	Study termination
RCV/SDA	Study termination
Sendai	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
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
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
M. arthritidis	6, 12, and 18 months, study termination
M. pulmonis	6, 12, and 18 months, 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	
GDVII	6 months
Mouse adenoma virus-FL	18 months
MCMV (mouse cytomegalovirus)	6, 12, and 18 months, study termination
Parvovirus	6, 12, and 18 months, study termination
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.

APPENDIX M
TOXICOKINETIC STUDIES
IN F344/N RATS AND B6C3F1 MICE

TOXICOKINETIC STUDIES IN F344/N RATS AND B6C3F1 MICE

INTRODUCTION

NTP toxicokinetic studies of bromochloroacetic acid were conducted to collect data necessary for understanding internal dose and time-dependent tissue concentrations of the parent compound and its metabolites, glyoxylic acid and oxalic acid. The study designs included analysis of blood following single intravenous administration and blood and urine following single gavage administration of bromochloroacetic acid. In addition, to provide information on the effects of repeated exposure, blood was analyzed following 14-day drinking water exposure and blood and urine were analyzed following 14-day drinking water exposure with a single gavage challenge dose of bromochloroacetic acid after the last day of the repeated dose period. The kinetics of the stereoisomers of bromochloroacetic acid were evaluated, using blood only, in a single dose intravenous study of this chemical. In addition, a separate study of the kinetics of the metabolite glyoxylic acid was conducted in rats by evaluating concentrations of glyoxylic acid in blood over time following a single intravenous administration of 50 mg/kg glyoxylic acid monohydrate in normal saline. Mice were not included in the glyoxylic acid study due to the high mortality observed at concentrations necessary to achieve measurable plasma concentrations.

MATERIAL AND METHODS

Bromochloroacetic Acid

Bromochloroacetic acid (lot II-37A) was obtained from Carbolabs, Inc (Woodbridge, CT), and was handled and characterized as reported in Appendix I. Study details for the set of bromochloroacetic acid toxicokinetic studies are provided in Table M1.

For the intravenous injection studies, male and female F344/N rats (up to 15/sex per group) and B6C3F1 mice (up to 27/sex per group) were given a single bolus intravenous injection of bromochloroacetic acid at 10 or 80 mg/kg for the rats and 100 mg/kg for the mice. Formulations were prepared at concentrations of 5, 25, and 40 mg/mL in 0.9% sodium chloride. The pH of the solutions was adjusted within the range of 6 to 8 with 0.1 N and 1 N sodium hydroxide solutions. The dose was delivered through an indwelling jugular catheter. For analysis, a 100 µL sample of the formulation, diluted into the analytical range with 0.9% sodium chloride, was combined with 35 µL of internal standard solution [5,000 µg/mL dichloroacetic acid in Milli-Q water (resistivity ≥18 megohm)]. Derivatizing agent (0.5 mL of 14% boron trifluoride in methanol) was added to each tube, and the samples were allowed to derivatize overnight at room temperature. The samples were diluted with Milli-Q water and methyl-*tert*-butyl ether, mixed, and the top layer separated by centrifugation. An aliquot of the top layer was injected onto gas chromatography/flame ionization detection (GC/FID) System A for analysis (Table M2).

For the gavage studies, male and female F344/N rats (up to 23/sex per group) and B6C3F1 mice (up to 45/sex per group) were given a single gavage administration of bromochloroacetic acid in Milli-Q water at dosages of 10, 40, or 100 mg/kg for the rats and 100, 200, or 400 mg/kg for the mice. Formulations were prepared at target concentrations of 2, 8, 10, 20, and 40 mg/mL in Milli-Q water. The pH of each solution was adjusted to be within the range of 6 to 8 with 0.1 N and 1 N sodium hydroxide solutions. For analysis, the formulations were diluted and an internal standard (2 mg/mL of bromoacetic acid solution) was added to a 1 mL aliquot of diluted formulation, mixed, and a subaliquot was injected onto high-performance liquid chromatography/ultraviolet detection (HPLC/UV) System B for quantitation.

For the stereoisomer studies, male and female F344/N rats (up to 15 per sex for the 10 mg/kg group and up to 5 per sex for the 80 mg/kg group) and B6C3F1 mice (up to 27 per sex) were given a single bolus intravenous injection of bromochloroacetic acid as described above.

The repeat dose drinking water studies included groups of male and female F344/N rats (up to 27/group) and B6C3F1 mice (up to 33/group) that were given a single gavage dose at the end of the 2-week exposure period. For the repeat dose period, these animals were provided a full amber glass drinking water bottle of bromochloroacetic acid in tap water at one of three concentrations *ad libitum* for 14 consecutive days. Formulations were prepared by diluting bromochloroacetic acid in tap water and adjusting into the pH range of 6 to 8, if needed, with a sodium hydroxide solution. For analysis, aliquots of the formulations were mixed with internal standard solution (2 mg/mL bromoacetic acid in tap water) and a subaliquot was injected onto System B. For the non-challenged drinking water groups, exposure continued through the fourteenth day of exposure and into the fifteenth day of exposure so that beginning on the fourteenth day of exposure, these groups of rats and mice had blood samples collected over a 24-hour period. For the challenged drinking water groups, exposure continued up through the fourteenth day of exposure and, on study day 14, the bromochloroacetic acid treated drinking water was replaced by untreated water; on study day 15, these animals were given a single gavage administration of bromochloroacetic acid in Milli-Q water.

Blood samples were collected using the retroorbital method for rats and cardiac puncture for mice. Animals were anesthetized with CO_2/O_2 prior to bleeding, and blood samples of approximately 1 to 2 mL for rats and the maximum obtainable (up to 1 mL) for mice were collected. For both routes, three animals/group were bled at each timepoint. Each blood sample was placed into a heparinized tube, gently inverted, and placed on wet ice until it was separated into plasma by centrifugation, which took place within 60 minutes after collection. The plasma samples were placed into cryovials and stored on dry ice until transferred to a –70° C freezer.

Plasma was analyzed for bromochloroacetic acid concentration using derivitization with gas chromatography and electron capture detection (GC/ECD) using System C. To 100 µL of each sample, 50 µL of a working internal standard (4 µg/mL of dichloroacetic acid in Milli-Q water) was added. Then 500 µL of 14% boron trifluoride-methanol complex was added, the samples were allowed to sit for 12 hours at 30° C, and then the samples were extracted with 2 mL of methyl-*tert*-butyl ether following centrifugation. Isomers were separated and quantitated using the same sample preparation procedure and GC/ECD System D. For analysis of plasma for the metabolites glyoxylic acid and oxalic acid, 200 µL of sample were combined with 50 µL of internal standard solution (50 µg/mL ^{13}C oxalic acid in Milli-Q water), 100 µL of acidified saturated sodium sulfate solution, and 500 µL derivatizing agent (as above), allowed to sit overnight, and injected onto GC/mass spectrometry (GC/MS) System E.

Urine samples were collected in several studies from different animals from those used for plasma collection. A plastic metabolism cage designed to separate urine and feces was used, and the urine cup was kept at room temperature during the collection period. Urine samples were transferred to plastic storage containers with screw-cap lids and stored refrigerated (approximately 5° C) until analyzed. Urine was analyzed for bromochloroacetic acid, glyoxylic acid, and oxalic acid using methods similar to those used for analyzing plasma above.

Glyoxylic Acid

Glyoxylic acid monohydrate (lot 04926BD) was obtained from Aldrich Chemical Company (St. Louis, MO), homogenized and repackaged in its original bottle under an argon atmosphere, and stored at room temperature. Identity was confirmed by comparing infrared and proton and carbon-13 nuclear magnetic resonance spectra with reference spectra in the literature. Several more peaks were present in the spectra than predicted for glyoxylic acid monohydrate, but analysis of additional commercially available samples of glyoxylic acid monohydrate and sodium glyoxylate by nuclear magnetic resonance spectroscopy indicated that commercially available glyoxylic acid monohydrate exists as a cyclic monomer. This observation was confirmed by HPLC/MS Systems F and G. Karl Fischer titrimetry was used to determine that the bulk material was approximately 20.1% water. Chromatographic purity was determined to be 100% using HPLC/UV System H.

Study details for the glyoxylic acid monohydrate toxicokinetic study are provided in Table M1. Glyoxylic acid was formulated at 25 mg/mL in physiological saline, and the concentration was confirmed using a validated method. After dosing, animals were anesthetized with CO_2/O_2 prior to bleeding. Blood samples of approximately 1 mL were collected using the retroorbital puncture method. Three rats were bled at each timepoint. Each blood sample was placed into a heparinized tube, gently inverted, and placed on wet ice until it was separated into plasma by centrifugation. Whole blood samples were centrifuged within 60 minutes after collection, and the plasma samples were placed into cryovials and stored on dry ice until transferred to a –70° C freezer.

For glyoxylic acid analysis in the intravenous study of glyoxylic acid monohydrate, the plasma samples were thawed to room temperature. Analysis was conducted on a 100 µL aliquot of plasma mixed with 100 µL of acetonitrile to precipitate the proteins, after centrifugation. A 50 µL aliquot of each sample was transferred to an autosampler vial; 50 µL of mobile phase was added to each vial, and a subaliquot was injected onto HPLC/MS System I.

TOXICOKINETICS

Glyoxylic acid and bromochloroacetic acid plasma concentration versus time data were evaluated using WinNonlin® (version 5.0.1; Pharsight Corporation, Mountain View, CA). The primary and secondary parameters were estimated using WinNonlin® software. One- and two-compartment models were tested based on the appearance of the plasma concentration time curve.

For one compartment: $C(t) = D \cdot k_{01}/V/(k_{01} - k_{10}) \cdot [exp(k_{10} \cdot t) - exp(k_{01} \cdot t)]$

For two compartments: $C(t) = [D/(\alpha - \beta)][(k_{21} - \beta)e^{-\beta t} - (k_{21} - \alpha)e^{-\alpha t}]$

Where: $\alpha = 0.5\{[k_{12} + k_{21} + k_{e1}] + [(k_{12} + k_{21} + k_{e1})^2 - 4k_{21}k_{10}]^{½}\}$, and

$\beta = 0.5\{[k_{12} + k_{21} + k_{e1}] - [(k_{12} + k_{21} + k_{e1})^2 - 4k_{21}k_{10}]^{½}\}$

C(t) is the plasma concentration at time *t*; *D* is dose; *V* is volume of distribution, and *k* is a rate constant (subscripts describe the compartment and direction). Parameters were estimated by nonlinear regression using a least-squares method and a weighting factor (1/Yhat^2 or 1/Yhat predicted). Goodness of fit was based on fitting tools generated by the WinNonlin® software and an evaluation of these tools.

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

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

And the *AUC* extrapolated to infinity was calculated as:

$$AUC_{infinity} = AUC_t + (C_f/k_{10})$$

Clearance (*Cl*) was computed by dividing the dose by the area under the concentration versus time curve extrapolated to infinity ($AUC_{infinity}$). Half lives ($t_{1/2}$) for the absorption and elimination phases were calculated as $0.693/k_{01}$ and $0.693/k_{10}$, respectively.

RESULTS

Studies in Rats

Bromochloroacetic Acid Intravenous Study

Bromochloroacetic acid was administered in a single intravenous injection of 10 or 80 mg/kg to groups of male and female F344/N rats, and plasma was collected at specific timepoints. Bromochloroacetic acid was measurable in plasma at the earliest postdose sample collection time (2 minutes) through the fifth collection time (40 minutes) after a single intravenous injection of bromochloroacetic acid at a dosage of 10 mg/kg in both sexes (Figures M1 and M2). The predose and all other postdose bromochloroacetic acid plasma concentrations were below a method limit of quantitation of 0.07 µg/mL. The predose sample result indicated that any background levels of bromochloroacetic acid would not interfere with measuring bromochloroacetic acid in the plasma that could be attributed to dosing. The percent relative standard deviation (RSD) values (30% or less) indicated there was good agreement among samples with measurable concentrations. A comparison of bromochloroacetic acid plasma concentrations at 10 and 80 mg/kg dosages revealed a flatter slope at the 80 mg/kg dosage, which indicated a capacity-limited elimination of bromochloroacetic acid at the higher dose. Toxicokinetic analysis was not performed on the 80 mg/kg data set because the experimental design only required a partial concentration time profile to be determined. The best fit was achieved with a one-compartment model, but different weightings were required to fit male and female rat intravenous injection data. Resulting toxicokinetic parameter estimates for male and female rats are provided in Table M3.

Bromochloroacetic Acid Gavage Study

Groups of rats were given a single gavage dose of 10, 40, or 100 mg/kg bromochloroacetic acid, and both plasma and urine were collected. In each group, bromochloroacetic acid was measurable in rat plasma at the earliest sample collection timepoint (2 minutes) and remained detectable in samples for all three dose groups taken at all subsequent timepoints up to and including the 90 and 120 minute timepoints, respectively, for the male and female 10 mg/kg groups, 240 and 360 minute timepoints for the male and female 40 mg/kg groups, and 420 minute timepoint for the male and female 100 mg/kg groups, although there were a few instances where one or two concentrations out of three were measurable at the next later timepoint. All predose plasma samples were below the limit of quantitation for the bromochloroacetic acid method, 0.075 µg/mL. All concentrations for the bromochloroacetic acid metabolites, glyoxylic acid and oxalic acid, in male and female F344/N rats administered 100 mg/kg bromochloroacetic acid were below the limit of quantitation (4.209 and 4.192 µg/mL, respectively) in plasma, showing that these two acids are minor metabolites or rapidly transformed. A one-compartment model with equal first-order absorption and elimination was used to obtain a best-fit curve for the bromochloroacetic acid plasma concentration time data sets. Table M4 presents the parameter estimates from this model and graphs of the observed versus modeled data are presented in Figures M3 for males and M4 for females.

Urinary excretion of bromochloroacetic acid was a minor pathway of elimination. For all groups, less than 0.2% of the administered bromochloroacetic acid was excreted in the urine. Peak urinary excretion occurred during the 2-to-4-hour collection interval for most groups. Data showing excreted amounts of bromochloroacetic acid in urine of treated rats are shown in Figures M5 for males and M6 for females.

Measurable predosing concentrations of glyoxylic acid and oxalic acid were found in the urine of rats of both sexes following bromochloroacetic acid administration by gavage. Using the predosing concentrations from all 11 animals (one did not produce urine), the background concentration was approximately 34.2 µg/mL. Male and female urinary glyoxylic acid increased after dosing with bromochloroacetic acid through the 24-to-48-hour collection time, except for the 40 mg/kg male rat group, which showed a very substantial increase in both glyoxylic acid and oxalic acid in the first 2-hour collection period following dosing, then a decrease through 4 to 8 hours and another increase through the 24-to-48-hour collection period. Glyoxylic acid and oxalic acid values in rat urine are shown in Figures M7 for males and M8 for females.

Bromochloroacetic Acid Drinking Water Study with or without Gavage Challenge

To evaluate the potential for bromochloroacetic acid to inhibit its own metabolism, a repeat exposure study of groups of F344/N rats was conducted by the drinking water route with and without a gavage challenge dose at the end of the dosing period. Rats were exposed to 40, 400, or 800 mg/L in tap water, which corresponded to target daily doses of 2.88, 28.8, or 57.6 mg/kg for male rats and 2.74, 27.4, or 54.9 mg/kg for female rats. The non-challenged groups of animals were used to collect blood samples at 3-hour intervals beginning at 9:00 AM on study day 14 and finishing at 6:00 AM on study day 15 (three animals/timepoint per exposure level), while remaining on bromochloroacetic acid-treated drinking water. Urine samples were not collected from non-challenged animals. The gavage-challenged groups were given untreated drinking water overnight on study day 14, and then on study day 15, they were given a single gavage dose of bromochloroacetic acid that was equivalent to one day's drinking water exposure. After the gavage administration, blood samples were collected at selected timepoints (three animals/timepoint per dose group). In addition, urine samples were collected from a separate group of gavage-challenged animals at specified intervals after the gavage-challenge was administered. Group mean water consumption values by study day were similar for all groups of a given sex (data not shown), indicating no taste aversion to increasing concentrations of bromochloroacetic acid in drinking water.

In the non-challenged set of animals, bromochloroacetic acid was measurable in plasma of the low-exposure group only at the 6:00 AM timepoint for males and only at the 3:00 and 6:00 AM timepoints for females, and were then only slightly above the limit of detection for the method (0.0750 µg/mL). For the male and female mid- and high-exposure groups, plasma bromochloroacetic acid was generally measurable at the 9:00 AM timepoint and then from 9:00 PM to 6:00 AM, whereas timepoints between 12:00 PM and 6:00 PM were generally below the limit of quantitation. Individual glyoxylic acid and oxalic acid plasma concentrations were either below the limit of quantitation (3.959 and 3.918 µg/mL, respectively) or not detected for all exposure groups. No toxicokinetic analysis was performed for the non-challenged groups; however, basic parameters are provided in Table M5.

Bromochloroacetic acid was measurable at the 2-minute timepoint for the female 2.74 mg/kg challenged group, whereas it was first measurable at the 5-minute timepoint for the other groups. Bromochloroacetic acid remained detectable at all subsequent timepoints up to and including the last sample collection timepoint (60 minutes for the low-dose groups, 240 minutes for the mid-dose groups, and 360 minutes for the high-dose groups). A one-compartment model with different rates for absorption and elimination provided the best fit for the data. Table M6 gives the parameter estimates generated by this model. Figures M9 and M10 show the concentration time curves for bromochloroacetic acid in plasma from the gavage-challenged animals. In addition, Figures M11 and M12 show that a comparison of area under the curve (AUC) versus dose indicates much higher exposures in the repeat administration studies. The effect of preconditioning can also be seen in that dose-normalized AUC values are much larger in the gavage-challenged drinking water groups. Plasma concentrations for the bromochloroacetic acid metabolites, glyoxylic acid and oxalic acid, were all below the limit of quantitation (3.959 and 3.918 µg/mL, respectively) or not detected.

Bromochloroacetic Acid Intravenous Study with (+) and (–) Isomer Determination

The separate kinetics of the optical isomers of bromochloroacetic acid in groups of male and female rats were evaluated using a single intravenous injection of 10 or 80 mg/kg racemic bromochloroacetic acid administered in physiological saline. The 80 mg/kg dose was chosen since there was a similar study in the literature (Schultz and Sylvester 2001); however, this dose seemed to produce saturation, and so this portion of the study was limited to a selected few timepoints and no toxicokinetic modeling. The 10 mg/kg dose was chosen for extensive evaluation at multiple timepoints. Blood samples were collected pre- and postdosing, and the isomers were measured using a validated method. Bromochloroacetic acid (+) isomer concentration time curves for the 10 mg/kg rat groups were described using a two-compartment model with bolus input and first-order elimination. The bromochloroacetic acid (+) isomer undergoes rapid distribution into the peripheral compartment following a single intravenous injection in male and female rats at a dose of 10 mg/kg (Figure M13). The bromochloroacetic acid (–) isomer concentrations following a single intravenous injection of 10 mg/kg declined much faster than the (+) isomer, thereby only allowing a noncompartmental analysis for the (–) isomer data sets. Estimates of toxicokinetic parameters for the bromochloroacetic acid (+) and (–) isomers in rats are summarized in Tables M7 and M8, respectively. These results demonstrated that the (–) isomer of bromochloroacetic acid was eliminated from the systemic circulation approximately 1.5 to 2.5 times faster than the (+) isomer.

Glyoxylic Acid Intravenous Study

To evaluate the kinetics of a single intravenous injection of glyoxylic acid in rats, 14 male and 14 female F344/N rats were injected with a single bolus of 50 mg/kg glyoxylic acid monohydrate. Blood samples were collected predosing and at eight time points postdosing. In the predosing samples, glyoxylic acid was below the limit of quantitation (1.98 µg/mL) for the method, indicating that background levels of glyoxylic acid would not interfere with measuring glyoxylic acid in plasma due to dosing. Glyoxylic acid was measurable in plasma at the earliest postdosing sample collection time (2 minutes) through the last collection time (30 minutes) (Figure M14). The RSD values for the data set were all less than 30%, indicating good agreement among samples.

The glyoxylic acid plasma concentration time profiles for male and female rats given a single intravenous injection of 50 mg/kg glyoxylic acid monohydrate had a biphasic decline. The shape of the curve was best described by a two-compartment pharmacokinetic model with first-order elimination. Table M9 shows the toxicokinetic parameters generated by this model. Glyoxylic acid was rapidly distributed into the peripheral compartment following intravenous injection. Glyoxylic acid elimination from the central compartment was very rapid. The terminal phase represented the glyoxylic acid distribution processes more than the elimination of glyoxylic acid. There were no apparent sex-related differences in the glyoxylic acid toxicokinetic parameters.

Studies in Mice

Bromochloroacetic Acid Intravenous Study

Bromochloroacetic acid was measurable in mouse plasma from the earliest sample collection time (2 minutes) through the last collection time (60 minutes) following a single intravenous injection of 100 mg/kg in B6C3F1 mice. A predose sample analysis indicated that bromochloroacetic acid levels were below a method limit of quantitation of 0.07 µg/mL and would not interfere with measuring bromochloroacetic acid attributed to dosing. RSDs for the data were higher than desired at 8% to 80%, but no reason could be found for this, and so these data were included in the toxicokinetic analysis. Observed and fitted bromochloroacetic acid concentration time profiles in this study are illustrated in Figure M15. The data were best fit with a one-compartment model with bolus input and first-order elimination, but the concentrations at the 50- and 60-minute timepoints suggested that this model did not completely characterize the observed concentration timepoints. A summary of toxicokinetic parameters is provided in Table M10.

Bromochloroacetic Acid Gavage Study

In the gavage study, mice given a single dose of 100, 200, or 400 mg/kg bromochloroacetic acid in saline showed measurable amounts of bromochloroacetic acid in plasma from the earliest sample collection timepoint (2 minutes) up to and including the last collection timepoint (90, 150, and 180 minutes for the 100, 200, and 400 mg/kg groups, respectively). The RSDs for some timepoints were very large, but no reason could be found for this, and so these data were included for toxicokinetic analysis. Plasma concentrations of glyoxylic acid in male and female mice administered 400 mg/kg bromochloroacetic acid were slightly above or below the limit of quantitation (4.2 µg/mL) for the method. For oxalic acid, all plasma concentrations were below the limit of quantitation (4.2 µg/mL) except for the 15- and 20-minute timepoints for two mice. A one-compartment model with first-order absorption and first-order elimination provided the best fit to the bromochloroacetic acid data. Figures M16 and M17 show the observed and fitted data, and toxicokinetic parameters are presented in Table M11.

Bromochloroacetic Acid Drinking Water Study with or without Gavage Challenge

Kinetics in a multiple exposure scenario were evaluated in male and female mice both with and without a gavage challenge at the end of the exposure period. Mice were exposed to bromochloroacetic acid in tap water at 40, 400, or 800 mg/L, which corresponded to target daily doses of 8, 80, or 160 mg/kg for male mice and 10, 100, or 200 mg/kg for female mice. The non-challenged set of animals was used to collect blood samples for analysis of bromochloroacetic acid, glyoxylic acid, and oxalic acid over a 24-hour period from 9:00 AM on study day 14 to 6:00 AM on study day 15 while remaining on bromochloroacetic acid-treated drinking water. Urine samples were not collected for non-challenged animals. Another set of animals at each exposure level was given untreated water overnight on study day 14 and then on study day 15 were given a single gavage administration of bromochloroacetic acid that was equivalent to one day's drinking water exposure. Blood samples for bromochloroacetic acid, glyoxylic acid, and oxalic acid analysis were collected at selected timepoints following gavage challenge. In addition, urine samples were collected from a separate group of gavage-challenged animals at specified time intervals after the gavage challenge was administered. Metabolites glyoxylic acid and oxalic acid were measured in urine. Group mean water consumption values by study day were similar for all groups of a given sex (data not shown), suggesting that there was no taste aversion to increasing concentrations of bromochloroacetic acid in the drinking water.

Bromochloroacetic acid concentrations were below the limit of quantitation (0.0750 µg/mL) for the method for the low exposure non-challenged groups of mice. For the male mid- and high-exposure groups, bromochloroacetic acid plasma concentrations were measurable from the 6:00 PM to the 12:00 AM time point. For the female mid-exposure group, plasma bromochloroacetic acid concentrations were measurable at the 9:00 AM time point and then from 9:00 PM to 3:00 AM, whereas for the female high-exposure group, concentrations were measurable at all of the time points except for 9:00 PM and 12:00 AM when the concentrations were below the limit of quantitation. Individual glyoxylic acid and oxalic acid concentrations in plasma were either below the limit of quantitation (3.959 and 3.918 µg/mL, respectively) or not detected for all exposure groups. No toxicokinetic analysis was performed for the non-challenged groups; however, basic observed parameters are provided in Table M12.

In the set of mice challenged with a gavage dose of bromochloroacetic acid following multiple exposures in the drinking water, plasma concentrations of bromochloroacetic acid were measurable at the earliest sample collection timepoint (2 minutes) in both males and females for all dosed groups (except for one mouse in the male 8 mg/kg group). For the low-dose groups, many of the bromochloroacetic acid plasma concentrations were near the limit of quantitation (0.0750 µg/mL) for the method for the males and below the limit of quantitation for the females. Plasma bromochloroacetic acid data from the mid- and high-dose groups were best fit with a one-compartment model with the same rates for absorption and elimination. Figures M18 and M19 show the observed and fitted plasma concentration time profiles for bromochloroacetic acid in mice, and Table M13 provides the toxicokinetic parameters generated using the fitted model. Figures M20 and M21 show AUC versus dose for the single versus multiple administration studies. Clearly, conditioning led to much higher exposures in the repeat-exposure groups.

Both groups had only two or three usable concentration timepoints for identifying the terminal linear phase. Plasma concentrations for glyoxylic acid and oxalic acid were generally below the limit of quantitation (3.959 µg/mL) for male and female low- and mid-exposure groups, but there were measurable glyoxylic acid concentrations at timepoints ranging from 5 to 60 minutes for the males and 5 to 20 minutes for the females in the high-exposure groups. All oxalic acid plasma concentrations were below the limit of quantitation (3.918 µg/mL) for all male and female exposure groups.

Bromochloroacetic Acid Intravenous Study with (+) and (–) Isomer Determination

The separate kinetics of the optical isomers of bromochloroacetic acid in groups of male and female mice were evaluated using a single intravenous injection of 100 mg/kg racemic bromochloroacetic acid administered in physiological saline. Blood samples were collected pre- and postdosing, and the isomers were measured using a validated method. Bromochloroacetic acid isomer concentration time curves (Figure M22) for the mouse were described using a one-compartment model with bolus input and first-order elimination. The parameter estimates generated with this model for the bromochloroacetic acid (+) isomer are provided in Table M14. Bromochloroacetic acid (–) isomer concentrations in mice declined much faster than the (+) isomer following a single intravenous injection of 100 mg/kg (Table M15).

REFERENCE

Schultz, I.R., and Sylvester, S.R. (2001). Stereospecific toxicokinetics of bromochloro- and chlorofluoroacetate: Effect of GST-ζ depletion. *Toxicol. Appl. Pharmacol.* **175**, 104-113.

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Single Intravenous Administration	Bromochloroacetic Acid Single Gavage Administration	Bromochloroacetic Acid Single Intravenous Administration (+) and (–) Isomers Determination
Study Laboratory		
Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)	Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)	Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Hilltop Lab Animals, Inc. (Scottdale, PA)	Taconic Farms, Inc. (Germantown, NY)	Hilltop Lab Animals, Inc. (Scottdale, PA)
Time Held Before Studies		
4 or 5 days	8 or 9 days	4 days
Average Age When Studies Began		
14 weeks	15 weeks	14 weeks
Dates of Dosing/Exposure		
Rats: April 23, 2003 Mice: April 29, 2003	Rats: June 25 (males) or 26 (females), 2003 Mice: July 9 (males) or 10 (females), 2003	Rats: March 28, 2006 Mice: May 2, 2006
Size of Study Groups		
Rats: 15 males and 15 females (10 mg/kg) 5 males and 5 females (80 mg/kg) Mice: 27 males and 27 females	Rats: 17 males and 17 females (10 mg/kg) 23 males and 23 females (40 mg/kg) 21 males and 21 females (100 mg/kg) Mice: 30 males and 30 females (100 mg/kg) 45 males and 45 females (200 mg/kg and 400 mg/kg)	Rats: 15 males and 15 females (10 mg/kg) 5 males and 5 females (80 mg/kg) Mice: 27 males and 27 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Animals were distributed randomly into groups of approximately equal initial mean body weights.	Animals were distributed randomly into groups of approximately equal initial mean body weights.
Diet		
NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*	NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*	NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Drinking Water Administration	Bromochloroacetic Acid Drinking Water Administration with Gavage Challenge	Glyoxylic Acid Monohydrate Single Intravenous Administration
Study Laboratory		
Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)	Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)	Battelle, Toxicology, Health and Life Sciences Division (Columbus, OH)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats
Animal Source		
Hilltop Lab Animals, Inc. (Scottdale, PA)	Hilltop Lab Animals, Inc. (Scottdale, PA)	Hilltop Lab Animals, Inc. (Scottdale, PA)
Time Held Before Studies		
17 or 18 days	18 or 19 days	4 days
Average Age When Studies Began		
12 weeks	12 weeks	14 weeks
Dates of Dosing/Exposure		
Rats: May 23 (males) or 24 (females), 2005 Mice: May 31 (males) or June 1 (females), 2005	Rats: drinking water May 23 (males) or 24 (females), 2005 followed by a single gavage dose on June 6 (males) or 7 (females), 2005 Mice: drinking water May 31 (males) or June 1 (females), 2005 followed by a single gavage dose on June 14 (males) or 15 (females), 2005	April 11, 2006
Size of Study Groups		
Rats: 27 males and 27 females Mice: 33 males and 33 females	Rats: 12 males and 12 females Mice: 24 males and 24 females	14 males and 14 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Animals were distributed randomly into groups of approximately equal initial mean body weights.	Animals were distributed randomly into groups of approximately equal initial mean body weights.
Diet		
NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*	NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*	NTP-2000 irradiated pelleted diet (Zeigler Brothers, Inc., Gardners, PA); available *ad libitum*

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Single Intravenous Administration	Bromochloroacetic Acid Single Gavage Administration	Bromochloroacetic Acid Single Intravenous Administration (+) and (–) Isomers Determination
Water		
Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*
Cages		
Polycarbonate solid-bottom with slotted feeders (Hazleton Systems, Inc., Aberdeen, MD)	Polycarbonate solid-bottom or metabolism (for urine collection) cages with slotted or cup feeders (Hazleton Systems, Inc., Aberdeen, MD)	Polycarbonate solid-bottom with slotted feeders (Hazleton Systems, Inc., Aberdeen, MD)
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses/Exposure Concentrations		
Rats: 10 or 80 mg/kg Mice: 100 mg/kg	Rats: 10, 40, or 100 mg/kg Mice: 100, 200, or 400 mg/kg	Rats: 10 or 80 mg/kg Mice: 100 mg/kg
Vehicle		
0.9% Aqueous sodium chloride	Milli-Q water	0.9% Aqueous sodium chloride
Dosing Volume		
Rats: 2 mL/kg Mice: 4 mL/kg	Rats: 5 mL/kg Mice: 10 mL/kg	Rats: 2 mL/kg Mice: 4 mL/kg
Type and Frequency of Observation		
Morbidity and mortality checks were performed twice daily. Animals were weighed on the day of dosing for calculation of the dosing volume.	Morbidity and mortality checks were performed twice daily. Animals were weighed on the day of dosing for calculation of the dosing volume.	Morbidity and mortality checks were performed twice daily. Animals were weighed on the day of dosing for calculation of the dosing volume.

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Drinking Water Administration	Bromochloroacetic Acid Drinking Water Administration with Gavage Challenge	Glyoxylic Acid Monohydrate Single Intravenous Administration
Water		
Tap water (Columbus municipal supply) via amber glass drinking water bottles, available *ad libitum*	Tap water (Columbus municipal supply) via amber glass drinking water bottles, available *ad libitum.* Urine collection animals received untreated tap water *ad libitum* overnight prior to the gavage challenge dose and during the 96-hour collection period.	Tap water (Columbus municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*
Cages		
Polycarbonate solid-bottom with slotted feeders (Hazleton Systems, Inc., Aberdeen, MD)	Polycarbonate solid-bottom or metabolism (for urine collection) cages with slotted or cup feeders (Hazleton Systems, Inc., Aberdeen, MD)	Polycarbonate solid-bottom with slotted feeders (Hazleton Systems, Inc., Aberdeen, MD)
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses/Exposure Concentrations		
40, 400, or 800 mg/L for 14 consecutive days	Drinking water: 40, 400, or 800 mg/L for 14 consecutive days except untreated tap water (*ad libitum*) overnight prior to gavage challenge dose. Gavage challenge: 2.88, 28.8, or 57.6 mg/kg (male rats); 2.74, 27.4, or 54.9 mg/kg (female rats); 8, 80, or 160 mg/kg (male mice); 10, 100, or 200 mg/kg (female mice)	50 mg/kg
Vehicle		
Tap water	Tap water (drinking water doses) or Milli-Q water (gavage challenge doses)	0.9% Aqueous sodium chloride
Dosing Volume		
Not applicable	Rats (gavage doses): 5 mL/kg Mice (gavage doses): 10 mL/kg	2 mL/kg
Type of Observation		
Morbidity and mortality checks were performed twice daily. Animals were weighed on study days 1, 8, and 14 (rats) or 15 (mice).	Morbidity and mortality checks were performed twice daily. Animals were weighed on study days 1, 8, and 15; day 15 body weights were used for calculation of the dosing volume for the gavage challenge dose.	Morbidity and mortality checks were performed twice daily. Animals were weighed on the day of dosing for calculation of the dosing volume.

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Single Intravenous Administration	Bromochloroacetic Acid Single Gavage Administration	Bromochloroacetic Acid Single Intravenous Administration (+) and (–) Isomers Determination
Postdosing Blood Collection Times		
Rats: 10 mg/kg - 0, 2, 5, 10, 20, 40, 60, 75, 90, and 120 minutes 80 mg/kg - 60, 120, and 180 minutes Mice: 0, 2, 5, 10, 20, 30, 40, 50, and 60 minutes	Rats: 0, 2, 5, 10, 15, 20, 45, 60 (10 and 40 mg/kg), 90, 120 (10 and 40 mg/kg), 180, 240 (40 mg/kg), 270 (100 mg/kg), 360 (40 and 100 mg/kg), and 420 minutes (100 mg.kg) Mice: 0, 2, 5, 10, 15, 20, 30 (100 and 200 mg/kg), 40, 60, 90, 120 (200 and 400 mg/kg), 150 (200 and 400 mg/kg), and 180 minutes (400 mg/kg)	Rats: 10 mg/kg - 0, 2, 5, 10, 20, 40, 60, 75, 90, and 120 minutes 80 mg/kg - 60, 120, and 180 minutes Mice: 0, 2, 5, 10, 20, 30, 40, 50, and 60 minutes
Urine Collection Intervals		
None	Rats (40 and 100 mg/kg) and mice (200 and 400 mg/kg): –24 to 0 hours (prior to dosing); then 0 to 2 hours, 2 to 4 hours, 4 to 8 hours, 8 to 24 hours, and 24 to 48 hours after dosing	None
Analyte(s)		
Plasma bromochloroacetic acid	Plasma and urine bromochloroacetic acid, glyoxylic acid, and oxalic acid concentrations	Plasma (+) and (–) bromochloroacetic acid concentrations

Table M1
Experimental Design and Materials and Methods in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate

Bromochloroacetic Acid Drinking Water Administration	Bromochloroacetic Acid Drinking Water Administration with Gavage Challenge	Glyoxylic Acid Monohydrate Single Intravenous Administration
Postdosing Blood Collection Times		
900, 1200, 1500, 1800, 2100, 000, 300, and 600 hours (clock time)	Rats: 2 (2.88 and 2.74 mg/kg), 5, 10, 15, 20 (2.88 and 2.74 mg/kg), 30, 45 (2.88 and 2.74 mg/kg), 60, 120 (28.8, 27.4, 57.6, and 54.9 mg/kg), 180 (28.8 and 27.4 mg/kg), 240 (28.8, 27.4, 57.6, and 54.9 mg/kg), and 360 (57.6 and 54.9 mg/kg) minutes Mice: 2, 5, 10, 15 (8 and 10 mg/kg), 20, 30 (8 and 10 mg/kg), 40 (80, 100, 160, and 200 mg/kg), 45 (8 and 10 mg/kg), 60, 90 (80 and 100 mg/kg), 120 (80, 100, 160, and 200 mg/kg), and 180 (160 and 200 mg/kg) minutes	0, 2, 5, 8, 11, 14, 18, 22, and 30 minutes
Urine Collection Intervals		
None	0 to 2 hours, 2 to 4 hours, 4 to 8 hours, 8 to 24 hours, 24 to 48 hours, 48 to 72 hours, and 72 to 96 hours after a single gavage challenge dose	None
Analyte(s)		
Plasma bromochloroacetic acid, glyoxylic acid, and oxalic acid concentrations	Plasma and urine bromochloroacetic acid, glyoxylic acid, and oxalic acid concentrations	Plasma glyoxylic acid concentrations

TABLE M2
Chromatographic Systems Used in the Toxicokinetic Studies of Bromochloroacetic Acid and Glyoxylic Acid Monohydrate in F344/N Rats and B6C3F1 Mice

System A	GC/FID (Agilent 6890, Palo Alto, CA) RTX-5 column (Restek, Bellefonte, PA), 30 m × 0.53 mm (ID), 1.5-µm film thickness, 1 µL sample injection; the oven program was 50° C for 1 minute, 12° C/minute to 150° C, 70° C/minute to 300° C for 6 minutes: carrier gas = helium (10 mL/minute).
System B	HPLC/UV (Waters, Milford, MA) with AQUA® C18 column (Phenomenex, Torrance, CA), 150 mm × 4.6 mm, 3-µm film thickness, with mobile phase A) of 80:20 0.1 M phosphoric acid/acetonitrile and mobile phase B) of 10:90 0.1 M phosphoric acid/acetonitrile, and program of 100% A for 2 minutes then to 100% B over 3 minutes, held at 100% B for 14 minutes and 10 µL sample injection and detection at 200 nm.
System C	GC/ECD (Agilent 6890) 1 µL aliquot was injected onto a VOCOL column (Supelco, St. Louis, MO), 60 m × 0.53 mm, 3-µm film thickness; the oven program was 45° C for 1 minute, then 5° C/minute to 100°C for 10 minutes and 20° C/minute to 220° C for 4 minutes: carrier gas flow rate of 10 mL/minute.
System D	GC/ECD (Agilent 6890) same as above except that a Beta-DEX™ column (Supelco), 30 m × 0.25 mm, 0.25-µm film thickness; the oven program was 45° C for 3 minutes, increased at 8°C/minute to 100° C, held for 5 minutes and again increased at 20° C/minute to 200° C and held for 10 minutes.
System E	GC/MS (Agilent 5973N) with RTX-5 column (Restek), 30 m × 0.32 mm, 1-µm film thickness; the oven program was 60° C for 2 minutes, then 5° C/minute to 105° C and 20° C/minute to 250° C and held for 2 minutes: helium carrier gas at 1.5 mL/minute and monitoring ions 59, 60, and 75.
System F	HPLC/MS (Sciex, Toronto Canada) system included a Sciex API 5000 using a direct infusion of 10 µL without an analytical column and mobile phase A) 4 mM ammonium formate in Milli-Q water with 0.1% formic acid and B) acetonitrile with 0.1% formic acid operated isocratically at 65% A:35% B.
System G	HPLC/MS (VG/Fisons, Manchester, UK) system included a Quattro LC using direct infusion of 50 µL with system included with isocratic mobile phase as above at a flow rate of 0.15 mL/minute.
System H	HPLC/UV (Waters) system included a Waters LCM1 detecting at 220 nm with a 20 µL sample injected onto a BETASIL CN (Thermo Fisher Scientific, Waltham, MA), 5µm, 250 mm × 4.6 mm ID analytical column using a flow rate of 1.0 mL/minute and isocratic mobile phase of 50 mM ammonium phosphate at pH 2.4.
System I	Sciex API 5000 mass spectrometer equipped with an Agilent HPLC system. A 2 µL sample was injected onto a Synergi™ Hydro-RP column (Phenomenex), 4 µm, 80 Å, 100 mm × 2.0 mm column. The mobile phase was A) 0.2% formic acid in Milli-Q water and B) 0.2% formic acid in acetonitrile with a linear program from A to B over 5 minutes and a flow rate of 300 µL/minute.

Plate A

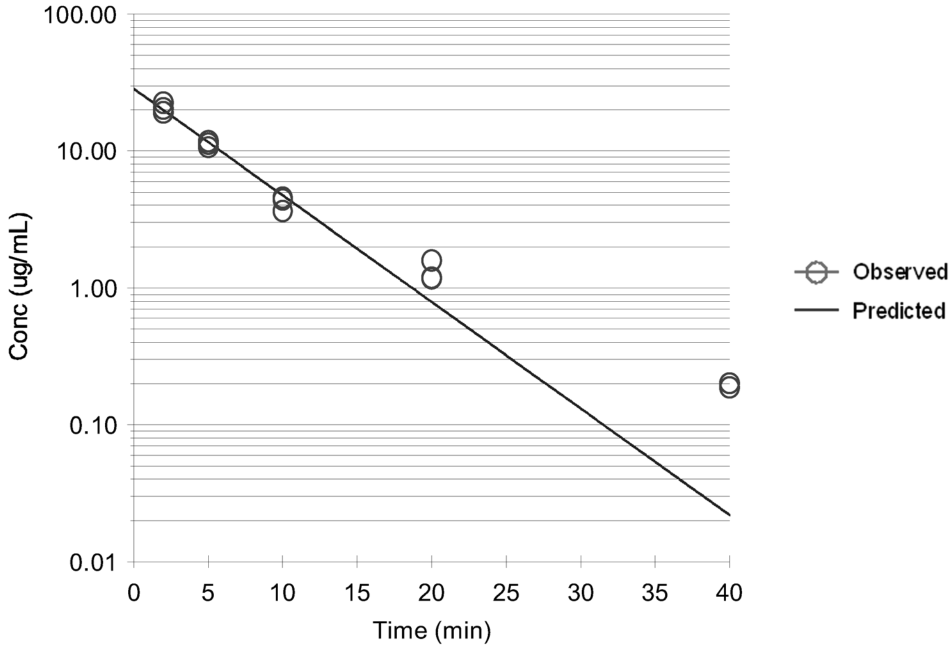

Plate B

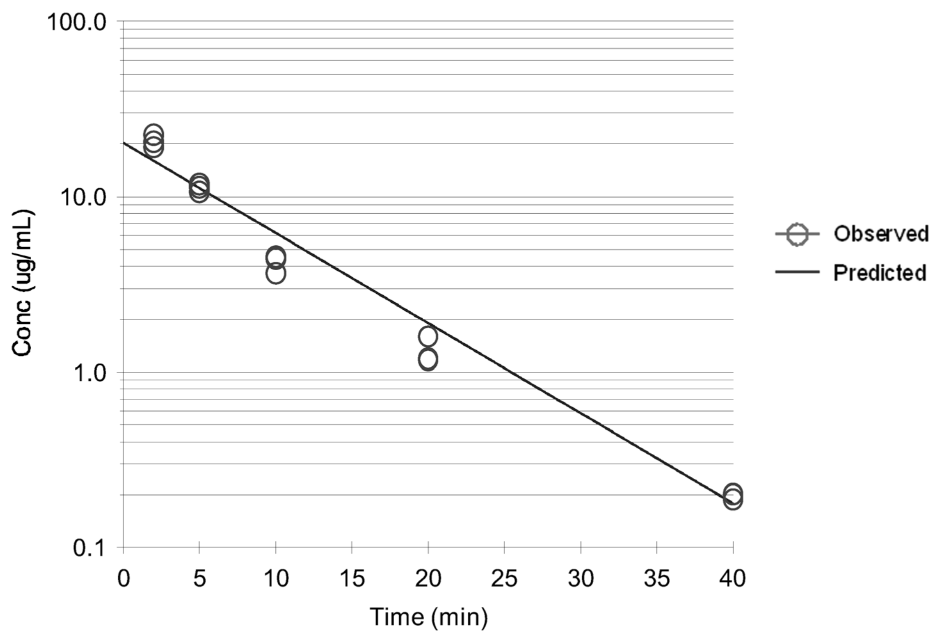

Figure M1
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male F344/N Rats Following a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid
Fitted using 1/Y Weighting (Plate A) and Fitted Using 1/Yhat2 Weighting (Plate B)

Plate A

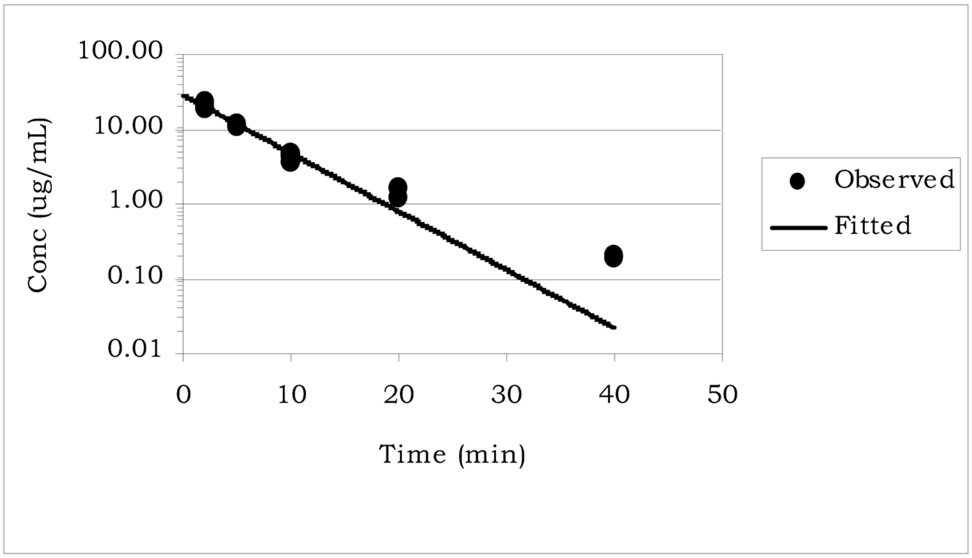

Plate B

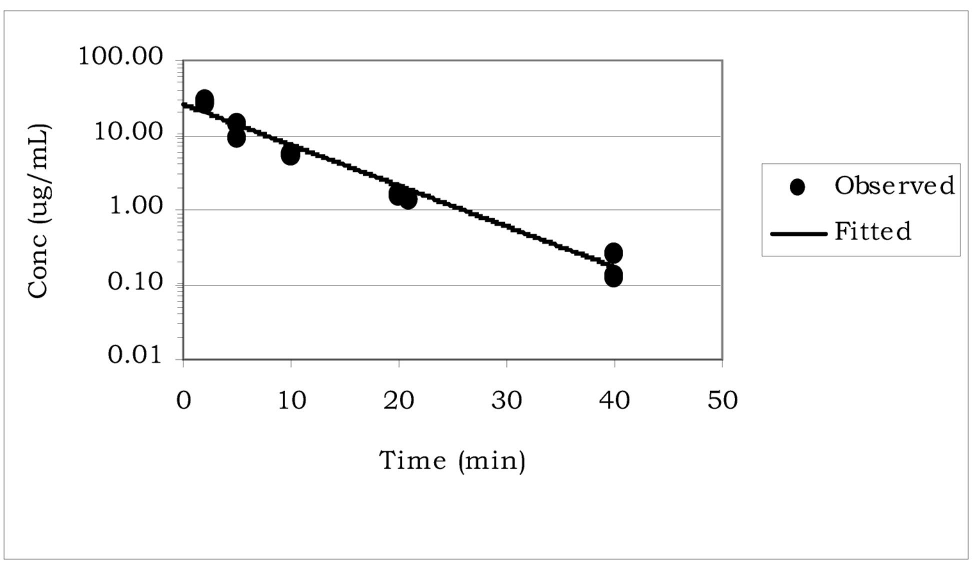

FIGURE M2
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for F344/N Rats Following a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid
Plate A (Male Rats) and Plate B (Female Rats)

TABLE M3
Bromochloroacetic Acid Toxicokinetic Parameters for F344/N Rats After a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid[a]

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	20.7 ± 1.0	27.5 ± 1.0
k_{10} (minute^{-1})	0.118 ± 0.005	0.125 ± 0.006
$t_{1/2}$ (minute)	5.86 ± 0.25	5.53 ± 0.27
AUC_{∞} (predicted) (µg/mL×minute)	171 ± 13	206 ± 19
Cl (mL/minute/kg)	58.5 ± 4.6	48.6 ± 4.6
AUC/Dose	17.1	20.6

[a] Based on a one-compartment model with a bolus input, first-order output, and 1/Yhat weighting; parameter estimates (± standard error) are reported to three significant figures.

TABLE M4
Bromochloroacetic Acid Toxicokinetic Parameters for F344/N Rats After a Single Gavage Administration of Bromochloroacetic Acid[a]

	10 mg/kg	40 mg/kg	100 mg/kg
Male			
k_{01} or k_{10} (minute^{-1})	0.0457 ± 0.0039	0.0188 ± 0.009	0.0163 ± 0.009
k_{01} or k_{10} half-life (minute)	15.2 ± 1.3	36.9 ± 1.8	42.6 ± 2.3
C_{max} (observed) (µg/mL)	0.532 ± 0.029	7.91 ± 0.73	38.5 ± 2.1
T_{max} (observed) (minute)	21.9 ± 1.9	53.2 ± 2.6	61.4 ± 3.4
AUC_{∞} (predicted) (µg/mL×minute)	28.2 ± 2.6	976 ± 61	4,690 ± 390
Cl (mL/minute/kg)	354 ± 37	41.0 ± 2.5	21.3 ± 1.8
Female			
k_{01} or k_{10} (minute^{-1})	0.056 ± 0.0035	0.0220 ± 0.0013	0.0172 ± 0.0010
k_{01} or k_{10} half-life (minute)	12.4 ± 0.8	31.5 ± 1.8	40.2 ± 2.3
C_{max} (observed) (µg/mL)	0.638 ± 0.052	11.1 ± 0.7	54.0 ± 4.4
T_{max} (observed) (minute)	15.0	20.0	45.0
AUC_{∞} (predicted) (µg/mL×minute)	29.1 ± 2.2	1,430 ± 110	7,050 ± 600
Cl (mL/minute/kg)	344 ± 26	27.9 ± 2.1	14.2 ±1.2

[a] Based on a one-compartment model with first-order absorption and elimination; parameter estimates (± standard error) are reported to three significant figures.

Plate A

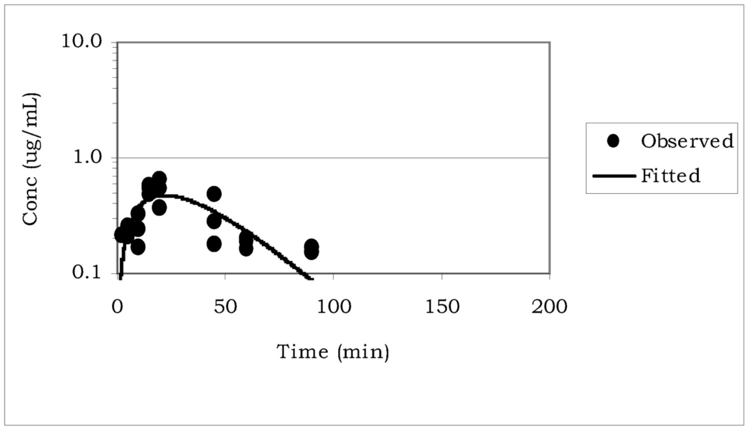

Plate B

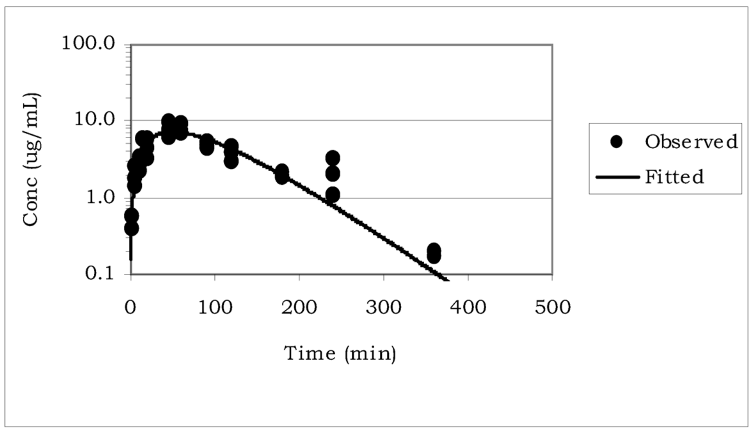

Plate C

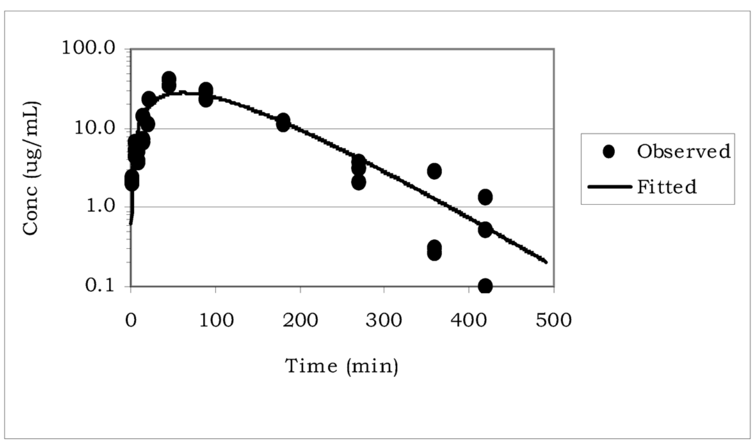

FIGURE M3
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male F344/N Rats After a Single Gavage Dose of 10 mg/kg (Plate A), 40 mg/kg (Plate B), or 100 mg/kg (Plate C) Bromochloroacetic Acid

Plate A

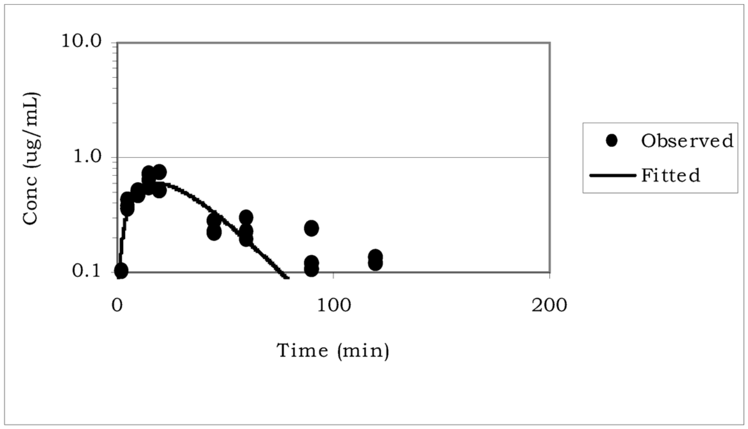

Plate B

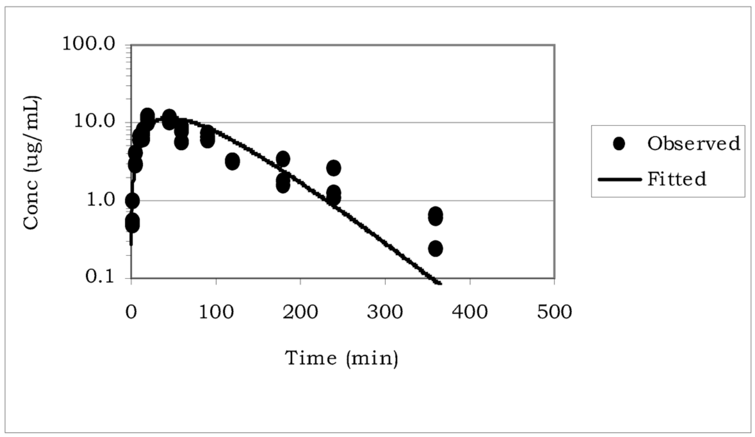

Plate C

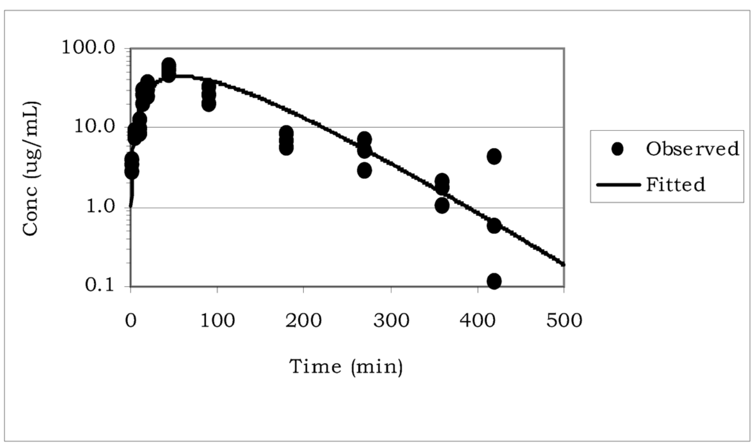

FIGURE M4
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Female F344/N Rats After a Single Gavage Dose of 10 mg/kg (Plate A), 40 mg/kg (Plate B), or 100 mg/kg (Plate C) Bromochloroacetic Acid

Plate A

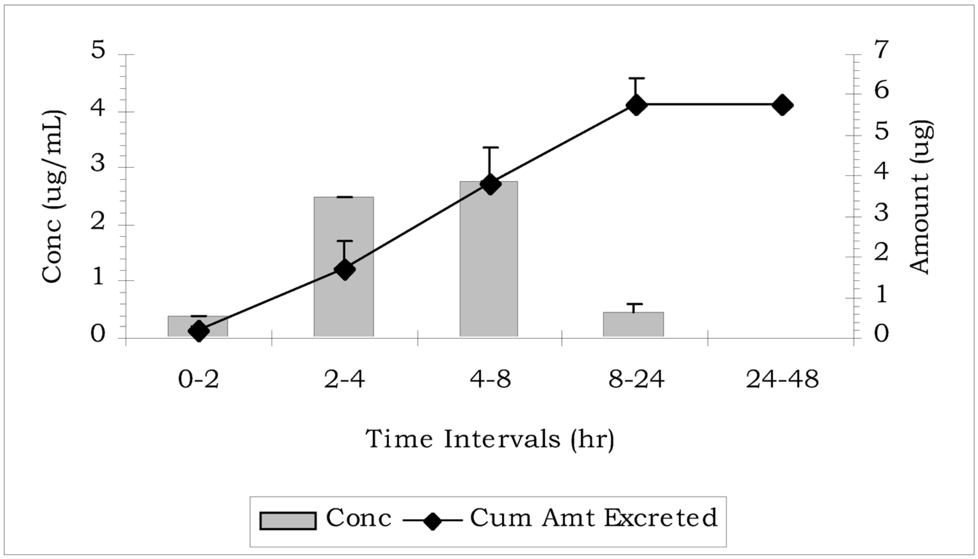

Plate B

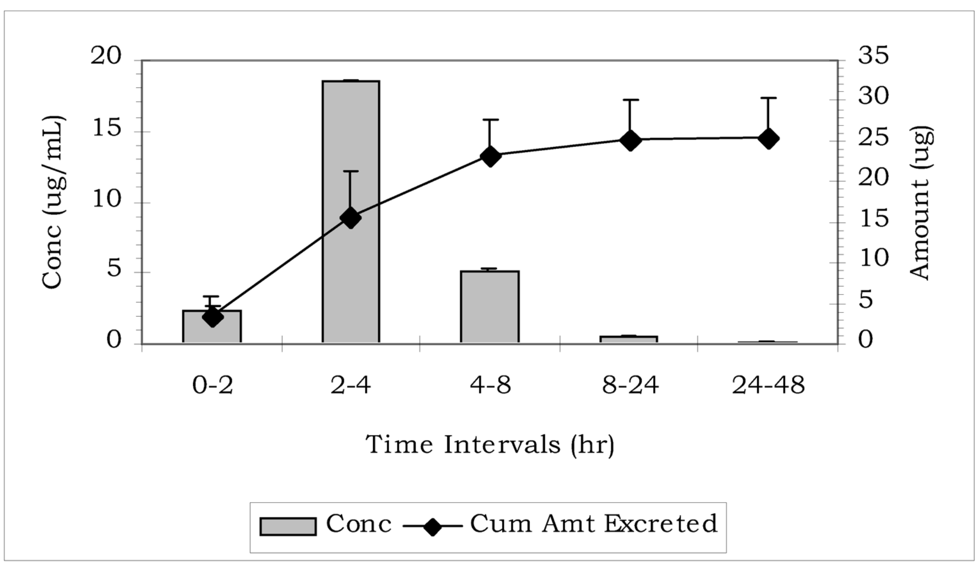

Figure M5
Bromochloroacetic Acid Concentrations in the Urine of Male F344/N Rats After a Single Gavage Dose of 40 mg/kg (Plate A) or 100 mg/kg (Plate B) Bromochloroacetic Acid

Plate A

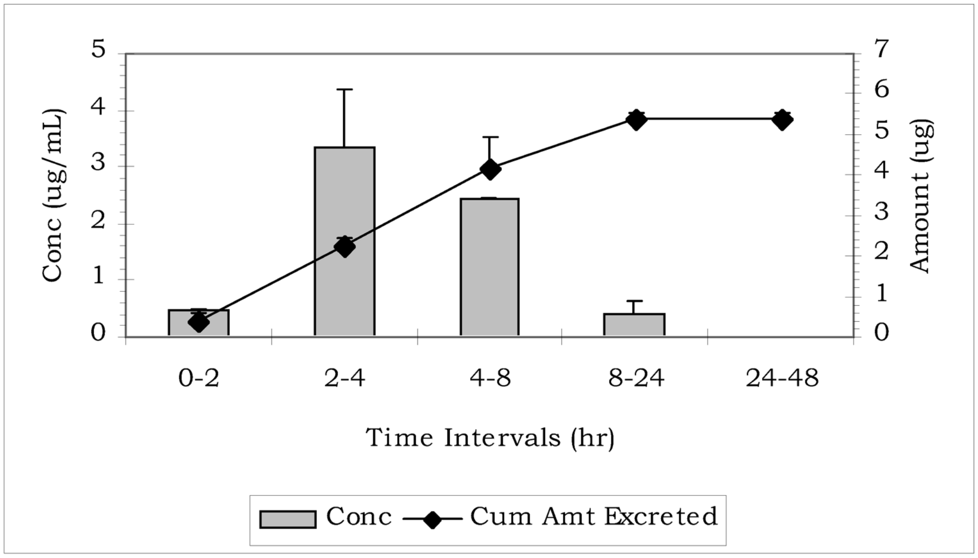

Plate B

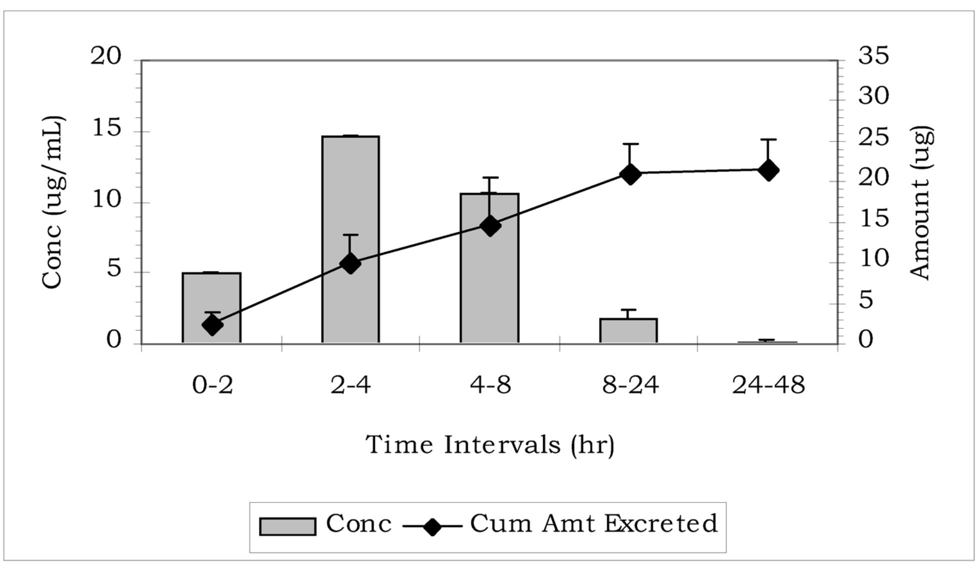

FIGURE M6
Bromochloroacetic Acid Concentrations in the Urine of Female F344/N Rats After a Single Gavage Dose of 40 mg/kg (Plate A) or 100 mg/kg (Plate B) Bromochloroacetic Acid

Plate A

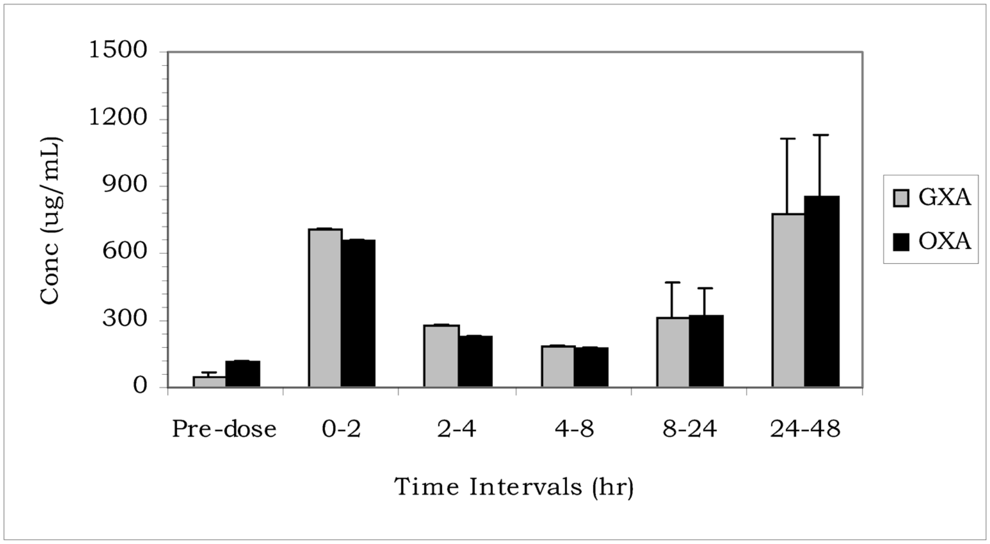

Plate B

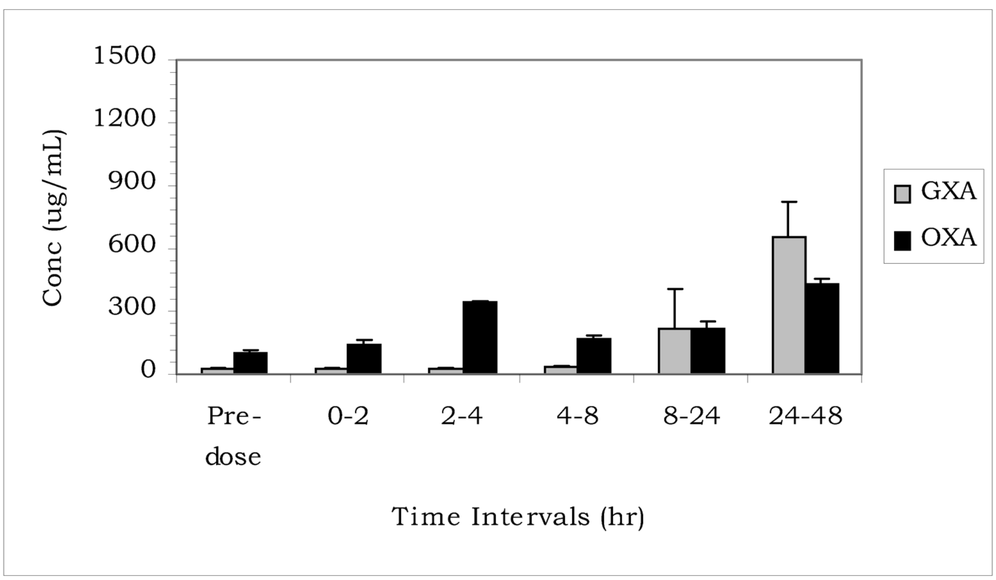

FIGURE M7
Concentrations of Glyoxylic Acid and Oxalic Acid in the Urine of Male F344/N Rats After a Single Gavage Dose of 40 mg/kg (Plate A) or 100 mg/kg (Plate B) Bromochloroacetic Acid

Plate A

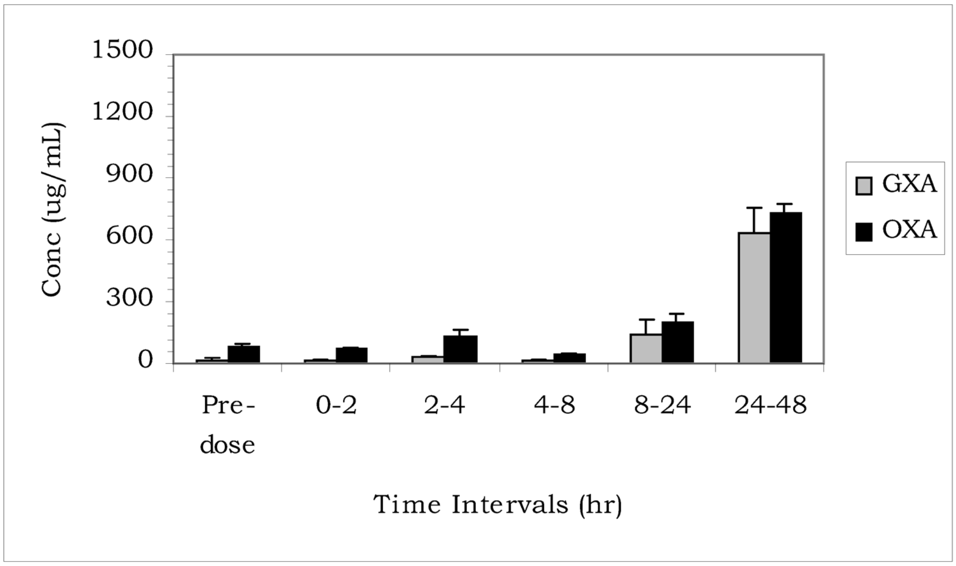

Plate B

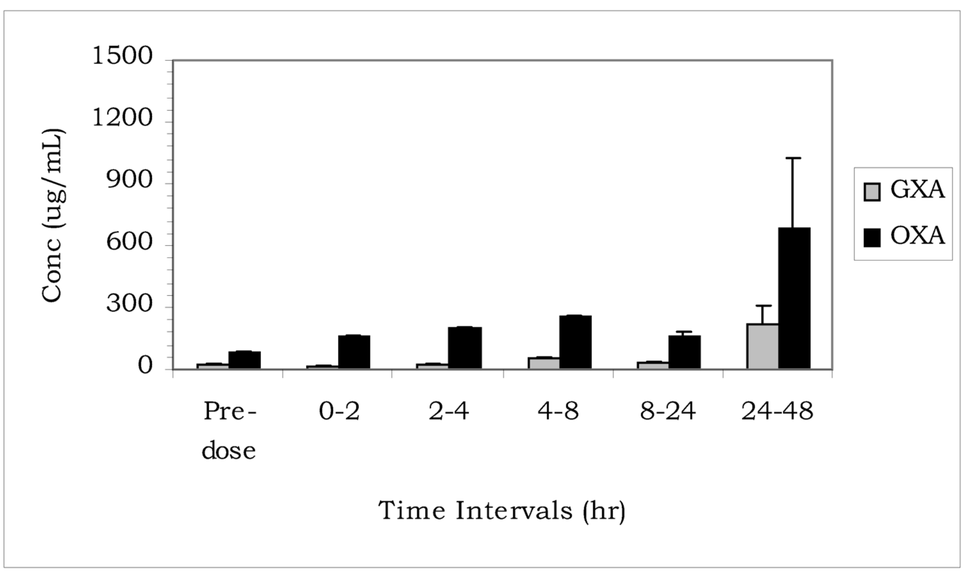

FIGURE M8
Concentrations of Glyoxylic Acid and Oxalic Acid in the Urine of Female F344/N Rats After a Single Gavage Dose of 40 mg/kg (Plate A) or 100 mg/kg (Plate B) Bromochloroacetic Acid

Table M5
Bromochloroacetic Acid Toxicokinetic Parameters for F344/N Rats in the Non-Challenged Drinking Water Group[a]

Male	2.88 mg/kg	28.8 mg/kg	57.6 mg/kg
C_{max} (observed) (µg/mL)	0.0801	2.66 ± 0.84	5.17 ± 4.02
T_{max} (observed) (minute)	6 AM	6 AM	6 AM
C_{max} (observed) (µg/mL)	BLOQ	BLOQ	BLOQ
T_{max} (observed) (minute)	NA	NA	NA
Female	**2.74 mg/kg**	**27.4 mg/kg**	**54.9 mg/kg**
C_{max} (observed) (µg/mL)	0.189 ± 0.026	3.84 ± 1.58	7.36 ± 1.30
T_{max} (observed) (minute)	6 AM	9 PM	6 AM
C_{max} (observed) (µg/mL)	BLOQ	BLOQ	BLOQ
T_{max} (observed) (minute)	NA	NA	NA

[a] BLOQ = below the limit of quantitation (0.075 µg/mL); NA = not applicable

Table M6
Bromochloroacetic Acid Toxicokinetic Parameters for F344/N Rats Given a Single Gavage Challenge Dose of Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days[a]

Male	2.88 mg/kg	28.8 mg/kg	57.6 mg/kg
k_{01} or k_{10} (minute^{-1})	0.224 ± 0.115	0.0709 ± 0.0138	0.145 ± 0.114
k_{01} or k_{10} half-life (minute)	3.09 ± 1.58	9.78 ± 1.90	4.78 ± 3.76
C_{max} (observed) (µg/mL)	0.352 ± 0.036	10.9 ± 0.6	43.8 ± 25.7
T_{max} (observed) (minute)	15.0	15.0	15.0
AUC_∞ (predicted) (µg/mL×minute)	26.9 ± 10.3	1,220 ± 70	3,700 ± 700
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	3.32	41.7	65.5
Cl_F (mL/minute/kg)	107 ± 41	23.6 ± 1.4	15.6 ± 2.9
Female	**2.74 mg/kg**	**27.4 mg/kg**	**54.9 mg/kg**
k_{01} or k_{10} (minute^{-1})	0.154 ± 0.038	0.102 ± 0.036	0.111 ± 0.036
k_{01} or k_{10} half-life (minute)	4.49 ± 1.10	6.82 ± 2.40	6.27 ± 2.06
C_{max} (observed) (µg/mL)	0.410 ± 0.050	15.0 ± 5.9	44.3 ± 8.6
T_{max} (observed) (minute)	20.0	15.0	30.0
AUC_∞ (predicted) (µg/mL×minute)	28.7 ± 3.6	1,380 ± 130	3,900 ± 380
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	6.39	46.0	66.7
Cl_F (mL/minute/kg)	95.3 ± 11.9	19.8 ± 1.9	14.1 ± 1.4

[a] Based on a one-compartment model with first order absorption and elimination; parameter estimates (± standard error) are reported to three significant figures.
[b] AUC_{last} was calculated using the trapezoidal method, which does not calculate a standard error.

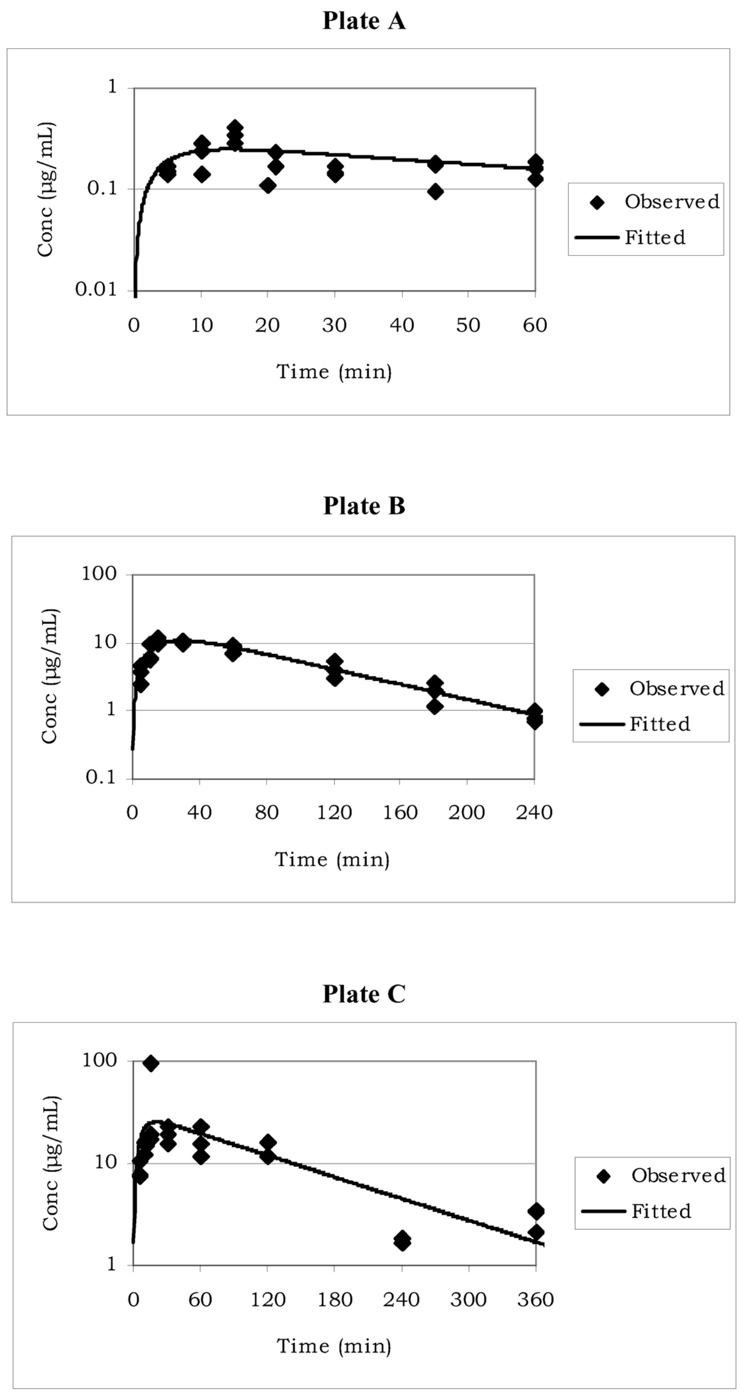

FIGURE M9
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male F344/N Rats After a Single Gavage Challenge Dose of 2.88 mg/kg (Plate A), 28.8 mg/kg (Plate B), or 57.6 mg/kg (Plate C) Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days

Plate A

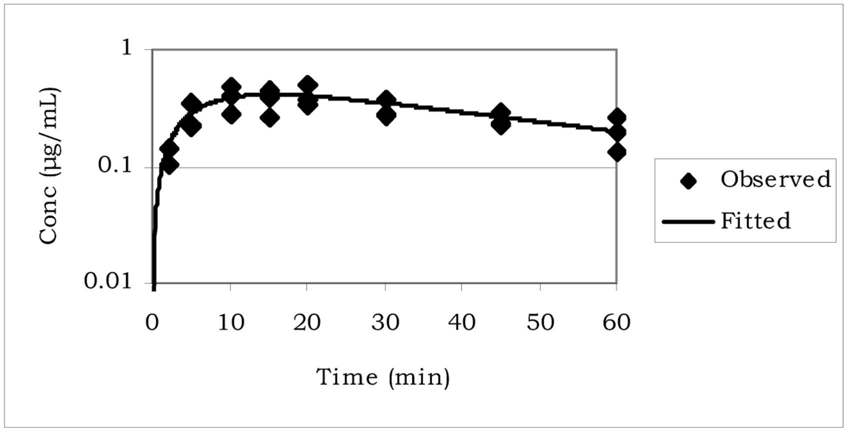

Plate B

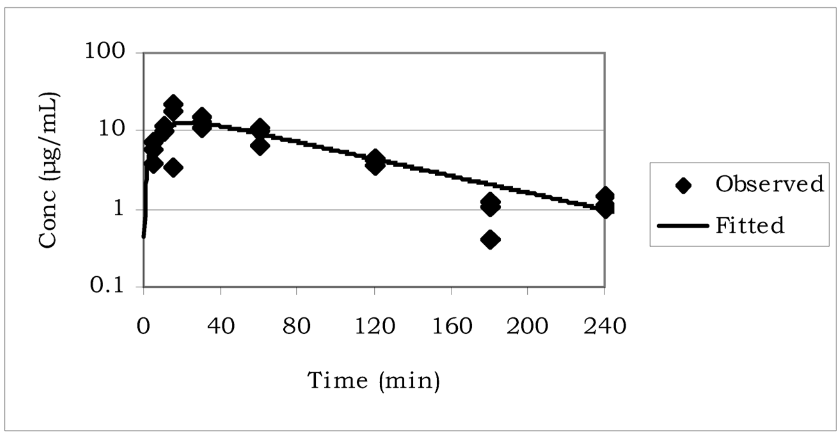

Plate C

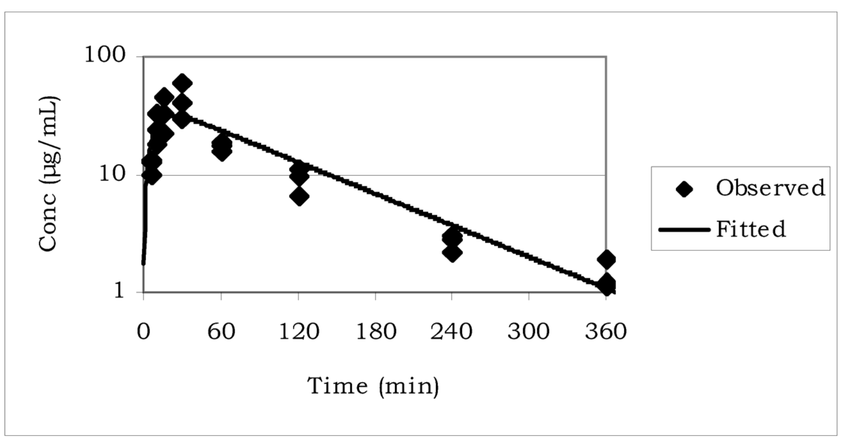

FIGURE M10
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Female F344/N Rats After a Single Gavage Challenge Dose of 2.74 mg/kg (Plate A), 27.4 mg/kg (Plate B), or 54.9 mg/kg (Plate C) Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days

Plate A

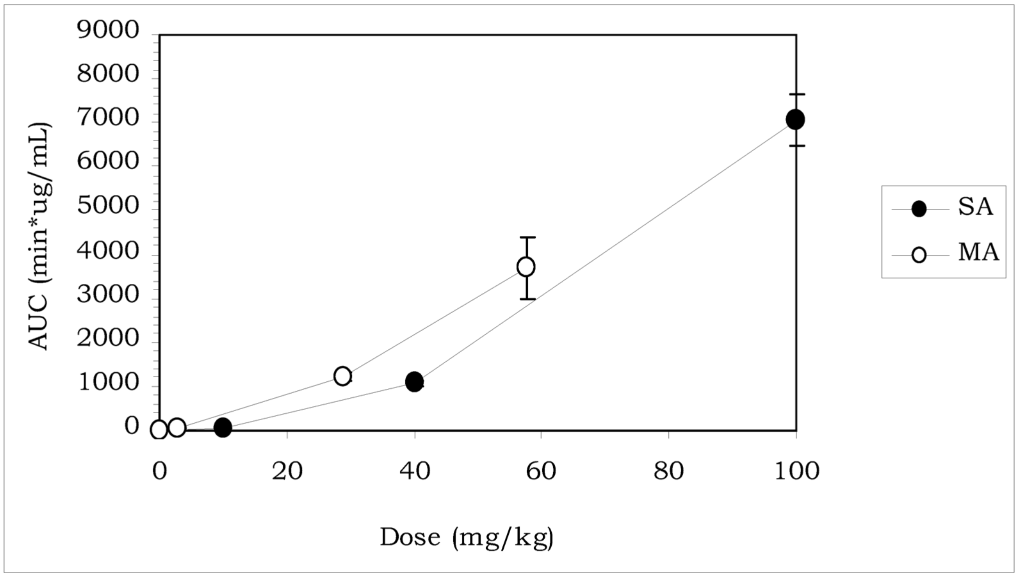

Plate B

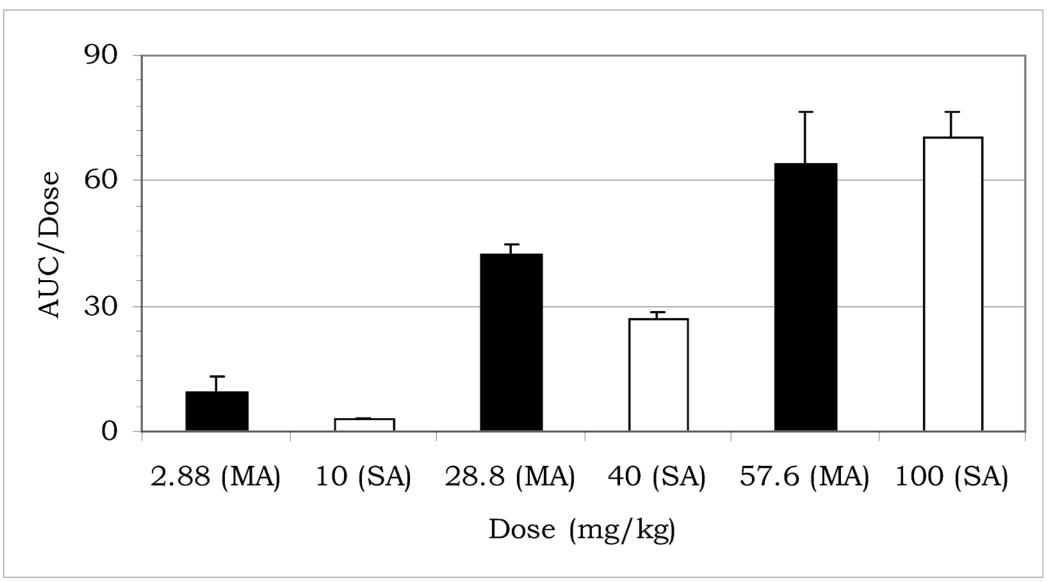

Figure M11
AUC for Male F344/N Rats After Receiving Bromochloroacetic Acid
(Dose versus AUC Plot - Plate A; Dose versus Dose-Normalized AUC Plot - Plate B)
(SA = Single Gavage Administration; MA = 14-Day Drinking Water Administration)

Plate A

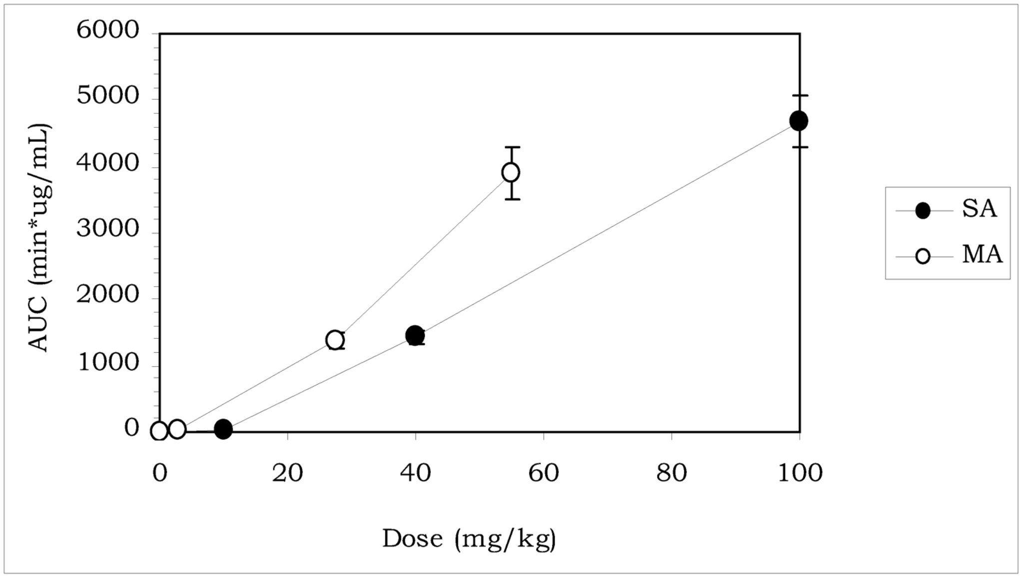

Plate B

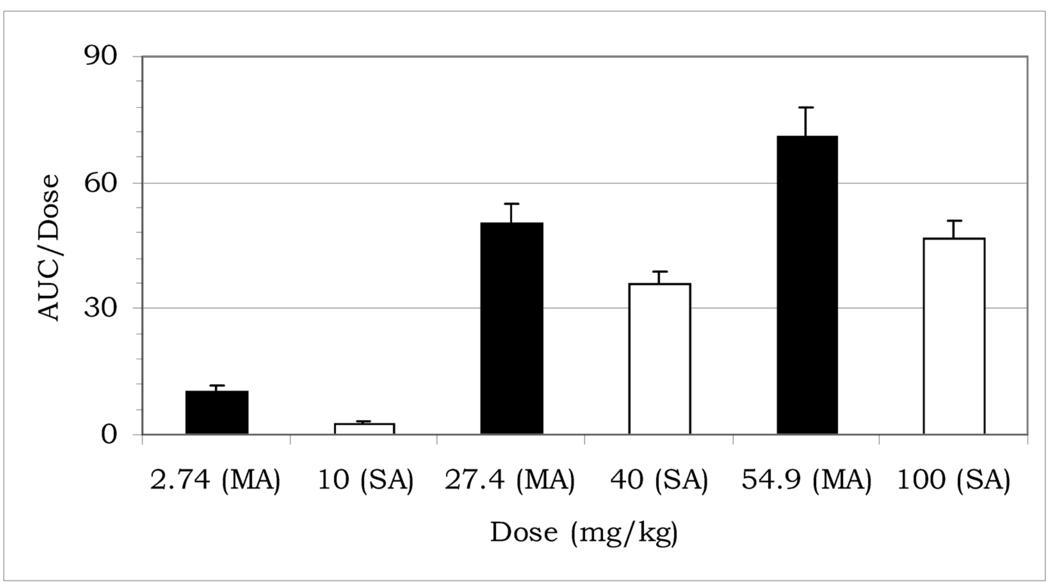

FIGURE M12
AUC for Female F344/N Rats After Receiving Bromochloroacetic Acid
(Dose versus AUC Plot - Plate A; Dose versus Dose-Normalized AUC Plot - Plate B)
(SA = Single Gavage Administration; MA = 14-Day Drinking Water Administration)

Plate A

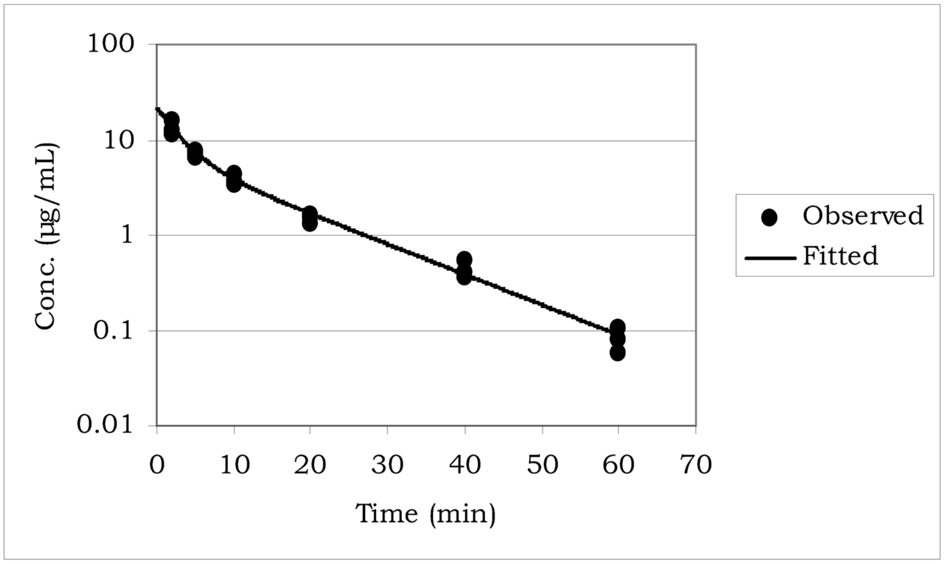

Plate B

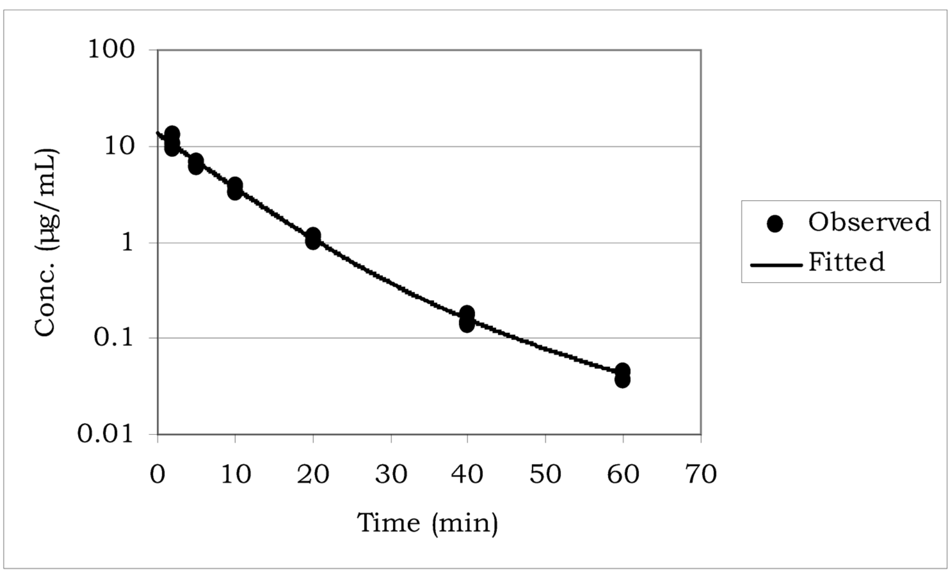

FIGURE M13
Bromochloroacetic Acid (+) Isomer Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats After a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid

Table M7
Bromochloroacetic Acid (+) Isomer Toxicokinetic Parameters for F344/N Rats After a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	11.7 ± 1.7	7.63 ± 1.10
k_{10} (minute^{-1})	0.156 ± 0.033	0.126 ± 0.007
$t_{1/2}$ (minute)	—	—
AUC_{∞} (predicted) (µg/mL×minute)	139 ± 8	108 ± 4
Cl (mL/minute/kg)	71.9 ± 4.3	92.9 ± 3.4
AUC/Dose	8.0	10.8

Table M8
Bromochloroacetic Acid (–) Isomer Toxicokinetic Parameters for F344/N Rats After a Single Intravenous Injection of 10 mg/kg Bromochloroacetic Acid

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	—	—
k_{10} (minute^{-1})	—	—
$t_{1/2}$ (minute)	—	—
AUC_{∞} (predicted) (µg/mL×minute)	79.4	48.5
Cl (mL/minute/kg)	126	206
AUC/Dose	7.9	4.8

Plate A

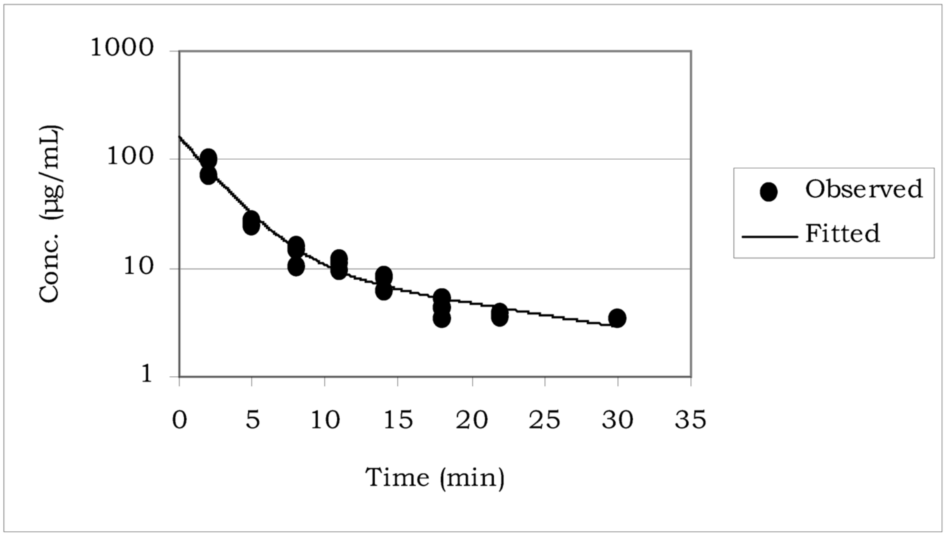

Plate B

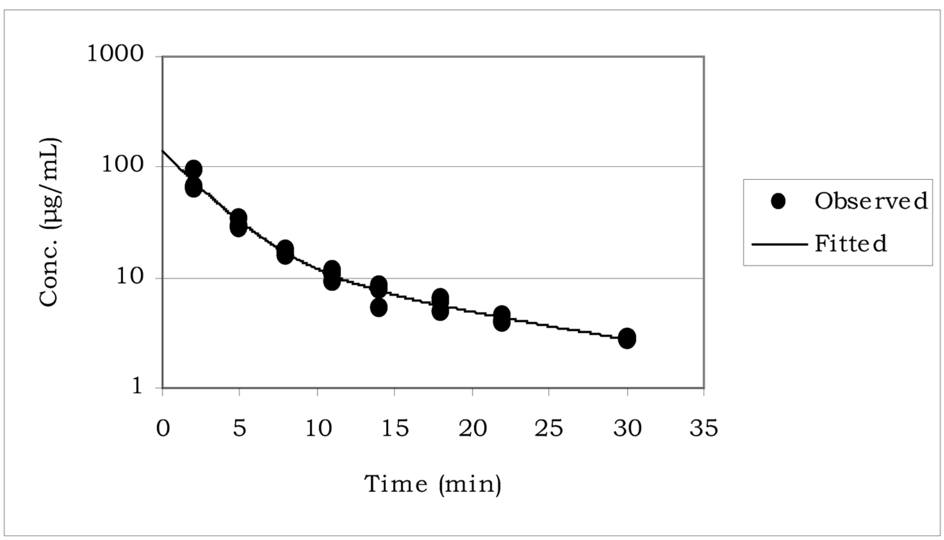

Figure M14
Glyoxylic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) F344/N Rats After a Single Intravenous Injection of 50 mg/kg Glyoxylic Acid

Table M9
Glyoxylic Acid Toxicokinetic Parameters for Male and Female F344/N Rats After a Single Intravenous Injection of 50 mg/kg Glyoxylic Acid[a]

Parameter	Male	Female
$C_{(2\ \text{minutes})}$ (observed) (µg/mL)	90.5 ± 10.3	74.1 ± 9.5
α half-life (minute)	1.81 ± 0.28	1.96 ± 0.22
β half-life (minute)	14.7 ± 4.3	12.5 ± 1.9
k_{10} (minute^{-1})	0.252 ± 0.039	0.226 ± 0.022
t_{10} half-life (minute)	2.75 ± 0.42	3.07 ± 0.29
AUC_{∞} (predicted) (µg/mL×minute)	648 ± 49	612 ± 26
Cl (mL/minute/kg)	77.1 ± 5.8	81.7 ± 3.5
AUC/Dose	13	12

[a] Based on a one-compartment model with a bolus input, first-order output, and 1/Yhat weighting; parameter estimates (± standard error) are reported to three significant figures.

Plate A

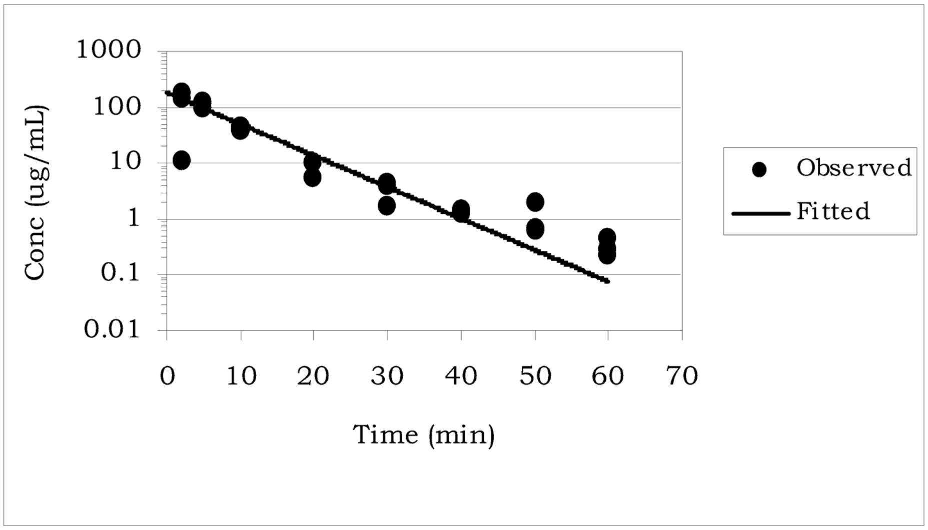

Plate B

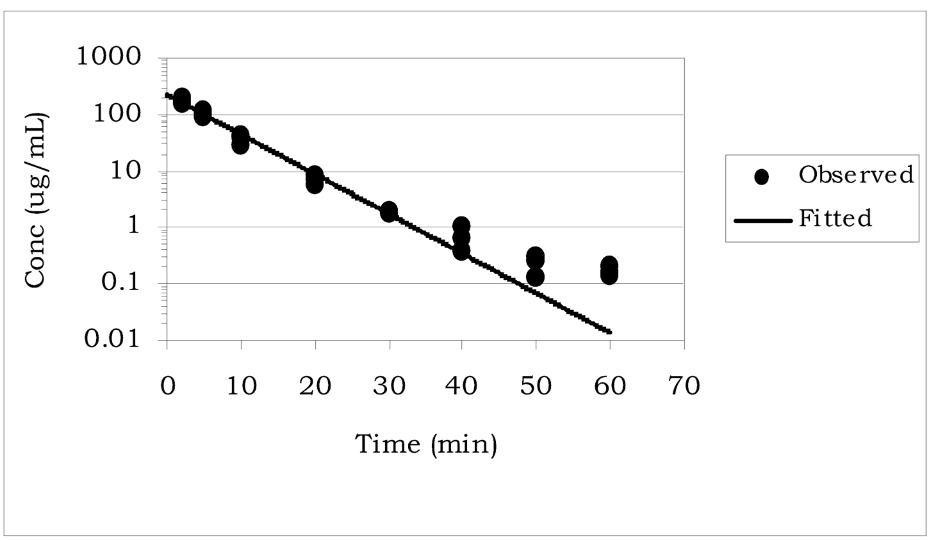

FIGURE M15
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice After a Single Intravenous Injection of 100 mg/kg Bromochloroacetic Acid

Table M10
Bromochloroacetic Acid Toxicokinetic Parameters for B6C3F1 Mice After a Single Intravenous Injection of 100 mg/kg Bromochloroacetic Acid[a]

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	110 ± 51	174 ± 13
k_{10} ($minute^{-1}$)	0.130 ± 0.016	0.186 ± 0.008
$t_{1/2}$ (minute)	5.34 ± 0.68	3.73 ± 0.16
AUC_{∞} (predicted) (µg/mL×minute)	1,400 ± 100	1,330 ± 40
Cl (mL/minute/kg)	71.3 ± 7.1	75.3 ± 2.2
AUC/Dose	140	133

[a] Based on a one-compartment model with a bolus input, first-order output, and 1/Yhat weighting; parameter estimates (± standard error) are reported to three significant figures.

Plate A

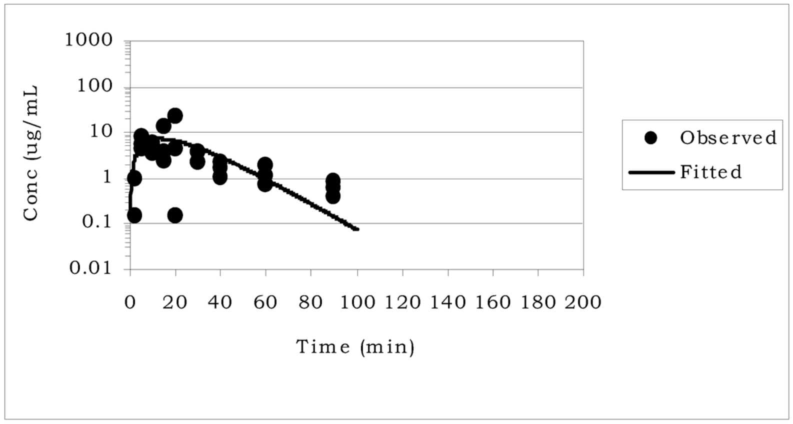

Plate B

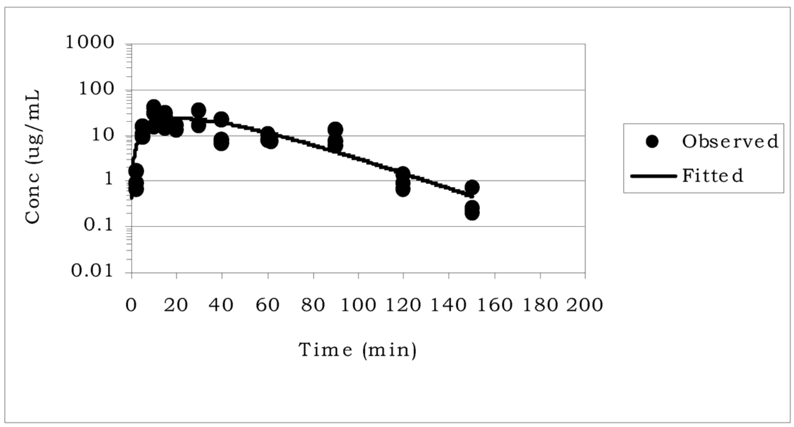

Plate C

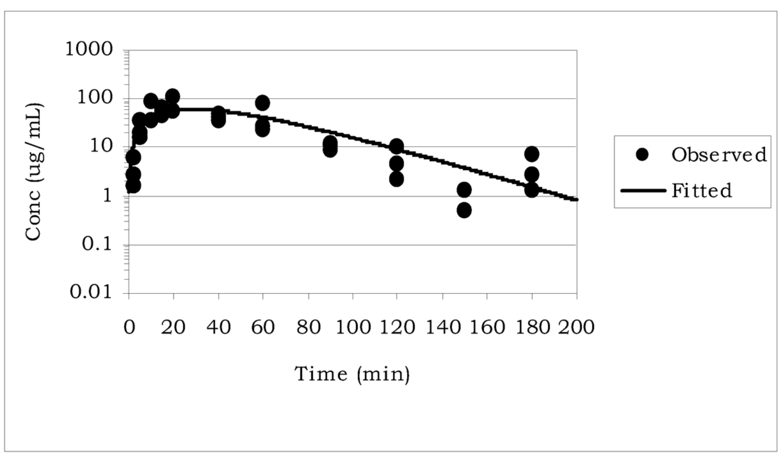

FIGURE M16
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male B6C3F1 Mice After a Single Gavage Dose of 100 mg/kg (Plate A), 200 mg/kg (Plate B), or 400 mg/kg (Plate C) Bromochloroacetic Acid

Plate A

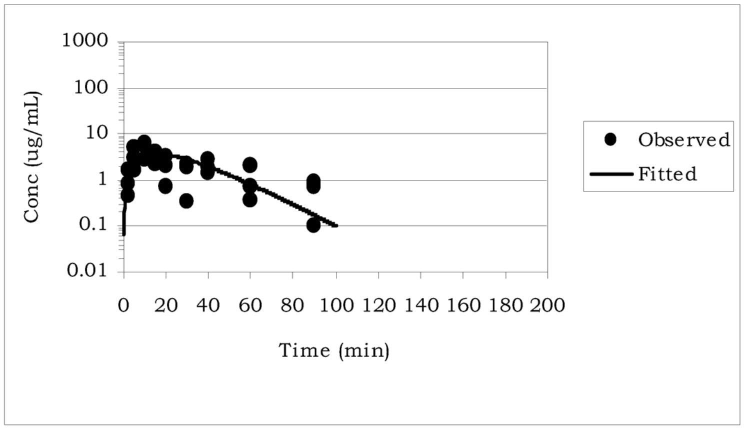

Plate B

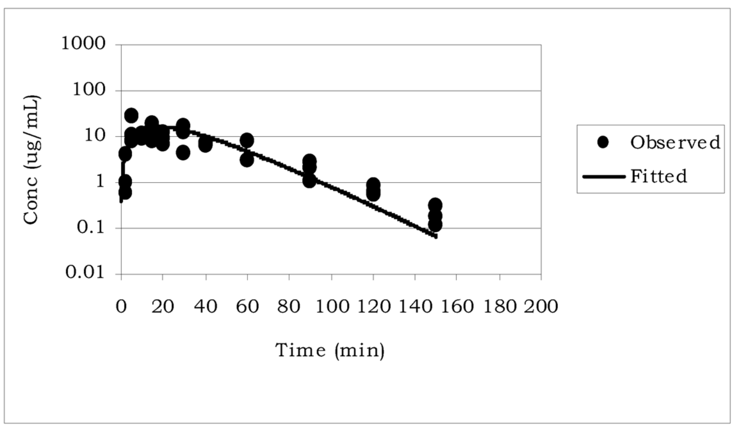

Plate C

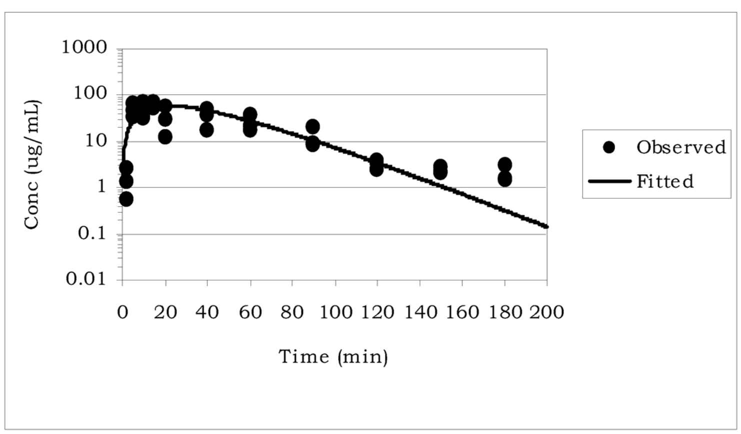

FIGURE M17
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Female B6C3F1 Mice After a Single Gavage Dose of 100 mg/kg (Plate A), 200 mg/kg (Plate B), or 400 mg/kg (Plate C) Bromochloroacetic Acid

TABLE M11
Bromochloroacetic Acid Toxicokinetic Parameters for B6C3F1 Mice After a Single Gavage Administration of Bromochloroacetic Acid[a]

	100 mg/kg	200 mg/kg	400 mg/kg
Male			
k_{01} or k_{10} (minute^{-1})	0.0762 ± 0.0130	0.0456 ± 0.0041	0.0367 ± 0.0028
k_{01} or k_{10} half-life (minute)	9.10 ± 1.56	15.2 ± 1.4	18.9 ± 1.4
C_{max} (observed) (µg/mL)	9.48 ± 7.26	28.2 ± 5.7	74.0 ± 18.7
T_{max} (observed) (minute)	20.0	30.0	20.0
AUC_{∞} (predicted) (µg/mL×minute)	260 ± 53	1,390 ± 160	4,630 ± 480
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	2.48	6.96	1.03
Cl_F (mL/minute/kg)	385 ± 79	144 ± 16	86.4 ± 9.0
Female			
k_{01} or k_{10} (minute^{-1})	0.0646 ± 0.0081	0.0579 ± 0.0056	0.0462 ± 0.0038
k_{01} or k_{10} half-life (minute)	10.7 ± 1.3	12.0 ± 1.2	15.0 ± 1.2
C_{max} (observed) (µg/mL)	4.96 ± 1.06	15.9 ± 6.5	66.0 ± 5.7
T_{max} (observed) (minute)	10.0	5.0	15.0
AUC_{∞} (predicted) (µg/mL×minute)	155 ± 23	768 ± 92	3,450 ± 390
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	1.52	3.4	7.72
Cl_F (mL/minute/kg)	646 ± 97	260 ± 31	116 ± 13

[a] Based on a one-compartment model with first-order absorption and elimination; parameter estimates (± standard error) are reported to three significant figures.

TABLE M12
Bromochloroacetic Acid Toxicokinetic Parameters for B6C3F1 Mice in the Non-Challenged Drinking Water Group[a]

Male	8 mg/kg	80 mg/kg	160 mg/kg
C_{max} (observed) (µg/mL)	BLOQ	0.732	1.09 ± 0.35
T_{max} (observed) (minute)	NA	12 AM	9 AM
C_{max} (observed) (µg/mL)	BLOQ	BLOQ	BLOQ
T_{max} (observed) (minute)	NA	NA	NA
Female	**10 mg/kg**	**100 mg/kg**	**200 mg/kg**
C_{max} (observed) (µg/mL)	BLOQ	0.993 ± 0.204	1.37
T_{max} (observed) (minute)	NA	9 PM	3 AM
C_{max} (observed) (µg/mL)	BLOQ	BLOQ	BLOQ
T_{max} (observed) (minute)	NA	NA	NA

[a] BLOQ = below the limit of quantitation (0.075 µg/mL); NA = not applicable

Plate A

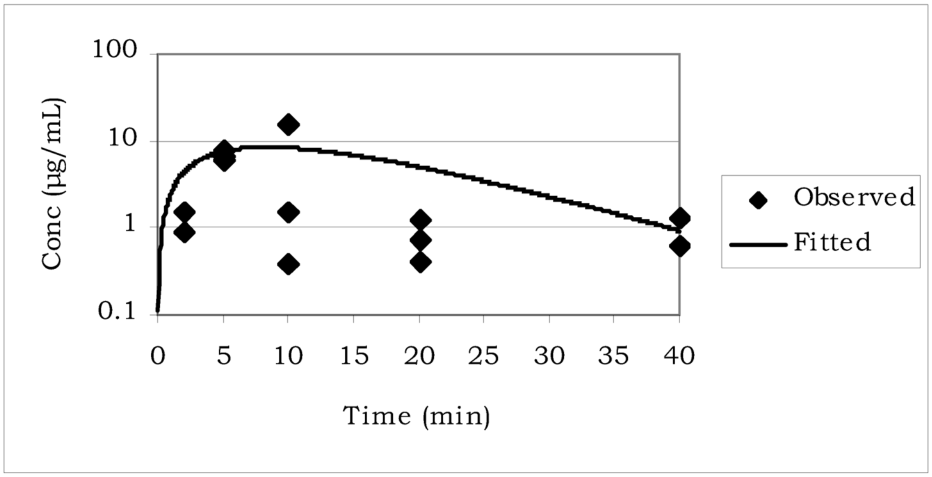

Plate B

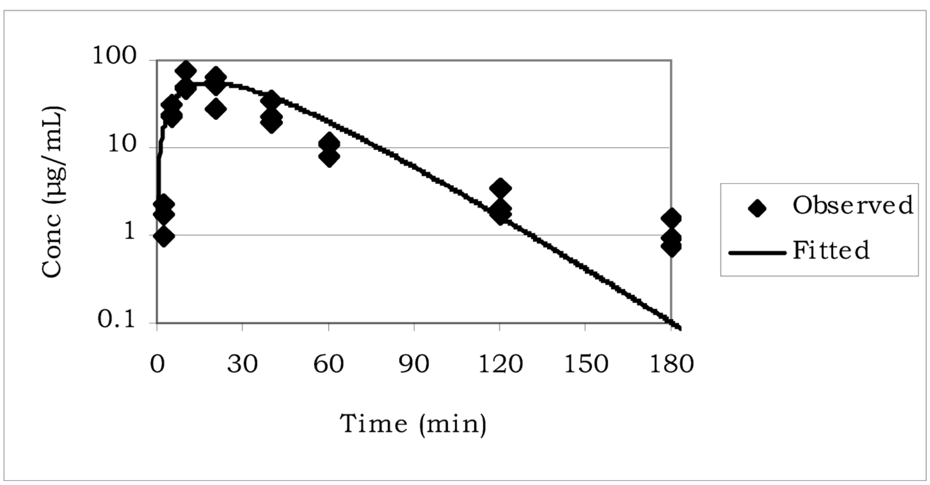

FIGURE M18
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Male B6C3F1 Mice After a Single Gavage Dose of 80 mg/kg (Plate A) or 160 mg/kg (Plate B) Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days

Plate A

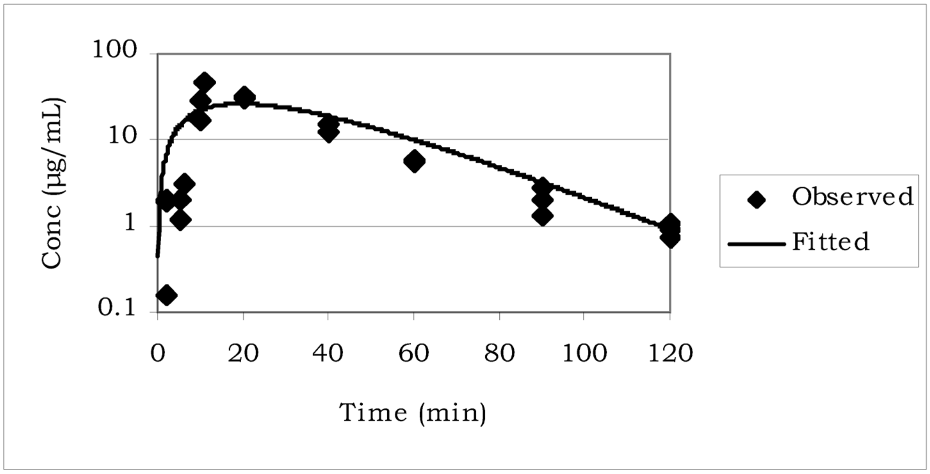

Plate B

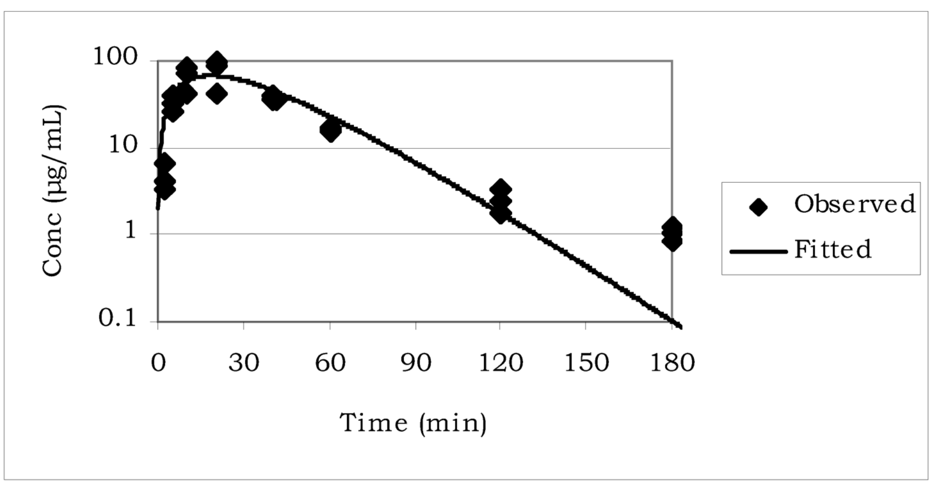

FIGURE M19
Bromochloroacetic Acid Plasma Concentration Time Profiles of Observed and Fitted Data for Female B6C3F1 Mice After a Single Gavage Dose of 100 mg/kg (Plate A) or 200 mg/kg (Plate B) Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days

TABLE M13
Bromochloroacetic Acid Toxicokinetic Parameters for B6C3F1 Mice Given a Single Gavage Challenge Dose of Bromochloroacetic Acid After Receiving Bromochloroacetic Acid in Drinking Water for 14 Days[a]

Male	80 mg/kg	160 mg/kg
k_{01} or k_{10} (minute^{-1})	0.120 ± 0.015	0.0530 ± 0.050
k_{01} or k_{10} half-life (minute)	5.8 ± 0.75	13.1 ± 1.2
C_{max} (observed) (µg/mL)	7.01 ± 0.48	59.0 ± 9.0
T_{max} (observed) (minute)	5.0	10
AUC_{∞} (predicted) (µg/mL×minute)	193 ± 38	2,820 ± 360
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	1.13	15.9
Cl_F (mL/minute/kg)	415 ± 83	56.7 ± 7.1
Female	**100 mg/kg**	**200 mg/kg**
k_{01} or k_{10} (minute^{-1})	0.0515 ± 0.0051	0.0543 ± 0.0045
k_{01} or k_{10} half-life (minute)	13.4 ± 1.3	12.8 ± 1.0
C_{max} (observed) (µg/mL)	32.4 ± 0.3	77.7 ± 17.4
T_{max} (observed) (minute)	20.0	20.0
AUC_{∞} (predicted) (µg/mL×minute)[b]	1,390 ± 190	3,400 ± 370
$AUC_{last}/Dosage$ [(µg/mL×minute)/(mg/kg)][b]	12.8	16.7
Cl_F (mL/minute/kg)	72.0 ± 9.6	58.8 ± 6.4

a Based on a one-compartment model with first-order absorption and elimination; parameter estimates (± standard error) are reported to three significant figures.

b AUC_{last} was calculated using the trapezoidal method, which does not calculate a standard error.

Plate A

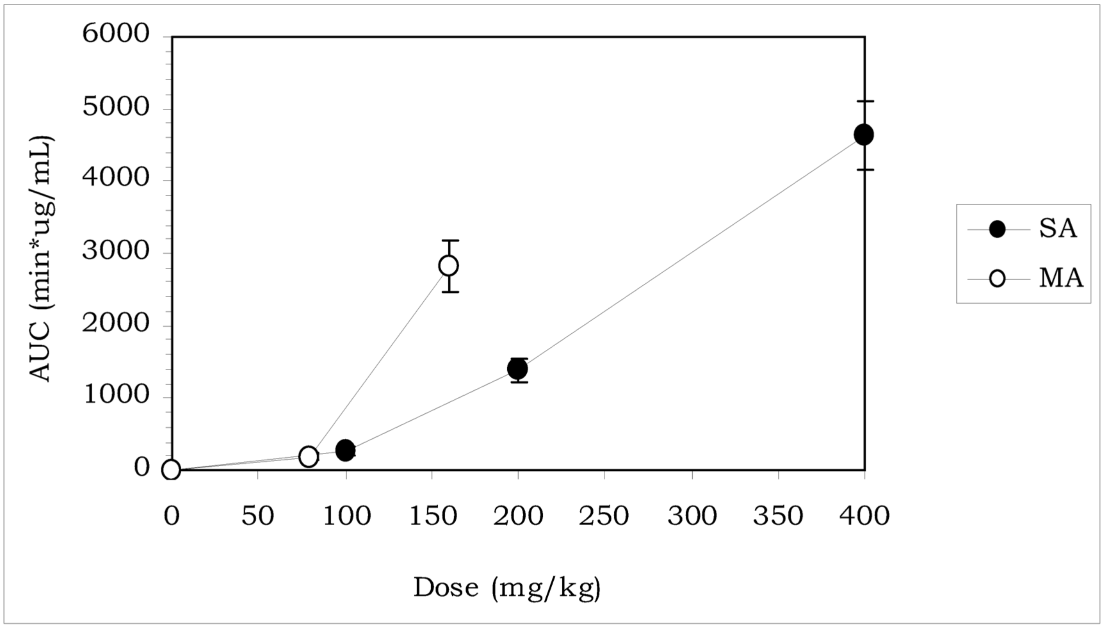

Plate B

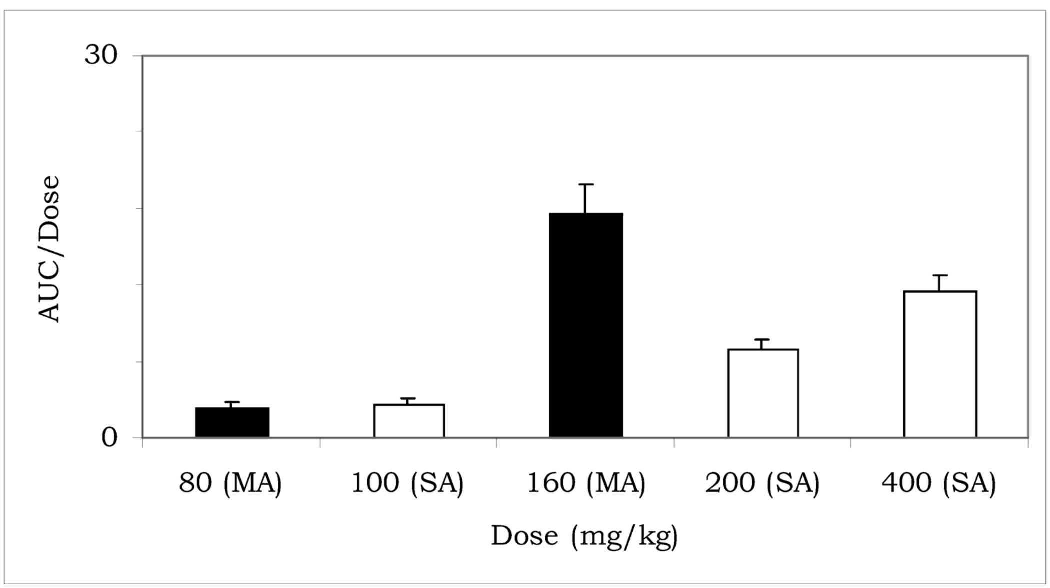

FIGURE M20
AUC for Male B6C3F1 Mice After Receiving Bromochloroacetic Acid
(Dose versus AUC Plot - Plate A; Dose versus Dose-Normalized AUC Plot - Plate B)
(SA = Single Gavage Administration; MA = 14-Day Drinking Water Repeated Administration)

Plate A

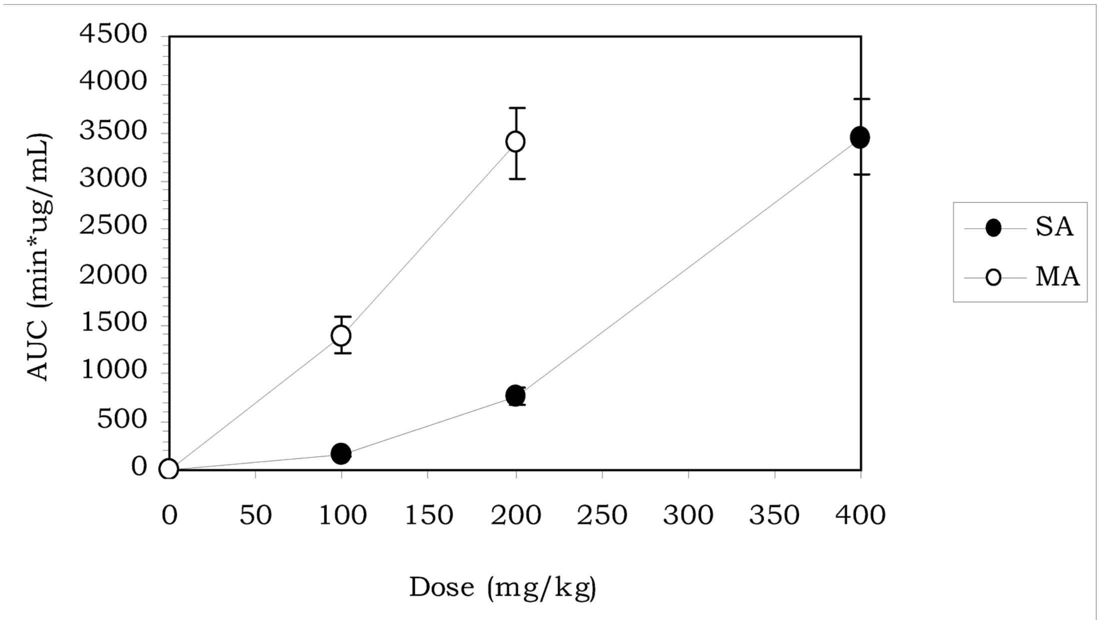

Plate B

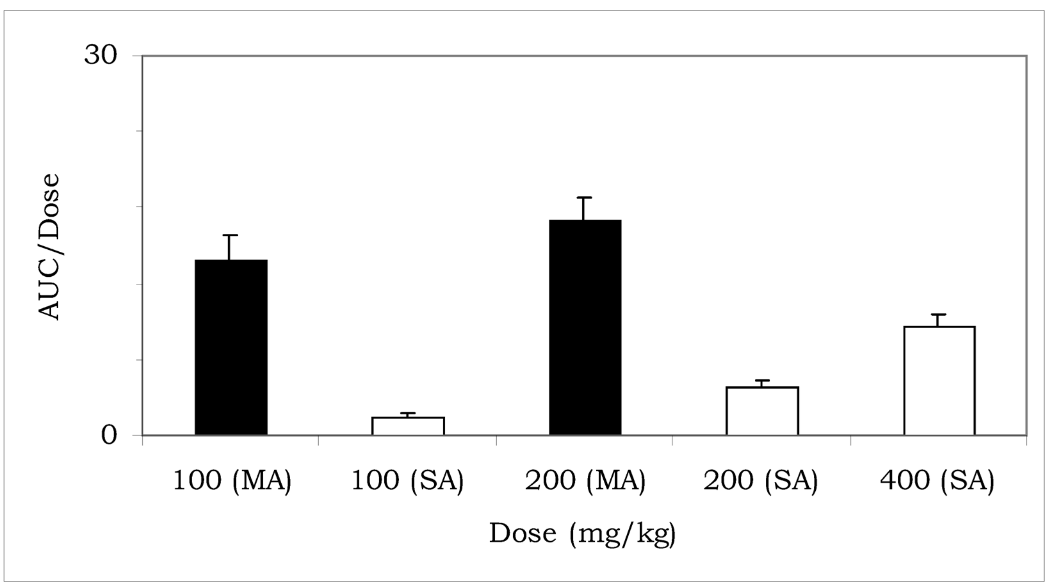

FIGURE M21
AUC for Female B6C3F1 Mice After Receiving Bromochloroacetic Acid
(Dose versus AUC Plot - Plate A; Dose versus Dose-Normalized AUC Plot - Plate B)
(SA = Single Gavage Administration; MA = 14-Day Drinking Water Repeated Administration)

Plate A

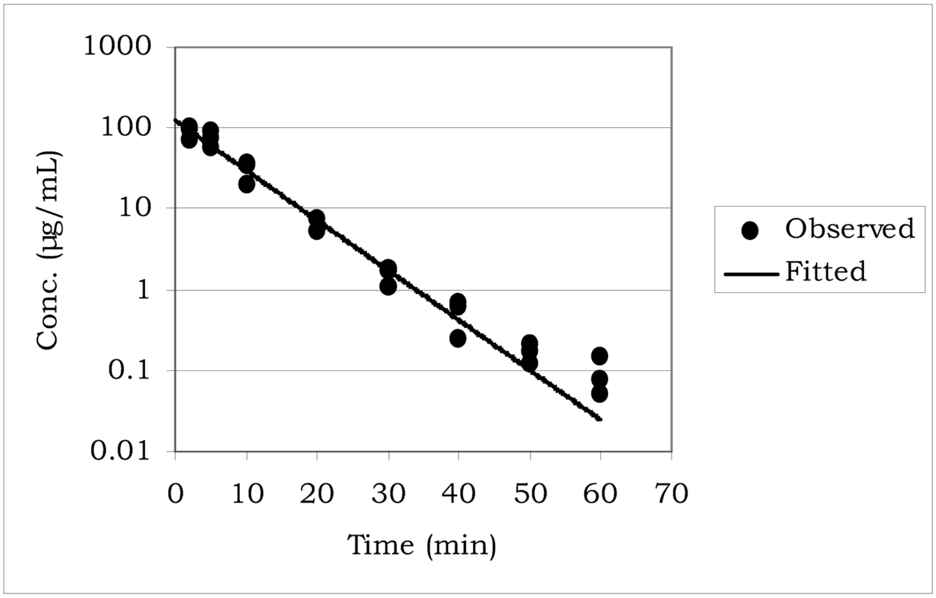

Plate B

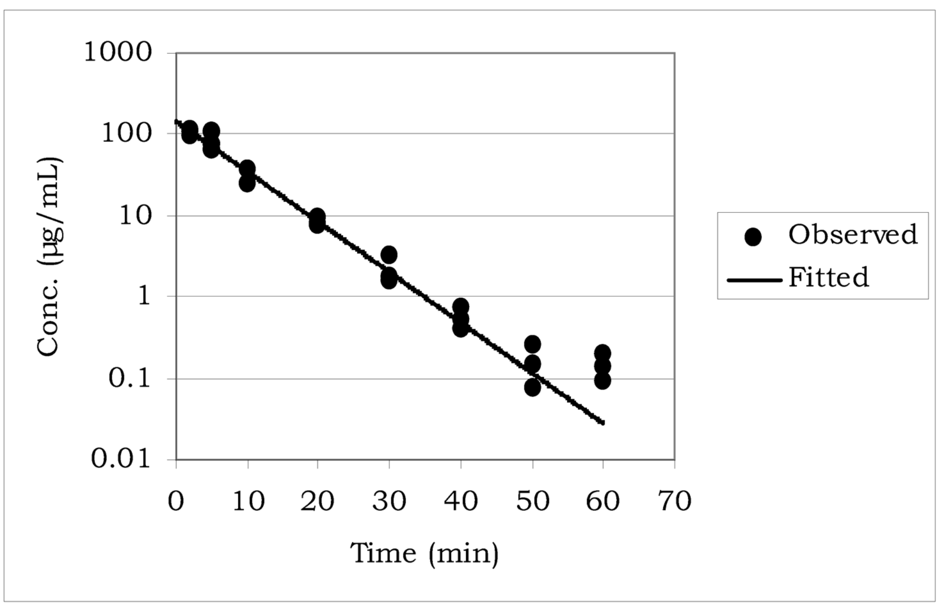

FIGURE M22
Bromochloroacetic Acid (+) Isomer Plasma Concentration Time Profiles of Observed and Fitted Data for Male (Plate A) and Female (Plate B) B6C3F1 Mice After a Single Intravenous Injection of 100 mg/kg Bromochloroacetic Acid

Table M14
Bromochloroacetic Acid (+) Isomer Toxicokinetic Parameters for B6C3F1 Mice After a Single Intravenous Injection of 100 mg/kg Bromochloroacetic Acid[a]

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	88.1 ± 9.5	105 ± 4
k_{10} ($minute^{-1}$)	0.142 ± 0.009	0.143 ± 0.007
$t_{1/2}$ (minute)	4.88 ± 0.32	4.86 ± 0.25
AUC_{∞} (predicted) (µg/mL×minute)	867 ± 45	1,010 ± 40
Cl (mL/minute/kg)	115 ± 6	98.9 ± 4.0
AUC/Dose	8.7	—

[a] Based on a one-compartment model with bolus input, first-order output, and 1/Yhat weighting; parameter estimates (± standard error) are reported to three significant figures.

Table M15
Bromochloroacetic Acid (–) Isomer Toxicokinetic Parameters for B6C3F1 Mice After a Single Intravenous Injection of 100 mg/kg Bromochloroacetic Acid[a]

Parameter	Male	Female
$C_{(2\ minutes)}$ (observed) (µg/mL)	91.2 ± 11.9	99.3 ± 2.8
k_{10} ($minute^{-1}$)	0.360 ± 0.031	0.384 ± 0.020
$t_{1/2}$ (minute)	1.92 ± 0.16	1.80 ± 0.09
AUC_{∞} (predicted) (µg/mL×minute)	529 ± 36	571 ± 23
Cl (mL/minute/kg)	189 ± 13	175 ± 7.0
AUC/Dose	5.3	5.7

[a] Based on a one-compartment model with bolus input, first-order output, and 1/Yhat weighting; parameter estimates (± standard error) are reported to three significant figures.

APPENDIX N
PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

PHYSIOLOGICALLY BASED PHARMACOKINETIC MODEL

INTRODUCTION

A physiologically based pharmacokinetic (PBPK) model was developed to describe the uptake, distribution, metabolism, and elimination of bromochloroacetate in F344/N rats and B6C3F1 mice. A PBPK model consists of a series of mass-balance differential equations that are formulated to represent in quantitative terms the complex physiological and biochemical processes that affect the behavior of the chemical in the intact animal. The animal is represented as being divided into separate organ compartments, including the site where the chemical enters the body and the sites where it is subsequently stored or metabolized. The organ compartments are connected by arterial and venous blood flow. The utility of a PBPK model that accurately represents the processes that regulate tissue dosimetry is its ability to characterize tissue concentrations of parent compound or metabolites resulting from different patterns of exposure. PBPK models can provide a biologically based approach for using tissue dose metrics rather than exposure concentrations to characterize dose-response relationships.

The current PBPK model is constructed around a novel description of the molecular interactions of bromochloroacetate and its metabolites with glutathione *S*-transferase$_{zeta}$ (GST-ζ), the enzyme primarily responsible for biotransformation of dihaloacetates (Tong *et al*., 1998). Parameters not available from the literature were estimated by fitting the model to data from current NTP toxicokinetic studies (Appendix M) using single-dose intravenous injection, single-dose oral gavage, and 2-week drinking water exposures followed by a single oral gavage challenge of racemic (+) and (–) bromochloroacetate.

MODEL DEVELOPMENT

The bromochloroacetate (BCA) PBPK model (Figure N1) has flow-limited compartments representing kidney, liver, and other aggregated tissues. The gastrointestinal tract is modeled as a lumen where absorption of gavage and drinking water doses occurs via a linear uptake rate directly into the hepatic portal vein. Intravenous doses are administered directly into the steady-state representation of venous blood.

The physiological parameters used in the rat and mouse models are summarized in Table N1. Mean body weights and average water consumption rates were study specific. Flow rates and tissue volumes are taken from Brown *et al*. (1997) and Davies and Morris (1993). Abbas and Fisher (1997) determined the partition coefficients for dichloroacetate; due to similarities in structure, behavior, and octanol:water partition coefficients amongst the dihaloacetates, the partition coefficients for dichloroacetate were used for bromochloroacetate (Table N2).

A key feature of this model is the inclusion of suicide inhibition in the description of metabolism. Suicide inhibition is characteristic of the entire class of dihaloacetates. This behavior is modeled by irreversible covalent binding of a bromochloroacetate intermediary metabolite, α-halocarboxymethylglutathione (α*h1*) to GST-ζ, which effectively inactivates the enzyme. It is assumed that an estimated percentage of the α-halocarboxymethylglutathione product formed from (–)bromochloroacetate releases to continue on the metabolic pathway to form glyoxalate (GXA) and subsequently oxalate (OXA). Conversely, it is assumed that all bound product formed from (+)bromochloroacetate will covalently bind. GST-ζ synthesis and degradation are described with rates determined in Anderson *et al*. (1999).

Amounts of both (+) and (–) isomers of bromochloroacetate, glyoxylate, and oxalate are tracked in all compartments of the model. Within the liver, seven additional compounds are accounted for including: glutathione-*S*-transferase zeta, GST-ζ bound with (+)bromochloroacetate, GST-ζ bound with (–)bromochloroacetate, α-halocarboxymethylglutathione produced from (+) bromochloroacetate bound to GST-ζ, α-halocarboxymethylglutathione produced from (–)bromochloroacetate bound to GST-ζ, free α-halocarboxymethylglutathione, and other glyoxylate-derived metabolites.

The model allows for stereospecific treatment of the (+) and (–) isomers of bromochloroacetate due to evidence of toxicokinetic behavioral differences between them (Schultz and Sylvester, 2001). Both of these isomers are capable of binding to GST-ζ, but due to the different conformations, binding is assumed to occur at different rates. In GST-ζ-depleted rats, the clearance of (+)bromochloroacetate is unaffected, while the clearance of (–)bromochloroacetate is clearly reduced (Schultz and Sylvester, 2001). In fact, the elimination kinetics of (–)bromochloroacetate in GST-ζ-depleted rats mimics that of (+)bromochloroacetate, indicating the presence of an additional metabolic pathway that transforms both isomers at equal rates (Schultz and Sylvester, 2001). *In vitro* studies with hepatic cytosol obtained from naive and GST-ζ-depleted rats demonstrated the glutathione dependence of this pathway. Although this secondary pathway is only a minor contributor for naive animals, after GST-ζ depletion, its role in bromochloroacetate elimination is more pronounced (Schultz *et al.*, 1999). This effect is incorporated into the model by including a secondary GST-ζ-independent metabolic pathway that clears both isomers at the same rate.

In addition to hepatic biotransformation, both bromochloroacetate isomers and glyoxylate may be excreted in the urine. Oxalate is cleared only by urinary elimination in the model. In rats, the removal of these compounds from the kidney tubule region is modeled using published values for urine flow. Published values for glomerular filtration describe the distribution of these compounds into kidney tissue and tubule regions (Davies and Morris, 1993). Motivated by the observations reported in Schultz *et al*. (1999), saturable reabsorption of bromochloroacetate from the kidney tubule region into kidney tissue was included for rats. For mice, a linear rate is used to describe the urinary elimination from the kidney. The metabolites glyoxalate and oxalate have endogenous sources separate from dihaloacetate biotransformation. The control data were used to estimate endogenous production rates of glyoxylate and oxalate. All non-bromochloroacetate-derived glyoxylate and oxalate were modeled as constant inputs to the cumulative urine output. These endogenous production rates are summarized in Table N3.

The NTP toxicokinetic data used for parameter estimation included blood time-course concentrations and urine content of bromochloroacetate and its metabolites glyoxylate and oxalate (Appendix M). In these studies, blood and urine samples were collected from male and female F344/N rats administered single intravenous injections of 10 or 80 mg racemic bromochloroacetate/kg body weight or single gavage doses of 10, 40, or 100 mg/kg. Data from 2-week drinking water studies with racemic bromochloroacetate concentrations of 40, 400, or 800 mg/L, followed by a gavage challenge dose of 2.88, 28.8, or 57.6 mg/kg, respectively, for male rats and 2.74, 27.4, or 54.9 mg/kg for female rats were also used for parameter estimation purposes. Similar samples were collected from male and female B6C3F1 mice administered single intravenous injections of 100 mg racemic bromochloroacetate/kg body weight or single gavage doses of 100, 200, or 400 mg/kg. Data from 2-week drinking water studies with racemic bromochloroacetate concentrations of 40, 400, or 800 mg/L, followed by a gavage challenge dose of 8, 80, or 160 mg/kg, respectively, for male mice and 10, 100, or 200 mg/kg for female mice were also used for parameter estimation purposes. Additionally, data from a study where male and female rats were administered single intravenous injections of 50 mg glyoxylate/kg body weight were used for parameter estimation of glyoxylate elimination kinetics. Two-week drinking water studies at the same exposure concentrations but without a gavage challenge were used for model verification purposes.

There were 11 unknown parameters in the model for rats and 12 unknown parameters for mice, with very little information to suggest the order of magnitude. Given the lack of indication for potential ranges of parameter values, the use of an optimizer proficient in locating global minimums was warranted. Therefore, a differential evolution optimization algorithm was first used to search the global parameter space for each of the four cases under study. The cost function computed the sum of squared errors between the simulated predictions and experimental measurements for blood and urine. The best parameters from the differential evolution algorithm were then used as the initial conditions in a simplex-based optimization routine in MATLAB® (The MathWorks, Inc., Natick, MA) to ensure convergence to the final parameter values (Table N4).

Definitions of Abbreviations

$A_{i,j}$ = Amount of compound *i* in tissue compartment *j* (mg)
V_j = Volume of tissue compartment *j* (L)
$C_{i,j}$ = Concentration of compound *i* in tissue compartment *j* (mg/L)
Q_j = Blood flow rate for tissue compartment *j*; if *j=urine*, then urine flow rate (L/hour)
$P_{i,j}$ = Tissue compartment *j*:blood partition coefficient for compound *i*
k_{abs} = Linear rate of absorption from stomach (hour^{-1})
k_f = Linear rate of metabolism (hour^{-1})
k_s = Rate of resynthesis (nmol/hour)
k_{de} = Degradation rate (hour^{-1})
k_{bind} = Rate of binding of BCA to GST-ζ (nmol^{-1}hour^{-1})
k_{trans} = Rate of metabolism from BCA to $\alpha h1$ (hour^{-1})
$k_{covalent\_Frac}$ = Fraction of bound $\alpha h1$ and GST-ζ product which covalently binds
k_{metab} = Rate of transformation from $\alpha h1$ to GXA (hour^{-1})
$k_{metabGXAtoOXA}$ = Rate of metabolism from GXA to OXA (hour^{-1})
$k_{metabGXAtoOther}$ = Rate of metabolism from GXA to other metabolites (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

The following equations represent the amount of bromochloroacetic acid in the stomach, kidney, kidney tubule, urine, other aggregated tissues, and arterial blood, respectively. Note that in the model each of these equations are included twice, once for (+)bromochloroacetate and once for (–)bromochloroacetate.

$$\frac{dA_{BCA,stomach}}{dt} = -k_{abs} \cdot A_{BCA,stomach} + drinkDoseBCA + oralDoseBCA$$

$$\frac{dA_{BCA,kidney}}{dt} = (Q_{kidney} - GFR) \cdot C_{BCA,art} - Q_{kidney} \cdot \frac{A_{BCA,kidney}}{V_{kidney} P_{BCA,kidney}} + \frac{T_{\max} C_{BCA,kidTub}}{K_t + C_{BCA,kidTub}}$$

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

$$\frac{dA_{BCA,urine}}{dt} = Q_{urine} \cdot \frac{A_{BCA,kidTub}}{V_{kidTub}}$$

$$\frac{dA_{BCA,Other}}{dt} = Q_{Other} (C_{BCA,art} - \frac{A_{BCA,Other}}{V_{Other} P_{BCA,Other}})$$

$$C_{BCA,art} = \frac{ivDoseBCA + \sum Q_j \frac{A_{BCA,j}}{V_j P_{BCA,j}}}{Q_{cardiac}}$$

The following equations represent the amount of glyoxylate in the kidney, kidney tubule, urine, other aggregated tissues, and arterial blood, respectively.

$$\frac{dA_{GXA,kidney}}{dt} = (Q_{kidney} - GFR) \cdot C_{GXA,art} - Q_{kidney} \cdot \frac{A_{GXA,kidney}}{V_{kidney} P_{GXA,kidney}}$$

$$\frac{dA_{GXA,kidTub}}{dt} = GFR \cdot C_{GXA,art} - \frac{dA_{GXA,urine}}{dt}$$

$$\frac{dA_{GXA,urine}}{dt} = Q_{urine} \cdot \frac{A_{GXA,kidTub}}{V_{kidTub}}$$

$$\frac{dA_{GXA,Other}}{dt} = Q_{Other} (C_{GXA,art} - \frac{A_{GXA,Other}}{V_{Other} P_{GXA,Other}})$$

$$C_{GXA,art} = \frac{ivDoseGXA + \sum Q_j \frac{A_{GXA,j}}{V_j P_{GXA,j}}}{Q_{cardiac}}$$

The following equations represent the amount of oxalate in the kidney, kidney tubule, urine, other aggregated tissues, and arterial blood, respectively.

$$\frac{dA_{OXA,kidney}}{dt} = (Q_{kidney} - GFR) \cdot C_{OXA,art} - Q_{kidney} \cdot \frac{A_{OXA,kidney}}{V_{kidney} P_{OXA,kidney}}$$

$$\frac{dA_{OXA,kidTub}}{dt} = GFR \cdot C_{OXA,art} - \frac{dA_{OXA,urine}}{dt}$$

$$\frac{dA_{OXA,urine}}{dt} = Q_{urine} \cdot \frac{A_{OXA,kidTub}}{V_{kidTub}}$$

$$\frac{dA_{OXA,Other}}{dt} = Q_{Other} (C_{OXA,art} - \frac{A_{OXA,Other}}{V_{Other} P_{OXA,Other}})$$

$$C_{OXA,art} = \frac{\sum Q_j \frac{A_{OXA,j}}{V_j P_{OXA,j}}}{Q_{cardiac}}$$

The following equations represent the amounts of GST-ζ, bromochloroacetic acid, bound bromochloroacetate and GST-ζ product, bound α-halocarboxymethylglutathione and GST-ζ product, free α-halocarboxymethylglutathione, inactivated GST-ζ product, glyoxylate, oxalate, and other glyoxylate metabolites in the liver.

$$\frac{dA_{GSTzeta,liver}}{dt} = k_s - k_{de} A_{GSTzeta,liver} - k_{bind} A_{BCA,liver} A_{GSTzeta,liver}$$

$$A_{GSTzeta,liver}(t) = A_{GSTzeta,liver}(t) + (1 - k_{covalent\_Frac}) A_{\alpha h1BoundGSTzeta,liver}(t)$$

$$\frac{dA_{BCA,liver}}{dt} = Q_{liver}(C_{BCA,art} - \frac{A_{BCA,liver}}{V_{liver} P_{BCA,liver}}) + k_{abs} \cdot A_{BCA,stomach} - k_{bind} A_{BCA,liver} A_{GSTzeta,liver} - k_f \cdot \frac{A_{BCA,liver}}{P_{BCA,liver}}$$

$$\frac{dA_{BCABoundGSTzeta,liver}}{dt} = k_{bind} A_{BCA,liver} A_{GSTzeta,liver} - k_{trans} A_{BCABoundGSTzeta,liver}$$

$$\frac{dA_{\alpha h1BoundGSTzeta,liver}}{dt} = k_{trans} A_{BCABoundGSTzeta,liver}$$

$$A_{\alpha h1BoundGSTzeta,liver}(t) = 0$$

$$\frac{dA_{\alpha h1,liver}}{dt} = -k_{metab} A_{\alpha h1,liver}$$

$$A_{\alpha h1,liver}(t) = A_{\alpha h1,liver}(t) + (1 - k_{covalent\_Frac}) A_{\alpha h1BoundGSTzeta,liver}(t)$$

$$A_{inactivated\_\alpha h1BoundGSTzeta,liver}(t) = A_{inactivated\_\alpha h1BoundGSTzeta,liver}(t) + k_{covalent\_Frac} A_{\alpha h1BoundGSTzeta,liver}(t)$$

$$\frac{dA_{GXA,liver}}{dt} = Q_{liver}(C_{GXA,art} - \frac{A_{GXA,liver}}{V_{liver} P_{GXA,liver}}) + k_{metab} A_{\alpha h1,liver} - (k_{metabGXAtoOXA} + k_{metabGXAtoOther}) A_{GXA,liver}$$

$$\frac{dA_{OXA,liver}}{dt} = Q_{liver}(C_{OXA,art} - \frac{A_{OXA,liver}}{V_{liver} P_{OXA,liver}}) + k_{metabGXAtoOXA} A_{OXA,liver}$$

$$\frac{dA_{OtherGXAmetab,liver}}{dt} = k_{metabGXAtoOther} A_{GXA,liver}$$

Results

Figures N2 through N15 show the results of simulations performed with the current PBPK model for bromochloroacetic acid as compared to the experimental data from the concurrent NTP toxicokinetic studies presented in Appendix M. Each figure represents one study and shows separate plots for outputs such as total bromochloroacetate blood concentration, total bromochloroacetate, glyoxylate, or oxalate cumulative urine amount, GST-ζ as percent of control, or isomer presence in blood as percent of the total bromochloroacetate in blood. PBPK model predictions with experimental data not used during model calibration for 2-week drinking water studies in male rats and female mice are given in Figures N16 and N17. The included figures illustrate results from male rats and female mice as indicated. The results for female rats and male mice are not presented but are respectively similar.

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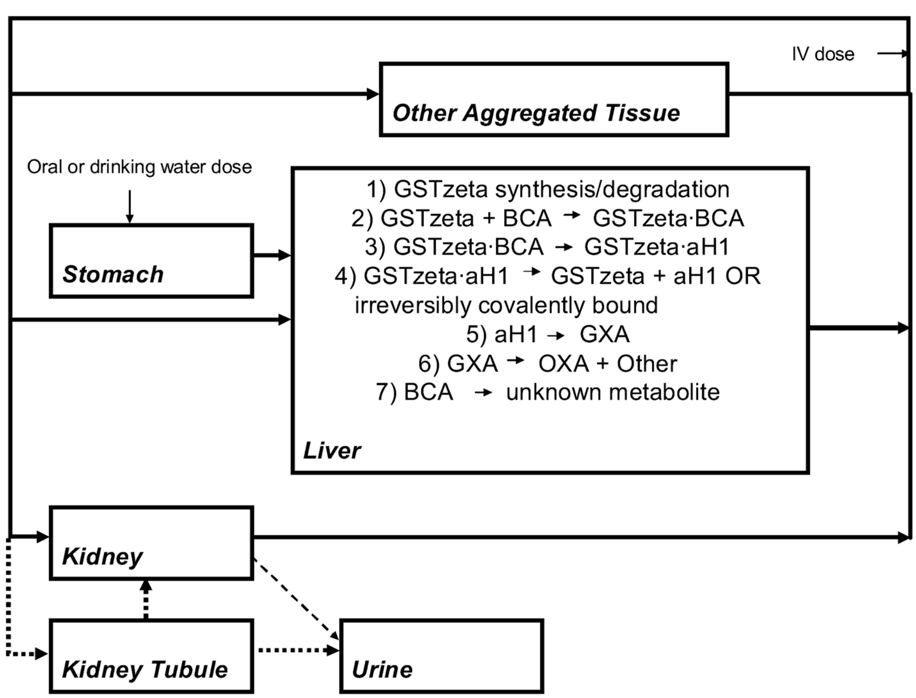


FIGURE N1
Physiologically Based Pharmacokinetic Model for F344/N Rats (Solid and Dotted Lines) and B6C3F1 Mice (Solid and Dashed Lines) Administered Bromochloroacetic Acid in Drinking Water, by Intravenous Injection, or by Oral Gavage

Table N1
Physiological Parameters for F344/N Rats and B6C3F1 Mice for the Physiologically Based Pharmacokinetic Model of Bromochloroacetic Acid[a]

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Parameter				
Body weight (kg)[b]	0.2335	0.1652	0.0290	0.0222
Cardiac output (L/hour per kg$^{0.75}$ body weight)	14.1	14.1	16.5	16.5
Urine flow (L/hour per kg body weight)[c]	0.00833	0.00833	0.00208	0.00208
Glomerular filtration (L/hour per kg body weight)[c]	0.3144	0.3144	0.84	0.84
Water consumption rate (mL/day per kg$^{0.75}$ body weight)[b]	48.2045	52.2441	52.8448	57.6526
Tissue Volume as Fraction of Body Weight				
Kidney tissue	0.0073	0.0073	0.0167	0.0167
Kidney tubule	0.000073	0.000073	0.000167	0.000167
Liver	0.0366	0.0366	0.0549	0.0549
Other aggregated tissue	0.7761	0.7761	0.7484	0.7484
Tissue Blood Flow as Fraction of Cardiac Output				
Kidney	0.141	0.141	0.091	0.091
Liver	0.183	0.183	0.161	0.161
Other aggregated tissue	0.676	0.676	0.748	0.748

a Except as noted, parameter estimates were taken from Brown *et al.* (1997).
b Computed as means from animals under study in the presented data
c Parameter estimate were taken from Davies and Morris (1993).

Table N2
Partition Coefficients for the Physiologically Based Pharmacokinetic Model of Bromochloroacetic Acid[a]

Tissue	Bromochloroacetic Acid Partition Coefficient	Glyoxylate Partition Coefficient	Oxalate Partition Coefficient
Kidney	0.74	1	1
Liver	1.08	1	1
Other aggregated tissue	0.4	1	1

a All coefficients are expressed as tissue:blood ratios; values for bromochloroacetic acid are the same as or derived from those provided for dichloroacetic acid by Abbas and Fisher (1997).

Table N3
Endogenous Production Rates of Glyoxylate and Oxalate in Urine of Untreated F344/N Rats and B6C3F1 Mice for the Physiologically Based Pharmacokinetic Model of Bromochloroacetic Acid

Sex and Species	Glyoxylate Production Rate (µg/hour)	Oxalate Production Rate (µg/hour)
Male F344/N rats	8.23	24.78
Female F344/N rats	6.40	19.00
Male B6C3F1 mice	0.85	9.96
Female B6C3F1 mice	0.89	8.27

Table N4
Parameter Estimates for F344/N Rats and B6C3F1 Mice from the Physiologically Based Pharmacokinetic Model of Bromochloroacetic Acid

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
k_{de} (hour^{-1})[a]	0.00875	0.00875	0.00875	0.00875
k_{abs} (hour^{-1})	0.97	1.4	3.3	3.6
$A_{GST\text{-}\zeta,liver}$(o) (mg)	48,200	8,460	19,400	19,300
$k_{bind\ (-)}$ (nmol^{-1} hour^{-1})	5.29	0.84	1.74	8.43
$k_{bind\ (+)}$ (nmol^{-1} hour^{-1})	0.0008	0.0018	0.011	0.010
k_{trans} (hour^{-1})	48	4.1	13.7	24.3
$k_{covalent\_Frac}$ (-)	0.00014	0.2	0.0013	0.011
$k_{covalent\_Frac}$ (+)[b]	1	1	1	1
k_f (hour^{-1})	6.4	21.4	26.1	21.5
k_{metab} (hour^{-1})[c]	50	50	50	50
$k_{metabGXAtoOXA}$ (hour^{-1})	162	1,000	3.5	3.7
$k_{metabGXAtoOther}$ (hour^{-1})	1,000	0.0013	117	150
T_{max} (mg/hour)	5.0	6.4	—	—
K_t (mg/L)	10.4	20	—	—
$k_{urine,BCA}$	—	—	0.14	0.43
$k_{urine,GXA}$	—	—	1.38	2.85
$k_{urine,OXA}$	—	—	14.9	2.01

[a] Values taken from Anderson *et al.* (1999).
[b] It is assumed that all metabolite product formed from (+)bromochloroacetic acid covalently binds to GST-ζ.
[c] Rate set to occur quickly

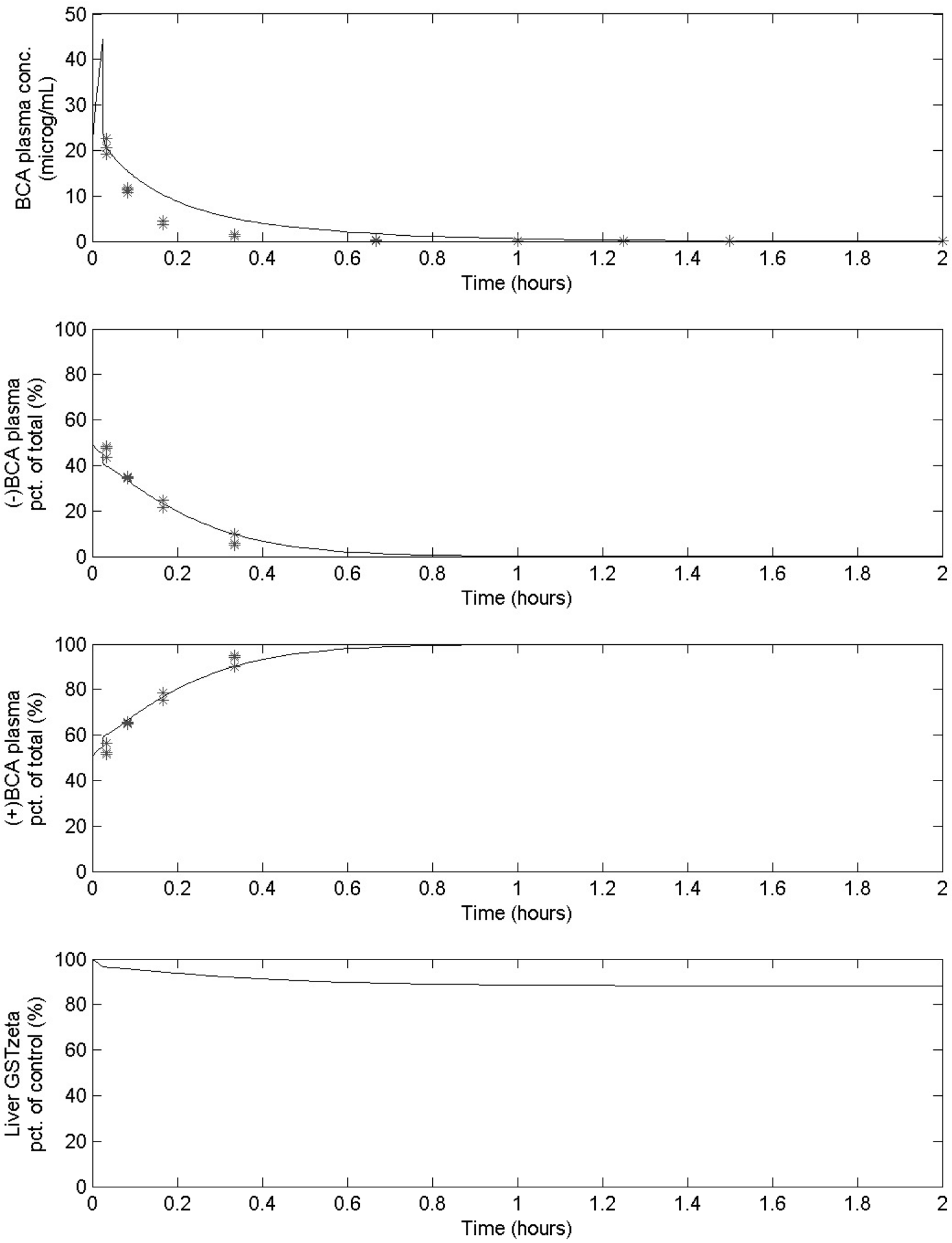


Figure N2
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Intravenous Injection of 10 mg/kg Racemic Bromochloroacetic Acid

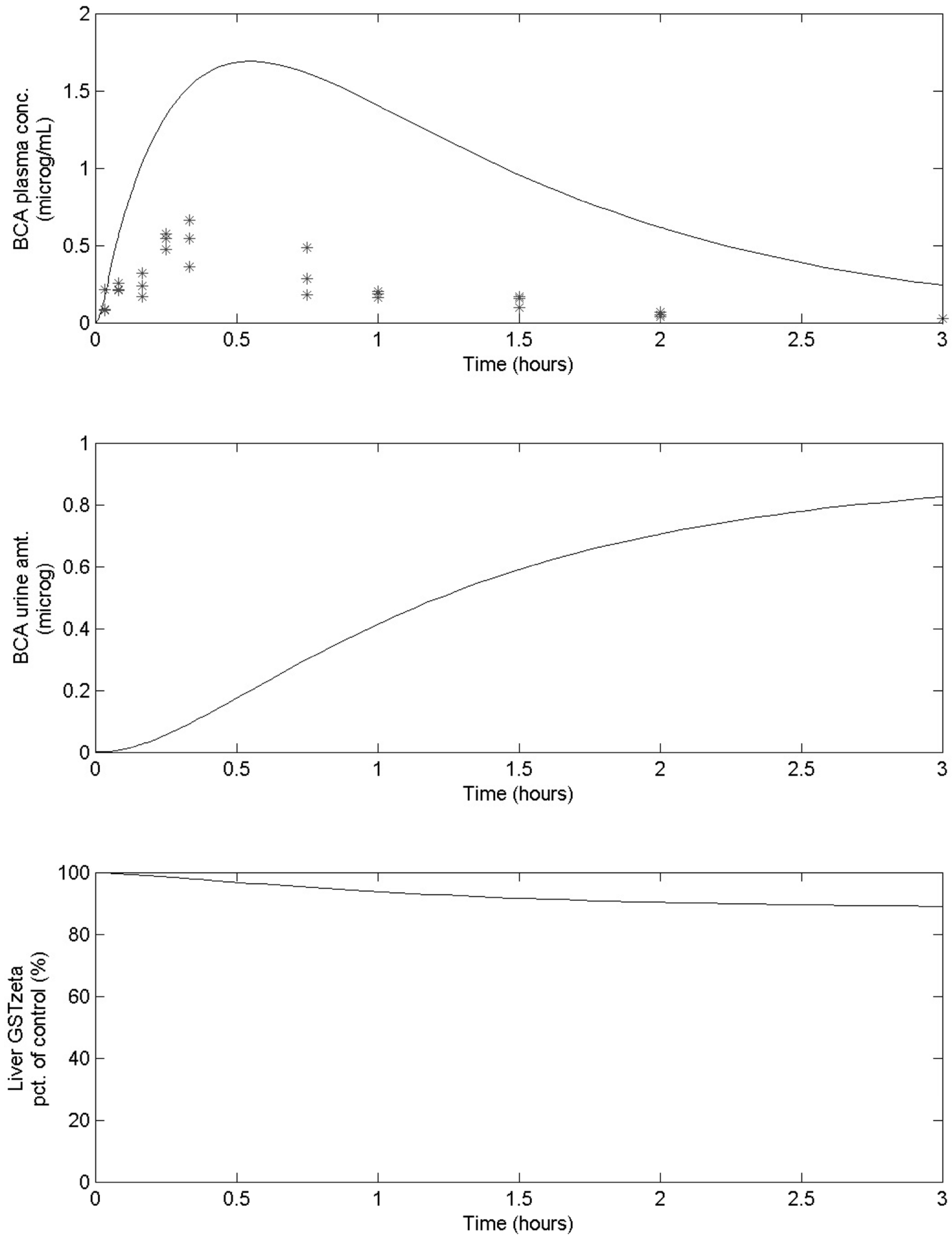


Figure N3
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 10 mg/kg Racemic Bromochloroacetic Acid

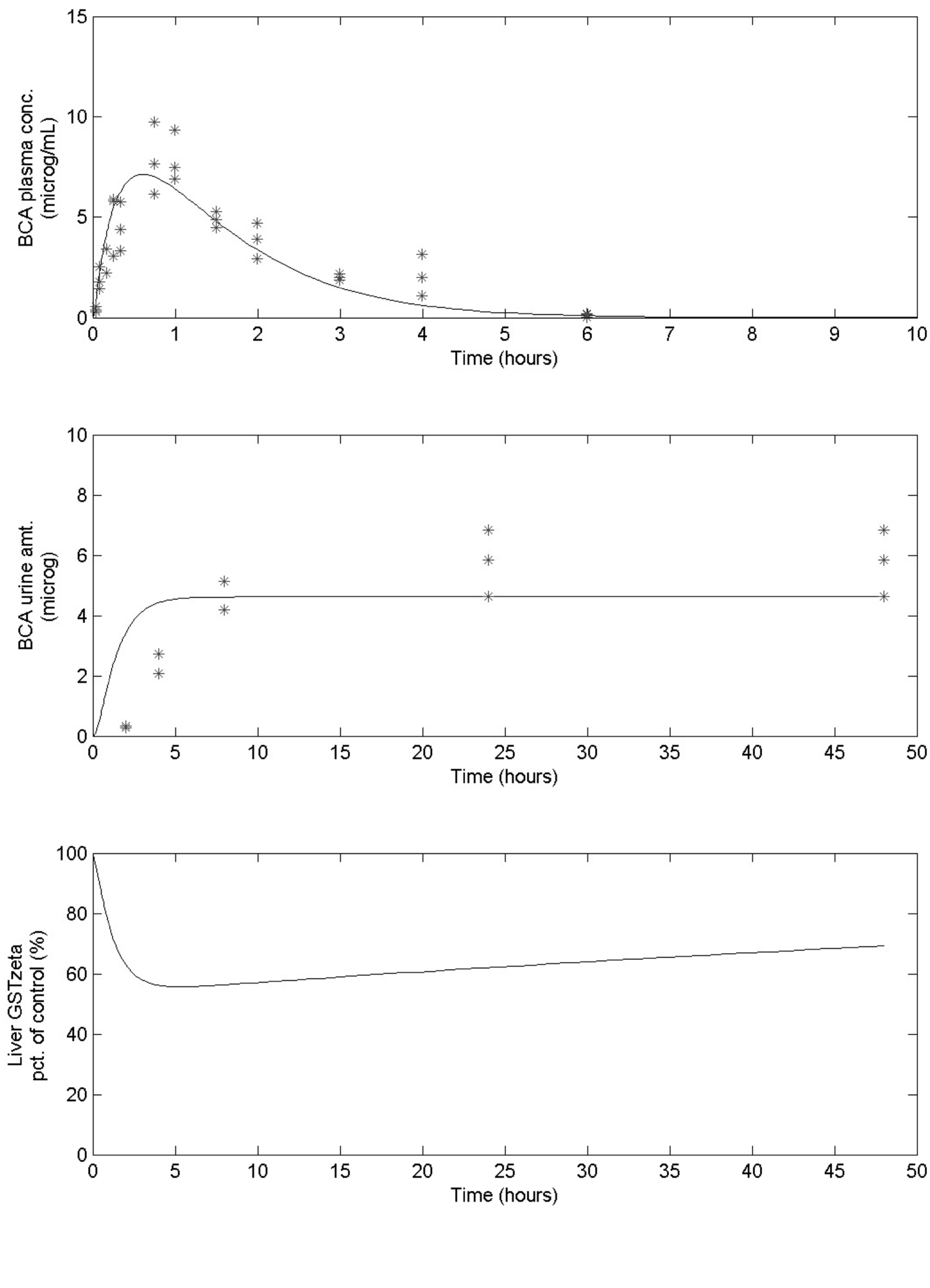


Figure N4
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 40 mg/kg Racemic Bromochloroacetic Acid

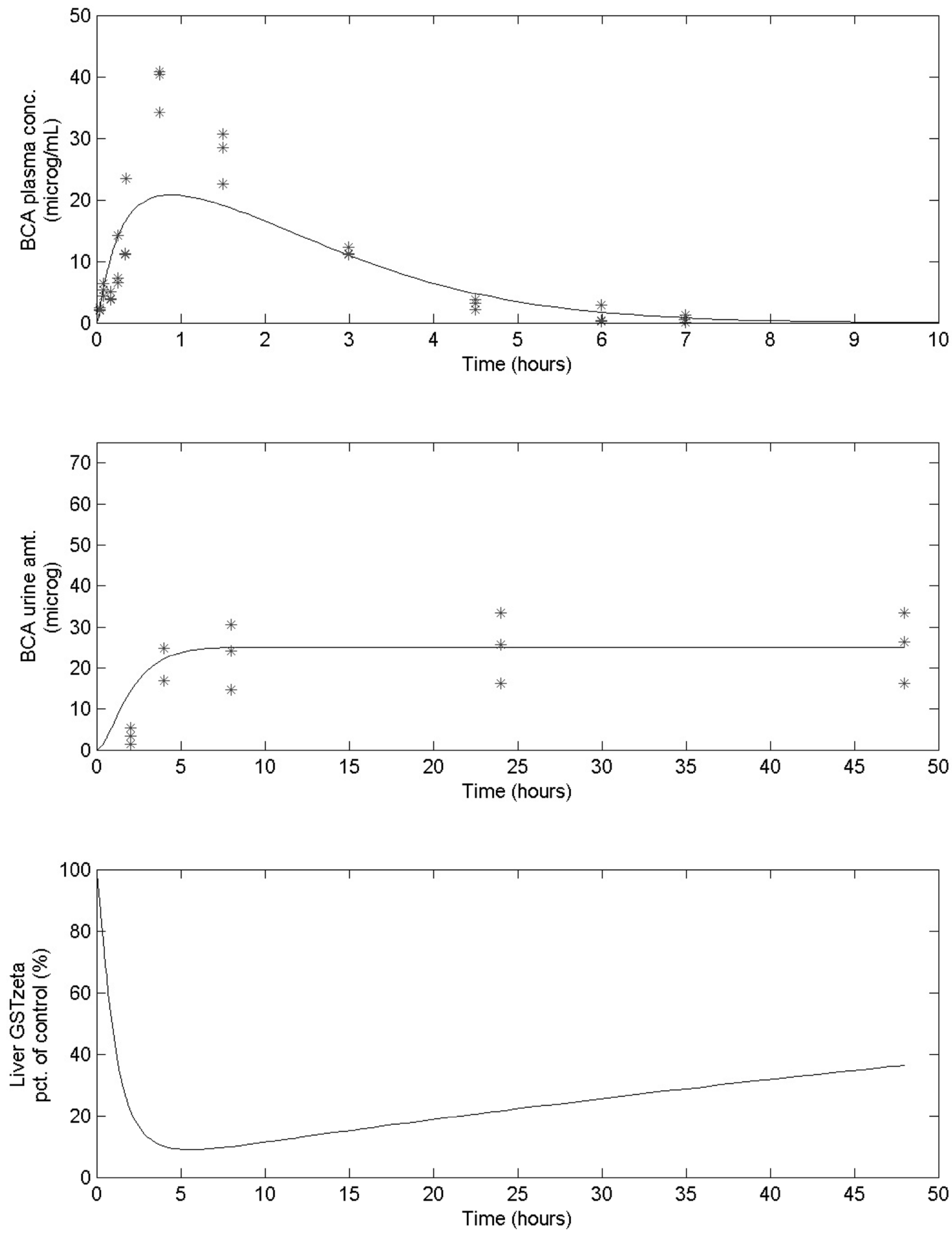


Figure N5
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 100 mg/kg Racemic Bromochloroacetic Acid

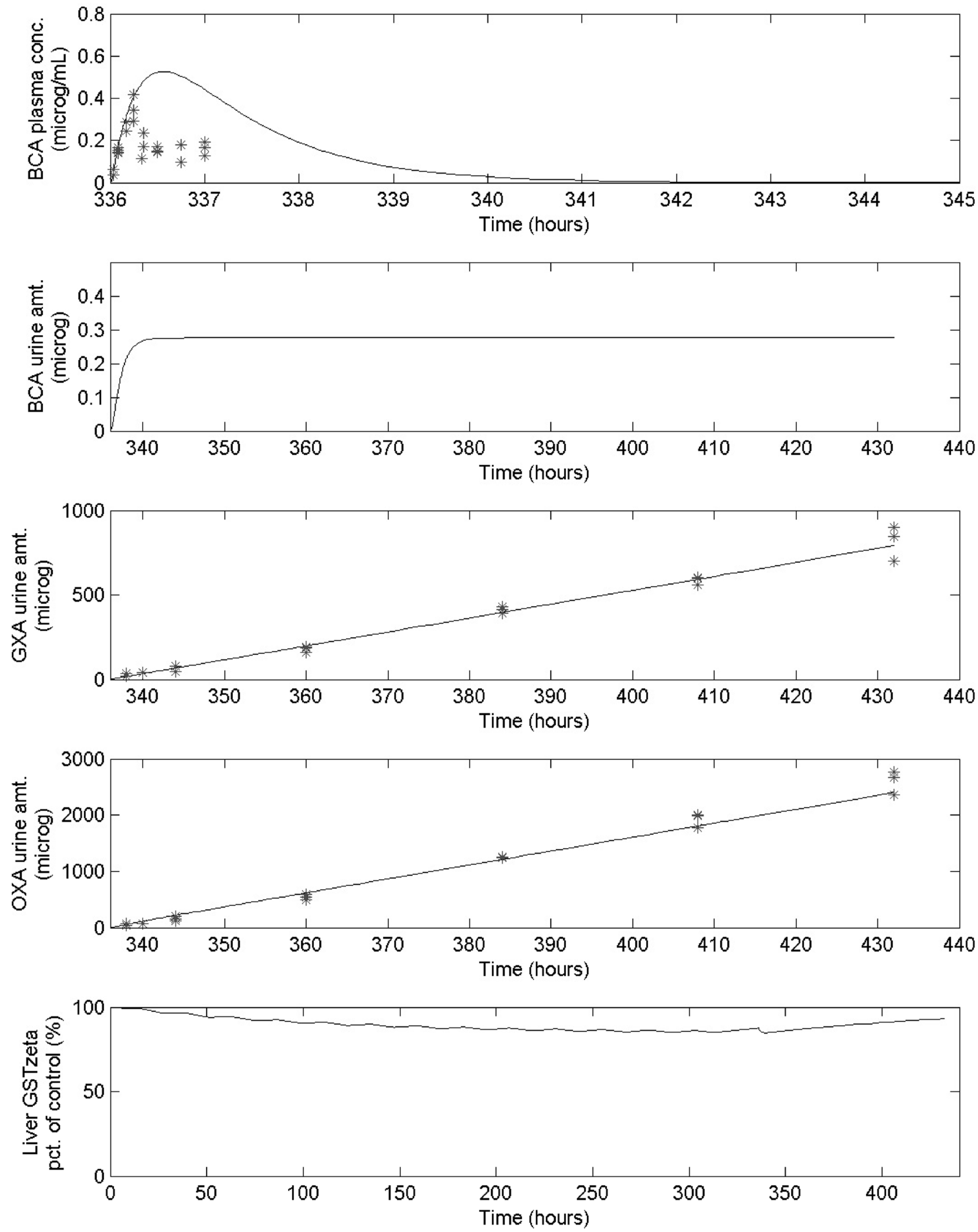


Figure N6
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 2.88 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 40 mg/L

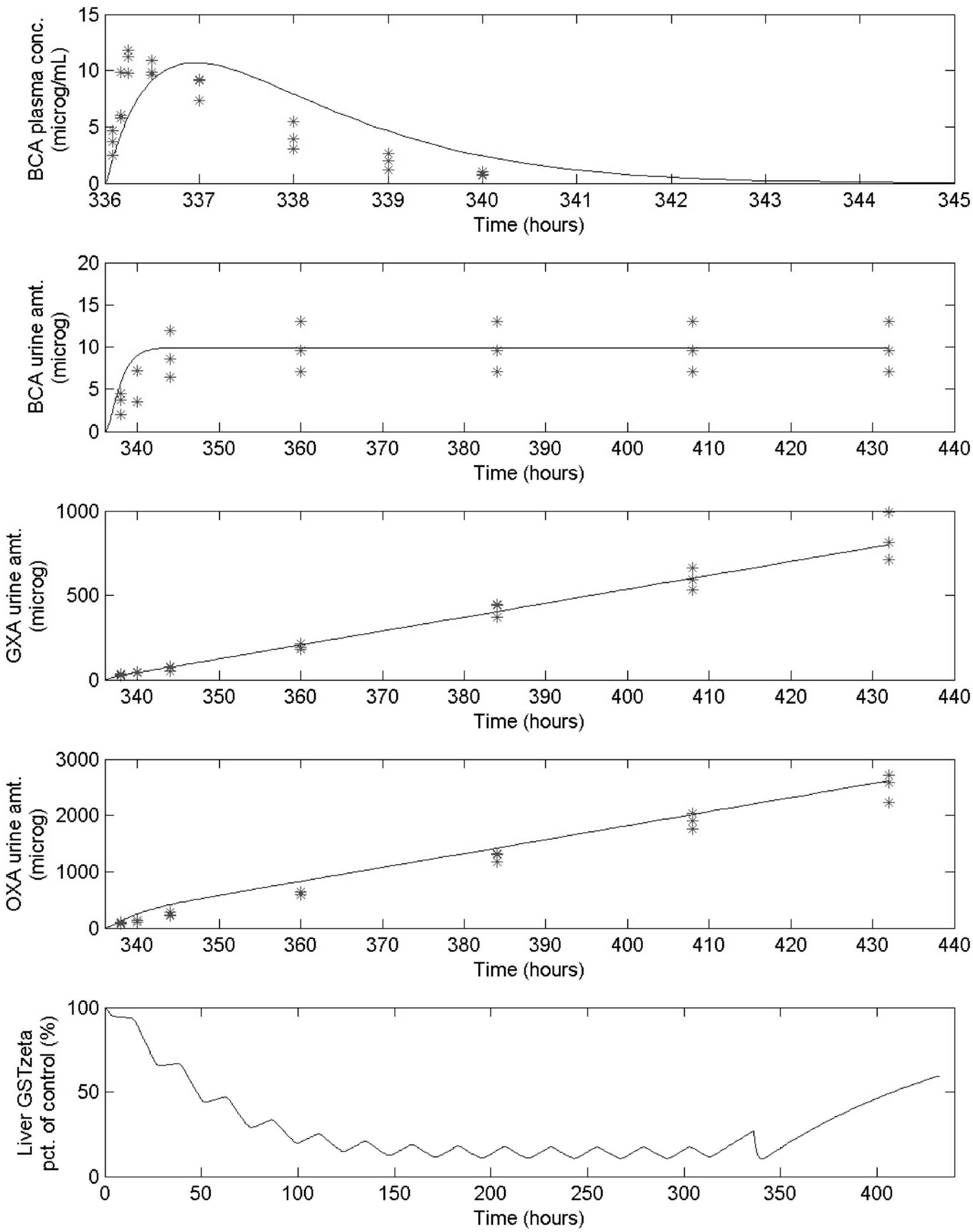


Figure N7
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 28.8 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 400 mg/L

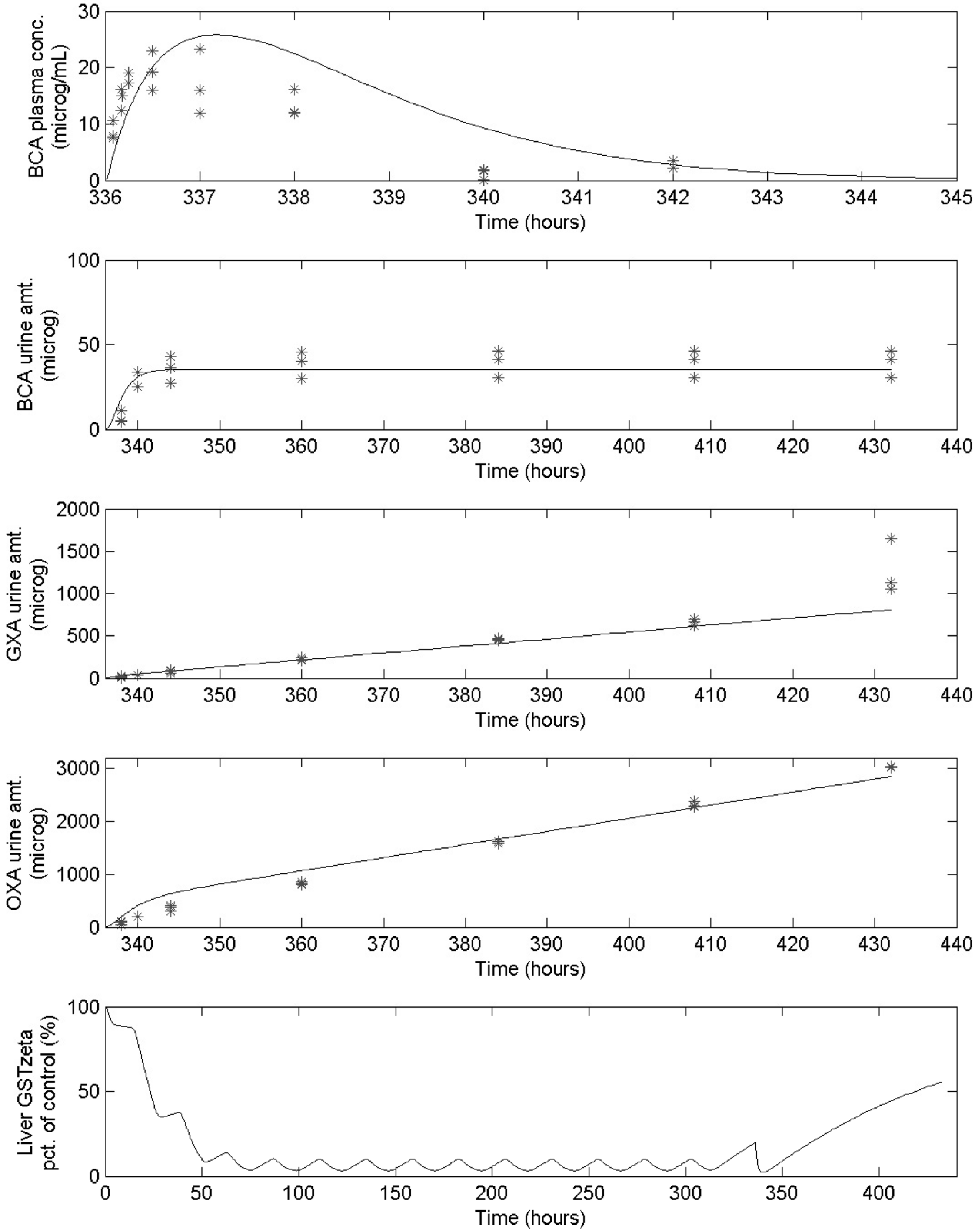


Figure N8
Data (Stars) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered a Single Gavage Dose of 57.6 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 800 mg/L

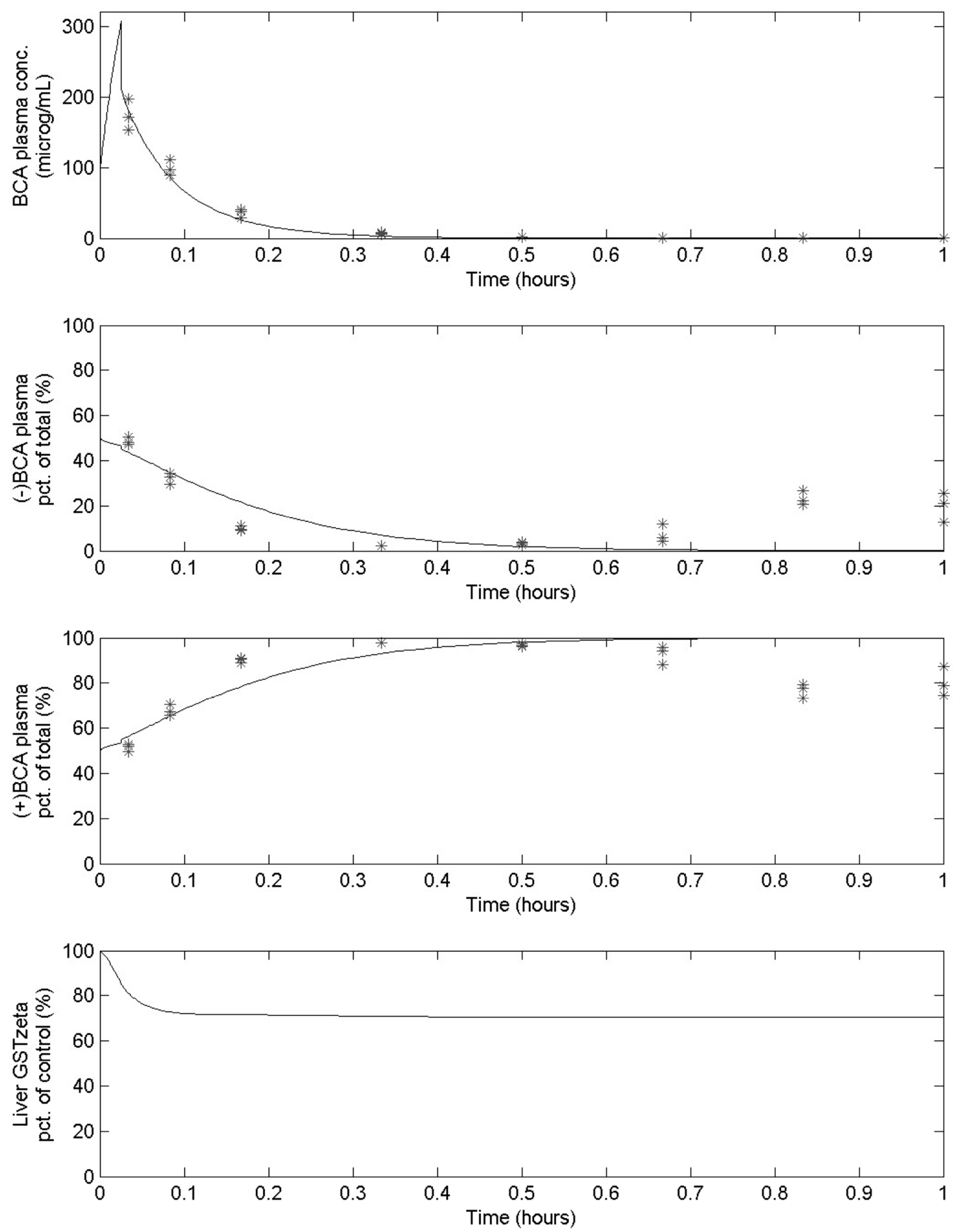


FIGURE N9
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Intravenous Injection of 100 mg/kg Racemic Bromochloroacetic Acid

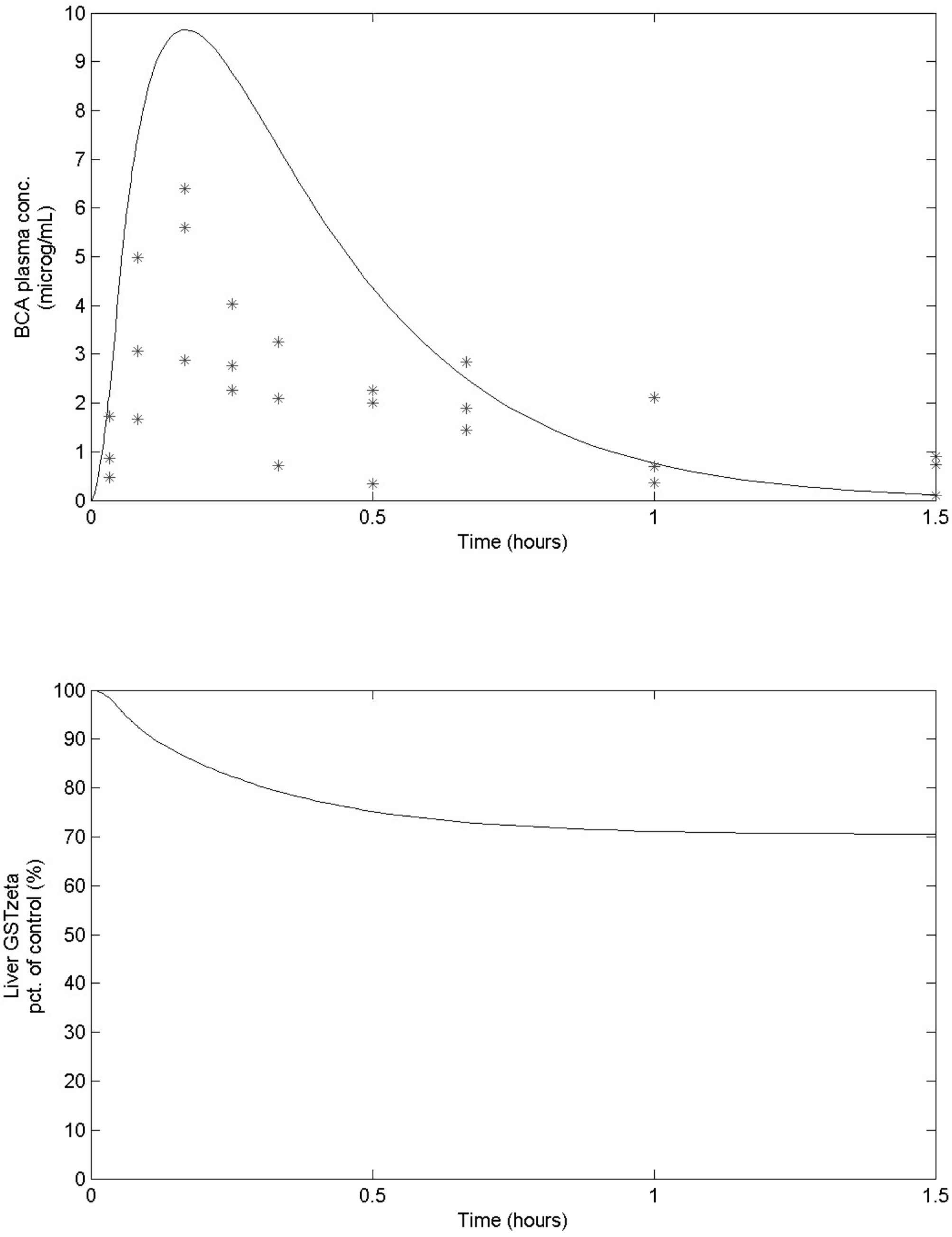


Figure N10
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 100 mg/kg Racemic Bromochloroacetic Acid

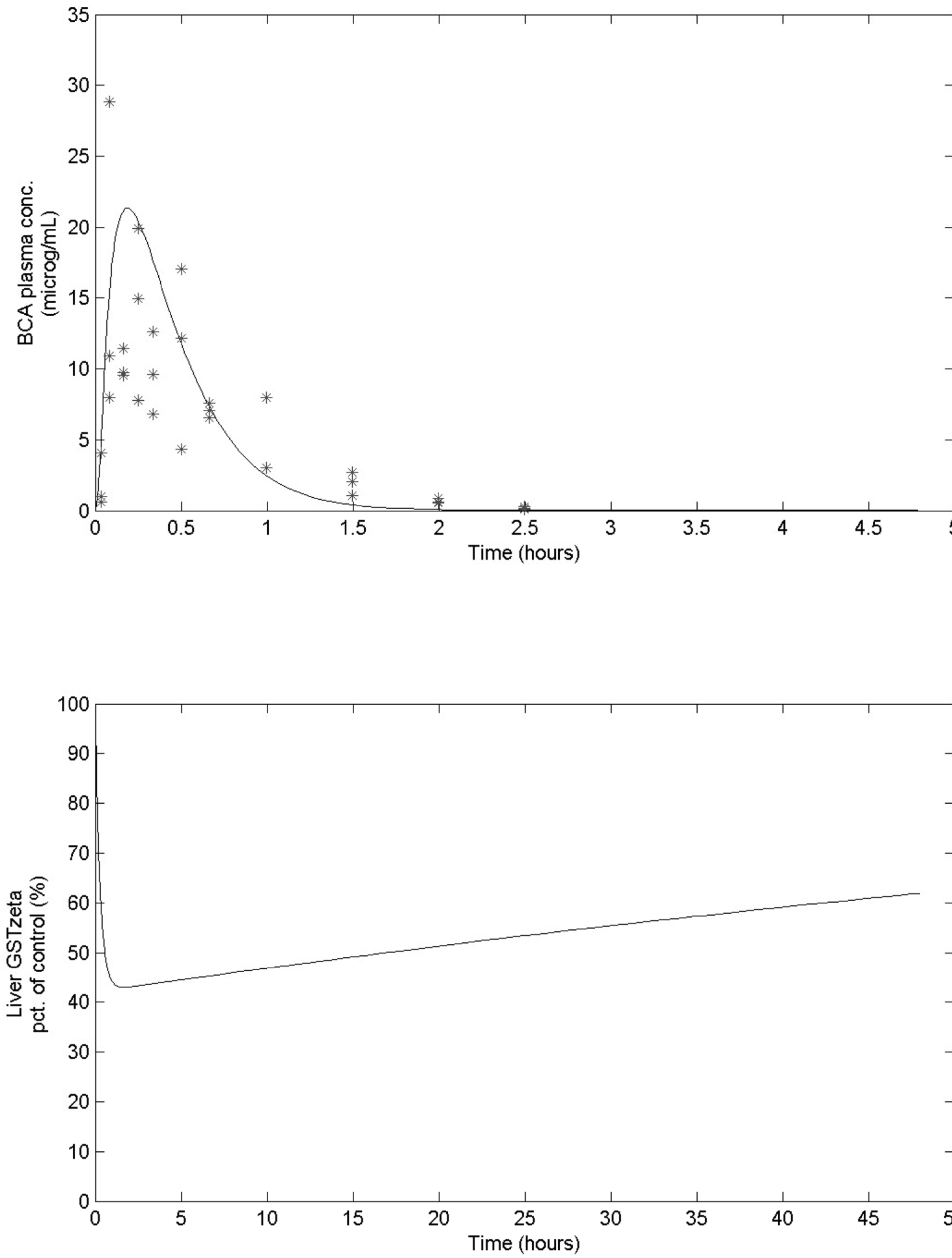


FIGURE N11
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 200 mg/kg Racemic Bromochloroacetic Acid

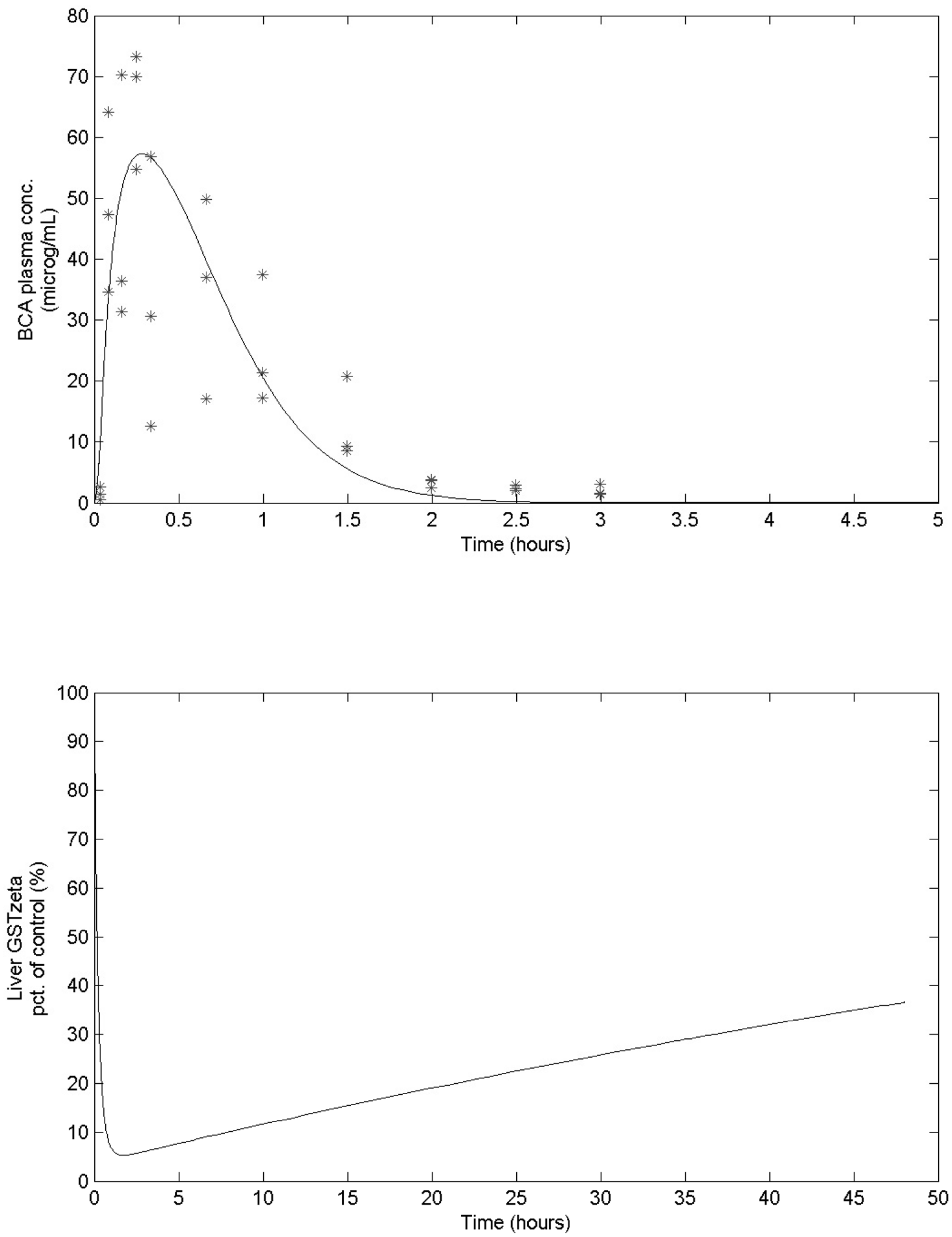


Figure N12
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 400 mg/kg Racemic Bromochloroacetic Acid

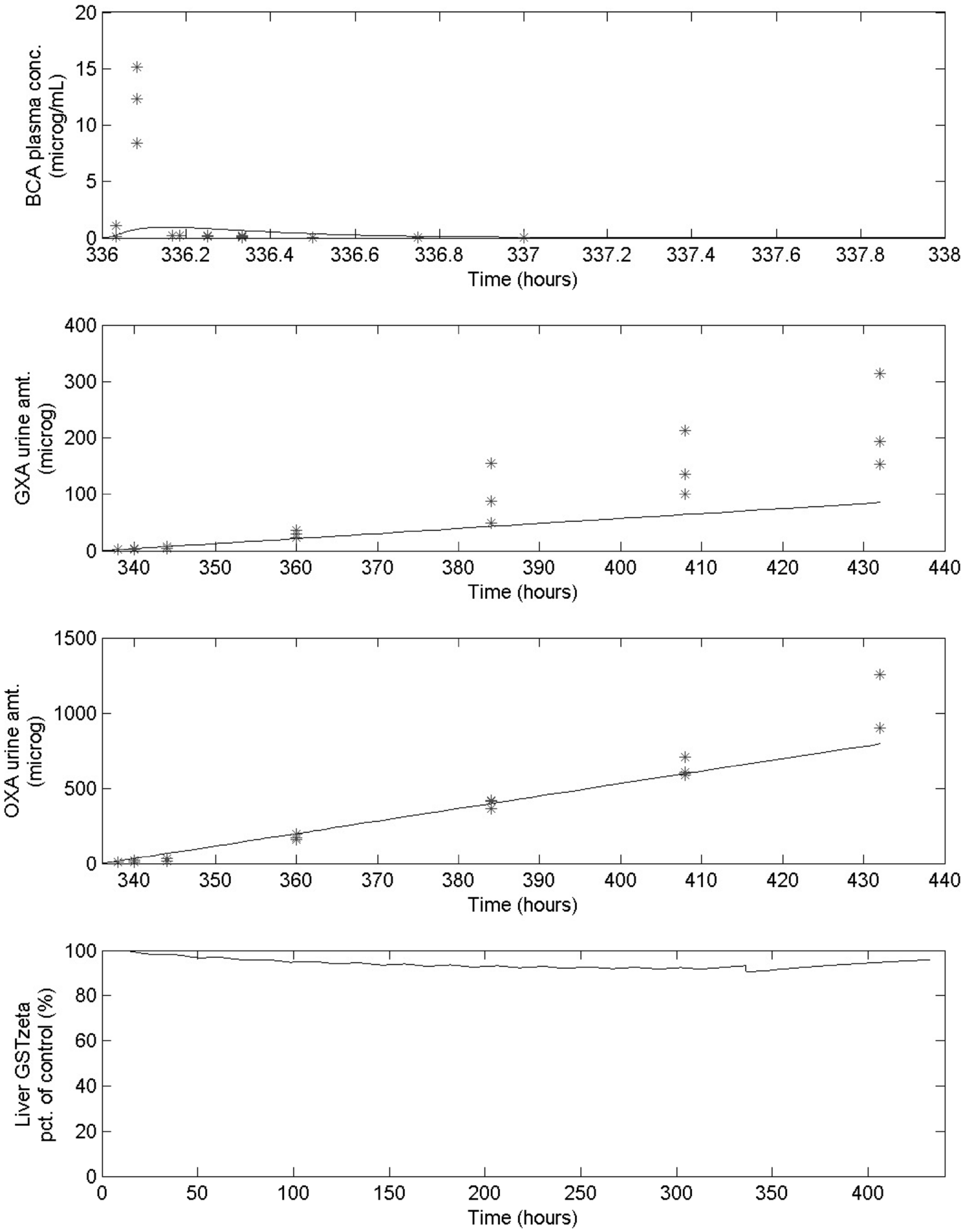


FIGURE N13
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 10 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 40 mg/L

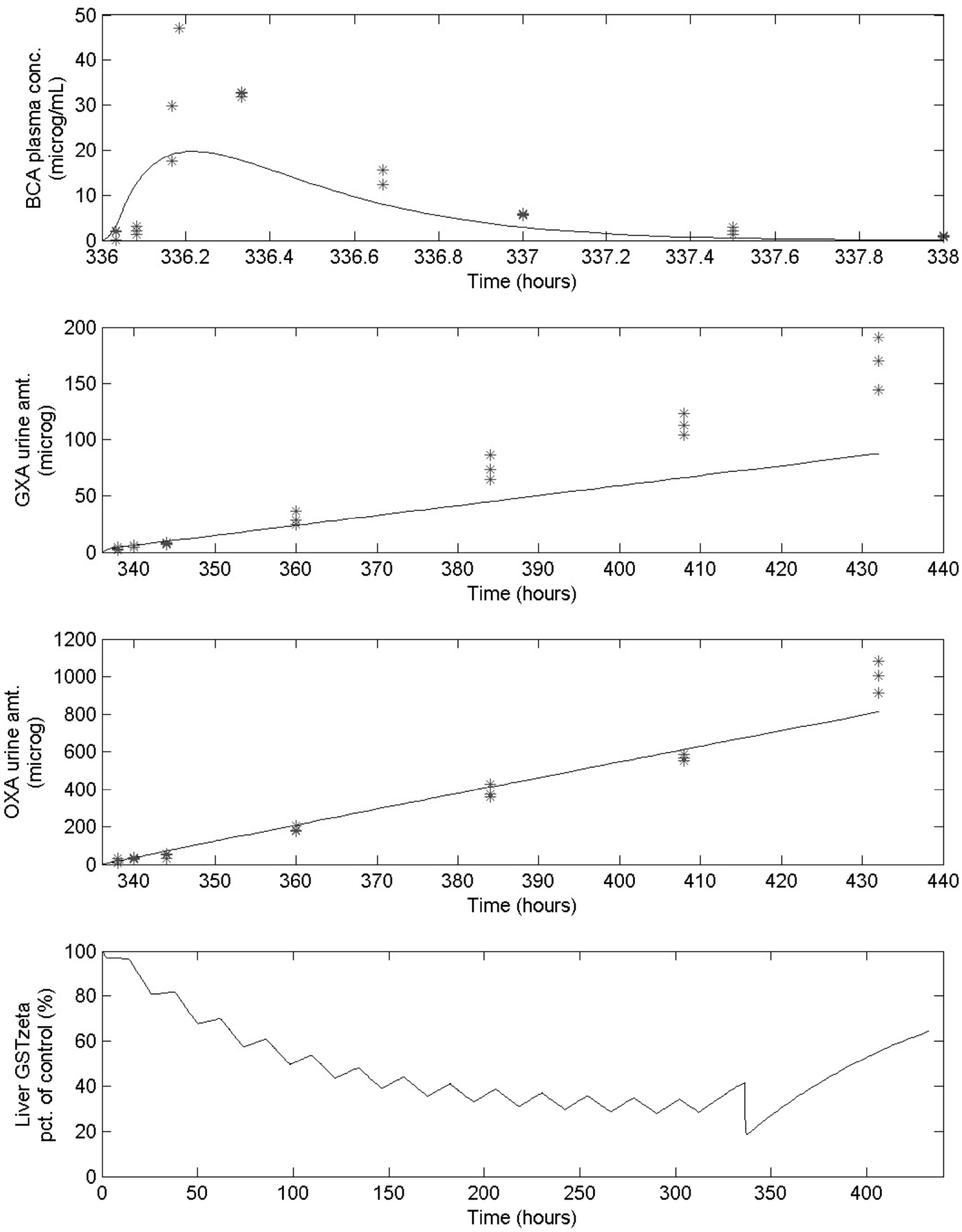


FIGURE N14
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 100 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 400 mg/L

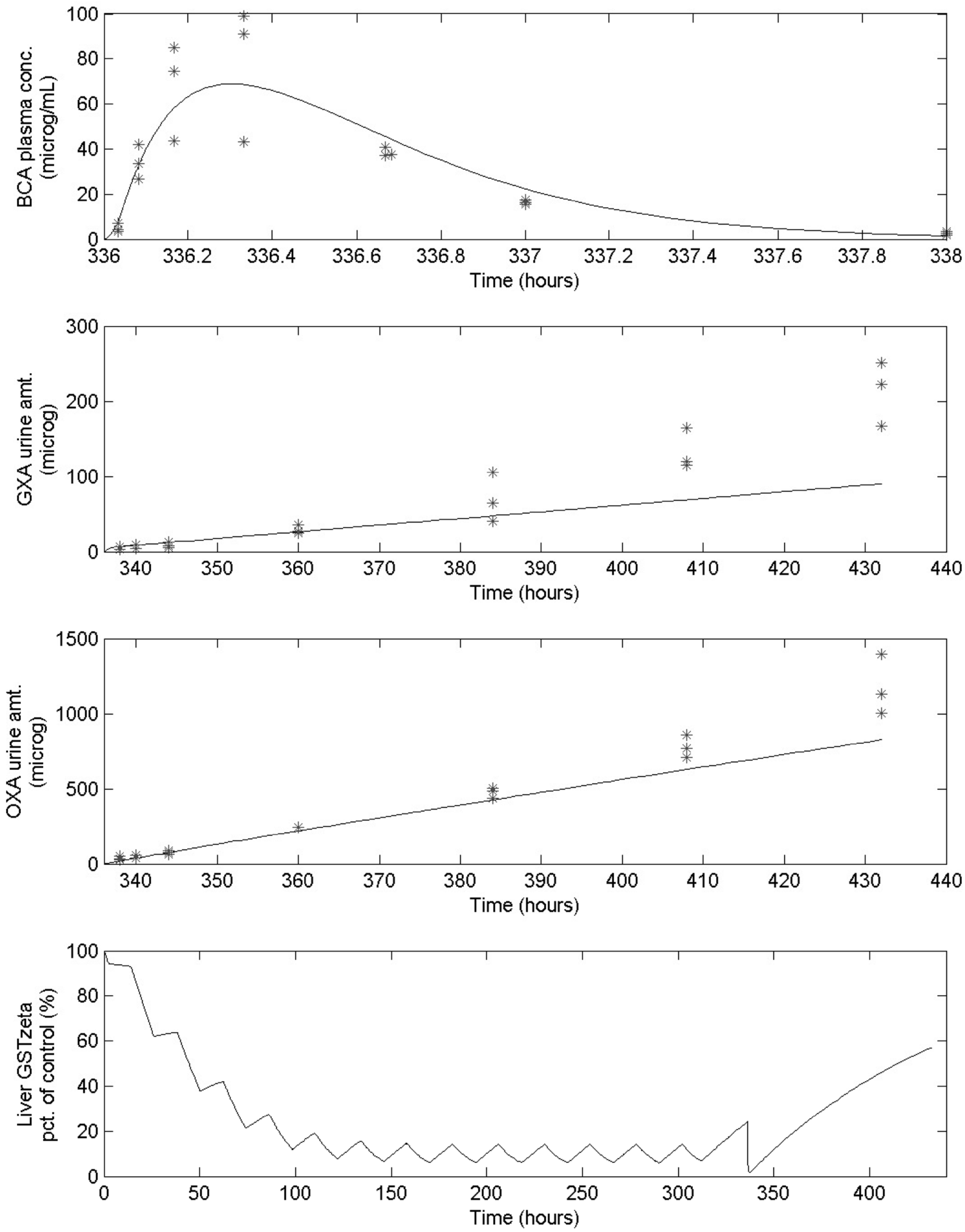


FIGURE N15
Data (Stars) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered a Single Gavage Dose of 200 mg/kg Racemic Bromochloroacetic Acid Following a 2-Week Drinking Water Exposure of 800 mg/L

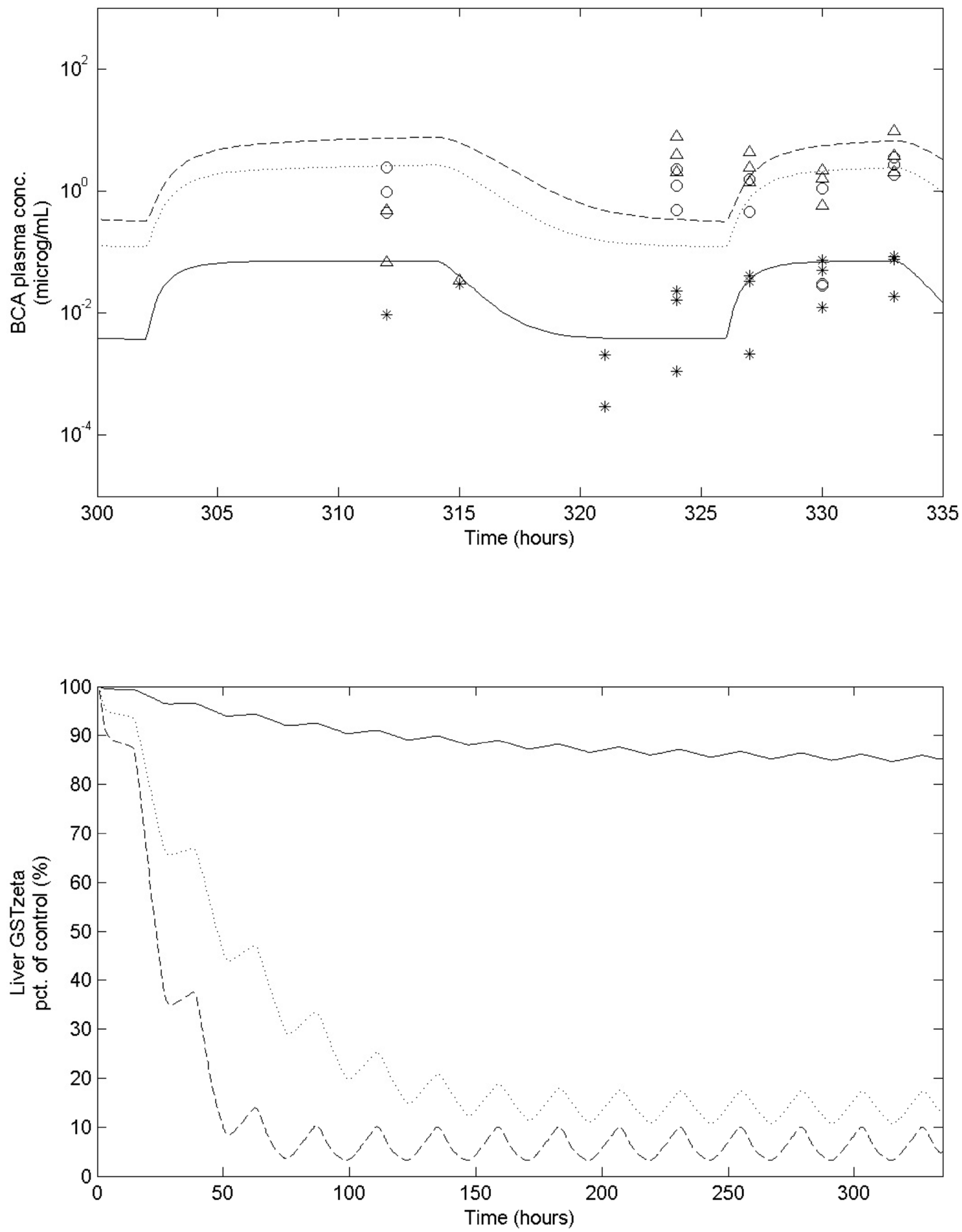
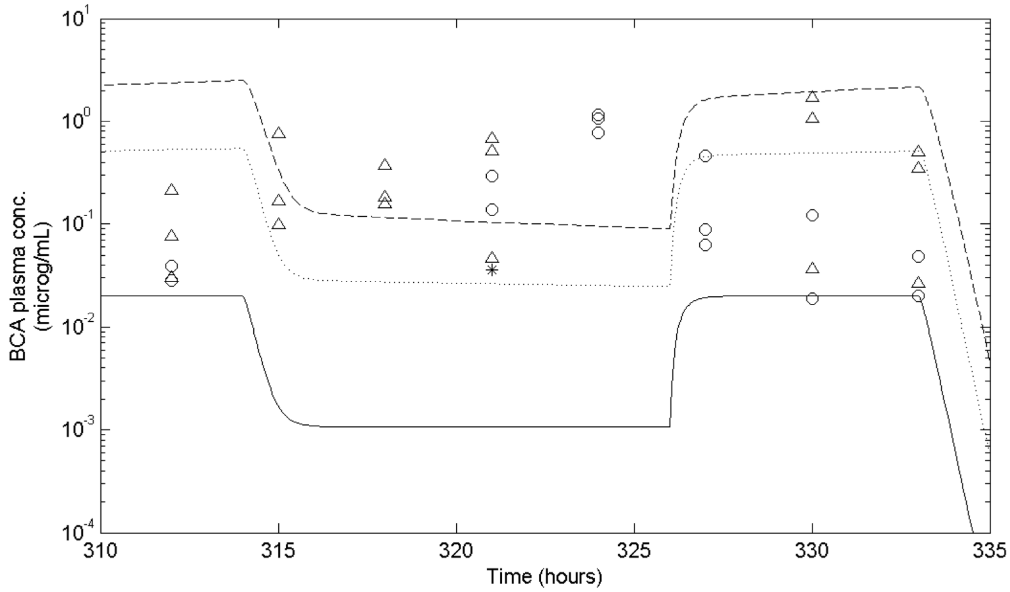


FIGURE N16
Data (Symbols) and PBPK Model Predictions (Lines) for Male F344/N Rats Administered 40 (Stars and Solid Lines), 400 (Circles and Dotted Lines), or 800 (Triangles and Dashed Lines) mg/L Racemic Bromochloroacetic Acid in Drinking Water for 2 Weeks



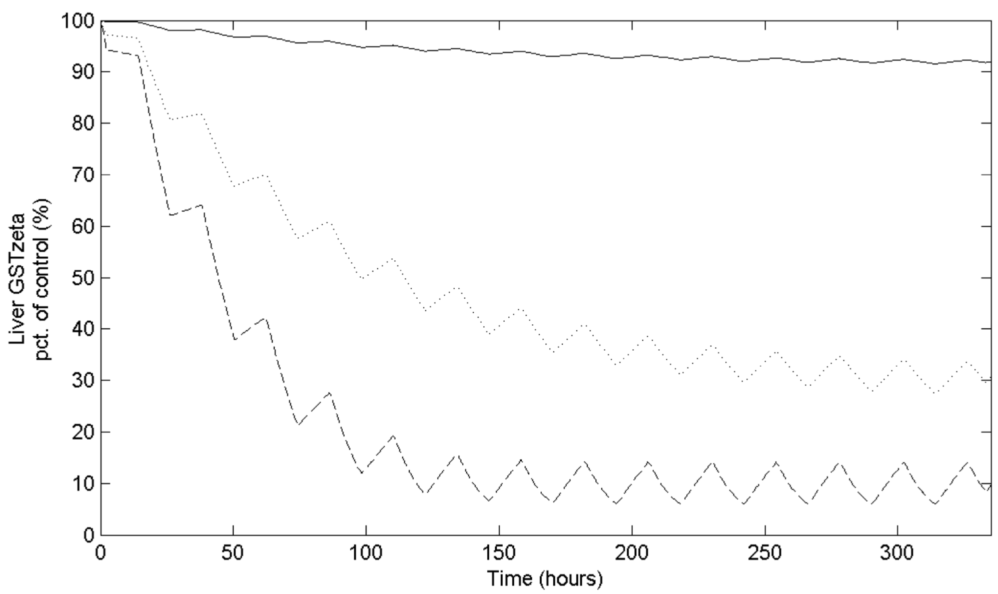


Figure N17
Data (Symbols) and PBPK Model Predictions (Lines) for Female B6C3F1 Mice Administered 40 (Stars and Solid Lines), 400 (Circles and Dotted Lines), or 800 (Triangles and Dashed Lines) mg/L Racemic Bromochloroacetic Acid in Drinking Water for 2 Weeks

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