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

NTP Genetically Modified Model Report on the

Toxicology Studies of Bromodichloromethane (CASRN 75-27-4) in Genetically Modified (FVB Tg.AC Hemizygous) Mice (Dermal, Drinking Water, and Gavage Studies) and Carcinogenicity Studies of Bromodichloromethane in Genetically Modified [B6.129-*Trp53*^{tm1Brd} (N5) Haploinsufficient] Mice (Drinking Water and Gavage Studies)

NTP GMM 05

MAY 2007

NTP REPORT ON THE

TOXICOLOGY STUDIES OF BROMODICHLOROMETHANE

(CAS NO. 75-27-4)

IN GENETICALLY MODIFIED (FVB Tg.AC HEMIZYGOUS) MICE

(DERMAL, DRINKING WATER, AND GAVAGE STUDIES)

AND CARCINOGENICITY STUDIES OF BROMODICHLOROMETHANE

IN GENETICALLY MODIFIED [B6.129-*Trp53*^{tm1Brd} (N5) HAPLOINSUFFICIENT] MICE

(DRINKING WATER AND GAVAGE STUDIES)

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

May 2007

NTP GMM 5

NIH Publication No. 07-4422

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 Genetically Modified Model (GMM) Report series began in 2005 with studies conducted by the NTP. The studies described in the GMM Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected agents in laboratory animals that have been genetically modified. These genetic modifications may involve inactivation of selected tumor suppressor functions or activation of oncogenes that are commonly observed in human cancers. This may result in a rapid onset of cancer in the genetically modified animal when exposure is to agents that act directly or indirectly on the affected pathway. An absence of a carcinogenic response may reflect either an absence of carcinogenic potential of the agent or that the selected model does not harbor the appropriate genetic modification to reduce tumor latency and allow detection of carcinogenic activity under the conditions of these subchronic studies. 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 GMM Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection *per se* is not an indicator of a substance's carcinogenic potential.

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

NTP GMM Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

G.A. Boorman, Ph.D., Study Scientist
R.A. Herbert, D.V.M., Ph.D., Study Pathologist
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
L.T. Burka, Ph.D.
R.S. Chhabra, Ph.D.
J.E. French, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
R.R. Maronpot, D.V.M.
S.D. Peddada, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
M.K. Vallant, B.S., M.T.
K.L. Witt, M.S.

Battelle Columbus Operations

Conducted studies and evaluated pathology findings

M.R. Hejtmancik, Ph.D., Principal Investigator
J.D. Johnson, Ph.D.
M.J. Ryan, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology review

M.H. Hamlin, II, D.V.M., Principal Investigator
J.C. Peckham, D.V.M., M.S., Ph.D.

Dynamac Corporation

Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides and prepared pathology report on 39-, 41-, and 42-week studies (July 16, 2002)

S.D. Rousselle, D.V.M., Chairperson
Pathology Associates, A Charles River Company
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
G. Pearse, B.V.M.&S.
National Toxicology Program
J.C. Peckham, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
D.C. Wolf, D.V.M., Ph.D.
United States Environmental Protection Agency

Constella Group, Inc.

Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.

Biotechnical Services, Inc.

Prepared Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
E.S. Rathman, M.S.
P.C. Rathman, B.S.E.
D.C. Serbus, Ph.D.

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SUMMARY

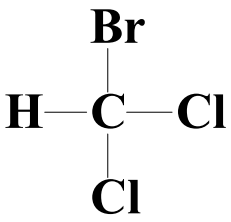
Background
Bromodichloromethane is a by-product of water disinfection. We tested if bromodichloromethane could cause cancer in two different strains of genetically modified mice.

Methods
We exposed male and female Tg.AC mice to bromodichloromethane for 6 or 9 months in three different ways: by painting solutions of the chemical dissolved in acetone on their backs, by giving the animals drinking water containing the chemical, or by depositing solutions of the chemical dissolved in corn oil directly into their stomachs through a tube (gavage). We also exposed p53 haploinsufficient mice to bromodichloromethane dissolved in drinking water or by gavage. Animals given the same solvents (acetone, drinking water, or corn oil), but without bromodichloromethane, served as the control groups. Tissues from 15 sites were examined for every animal.

Results
Exposure to bromodichloromethane through the skin had no effect on male or female Tg.AC mice. Male Tg.AC and p53 mice given bromodichloromethane by drinking water or gavage had increased rates of kidney renal tubule degeneration, and male and female p53 mice exposed by those two means had increased rates of fatty changes in the liver. No increases in tumors were seen in males or females from either strain of mice exposed by any of these routes.

Conclusions
We conclude that bromodichloromethane did not cause cancer in the genetically modified mice in these studies. This chemical did cause cancer in other studies with different rodents, and thus these genetically modified mice may not be as sensitive for detecting cancer-causing compounds.

ABSTRACT



BROMODICHLOROMETHANE

CAS No. 75-27-4

Chemical Formula: $CHBrCl_2$ Molecular Weight: 163.83

Synonyms: Dichlorobromomethane, monobromodichloromethane

Bromodichloromethane is a by-product of the chlorination of drinking water. It is formed by the halogen substitution and oxidation reactions of chlorine and naturally occurring organic matter (e.g., humic or fluvic acids) in water containing bromide. Bromodichloromethane was nominated to the NTP by the United States Environmental Protection Agency for toxicology and carcinogenicity studies. Male and female Tg.AC hemizygous mice received bromodichloromethane (at least 98% pure) by dermal application for 26 or 39 weeks, in drinking water for 26 or 42 weeks, or by gavage for 26 or 41 weeks. p53 Haploinsufficient mice received bromodichloromethane in drinking water for 26 or 42 weeks or by gavage for 26 or 41 weeks. Genetic toxicology studies were conducted in mouse peripheral blood erythrocytes.

26- and 39-Week Dermal Studies in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were dermally administered 0, 64, 128, or 256 mg bromodichloromethane/kg body weight in acetone, 5 days per week for 26 weeks, and groups of 10 male and 10 female Tg.AC hemizygous mice were dermally administered the same doses 5 days per week for 39 weeks. The survival and mean body and organ weights of all dosed groups of males and females were similar to those of the vehicle controls. There were no statistically or biologically significant increases in the incidences of neoplasms or nonneoplastic lesions.

26- and 42-Week Drinking Water Studies in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were exposed to drinking water containing 0, 175, 350, or 700 mg/L bromodichloromethane for 26 weeks (equivalent to average daily doses of approximately 20, 36, or 61 mg bromodichloromethane/kg body weight to males and 31, 61, or 130 mg/kg to females). The survival of exposed males and females was similar to that of the control groups. Mean body weights of males exposed to 350 or 700 mg/L were less than those of the controls during most of the study. Mean body weights of

175, 350, and 700 mg/L females were greater than those of the controls after weeks 10, 22, and 23, respectively. In exposed males, water consumption declined with increasing exposure concentration. Water consumption by exposed females was less at the beginning of the study, but was similar to that by controls at the end of the study. The decreased water consumption was related to poor palatability. Absolute heart and right kidney weights of exposed males were significantly less than those of the control group. The incidences of hepatocyte fatty change and hypertrophy in 350 and 700 mg/L females and cytoplasmic vacuolization in 700 mg/L females were significantly greater than those in the control group. Incidences of renal tubule dilatation in males exposed to 175 mg/L or greater, renal tubule hypertrophy in 350 and 700 mg/L males, and nephropathy and renal tubule degeneration in 700 mg/L males were also increased.

Groups of 10 male and 10 female Tg.AC hemizygous mice were exposed to drinking water containing 0, 175, 350, or 700 mg/L bromodichloromethane for 42 weeks (equivalent to average daily doses of approximately 18, 33, or 64 mg/kg to males and 28, 49, or 111 mg/kg to females). The survival of exposed males and females was similar to that of the control groups. Mean body weights of 350 and 700 mg/L males were less than those of the controls at the end of the study. Due to poor palatability, water consumption decreased with increasing exposure concentration. Absolute right kidney weights of 350 and 700 mg/L males were significantly less than those of the control group. The incidences of hepatocyte fatty change in all exposed groups of females, renal tubule dilatation in all exposed groups of males, and nephropathy in 700 mg/L males were significantly increased.

26- and 41-Week Gavage Studies in Tg.AC Hemizygous Mice

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 25, 50, or 100 mg bromodichloromethane/kg body weight in corn oil by gavage, 5 days per week for 26 weeks. The survival of dosed males and females was similar to that of the vehicle control groups. Mean body weights of dosed females were generally greater than those of the vehicle controls at the end of the study. The incidence of multiple squamous cell papilloma of the forestomach in 100 mg/kg females was significantly greater than that in the vehicle controls. The incidences of hepatocyte fatty change in all dosed groups of females, hepatocyte cytoplasmic vacuolization in 25 and 50 mg/kg females, renal tubule hypertrophy in 100 mg/kg females, and renal tubule degeneration in 100 mg/kg males were significantly increased.

Groups of 10 male and 10 female Tg.AC hemizygous mice were administered 0, 25, 50, or 100 mg/kg in corn oil by gavage, 5 days per week for 41 weeks. The survival of dosed males and females was similar to that of the control groups. Mean body weights of 25 mg/kg males and 100 mg/kg females were greater than those of the vehicle controls at the end of the study. The incidences of multiple squamous cell papilloma of the forestomach in 25 and 100 mg/kg females and of all squamous cell papillomas of the forestomach in 100 mg/kg females were significantly greater than those of the vehicle controls. The incidences of hepatocyte cytoplasmic vacuolization in 50 mg/kg females and hepatocyte fatty change in 50 and 100 mg/L females were significantly increased; the incidences of renal tubule degeneration in 100 mg/kg males was also significantly greater than that in the vehicle control group.

26- and 42-week Drinking Water Studies in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were exposed to drinking water containing 0, 175, 350, or 700 mg/L bromodichloromethane for 26 weeks (equivalent to average daily doses of approximately 16, 31, or 65 mg/kg to males and 26, 50, or 100 mg/kg to females). The survival of exposed males and females was similar to that of the control groups. Mean body weights of 350 and 700 mg/L males were less than those of the controls throughout most of the study. Mean body weights of 175, 350, and 700 mg/L females were less than control body weights after weeks 15, 23, and 18, respectively. In exposed males, water consumption declined with increasing exposure concentration. Water consumption by exposed females was similar to that by controls by the end of the study. The absolute heart weight of 700 mg/L males and absolute right kidney and liver weights of 350 and 700 mg/L males were significantly less than those of the control group. The incidences of renal tubule dilatation in all exposed groups of males, renal tubule degeneration in 350 and 700 mg/L males, and the incidence of fatty change in hepatocytes of 700 mg/L females were significantly greater than those in the control groups.

Groups of 10 male and 10 female p53 haploinsufficient mice were exposed to drinking water containing 0, 175, 350, or 700 mg/L for 42 weeks (equivalent to approximately 14, 30, or 55 mg/kg to males and 22, 43, or 98 mg/kg to females). The survival of exposed males and females was similar to that in the control groups. Mean body weights of males exposed to 350 or 700 mg/L were less than those of the controls. Mean body weights in 700 mg/L females were less during the last three weeks of the study. Water consumption by exposed males was less than that by controls. The absolute right kidney weights in 350 and 700 mg/L males were significantly less than those of the control group. The incidences of renal tubule degeneration in 350 and 700 mg/L males were significantly greater than that in the control group.

26- and 41-Week Gavage Studies in p53 Haploinsufficient Mice

Groups of 15 male and 15 female p53 haploinsufficient mice were administered 0, 25, 50, or 100 mg bromodichloromethane/kg body weight in corn oil by gavage for 26 weeks. The survival of dosed males and females was similar to that of the vehicle control groups. The mean body weights of males administered 50 or 100 mg/kg and females administered 50 mg/kg were less than those of the vehicle controls during most of the study. The absolute heart, right kidney, and right testis weights in 100 mg/kg males were significantly less than those of the vehicle controls. The absolute liver weight of 100 mg/kg females was significantly greater. The incidences of fatty change in hepatocytes of 100 mg/kg females and renal tubule degeneration in 100 mg/kg males were significantly greater than those in the vehicle control groups.

Groups of 10 male and 10 female p53 haploinsufficient mice were administered 0, 25, 50, or 100 mg/kg in corn oil by gavage for 41 weeks. The survival of dosed males and females was similar to that of the vehicle control groups. Mean body weights of 50 and 100 mg/kg males were less than those of the vehicle controls throughout the study and those of 25, 50, and 100 mg/kg females were less after weeks 9, 14, and 24, respectively. The absolute liver weight of 100 mg/kg females was increased with respect to the vehicle controls, and the absolute heart and right kidney weights of 100 mg/kg males were decreased. The incidences of hepatocyte fatty change in 100 mg/kg males and females and renal tubule degeneration and nephropathy in 100 mg/kg males were significantly greater than those in the vehicle controls.

Genetic Toxicology

Peripheral blood micronucleus tests on male and female Tg.AC hemizygous and p53 haploinsufficient mice exposed to bromodichloromethane in drinking water, by dermal application, and by gavage for 26 weeks yielded mixed results but no clearly positive responses. Results in Tg.AC hemizygous mice were judged to be equivocal for both males and females in the drinking water study, equivocal in males and negative in females treated by dermal application, and negative in males and females treated by gavage. For the micronucleus studies in p53 haploinsufficient mice, the drinking water route gave equivocal results in males and negative results in females; gavage administration gave negative results in both males and females.

Conclusions

Under the conditions of these drinking water studies, there was *no evidence of carcinogenic activity** of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 175, 350, or 700 mg/L for 26 or 42 weeks.

Under the conditions of these gavage studies, there was *no evidence of carcinogenic activity** of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 25, 50, or 100 mg/kg body weight 5 days per week for 26 or 41 weeks.

In both the drinking water and the gavage studies in p53 haploinsufficient mice, there were increased incidences of renal tubule degeneration in male mice and fatty change of the hepatocyte in female mice exposed to bromodichloromethane.

No treatment-related neoplasms or nonneoplastic lesions were seen in male or female Tg.AC hemizygous mice exposed dermally to 64, 128, or 256 mg bromodichloromethane/kg body weight 5 days per week for 26 or 39 weeks.

No treatment-related neoplasms were seen in male or female Tg.AC hemizygous mice exposed by drinking

water to 175, 350, or 700 mg bromodichloromethane/L for 26 or 42 weeks.

No treatment-related neoplasms were seen in male Tg.AC hemizygous mice exposed by gavage to 25, 50, or 100 mg bromodichloromethane/kg body weight 5 days per week for 26 or 41 weeks. An increased incidence of multiple forestomach papillomas was seen in female Tg.AC hemizygous mice exposed to bromodichloromethane by gavage for 26 or 41 weeks.

In the drinking water and gavage studies in Tg.AC hemizygous mice, there were increased incidences of nephropathy and/or renal tubule degeneration in male mice and fatty change and/or cytoplasmic vacuolization of the hepatocyte in female mice exposed to bromodichloromethane.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Technical Reports Review Subcommittee comments and the public the discussion on this Report appears on page 15.

Summary of the 26- and 39-Week Dermal and Genetic Toxicology Studies of Bromodichloromethane in Tg.AC Hemizygous Mice

	Male		Female	
	26-Week	**39-Week**	**26-Week**	**39-Week**
Applied dermally in acetone	0, 64, 128, and 256 mg/kg	0, 64, 128, and 256 mg/kg	0, 64, 128, and 256 mg/kg	0, 64, 128, and 256 mg/kg
Body weights	Dosed groups similar to vehicle control group	Dosed groups similar to vehicle control group	Dosed groups similar to vehicle control group	Dosed groups similar to vehicle control group
Survival rates	13/15, 14/15, 15/15, 13/15	6/10, 8/10, 9/10, 8/10	11/15, 10/15, 12/15, 10/15	5/10, 4/10, 7/10, 5/10
Nonneoplastic effects	None	None	None	None
Neoplastic effects	None	None	None	None
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		equivocal in males, negative in females		

Summary of the 26- and 42-Week Drinking Water and Genetic Toxicology Studies of Bromodichloromethane in Tg.AC Hemizygous Mice

	Male		Female	
	26-Week	**42-Week**	**26-Week**	**42-Week**
Concentrations in water	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L
Body weights	350 and 700 mg/L groups less than control group	350 and 700 mg/L groups less than control group	Exposed groups greater than control group	Exposed groups similar to control group
Survival rates	13/15, 12/15, 12/15, 14/15	6/10, 9/10, 8/10, 9/10	10/15, 13/15, 11/15, 13/15	5/10, 8/10, 4/10, 4/10
Nonneoplastic effects	Kidney: nephropathy (4/15, 3/15, 4/15, 11/15); renal tubule degeneration (0/15, 4/15, 4/15, 9/15)	Kidney: nephropathy (4/10, 7/10, 8/10, 9/10)	Liver: hepatocyte fatty change (0/15, 4/15, 8/15, 10/15); hepatocyte cytoplasmic vacuolization (2/15, 5/15, 4/15, 8/15)	Liver: hepatocyte fatty change (0/10, 6/10, 6/10, 6/10)
Neoplastic effects	None	None	None	None
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		equivocal in males and females		

Summary of the 26- and 41- Week Gavage and Genetic Toxicology Studies of Bromodichloromethane in Tg.AC Hemizygous Mice

	Male		Female	
	26-Week	41-Week	26-Week	41-Week
Oral doses in in corn oil	0, 25, 50, and 100 mg/kg	0, 25, 50, and 100 mg/kg	0, 25, 50, and 100 mg/kg	0, 25, 50, 100 mg/kg
Body weights	Dosed groups similar to vehicle control group	25 mg/kg group greater than vehicle control group	Dosed groups greater than vehicle control group	100 mg/kg group greater than vehicle control group
Survival rates	13/15, 14/15, 12/15, 15/15	6/10, 6/10, 6/10, 8/10	11/15, 14/15, 13/15, 13/15	7/10, 9/10, 9/10, 7/10
Nonneoplastic effects	Kidney: renal tubule degeneration (0/15, 0/15, 0/15, 4/15)	Kidney: renal tubule degeneration (0/10, 0/10, 0/10, 6/10)	Liver: hepatocyte fatty change (0/15, 5/15, 8/15, 7/15); hepatocyte cytoplasmic vacuolization (0/15, 6/15, 4/15, 3/15)	Liver: hepatocyte fatty change (0/10, 2/10, 8/10, 5/10); hepatocyte cytoplasmic vacuolization (6/10, 9/10, 10/10, 9/10)
Neoplastic effects	None	None	Forestomach: multiple squamous cell papilloma (3/15, 5/15, 6/15, 11/15)	Forestomach: multiple squamous cell papilloma (1/10, 6/10, 5/10, 9/10)
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		negative in males and females		

Summary of the 26- and 42-Week Drinking Water and Genetic Toxicology Studies of Bromodichloromethane in p53 Haploinsufficient Mice

	Male		Female	
	26-Week	**42-Week**	**26-Week**	**42-Week**
Concentrations in water	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L	0, 175, 350, and 700 mg/L
Body weights	350 and 700 mg/L groups less than control group	350 and 700 mg/L groups less than control group	Exposed groups less than control group	700 mg/L group less than control group
Survival rates	15/15, 15/15, 15/15, 15/15	9/10, 10/10, 9/10, 7/10	15/15, 15/15, 14/15, 15/15	9/10, 9/10, 10/10, 8/10
Nonneoplastic effects	Kidney: renal tubule degeneration (0/15, 0/15, 9/15, 12/15)	Kidney: renal tubule degeneration (0/10, 0/10, 6/10, 10/10)	Liver: hepatocyte fatty change (0/15, 1/15, 1/15, 10/15)	None
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		equivocal in males, negative in females		

Summary of the 26- and 41-Week Gavage and Genetic Toxicology Studies of Bromodichloromethane in p53 Haploinsufficient Mice

	Male		Female	
	26-Week	**41-Week**	**26-Week**	**41-Week**
Concentrations in water	0, 25, 50, and 100 mg/kg	0, 25, 50, and 100 mg/kg	0, 25, 50, and 100 mg/kg	0, 25, 50, and 100 mg/kg
Body weights	50 and 100 mg/kg groups less than vehicle control group	50 and 100 mg/kg groups less than the vehicle control group	50 mg/kg group less than vehicle control group	Dosed groups less than the vehicle control group
Survival rates	15/15, 15/15, 15/15, 15/15	10/10, 9/10, 10/10, 10/10	15/15, 14/15, 14/15, 14/15	9/10, 9/10, 8/10, 9/10
Nonneoplastic effects	Kidney: renal tubule degeneration (0/15, 0/15, 0/15, 4/15)	Kidney: renal tubule degeneration (0/10, 1/10, 0/10, 10/10)	Liver: hepatocyte, fatty change (2/15, 2/15, 3/15, 11/15)	Liver: hepatocyte, fatty change (3/10, 3/10, 6/10, 9/10)
Neoplastic effects	None	None	None	None
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	No evidence
Genetic toxicology				
Micronucleated erythrocytes				
Mouse peripheral blood *in vivo:*		negative in males and females		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

Five categories of evidence of carcinogenic activity are used in the 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 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 Report on bromodichloromethane on September 27, 2005, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

Charlene A. McQueen, Ph.D., Chairperson
College of Pharmacy
University of Arizona
Tucson, AZ

Diane F. Birt, Ph.D., Principal Reviewer
Department of Food Science & Human Nutrition
Iowa State University
Ames, IA

Michael R. Elwell, D.V.M., Ph.D.
Pathology, Drug Safety Evaluation
Pfizer Global Research and Development
Groton, CT

Thomas A. Gasiewicz, Ph.D.
Department of Environmental Medicine
Environmental Health Sciences Center
University of Rochester School of Medicine
Rochester, NY

John P. Giesy, Jr., Ph.D.
Department of Zoology
Michigan State University
East Lansing, MI

Shuk-Mei Ho, Ph.D.*
Department of Surgery, Division of Urology
University of Massachusetts Medical School
Worcester, MA

Stephen M. Roberts, Ph.D.
Center for Environmental & Human Toxicology
University of Florida
Gainesville, FL

Mary Vore, Ph.D., Principal Reviewer
Graduate Center for Toxicology
University of Kentucky
Lexington, KY

Special Ad Hoc Reviewers

Kenny Crump, Ph.D.
Environ International
Ruston, LA

Prescott Deininger, Ph.D.*
Tulane University Medical Center
New Orleans, LA

Harish Sikka, Ph.D.
Environmental Toxicology and Chemistry Laboratory
State University of New York College at Buffalo
Buffalo, NY

Keith Soper, Ph.D.
Merck Research Laboratories
West Point, PA

Vernon Walker, Ph.D.*
Lovelace Respiratory Institute
Albuquerque, NM

* Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On September 27, 2005, the draft Report on the toxicology and carcinogenesis studies of bromodichloromethane 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 (NIEHS), Research Triangle Park, NC.

Dr. G.A. Boorman, NIEHS, introduced the studies of the water disinfection by-product bromodichloromethane in Tg.AC hemizygous and p53 haploinsufficient mice by different routes of administration. The proposed conclusions were:

Under the conditions of these drinking water studies, there was *no evidence of carcinogenic activity* of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 175, 350, or 700 mg/L for 26 or 42 weeks.

Under the conditions of these gavage studies, there was *no evidence of carcinogenic activity* of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 25, 50, or 100 mg/kg body weight 5 days per week for 26 or 41 weeks.

In both the drinking water and the gavage studies in p53 haploinsufficient mice, there were increased incidences of renal tubule degeneration in male mice and fatty change of the hepatocyte in female mice exposed to bromodichloromethane.

No treatment-related neoplasms or nonneoplastic lesions were seen in male or female Tg.AC hemizygous mice exposed dermally to 64, 128, or 256 mg bromodichloromethane/kg body weight 5 days per week for 26 or 39 weeks.

No treatment-related neoplasms were seen in male or female Tg.AC hemizygous mice exposed by drinking water to 175, 350, or 700 mg bromodichloromethane/L for 26 or 42 weeks.

No treatment-related neoplasms were seen in male Tg.AC hemizygous mice exposed by gavage to 25, 50, or 100 mg bromodichloromethane/kg body weight 5 days per week for 26 or 41 weeks. An increased incidence of multiple forestomach papillomas was seen in female Tg.AC hemizygous mice exposed to bromodichloromethane by gavage for 26 or 41 weeks.

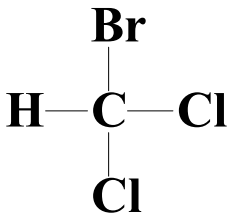
In the drinking water and gavage studies in Tg.AC hemizygous mice, there were increased incidences of nephropathy and/or renal tubule degeneration in male mice and fatty changes and/or cytoplasmic vacuolization of the hepatocyte in female mice exposed to bromodichloromethane.

Dr. Birt, the first principal reviewer, thought the studies were well designed and conducted. She suggested adding details about the animal sources and also historical data, particularly regarding forestomach papillomas.

Dr. Vore, the second principal reviewer, also felt the study was well conducted, and she agreed with the conclusions.

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

INTRODUCTION



BROMODICHLOROMETHANE

CAS No. 75-27-4

Chemical Formula: $CHBrCl_2$ Molecular Weight: 163.83

Synonyms: Dichlorobromomethane, monobromodichloromethane

Chemical and Physical Properties

Bromodichloromethane, a clear, colorless liquid with a density of 1.980 g/mL at 20° C, is one of several trihalomethanes formed when organic substances in water react with chlorine or bromine (Stevens *et al*., 1976; Hoehn *et al*., 1978; Rook, 1980). Bromodichloromethane as a halogenated organic molecule is included in the trihalomethane class of chemicals formed as a by-product when drinking water supplies are disinfected by chlorination (Rook, 1974).

Production, Use, and Human Exposure

The U.S. Environmental Protection Agency has established a maximum contaminant level of 0.080 mg/L for total trihalomethanes in community water systems serving more than 10,000 persons (40 CFR § 141.64). The presence of trihalomethanes in drinking water is believed to pose a risk to humans because chloroform, another trihalomethane found in drinking water, is carcinogenic in rats and mice (IARC, 1999a). Bromodichloromethane when administered in corn oil by oral gavage is carcinogenic for rats and mice (NTP, 1987). Trihalomethanes are widespread in the environment, not only in water supplies but also in swimming pools, soft drinks, and dump sites (NTP, 1987). The mean concentration of bromodichloromethane in chlorinated water supplies in the United States is 0.017 mg/L (range: 0 to 0.125 mg/L; *Fed. Regist.*, 1979). Assuming that the average daily water consumption for an adult human male weighing 70 kg is 2 L per day, intake of bromodichloromethane could reach a maximum daily consumption of 4.0 pg/kg per day.

Absorption, Distribution, and Excretion

Bromodichloromethane was administered in corn oil by gavage to male Sprague-Dawley rats at 100 mg/kg (16 pCi/kg) and to male $B6C3F_1$ mice at 150 mg/kg (32 pCi/kg; Mink *et al*., 1986). Urine and expired gas were monitored for radioactivity, and tissue distribution was determined. Eight hours after administration of bromodichloromethane, the percentage of radioactivity recovered as expired carbon dioxide was 14% in rats and

81% in mice; the percentage of unmetabolized compound in expired air was 41% in rats and 7% in mice. The percentage of recovered label at 8 hours in expired air, urine, and tissues was 63% for rats and 93% for mice. Radioactivity was found in the liver, kidney, and stomach. These studies indicate that mice metabolize bromodichloromethane at a faster rate than do rats. Similar studies with chloroform, chlorodibromomethane, and bromoform indicated that mice also metabolize these trihalomethanes at a faster rate than do rats (Mink *et al*., 1986). In another series of experiments, bromodichloromethane, chloroform, chlorodibromomethane, bromoform, and iodoform were administered intraperitoneally in corn oil to male Sprague-Dawley rats at 1 mmol/kg body weight; blood samples were collected from the tail vein, and the amount of total carbon monoxide was measured. The highest blood carbon monoxide levels were observed after iodoform and bromoform administration; chlorodibromomethane, bromodichloromethane, and chloroform were metabolized at slower rates (Anders *et al*., 1978). Bromodichloromethane given in corn oil shows a complex blood profile that may reflect discontinuous stomach emptying into the small intestine (Lilly *et al*., 1998). While several P450 enzymes can metabolize bromodichloromethane, CYP2E1 appears to be the dominant hepatic CYP isoenzyme for metabolism of bromodichloromethane in both rats and humans at concentrations found in the drinking water (Allis and Zhao, 2002; Zhao and Allis, 2002).

TOXICITY

Experimental Animals

The following oral LD_{50} values have been reported for bromodichloromethane: 450 mg/kg, male ICR mice; 900 mg/kg, female ICR mice; 916 mg/kg, male Sprague-Dawley rats; 969 mg/kg, female Sprague-Dawley rats; 450 mg/kg, male CD-1 mice; and 900 mg/kg, female CD-1 mice (Bowman *et al*., 1978; Chu *et al*., 1982). Clinical signs associated with bromodichloromethane administration at LD_{50} or higher doses included piloerection, sedation, flaccid muscle tone, ataxia, prostration, and enlargement and congestion of the liver and kidneys. Bromodichloromethane administered in corn oil by gavage for 14 consecutive days to 10 male CD-1 mice at 148 mg/kg per day caused focal inflammation of the liver and intratubular mineralization and epithelial hyperplasia of the kidney; however, no effect on body weight gain was seen (Condie *et al*., 1983). Kidney function was judged to be impaired because uptake of *p*-aminohippurate in renal cortical slices was decreased. No dose-related changes were seen in blood urea nitrogen or serum creatinine levels; serum glutamic-pyruvic transaminase activity (SGPT) was elevated. Munson *et al*. (1982) conducted a 14-day study in male and female CD-1 mice in which bromodichloromethane was administered by gavage in a solution of 10% emulphor at levels of 50 to 250 mg/kg per day. At the highest dose, liver weight, serum glutamic-oxaloacetic transaminase and SGPT activities, and blood urea nitrogen levels increased; body weight gain, serum glucose levels, and spleen weight decreased. Four days before sacrifice, a separate group of mice was immunized with sheep erythrocytes. The mice were sacrificed, and spleen cell suspensions were prepared and assayed for antibody-forming cells; at 250 mg/kg, antibody-forming cells were decreased, suggesting impairment of the immune system. Histopathologic evaluation of tissues was not reported. Bromodichloromethane administered to male and female Sprague-Dawley rats for 90 days in drinking water at levels up to 2,500 ppm (resulting in doses of approximately 100 mg/kg body weight and 135 mg/kg body weight) produced mild toxicity in the liver and decreased body weight gain (Chu *et al*., 1982). The vacuolar changes observed in the liver, interpreted as fatty infiltration, were reversed after a 90-day recovery period.

Humans

There is no information on the acute toxicity of bromodichloromethane for humans.

REPRODUCTIVE TOXICITY

Experimental Animals

Bromodichloromethane was given to pregnant Sprague-Dawley rats in corn oil by gavage on days 6 through 15 of gestation at doses of 0, 50, 100, or 200 mg/kg per day (Ruddick *et al*., 1983). At the highest doses, maternal body weight gain was decreased, but no teratogenic effects were observed. A mixture of trihalomethanes and 15 other organic substances concentrated from water was given in dimethyl sulfoxide by gavage to pregnant CD-1 mice at 51, 170, or 510 mg/kg per day on days 7 through 14 of gestation; no indication of fetal toxicity was observed (Kavlock *et al*., 1979). By weight, the mixture contained 69% chloroform, 16% bromodichloromethane, 10% chlorodibromomethane, and 4% bromoform. Bromodichloromethane appears to have

greater reproductive toxicity when administered in corn oil than when administered in an aqueous vehicle (Narotsky *et al*., 1997). However, even in a two-generation study, the oral exposure required to produce toxicity in rats is several orders of magnitude greater than human drinking water exposure (Christian *et al*., 2002).

Humans

There have been a series of studies evaluating the potential for trihalomethanes in the drinking water to adversely affect pregnancy outcomes. A study in Sweden evaluating different chlorination methods did not find an effect on delivery outcomes (Källén and Robert, 2000), but the study was not based on the amount of by-products in the drinking water. Other studies have found a weak association between low birth weights and the levels of trihalomethanes in the drinking water (Källén and Robert, 2000; Bove *et al*., 2002; Aggazzotti *et al*., 2004). Other studies have shown increased adverse birth outcomes in municipalities that chlorinate their drinking water compared with those that do not chlorinate (Yang, 2004).

CARCINOGENICITY

Experimental Animals

There have been several rodent studies evaluating the potential carcinogenicity of bromodichloromethane. Bromodichloromethane or chloroform was administered in drinking water to male and female Wistar rats for up to 180 weeks (Tumasonis *et al*., 1985). During the first 72 weeks of the study, bromodichloromethane was administered at concentrations of 0 or 1.2 mL (2.4 g) per liter of drinking water; at week 72, the concentration was halved because of a gradual increase in water intake. The dose of bromodichloromethane was estimated at 150 mg/kg per day in female rats and 200 mg/kg per day in male rats. The liver and grossly observable lesions were examined. An increased incidence of hepatic neoplastic nodules was found in females (but not in males) when bromodichloromethane was administered throughout the lifespan [males: control, 5/22 (23%); dosed, 6/47 (13%); females: control, 0/18; dosed 17/53 (32%)].

NTP studies showed increased kidney cancer in F344/N rats and male B6C3F_1 mice plus increased incidences of liver cancer in female mice following bromodichloromethane exposure by oral gavage in corn oil (NTP, 1987). However, most attention was on the increased incidences of adenocarcinoma of the large intestine in male and female rats following oral gavage exposure to bromodichloromethane (males: vehicle control, 0/50; 50 mg/kg, 11/50; 100 mg/kg, 38/50; females: 0/46, 0/50, 6/47) because colon and rectal neoplasms have been associated with trihalomethane exposure in drinking water in humans (Gottlieb and Carr 1982; NTP, 1987; King *et al*., 2000). In another study, bromodichloromethane in the drinking water did not cause colon cancer in male rats or male mice (George *et al*., 2002). Exposure-related cancers were not found at any site in the male mice, but the authors report a marginal increase in benign liver cancer but only at the lowest dose. The NTP (2006) conducted toxicology and carcinogenesis studies of bromodichloromethane at concentrations of 0, 175, 350, or 700 mg/L in drinking water for 2 years in male F344/N rats and female B6C3F_1 mice. There was no evidence of increased incidences of neoplasms in the exposed rats or mice compared to the control groups.

Male and female Long-Evans rats from a mutant Tsc2 (Eker rats) exposed to 70 and 700 mg/L of bromodichloromethane in the drinking water failed to show an increase in hyperplasia in either the urinary bladder (another potential human cancer associated with chlorinated water) or colon epithelium (McDorman *et al*., 2003). However, these rats did show a nonstatistical increase in aberrant crypt foci in the colon. Bromodichloromethane both in the drinking water and by oral gavage in corn oil produced a marginal increase in aberrant crypt foci that was significant only for the drinking water group (Geter *et al*., 2004).

Bromodichloromethane was administered as a microencapsulated preparation in feed to Wistar rats at concentrations of 0, 140, 550, or 2,200 ppm (w/w) bromodichloromethane for 24 months (Aida *et al*., 1992). Histologic findings included cholangiofibrosis and/or fibrosis in the liver of males and females in the 2,200 ppm group at 6, 12, 18, and 24 months. Tumors of the liver were not increased in dosed rats.

Humans

Exposure to chlorinated drinking water and trihalomethanes has been associated with increased rates of various cancers in humans. Several studies have noted an increase in colon or rectal cancers with exposure to chlorination by-products (Young *et al*., 1987; Hildesheim *et al*., 1998). Modeling human exposure to drinking water disinfection by-products is difficult at

best, and the epidemiology results are often not consistent across sexes (usually only males) or cancer sites (increase in rectal cancer and not colon cancer in one study, increase in colon but not rectal cancer in a second study), while other studies fail to show a cancer effect (Young *et al*., 1987).

More human studies have evaluated the effect of chlorinated water on bladder cancer. Some studies have shown an association (Villanueva *et al*., 2003; Chevrier *et al*., 2004); however, reconstructing accurate and specific disinfection by-product exposure histories over long periods of time is difficult. Generally the studies suggest an association between increasing rates of bladder cancer and increasing chlorinated water exposure (McGeehin *et al*., 1993) but no association between total trihalomethane exposure and bladder cancer risk.

Genetic Toxicity

The mutagenicity data for bromodichloromethane were reviewed by IARC (1999b). The data from a large number of tests show mixed results that may, in some cases, be directly related to inadequate exposures to this volatile chemical. A summary of the most significant observations follows.

Bromodichloromethane was mutagenic in *Salmonella typhimurium* strains sensitive to base substitution mutations, such as TA100 and TA1535, when tested with protocols that controlled for volatility (Simmon *et al.,* 1977; Pegram *et al.,* 1997; DeMarini *et al.,* 1997). Bromodichloromethane was not mutagenic at the tk locus in mouse lymphoma L5178Y cells treated without exogenous liver activation enzymes (S9), but with induced rat liver S9, a highly significant dose-related induction of mutant colonies was seen (McGregor *et al.,* 1988).

Bromodichloromethane (maximum dose, 1 mmol/kg) induced chromosome aberrations in bone marrow cells of Long-Evans rats 12 hours after a single intraperitoneal injection, but not after 5 days of oral administration (Fujie *et al.,* 1990). Male ICR/SJ mice treated by oral gavage once daily for 4 days with doses of 25, 50, or 100 mg/kg bromodichloromethane showed elevated frequencies of sister chromatid exchanges in bone marrow samples (Fujie *et al.,* 1993). Bromodichloromethane did not induce micronuclei in bone marrow cells of male ddy mice given one or four daily intraperitoneal injections of up to 500 mg/kg or 200 mg/kg for single or multiple injections, respectively (Hayashi *et al.,* 1988). However, bromodichloromethane did induce a small but significant increase in micronucleated erythrocytes in peripheral blood of C57BL/6 and FVB/N p53 heterozygous mice exposed by inhalation to 15 ppm for 13 weeks (Torti *et al.,* 2002). *In vitro*, bromodichloromethane induced chromosomal aberrations in cultured Chinese hamster lung fibroblasts incubated for 48 hours, in tightly capped flasks, in the presence or absence of rat liver S9 (Matsuoka *et al.,* 1996); tests with bromodichloromethane for chromosomal aberration induction in Chinese hamster ovary cells incubated with loose caps were negative (Anderson *et al.,* 1990).

Bromodichloromethane administered by gavage did not induce unscheduled DNA synthesis, a measure of DNA damage, in the livers of Sprague-Dawley rats after single doses of 135 or 450 mg/kg (Stocker *et al.,* 1997). Furthermore, no induction of DNA strand breaks was noted in kidney cells of F344 rats after 7 days of exposure to 0.75 or 1.5 mmol/kg (Potter *et al.,* 1996). *In vitro*, bromodichloromethane induced a dose-related increase in DNA damage in *Escherichia coli* in the absence of exogenous metabolic activation; the response was enhanced markedly in experiments conducted with rat liver S9 (Le Curieux *et al.,* 1995). Bromodichloromethane was more potent than other trihalomethanes and methylene chloride at inducing DNA strand breaks in cultured human lung epithelial cells (Landi *et al.,* 2003).

Background on Genetically Altered Mice

Mutation and/or deletions of tumor suppressor genes or activation of protooncogenes can disrupt cell function and predispose an animal to cancer. In the current studies, two genetically altered mouse models with either a loss of heterozygosity in a critical cancer gene (*Trp53*) or a gain of oncogene function (Ha-*ras*) were used to determine how these animals would respond to bromodichloromethane exposure. The Tg.AC hemizygous and p53 haploinsufficient mice have been shown to be susceptible to the rapid development of cancer and are being evaluated by the National Institute of Environmental Health Sciences (NIEHS) and the NTP

as models for identifying chemical toxicity and/or chemical carcinogenic processes (Tennant *et al.,* 1996; Pritchard *et al.,* 2003).

FVB/N-TgN (v-Ha-ras)Led (Tg.AC) Hemizygous Mouse Model

The Tg.AC hemizygous transgenic mouse model has been evaluated as a reporter phenotype (skin papillomas) in response to either genotoxic or nongenotoxic carcinogens, including tumor promoters (Spalding *et al.,* 1993, 1999; Tennant *et al.,* 1999). The Tg.AC strain of mice are hemizygous for a mutant v-Ha-*ras* transgene. The model was developed by Leder *et al.* (1990) with an inducible zeta-globin promoter driving the expression of a mutated (point mutation in codons 12 and 59) v-Ha-*ras* oncogene and is regarded as a genetically initiated model. With the exception of bone marrow, constitutive expression of the transgene cannot be detected in adult tissues. The transgene is usually transcriptionally silent until activated by certain treatments including full-thickness wounding, ultraviolet irradiation, or exposure to some chemicals (Cannon *et al.,* 1997; Trempus *et al.,* 1998). Point mutations in the Ha-*ras* gene are believed to be early events in the induction of skin papillomas and malignancies. Topical application of carcinogens to the shaved dorsal surface of Tg.AC hemizygous mice induces epidermal squamous cell papillomas or carcinomas, a reporter phenotype that defines the activity of the chemical. 12-*O*-tetradecanoylphorbol-13-acetate (TPA) has been used as a positive control in NIEHS Tg.AC mouse studies to confirm the mice are responsive to carcinogens because it has been found that a subset of Tg.AC mice may revert and become nonresponsive to a tumor promoter (Honchel *et al.,* 2001). The oral route of administration can also generate tumor responses in the skin of Tg.AC hemizygous mice and lead to squamous cell papillomas and/or carcinomas of the forestomach. To date, the appearance of either spontaneous or induced tumors has been shown to involve transgene expression. However, the mechanism of response by the Tg.AC hemizygous mouse model to chemical carcinogens is not yet understood.

In NIEHS studies, mice are exposed beginning at 2 months of age for a total of 6 to 9 months. Cutaneous papillomas at various sites have been reported at 3.7% and 3.8% incidence in 33-week-old control male and female Tg.AC hemizygous mice, respectively (Mahler *et al.,*1998). Cutaneous papillomas occurring at sites such as the lip, pinnae, prepuce, and vulva suggest a possible relationship to grooming and chronic irritation. Up to 32% of Tg.AC homozygous and heterozygous male or female mice can develop odontogenic tumors as early as 33 weeks (Wright *et al.,* 1995; Mahler *et al.,* 1998). A number of different tumor types occur in untreated Tg.AC hemizygous mice at an incidence of greater than 3% including odontogenic tumors, forestomach papillomas, cutaneous papillomas, alveolar/bronchiolar adenomas, salivary gland duct carcinomas, and erythroleukemia (Mahler *et al.,*1998). In the FVB mouse (the background strain for the Tg.AC hemizygous mouse), alveolar/bronchiolar neoplasms occur at 14 months of age (Mahler *et al.,* 1996).

The Tg.AC hemizygous mouse model was used in the current Report for the studies of bromodichloromethane because this model has been reported to detect both nongenotoxic and genotoxic carcinogens (Spalding *et al.,* 1993; Tennant *et al.,* 1995*,* 1996; Pritchard, 2003). Tg.AC mice may spontaneously lose a portion of the zeta-globlin promoter sequence making them unresponsive to TPA-initated tumors (Thompson *et al.,* 1998). Therefore, TPA positive controls were included for Tg.AC studies.

B6.129-Trp53^{tm1Brd} (N5) Mouse Model

The heterozygous B6.129-*Trp53* (N12)$^{tmlBrd(+/-)}$ mouse (on a B6.129S7 background) was developed by Donehower *et al.* (1992). A null mutation was introduced into one p53 allele by homologous recombination in murine embryonic stem cells. Insertion of a neo cassette resulted in deletion of a 450-base pair gene fragment containing 106 nucleotides of exon 5 and approximately 350 nucleotides of intron 4.

Trp53, a nuclear protein, plays an essential role in the regulation of the cell cycle, specifically in the transition from G_0 to G_1, as well as G_2 to M, and the spindle apparatus. The p53 protein is labile and exists at very low concentrations in normal cells; mutants of p53 are expressed in high amount in DNA damaged cells or a variety of transformed cell lines and are believed to contribute to transformation and malignancy. The p53 protein is a DNA-binding protein containing DNA-binding, oligomerization, and transcription activation domains. Many amino acid residues may be phosphorylated or acetylated, which may determine p53 function. It is postulated to bind as a tetramer to a p53-binding site and activate expression of downstream genes that inhibit growth and/or invasion or promote apoptosis, functioning as a tumor suppressor. This protein is critical to

tumor suppression in humans and rodents. Mutants of p53 that fail to bind the consensus DNA binding site frequently occur in human cancers, and are unable to function as tumor suppressors. Alterations of the *Trp53* gene occur not only as somatic mutations in human malignancies, but also as germline mutations in some cancer-prone families with Li-Fraumeni syndrome.

The mouse heterozygous for a p53 null allele (+/–) has only a single functional wild-type p53 allele which provides a target for mutagens. The p53 tumor suppressor gene is one of the most common sites for mutations and gene alterations in human cancer (Harris, 1996a,b,c).

Heterozygous $p53^{(+/-)}$ mice develop normally and like humans and other mammals, develop cancer (primarily lymphomas or sarcomas) with age, but often with decreased latency.

Study Rationale

The purpose of this study was twofold. Given the hundreds of potentially hazardous disinfection by-products (Bull *et al.,* 1995), usually at very low concentrations and occurring as mixtures, a more efficient process for determining safety of chemicals and chemical mixtures found in finished drinking water is needed (Boorman *et al.,* 1999). One objective of this study was to help determine whether the use of genetically modified mice could reduce study length and increase sensitivity for determining potential hazards of drinking water disinfection by-products compared to traditional rodent bioassays. This study was one of three studies to investigate the Tg.AC hemizygous and p53 haploinsufficient mouse models using disinfection by-products that had been extensively studied using traditional rodent models. The results of dichloroacetic acid (NTP, 2007a) and sodium bromate (NTP, 2007b) are being reported separately. The second objective was to determine whether the use of genetically modified mice could provide more insight on the apparent discrepancy in carcinogenicity results between studies where bromodichloromethane is given in the drinking water and where it is given by oral gavage.

This Report focuses on Tg.AC hemizygous and p53 haploinsufficient strains that were exposed to bromodichloromethane in the drinking water and also in corn oil by gavage for up to 42 weeks. The Tg.AC hemizygous mouse strain was also exposed to bromodichloromethane by the dermal route, which if predictive, could prove to be the most efficient screening procedure for drinking water mixtures.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Bromodichloromethane

A single lot of bromodichloromethane (14522LS) was obtained from Aldrich Chemical Co. (Milwaukee, WI) for use in the 26-, 39-, 41-, and 42-week studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Memorial Institute (Columbus, OH), and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the bromodichloromethane studies are on file at the National Institute of Environmental Health Sciences.

Lot 14522LS, a clear, colorless liquid, was identified as bromodichloromethane using infrared (IR) spectroscopy. The purity of lot 14522LS was determined using gas chromatography (GC). GC by one system indicated one major peak and three impurity peaks with a combined peak area of 1.9% relative to the major peak area. GC by a second system indicated a purity of 98.4% relative to a frozen reference standard of the same lot. The overall purity of lot 14522LS was determined to be 98% or greater.

Stability studies of another lot of bulk chemical (02107TG) were performed using GC. These studies indicated that bromodichloromethane was stable as a bulk chemical for at least 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at or below –20° C, protected from light, in heat-sealed glass ampules with potassium carbonate stabilizer. Stability of lot 14522LS was monitored during the studies using GC. No degradation of the bulk chemical was detected.

12-*O*-Tetradecanoylphorbol-13-acetate (TPA)

12-*O*-tetradecanoylphorbol-13-acetate was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO) in one lot (48H1178) that was used in the 26-week studies in Tg.AC hemizygous mice. Identity and purity analyses were performed by Research Triangle Institute (Research Triangle Park, NC).

Lot 48H1178, a white crystalline powder, was identified as 12-*O*-tetradecanoylphorbol-13-acetate using IR and proton nuclear magnetic resonance (NMR) spectrometry. All spectra were consistent with the structure of 12-*O*-tetradecanoylphorbol-13-acetate. The purity of lot 48H1178 was determined using high performance liquid chromatography. This analysis indicated one major peak and one impurity peak with an area equal to approximately 0.11% of the total integrated peak area. The overall purity of lot 48H1178 was determined to be greater than 99%.

Acetone

USP-grade acetone was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in three lots (NV0163, OG0513, OX0312) that were used during the 26- and 39-week dermal studies. Identity and purity analyses were performed by the study laboratory.

The identity of each lot was determined by IR spectroscopy. The purity of all lots was determined using GC. These analyses did not indicate any impurities with relative peak areas greater than 0.1% of the major peak. The overall purity of all lots used was determined to be greater than 99%. No degradation of the acetone was detected.

Corn Oil

USP-grade corn oil was obtained from Spectrum Chemicals and Laboratory Products in six lots (OT0213, OU0101, OV0137, OH0409, PN0012, PO0173) that were used during the 26- and 41-week gavage studies. The study laboratory analyzed peroxide levels in bulk corn oil; potentiometric titration demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

Preparation and Analysis of Dose Formulations

Dermal Studies

The dose formulations were prepared approximately every 4 weeks by mixing bromodichloromethane with USP-grade acetone to give the required concentration (Table I2). The dose formulations were stored at room temperature in amber glass bottles with Teflon®-lined lids for up to 39 days. A positive control dose formulation of TPA was prepared twice during the studies by adding the appropriate amount of TPA to acetone; the formulations were stored at approximately 5° C in amber glass bottles for up to 6 months.

Stability studies of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 µg/mL dose formulations were performed by the study laboratory using GC. Stability was confirmed for dose formulations stored in amber glass bottles with Teflon®-lined lids for up to 39 days at room temperature.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC. During the 26- and 39-week studies, dose formulations were analyzed four times (Table I3). All 12 dose formulations for Tg.AC hemizygous mice were within 10% of the target concentration. Animal room samples of these dose formulations were also analyzed; all nine animal room samples were within 10% of the target concentration.

Drinking Water Studies

The dose formulations were prepared every 1 to 3 weeks by mixing bromodichloromethane with tap water (Table I2). Formulations were stored in glass bottles with Teflon®-lined lids at 5° C for up to 35 days. Positive control dose formulations of TPA were prepared and stored as described for the dermal studies.

Stability studies of 0.75, 0.9, 0.8, 1.0, 1.05, and 1.2 µg/mL dose formulations were performed by the study laboratory using GC. Stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles at 5° C.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC. During the 26- and 42-week studies, dose formulations were analyzed four times. All 12 dose formulations for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target concentration (Table I4). Animal room samples of these dose formulations were also analyzed; three of nine Tg.AC hemizygous mouse animal room samples and none of the nine p53 haploinsufficient mouse animal room samples were within 10% of the target concentration. These low results were attributed to the volatility and hydrophobic nature of bromodichloromethane.

Gavage Studies

The dose formulations were prepared approximately every 4 weeks by mixing bromodichloromethane with USP-grade corn oil to give the required concentrations (Table I2). Dose formulations were stored in amber glass bottles with Teflon®-lined lids and refrigerated for up to 35 days. The positive control dose formulations of TPA were prepared and stored as described for the dermal studies.

Stability studies of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 µg/mL dose formulations were performed by the study laboratory using GC. Stability was confirmed for at least 21 days for dose formulations stored in glass bottles protected from light at room temperature.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC. During the 26- and 41-week studies, dose formulations were analyzed five times. All 12 dose formulations used in the studies for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target concentration (Table I5). Animal room samples of these dose formulations were also analyzed; eight of nine animal room samples were within 10% of the target concentration.

Study Designs

Dermal Studies

Groups of 15 male and 15 female Tg.AC hemizygous mice were administered 0, 64, 128, or 256 mg bromodichloromethane/kg body weight in 3.3 mL acetone/kg body weight 5 days per week for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous mice were administered the same doses for 39 weeks. Vehicle control mice were administered acetone only. Doses were applied to the clipped dorsal skin from the mid-back to the interscapular area.

Drinking Water Studies

Groups of 15 male and 15 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to 0, 175, 350, or 700 mg bromodichloromethane/L drinking water for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous and p53 haploinsufficient mice were exposed to the same concentrations for 42 weeks.

Gavage Studies

Groups of 15 male and 15 female Tg.AC hemizygous and p53 haploinsufficient mice were administered 0, 25, 50, or 100 mg bromodichloromethane/kg body weight in corn oil by gavage, 5 days per week for 26 weeks. Groups of 10 male and 10 female Tg.AC hemizygous and p53 haploinsufficient mice were administered the same doses for 41 weeks. Vehicle control mice were administered corn oil only.

Positive Control Mice

For each route of administration, positive control groups of 15 male and 15 female Tg.AC hemizygous mice were administered 1.25 µg TPA in 100 µL acetone (12.5 µg TPA/L solution), three times per week for 26 weeks. The positive TPA controls are included because it has been shown that a subset of Tg.AC mice may revert and become non-responsive to tumor promoters (Honchel *et al.*, 2001). The TPA solution was applied to the clipped dorsal skin from the mid-back to the interscapular area. Positive control mice were removed from study after the appearance of 20 or more skin papillomas and discarded.

Source and Specification of Animals

Male and female FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous and B6.129-*Trp53*^{tm1Brd}(N5) haploinsufficient mice were obtained from Taconic Laboratory Animals and Services (Germantown, NY) for use in the 26-, 39-, 41-, and 42-week studies. Mice were quarantined for 11 to 14 days before the beginning of the studies. Five male and five female mice per strain were randomly selected for parasite evaluation and gross observation of disease. Mice were approximately 6 weeks old at the beginning of the studies. Blood samples were collected from five male and five female sentinel mice from each study at 4 and 26 weeks, from five male and five female mice from the highest-surviving groups from each study at study termination, and from moribund mice from the dermal and drinking water studies after June 5, 2000. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.,* 1986; Rao *et al.,* 1989a,b). All results were negative.

Animal Maintenance

Mice were housed individually. Feed and water were available *ad libitum*. Water consumption was measured weekly by cage (drinking water studies only). Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings and body weights were recorded initially, weekly, and at the end of the studies. Clinical findings for dermal and gavage study mice were recorded postdosing.

In-life observations of papilloma formation on the skin were recorded weekly using the Toxicology Data Management System (TDMS). A papilloma was initially recorded as a mass. The observation "papilloma" was not entered into TDMS for a given animal until the first-observed mass was documented for 3 consecutive weeks. At the third observation, a mass (wart-like in appearance) was entered as a papilloma. Any new mass(es) appearing after the 3-week confirmation period for a given animal at a different site was entered into TDMS first as a mass until the third week, when it was entered as a papilloma. In a few instances, a papilloma that had been previously observed was missing, and therefore not recorded. Reappearance of a mass at a later time was entered into TDMS as a mass until the third observation week, when it was called a papilloma.

At the end of the 26-week studies, blood for hematology analysis was collected from the retroorbital sinus of all mice (except positive controls) under carbon dioxide anesthesia. Samples for hematology analysis were placed in microcollection tubes (Sarstedt, Inc., Nümbrecht, Germany) coated with potassium EDTA. Hematocrit; erythrocyte, platelet, and leukocyte counts; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with a Cell-Dyn® hematology analyzer (Abbott Diagnostics, Santa Clara, CA). Hemoglobin concentrations were determined photometrically using a cyanmethemoglobin procedure. Differential leukocyte counts were determined microscopically from blood smears stained with a modified Wright-Giemsa stain. A Miller Disc was used to determine reticulocyte counts from smears prepared with blood stained with new methylene blue. Mean cell

volumes were determined from average red blood cell impedance pulse heights. The parameters measured are listed in Table 1.

Necropsies and microscopic examinations were performed on all mice except positive controls. The heart, right kidney, liver, lung, right testis, and thymus were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. The quality assessment pathologist examined all tumors and all slides from potential target organs, which included the kidney and liver of Tg.AC hemizygous and p53 haploinsufficient mice in the 39-week dermal, 42-week drinking water, and 41-week gavage studies. The forestomach of Tg.AC hemizygous mice in the 41-week gavage study was also examined.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell *et al.* (1986).

The 26-week studies had not undergone a quality assessment review prior to completion of the pathology review for the 39-, 41-, and 42-week studies. For the 26-week studies, a quality assessment pathologist evaluated all tumor diagnoses from all animals and all potential target organs (both genders, both strains, all routes of administration), which included the liver, kidney, forestomach, and skin, using terminology and diagnostic criteria defined by the Pathology Working Group for the 39-, 41-, and 42-week studies in order to maintain diagnostic consistency between the studies. The quality assessment pathologist and two NTP pathologists met to review selected examples of lesions related to chemical administration and to address any disagreements in the diagnoses made by the laboratory and quality assessment pathologists. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, the quality assessment pathologist, and the NTP pathologists.

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

Dermal Studies	Drinking Water Studies	Gavage Studies
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)
Strain and Species		
FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mice	FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mice and B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice	FVB/N-TgN(v-Ha-*ras*)Led (Tg.AC) hemizygous mice and B6.129-*Trp53*^{tm1Brd} (N5) haploinsufficient mice
Animal Source		
Taconic Laboratory Animals and Services, (Germantown, NY)	Taconic Laboratory Animals and Services, (Germantown, NY)	Taconic Laboratory Animals and Services, (Germantown, NY)
Time Held Before Studies		
14 days	Tg.AC mice: 11 days p53 mice: 12 days	Tg.AC mice: 13 days p53 mice: 14 days
Average Age When Studies Began		
6 weeks	Tg.AC mice: 5 weeks p53 mice: 6 (males) or 7 (females) weeks	Tg.AC mice: 5 weeks p53 mice: 6 (males) or 7 (females) weeks
Date of First Dose or Exposure		
August 19, 1999	Tg.AC mice: August 30, 1999 p53 mice: August 31, 1999	Tg.AC mice: September 15, 1999 p53 mice: September 16, 1999
Duration of Dosing or Exposure		
26 or 39 weeks	26 or 42 weeks	26 or 41 weeks
Date of Last Dose or Exposure		
26-Week Studies February 16, 2000 (males) February 17, 2000 (females)	*26-Week Studies* Tg.AC mice: February 28, 2000 (males) February 29, 2000 (females) p53 mice: March 1, 2000 (males) March 2, 2000 (females)	*26-Week Studies* Tg.AC mice: March 13, 2000 (males) March 14, 2000 (females) p53 mice: March 15, 2000 (males) March 16, 2000 (females)
39-Week Studies May 17, 2000	*42-Week Studies* Tg.AC mice: June 20, 2000 (males and females) p53 mice: June 21, 2000 (males) June 22, 2000 (females)	*41-Week Studies* Tg.AC mice: June 27, 2000 (males and females) p53 mice: June 28, 2000 (males) June 29, 2000 (females)

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

Dermal Studies	Drinking Water Studies	Gavage Studies
Necropsy Dates		
26-Week Studies February 17, 2000 (males) February 18, 2000 (females)	*26-Week Studies* Tg.AC mice: February 28, 2000 (males) February 29, 2000 (females) p53 mice: March 1, 2000 (males) March 2, 2000 (females)	*26-Week Studies* Tg.AC mice: March 14, 2000 (males) March 15, 2000 (females) p53 mice: March 16, 2000 (males) March 17, 2000 (females)
39-Week Studies May 18, 2000	*42-Week Studies* Tg.AC mice: June 20, 2000 p53 mice: June 21, 2000 (males) June 22, 2000 (females)	*41-Week Studies* Tg.AC mice: June 28, 2000 p53 mice: June 29, 2000 (males) June 30, 2000 (females)
Average Age at Necropsy		
26-Week Studies 32 weeks	*26-Week Studies* Tg.AC mice: 31 weeks (males) 32 weeks (females) p53 mice: 32 weeks (males) 33 weeks (females)	*26-Week Studies* Tg.AC mice: 31 weeks (males) 32 weeks (females) p53 mice: 33 weeks
39-Week Studies 45 weeks	*42-Week Studies* Tg.AC mice: 48 weeks p53 mice: 48 weeks (males) 49 weeks (females)	*41-Week Studies* Tg.AC mice: 46 weeks p53 mice: 48 weeks
Size of Study Groups		
26-Week Studies 15 males and 15 females *39-Week Studies* 10 males and 10 females	*26-Week Studies* 15 males and 15 females *42-Week Studies* 10 males and 10 females	*26-Week Studies* 15 males and 15 females *41-Week Studies* 10 males and 10 females
Method of Distribution		
Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as dermal studies	Same as dermal studies
Animals per Cage		
1	1	1
Method of Animal Identification		
Tail tattoo	Same as dermal studies	Same as dermal studies
Diet		
Irradiated NTP-2000 open formula pelleted diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*, changed weekly	Same as dermal studies	Same as dermal studies

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

Dermal Studies	Drinking Water Studies	Gavage Studies
Water		
Tap water (Columbus, OH municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI), available *ad libitum*	Tap water (Columbus, OH, municipal supply) via amber glass bottles (Supelco, Bellefonte, PA) with stainless steel double ball bearing sipper tubes (Ancare, Bellmore, NY) and Teflon®-coated septa, available *ad libitum*, changed twice weekly	Same as dermal studies
Cages		
Polycarbonate (Lab Products Corp., Maywood, NJ), changed once weekly	Same as dermal studies	Same as dermal studies
Bedding		
Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Maywood, NJ), changed weekly	Same as dermal studies	Same as dermal studies
Rack Filters		
Dupont spun-bonded polyester (Snow Filtration Co., Cincinnati, OH), changed once every 2 weeks	Same as dermal studies	Same as dermal studies
Racks		
Stainless steel racks (Lab Products Corp., Maywood, NJ), changed once every 2 weeks	Same as dermal studies	Same as dermal studies
Animal Room Environment		
Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Doses or Exposure Concentrations		
0, 64, 128, or 256 mg/kg bromodichloromethane dermally in acetone at a volume of 3.3 mL/kg body weight for 5 days per week or 1.25 µg TPA three times/week	0, 175, 350, or 700 mg/L bromodichloromethane per day or 1.25 µg TPA applied dermally three times/week	0, 25, 50, or 100 mg/kg bromodichloromethane in corn oil by gavage in a volume of 10 mL/kg body weight 5 days per week or 1.25 µg TPA applied dermally three times/week
Type and Frequency of Observation		
Observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Clinical findings were recorded after dosing.	Observed twice daily; animals were weighed and clinical findings were recorded initially, weekly, and at the end of the studies. Water consumption was recorded weekly.	Same as dermal studies

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

Dermal Studies	Drinking Water Studies	Gavage Studies
Method of Sacrifice CO_2 asphyxiation	Same as dermal studies	Same as dermal studies
Necropsy Necropsies were performed on all animals (except positive controls). Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Same as dermal studies	Same as dermal studies
Clinical Pathology Blood was collected from the retroorbital sinus of all mice (except positive controls) at the end of the 26-week study for hematology. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; erythrocyte and platelet morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials	Same as dermal studies	Same as dermal studies
Histopathology Histopathology was performed on all animals (except positive controls). In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, large intestine (colon and cecum), small intestine (duodenum, ileum, jejunum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, ovary, pituitary gland, skin, spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.	Histopathology was performed on all animals (except positive controls). In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, large intestine (colon and cecum), small intestine (duodenum, ileum, jejunum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), ovary, pituitary gland, spleen, stomach (forestomach), testis with epididymis, thymus, thyroid gland, and uterus.	Same as drinking water studies

Statistical Methods

Survival Analyses

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

Calculation and Analysis of Lesion Incidences

The incidences of lesions are presented in Appendixes A, B, C, D, and E as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. The Fisher exact test (Gart *et al.,* 1979), a procedure based on the overall proportion of affected animals, was used to determine significance.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed or 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 data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance with the Mann-Whitney U test (Hollander and Wolfe, 1973).

Quality Assurance Methods

The 26-, 39-, 41-, and 42-week studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the these studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP 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 Report.

Genetic Toxicology

Mouse Peripheral Blood Micronucleus Test Protocol

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the termination of the 26-week studies, peripheral blood samples were obtained from male and female Tg.AC hemizygous and p53 haploinsufficient mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromatic erythrocytes (NCEs) in each of up to 15 mice per dose or 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 dose or exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed or 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 dosed or exposed group is less than or equal to 0.025 divided by the number of dosed or exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials. Ultimately, the scientific staff determines the final call after considering the results of statistical analyses, reproducibility of any effects observed, and the magnitudes of those effects. Because these studies were not repeated, the results of the single micronucleus trials in these mice were accepted without replication.

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 tables in the Abstract of this Report present a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

RESULTS

26-Week Dermal Study in Tg.AC Hemizygous Mice

Dose Selection Rationale

The bromodichloromethane doses administered in this 26-week dermal study (64, 128, and 256 mg/kg) were the three highest doses administered in a 2-week NTP range-finding study in FVB/N mice. In the range-finding study, these doses were not found to produce serious survival or toxicity effects and thus would be appropriate for longer term studies. Because 256 mg/kg was more than twice the gavage dose and expected drinking water dose, this was considered a reasonable high dose for the dermal study.

Positive Control Tg.AC Hemizygous Mice

12-*O*-Tetradecanoylphorbol-13-acetate (TPA) (1.25 µg) was dermally administered to groups of 15 males and 15 females three times weekly. Eighty-seven percent of males and all females developed more than 20 skin papillomas each by week 24 (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.,* 2001).

Survival

Estimates of 26-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 2. The survival of dosed males and females was similar to that of the vehicle controls.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of dosed groups of males and females were similar to those of the vehicle controls (Figure 1 and Tables 3 and 4). There were no clinical findings related to bromodichloromethane administration. Organ weights of treated males and females were similar to those of the vehicle controls (Table H1).

Hematology

The hematology data for Tg.AC hemizygous mice in the 26-week dermal study of bromodichloromethane are listed in Table G1. A very minimal (2%) decrease in mean cell hemoglobin concentration was identified in 256 mg/kg female mice; the value was not below what would be considered an acceptable reference limit and was not considered clinically or toxicologically relevant. No changes occurred in other variables.

Pathology and Statistical Analyses

There were no statistically or biologically significant increases in the incidences of neoplasms or nonneoplastic lesions in Tg.AC hemizygous mice dermally administered bromodichloromethane for 26 weeks. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables A1, A2, A3, and A4.

TABLE 2
Survival of Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Male				
Animals initially in study	15	15	15	15
Moribund	2	1	0	0
Natural deaths	0	0	0	2
Animals surviving to study termination	13	14	15	13
Percent probability of survival at end of study[a]	87	93	100	87
Mean survival (days)[b]	176	180	183	177
Survival analysis[c]	P=1.000	P=0.984N	P=0.464N	P=1.000N
Female				
Animals initially in study	15	15	15	15
Moribund	2	3	1	4
Natural deaths	2	2	2	1
Animals surviving to study termination	11	10	12	10
Percent probability of survival at end of study	73	67	80	67
Mean survival (days)	165	167	172	164
Survival analysis	P=0.966	P=1.000	P=0.945N	P=1.000

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

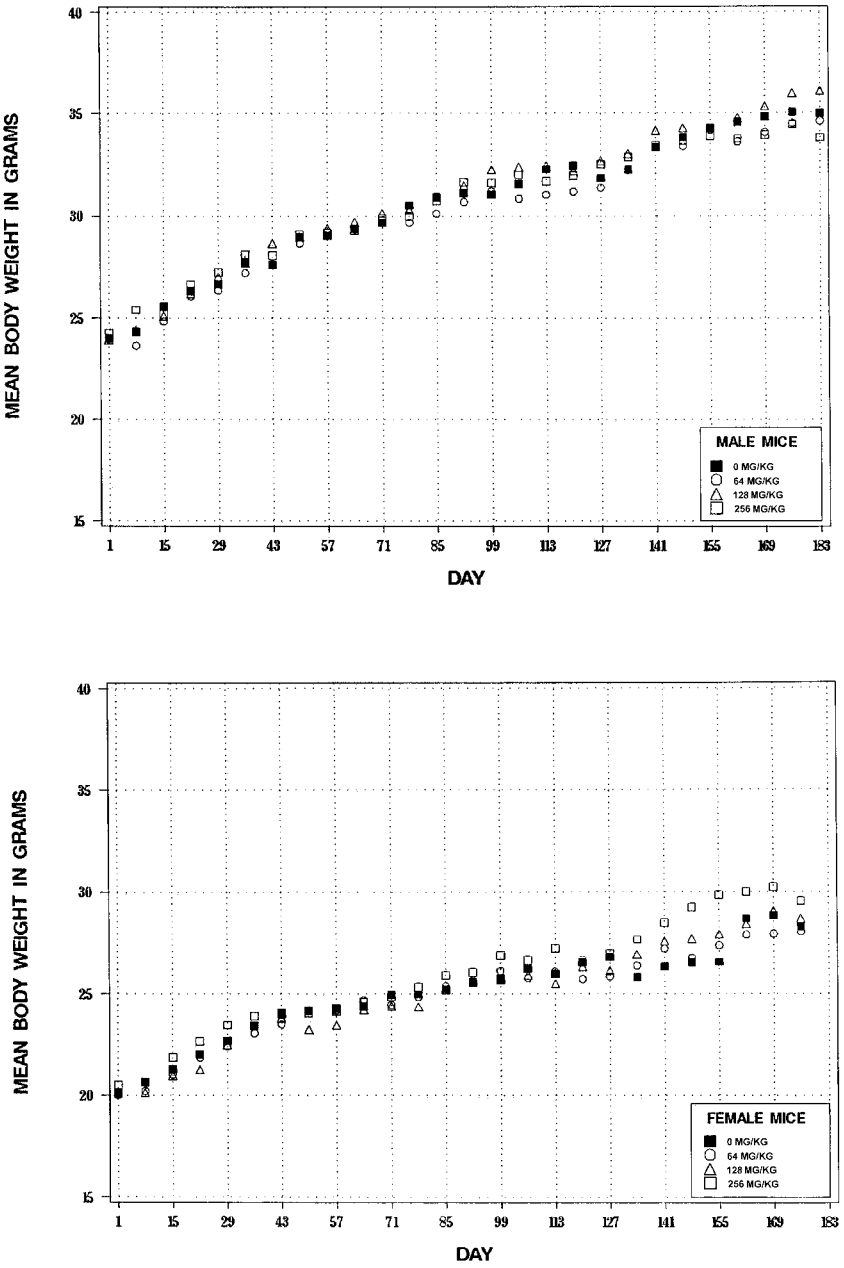


FIGURE 1
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Bromodichloromethane Dermally for 26 Weeks

TABLE 3
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

Weeks on Study	Vehicle Control		64 mg/kg			128 mg/kg			256 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.0	15	23.9	100	15	23.9	100	15	24.3	101	15
2	24.3	15	23.6	97	15	24.4	100	15	25.4	105	15
3	25.5	15	24.8	97	15	25.1	98	15	25.2	99	15
4	26.3	15	26.0	99	15	26.2	100	15	26.6	101	15
5	26.7	15	26.3	99	15	27.0	101	15	27.2	102	15
6	27.7	15	27.2	98	15	27.8	100	15	28.1	101	15
7	27.6	15	27.7	100	15	28.6	104	15	28.1	102	15
8	29.0	15	28.6	99	15	29.0	100	15	29.1	100	15
9	29.0	15	29.0	100	15	29.4	101	15	29.1	100	15
10	29.4	15	29.3	100	15	29.7	101	15	29.3	100	15
11	29.7	15	29.7	100	15	30.1	101	15	29.9	101	15
12	30.5	15	29.7	97	15	30.3	99	15	30.0	98	15
13	30.9	15	30.1	97	15	30.9	100	15	30.7	99	15
14	31.1	15	30.7	99	15	31.5	101	15	31.6	102	15
15	31.1	15	31.2	100	15	32.3	104	15	31.6	102	15
16	31.6	15	30.8	98	15	32.4	103	15	32.0	101	15
17	32.3	14	31.0	96	15	32.5	101	15	31.7	98	15
18	32.4	14	31.2	96	15	32.2	99	15	32.0	99	14
19	31.9	14	31.4	98	15	32.7	103	15	32.5	102	14
20	32.3	14	32.2	100	14	33.0	102	15	32.9	102	14
21	33.3	14	33.4	100	14	34.1	102	15	33.4	100	14
22	33.8	13	33.4	99	14	34.3	102	15	33.6	99	14
23	34.3	13	34.2	100	14	34.3	100	15	33.9	99	13
24	34.6	13	33.6	97	14	34.8	101	15	33.7	97	13
25	34.8	13	34.1	98	14	35.3	101	15	33.9	97	13
26	35.1	13	34.5	98	14	36.0	103	15	34.4	98	13
Mean for weeks											
1-13	27.7		27.4	99		27.9	100		27.9	101	
14-26	33.0		32.4	98		33.5	102		32.9	100	

TABLE 4
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

Weeks on Study	Vehicle Control		64 mg/kg			128 mg/kg			256 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.1	15	20.0	100	15	20.2	101	15	20.5	102	15
2	20.6	15	20.2	98	15	20.1	98	15	20.7	101	15
3	21.3	15	21.0	99	15	21.0	99	15	21.9	103	15
4	22.0	15	21.8	99	15	21.3	97	15	22.7	103	15
5	22.7	15	22.4	99	15	22.5	99	15	23.5	104	15
6	23.4	15	23.0	98	15	23.4	100	15	23.9	102	15
7	24.0	15	23.5	98	15	23.8	99	15	24.0	100	15
8	24.2	15	24.2	100	15	23.2	96	15	24.1	100	15
9	24.3	15	24.2	100	15	23.5	97	15	24.1	99	15
10	24.4	15	24.7	101	15	24.2	99	15	24.6	101	15
11	25.0	15	24.4	98	15	24.4	98	15	24.8	99	15
12	25.0	14	24.8	99	15	24.3	97	15	25.3	101	14
13	25.2	14	25.4	101	15	25.2	100	15	25.9	103	14
14	25.6	14	25.7	100	15	25.7	100	15	26.1	102	14
15	25.8	13	26.1	101	14	25.7	100	15	26.9	104	14
16	26.2	13	25.8	99	14	25.9	99	15	26.7	102	13
17	26.0	13	26.1	100	14	25.5	98	14	27.2	105	13
18	26.5	13	25.7	97	13	26.3	99	14	26.6	100	13
19	26.8	13	25.8	96	12	26.1	97	13	26.9	100	13
20	25.8	12	26.4	102	12	26.9	104	12	27.7	107	13
21	26.4	12	27.2	103	12	27.6	105	12	28.5	108	13
22	26.5	12	26.7	101	12	27.7	105	12	29.2	110	11
23	26.6	12	27.4	103	11	27.9	105	12	29.8	112	11
24	28.7	11	27.9	97	11	28.4	99	12	30.0	105	10
25	28.8	11	27.9	97	11	29.1	101	12	30.2	105	10
26	28.3	11	28.0	99	11	28.7	101	12	29.5	104	10
Mean for weeks											
1-13	23.2		23.0	99		22.9	98		23.5	101	
14-26	26.8		26.7	100		27.0	101		28.1	105	

39-Week Dermal Study in Tg.AC Hemizygous Mice

Survival

Estimates of 39-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 5. The survival of dosed males and females was similar to that of the vehicle controls.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of dosed groups of males and females were similar to those of the vehicle controls (Figure 2 and Tables 6 and 7). There were no clinical findings related to bromodichloromethane administration. Absolute heart weights of 128 and 256 mg/kg females and the absolute lung weight of 256 mg/kg females were greater than those of the vehicle controls (Table H2). No differences in organ weights were observed in males.

Pathology and Statistical Analyses

There were no statistically or biologically significant increases in the incidences of neoplasms or nonneoplastic lesions in Tg.AC hemizygous mice dermally administered bromodichloromethane for 39 weeks. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables A5, A6, A7, and A8.

Table 5
Survival of Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Male				
Animals initially in study	10	10	10	10
Moribund	3	0	1	1
Natural deaths	1	2	0	1
Animals surviving to study termination	6	8	9	8
Percent probability of survival at end of study[a]	60	80	90	80
Mean survival (days)[b]	244	243	273	262
Survival analysis[c]	P=0.424N	P=0.730N	P=0.242N	P=0.566N
Female				
Animals initially in study	10	10	10	10
Moribund	4	4	3	4
Natural deaths	1	2	0	1
Animals surviving to study termination	5	4	7	5
Percent probability of survival at end of study	50	40	70	50
Mean survival (days)	199	186	261	204
Survival analysis	P=0.849N	P=0.879	P=0.472N	P=1.000N

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

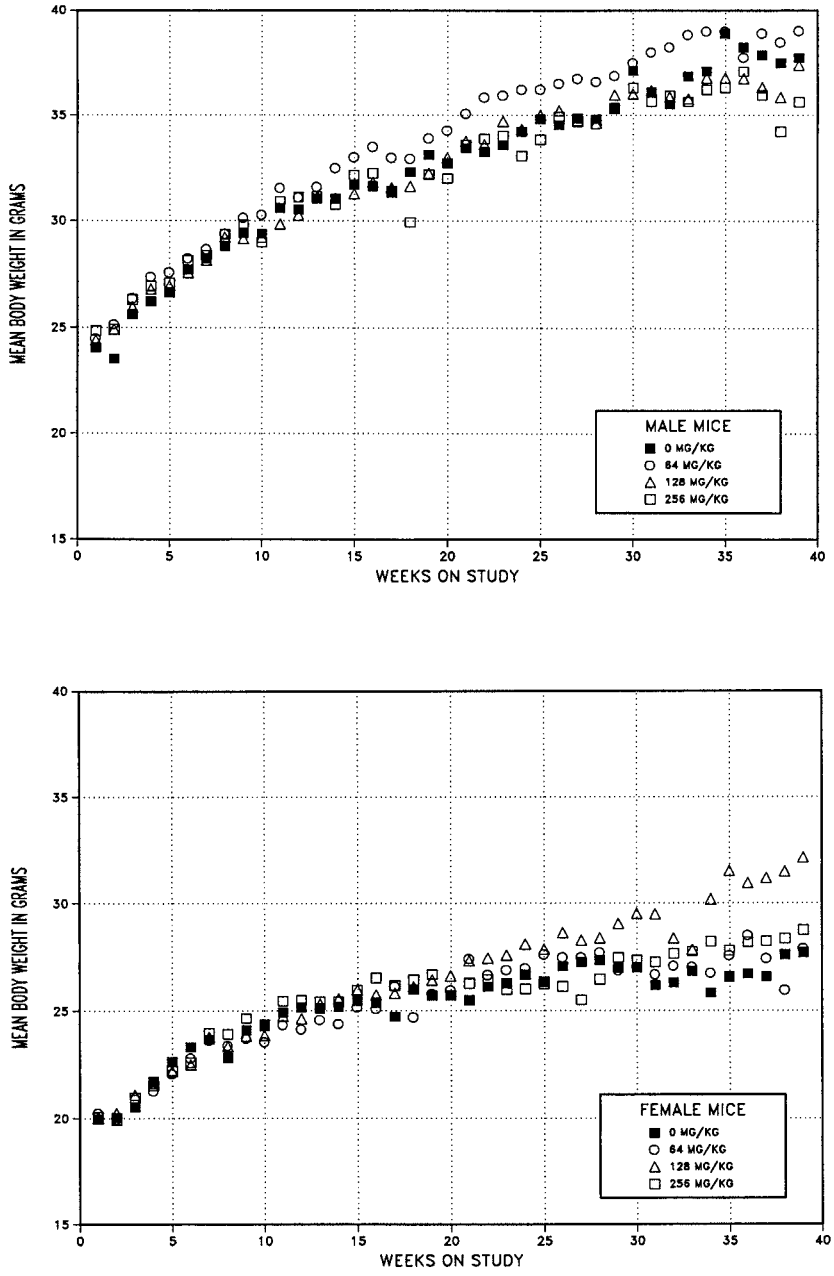


FIGURE 2
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Bromodichloromethane Dermally for 39 Weeks

TABLE 6
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

Weeks on Study	Vehicle Control		64 mg/kg			128 mg/kg			256 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.1	10	24.5	102	10	24.4	101	10	24.9	103	10
2	23.5	10	25.2	107	10	24.9	106	10	24.9	106	10
3	25.6	10	26.4	103	10	26.0	102	10	26.3	103	10
4	26.2	10	27.4	105	10	26.8	102	10	27.0	103	10
5	26.6	10	27.6	104	10	27.0	102	10	27.1	102	10
6	27.7	10	28.2	102	10	27.6	100	10	28.2	102	10
7	28.2	10	28.7	102	10	28.2	100	10	28.4	101	10
8	28.8	10	29.4	102	10	29.2	101	10	29.4	102	10
9	29.4	10	30.2	103	10	29.2	99	10	29.8	101	10
10	29.4	10	30.3	103	10	29.2	99	10	29.0	99	10
11	30.6	10	31.6	103	10	29.9	98	10	30.9	101	10
12	30.5	10	31.1	102	10	30.3	99	10	31.1	102	10
13	31.1	10	31.6	102	10	31.1	100	10	31.2	100	10
14	31.1	10	32.5	105	9	31.1	100	10	30.8	99	10
15	31.7	10	33.0	104	9	31.3	99	10	32.2	102	10
16	31.6	10	33.5	106	9	31.8	101	10	32.3	102	10
17	31.3	10	33.0	105	9	31.6	101	10	31.4	100	10
18	32.3	10	32.9	102	9	31.6	98	10	29.9	93	10
19	33.1	10	33.9	102	9	32.2	97	10	32.2	97	10
20	32.7	10	34.3	105	9	33.0	101	10	32.0	98	10
21	33.4	10	35.1	105	9	33.8	101	10	33.6	101	10
22	33.3	10	35.9	108	8	33.6	101	10	33.9	102	10
23	33.6	9	36.0	107	8	34.7	103	10	34.0	101	10
24	34.2	9	36.2	106	8	34.3	100	10	33.1	97	10
25	34.8	9	36.2	104	8	35.0	101	10	33.8	97	9
26	34.5	9	36.5	106	8	35.2	102	10	34.9	101	9
27	34.9	9	36.7	105	8	34.8	100	10	34.7	99	9
28	34.8	9	36.6	105	8	34.6	99	10	34.6	99	9
29	35.3	9	36.9	105	8	36.0	102	10	35.4	100	9
30	37.1	8	37.5	101	8	36.0	97	10	36.3	98	9
31	36.1	7	38.0	105	8	36.2	100	10	35.7	99	9
32	35.5	7	38.2	108	8	35.9	101	10	35.9	101	9
33	36.9	7	38.8	105	8	35.8	97	10	35.6	97	9
34	37.1	7	39.0	105	8	36.8	99	10	36.2	98	9
35	38.9	6	39.0	100	8	36.8	95	10	36.3	93	9
36	38.2	6	37.7	99	8	36.8	96	10	37.1	97	9
37	37.9	6	38.9	103	8	36.3	96	10	36.0	95	9
38	37.5	6	38.5	103	8	35.9	96	10	34.2	91	9
39	37.7	6	39.0	103	8	37.4	99	9	35.6	94	8
Mean for weeks											
1-13	27.8		28.6	103		28.0	101		28.3	102	
14-39	34.8		36.3	104		34.6	99		34.1	98	

TABLE 7
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

Weeks on Study	Vehicle Control		64 mg/kg			128 mg/kg			256 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.0	10	20.2	101	10	20.0	100	10	20.1	101	10
2	20.1	10	19.9	99	10	20.3	101	10	19.9	99	10
3	20.5	10	20.9	102	10	21.1	103	10	21.0	102	10
4	21.7	10	21.3	98	10	21.6	100	10	21.5	99	10
5	22.6	10	22.1	98	10	22.2	98	10	22.2	98	10
6	23.3	10	22.8	98	10	22.5	97	10	22.6	97	10
7	23.7	10	23.6	100	10	23.8	100	10	24.0	101	9
8	22.8	10	23.4	103	10	23.4	103	10	23.9	105	9
9	24.1	9	23.7	98	10	23.8	99	10	24.7	103	9
10	24.3	9	23.5	97	10	23.9	98	10	24.4	100	9
11	24.9	9	24.3	98	10	24.8	100	10	25.5	102	9
12	25.2	9	24.1	96	10	24.6	98	10	25.5	101	9
13	25.1	9	24.6	98	9	25.3	101	10	25.4	101	9
14	25.2	9	24.4	97	9	25.6	102	10	25.4	101	9
15	25.5	7	25.2	99	7	26.0	102	10	26.0	102	8
16	25.4	7	25.1	99	6	25.7	101	10	26.5	104	8
17	24.7	7	26.1	106	6	25.8	105	10	26.2	106	8
18	26.0	6	24.7	95	6	26.1	100	10	26.4	102	8
19	25.7	6	25.8	100	6	26.4	103	10	26.7	104	8
20	25.7	6	25.9	101	6	26.6	104	10	25.7	100	7
21	25.5	6	27.4	108	6	27.3	107	10	26.3	103	7
22	26.1	6	26.7	102	6	27.4	105	10	26.4	101	7
23	26.3	6	26.9	102	6	27.6	105	10	26.0	99	7
24	26.7	6	27.0	101	6	28.1	105	10	26.0	97	7
25	26.3	6	27.6	105	6	27.9	106	10	26.2	100	7
26	27.1	6	27.5	102	6	28.6	106	10	26.1	96	7
27	27.3	6	27.5	101	6	28.3	104	10	25.5	93	7
28	27.4	6	27.7	101	5	28.4	104	10	26.5	97	6
29	27.0	6	26.9	100	4	29.1	108	10	27.5	102	6
30	27.0	6	27.0	100	4	29.5	109	10	27.4	102	6
31	26.2	6	26.7	102	4	29.5	113	10	27.3	104	6
32	26.3	6	27.1	103	4	28.4	108	10	27.7	105	5
33	26.8	6	27.0	101	4	27.8	104	10	27.8	104	5
34	25.8	6	26.7	104	4	30.2	117	8	28.2	109	5
35	26.6	6	27.6	104	4	31.5	118	7	27.8	105	5
36	26.7	6	28.5	107	4	30.9	116	7	28.2	106	5
37	26.6	6	27.4	103	4	31.2	117	7	28.2	106	5
38	27.6	5	26.0	94	4	31.5	114	7	28.4	103	5
39	27.7	5	27.9	101	4	32.2	116	7	28.8	104	5
Mean for weeks											
1-13	22.9		22.6	99		22.9	100		23.1	101	
14-39	26.4		26.7	101		28.4	108		26.9	102	

26-Week Drinking Water Study in Tg.AC Hemizygous Mice

Dose Selection Rationale

The exposure concentrations used in this 26-week drinking water study (175, 350, and 700 mg/L) were the same as those used in a previous 2-year drinking water study of bromodichloromethane in male F344/N rats and female $B6C3F_1$ mice (NTP, 2006a).

Positive Control Tg.AC Hemizygous Mice

TPA (1.25 µg) was dermally administered to groups of 15 males and 15 females three times weekly. All males and females developed more than 20 skin papillomas each by week 20 (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.,* 2001).

Survival

Estimates of 26-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 8. The survival of exposed males and females was similar to that of the control groups.

Body Weights, Water and Compound Consumption, Clinical Findings, and Organ Weights

Mean body weights of males exposed to 350 mg/L were less than those of the controls after week 9 and those of 700 mg/L males were less after week 5 (Tables 9 and 10 and Figure 3). Mean body weights of 175, 350, and 700 mg/L females were greater than those of the controls after weeks 10, 22, and 23, respectively. Water consumption declined with increasing exposure concentration at the beginning of the study due to poor palatability (Tables J1 and J2). Females recovered from this taste aversion by the end of the study. While water consumption by exposed males did improve, it was still lower than controls at the end of the study. Drinking water concentrations of 175, 350, or 700 mg/L delivered average daily doses of approximately 20, 36, or 61 mg bromodichloromethane/kg body weight to males and 31, 61, or 130 mg/kg to females. No clinical findings related to bromodichloromethane exposure were observed. Absolute heart and right kidney weights of exposed males were significantly less than those of the control group (Table H3). The weights of these organs decreased with increasing dose, mirroring a similar pattern in overall body weight.

Hematology

The hematology data for Tg.AC hemizygous mice in the 26-week drinking water study of bromodichloromethane are listed in Table G2. An apparent dose-related decrease in platelet counts occurred in 350 (7% decrease) and 700 (13% decrease) mg/L male mice; the values were not below what would be considered an acceptable reference limit, and the relevance was unknown. A treatment-related, but not exposure concentration-related, decrease in neutrophil counts occurred in 350 and 700 mg/L male mice; the relevance of this finding was questionable and may reflect a slightly higher than expected neutrophil count in the control males. No changes occurred in other variables.

TABLE 8
Survival of Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
Animals initially in study	15	15	15	15
Moribund	2	3	3	1
Animals surviving to study termination	13	12	12	14
Percent probability of survival at end of study[a]	87	80	80	93
Mean survival (days)[b]	178	167	177	181
Survival analysis[c]	P=0.635N	P=0.922	P=1.000	P=0.984N
Female				
Animals initially in study	15	15	15	15
Moribund	1	0	3	1
Natural deaths	4	2	1	1
Animals surviving to study termination	10	13	11	13
Percent probability of survival at end of study	67	87	73	87
Mean survival (days)	155	170	168	181
Survival analysis	P=0.343N	P=0.381N	P=0.855N	P=0.297N

[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 exposure group is indicated by **N**.

TABLE 9
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.1	15	22.6	98	15	22.6	98	15	22.7	98	15
2	24.6	15	24.1	98	15	23.2	94	15	20.8	85	15
3	26.1	15	25.7	99	15	25.0	96	15	23.9	92	15
4	26.8	15	26.7	100	15	25.8	96	15	25.9	97	15
5	27.8	15	27.7	100	15	27.3	98	15	26.5	95	15
6	29.4	15	28.9	98	15	28.1	96	15	27.3	93	15
7	30.0	15	29.4	98	15	28.6	95	15	27.4	91	15
8	30.2	15	29.9	99	15	28.9	96	15	28.1	93	15
9	31.0	15	30.5	98	15	29.5	95	15	28.7	93	15
10	32.0	15	31.1	97	15	30.1	94	15	29.3	92	15
11	32.8	15	31.4	96	15	30.0	92	15	30.2	92	15
12	32.6	15	32.2	99	14	30.6	94	15	30.7	94	15
13	34.2	15	33.1	97	14	31.4	92	15	31.8	93	15
14	34.9	15	33.5	96	14	31.6	91	15	32.1	92	15
15	35.2	15	33.6	96	14	31.3	89	15	32.1	91	15
16	35.7	15	34.5	97	13	31.9	89	15	33.1	93	15
17	35.8	15	34.7	97	13	32.0	89	15	32.8	92	15
18	35.9	15	35.0	98	13	32.1	89	15	33.1	92	15
19	35.6	15	34.6	97	13	32.7	92	15	32.9	92	15
20	36.9	14	35.4	96	13	33.5	91	15	32.7	89	15
21	37.6	14	36.4	97	12	34.7	92	14	32.6	87	15
22	38.4	14	37.9	99	12	34.9	91	14	33.2	87	15
23	39.2	14	38.7	99	12	35.6	91	14	34.9	89	14
24	39.6	13	39.9	101	12	35.9	91	14	36.2	91	14
25	40.2	13	40.2	100	12	37.3	93	12	35.9	89	14
26	41.0	13	40.4	99	12	38.3	93	12	36.5	89	14
Mean for weeks											
1-13	29.3		28.7	98		27.8	95		27.2	93	
14-26	37.4		36.5	98		34.0	91		33.7	90	

TABLE 10
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.2	15	19.1	100	15	19.0	99	15	18.9	98	15
2	20.0	15	20.3	102	15	20.2	101	15	20.2	101	15
3	21.2	15	21.4	101	15	21.3	101	15	21.4	101	15
4	22.3	15	22.3	100	15	22.3	100	15	22.3	100	15
5	23.2	15	23.1	100	15	22.7	98	15	22.7	98	15
6	24.2	15	24.1	100	15	23.6	98	14	23.8	98	15
7	24.6	15	24.3	99	15	23.9	97	14	23.8	97	15
8	24.5	15	24.7	101	15	24.4	100	14	24.0	98	15
9	24.8	14	25.8	104	15	24.8	100	14	25.0	101	15
10	25.4	13	25.6	101	15	25.1	99	14	25.3	100	15
11	24.5	13	27.1	111	15	25.5	104	14	25.8	105	15
12	25.3	13	27.2	108	13	26.2	104	14	26.2	104	15
13	26.3	13	28.2	107	13	26.7	102	14	26.8	102	15
14	26.4	13	28.5	108	13	26.9	102	14	26.8	102	15
15	26.6	13	29.0	109	13	27.8	105	14	27.9	105	15
16	27.6	13	29.5	107	13	28.0	101	14	28.1	102	15
17	27.0	11	29.1	108	13	27.8	103	14	28.2	104	15
18	27.5	11	30.4	111	13	27.8	101	14	28.4	103	15
19	27.9	11	30.5	109	13	29.0	104	13	28.6	103	15
20	29.3	11	31.0	106	13	29.8	102	13	29.5	101	15
21	29.2	11	31.6	108	13	29.9	102	13	30.1	103	15
22	29.5	10	31.9	108	13	30.7	104	13	30.7	104	15
23	29.9	10	33.1	111	13	31.8	106	13	31.2	104	15
24	30.2	10	33.9	112	13	32.1	106	13	32.5	108	14
25	28.5	10	34.6	121	13	33.5	118	11	32.9	115	13
26	29.9	10	34.9	117	13	33.9	113	11	33.2	111	13
Mean for weeks											
1-13	23.5		24.1	103		23.5	100		23.6	100	
14-26	28.4		31.4	110		29.9	105		29.9	105	

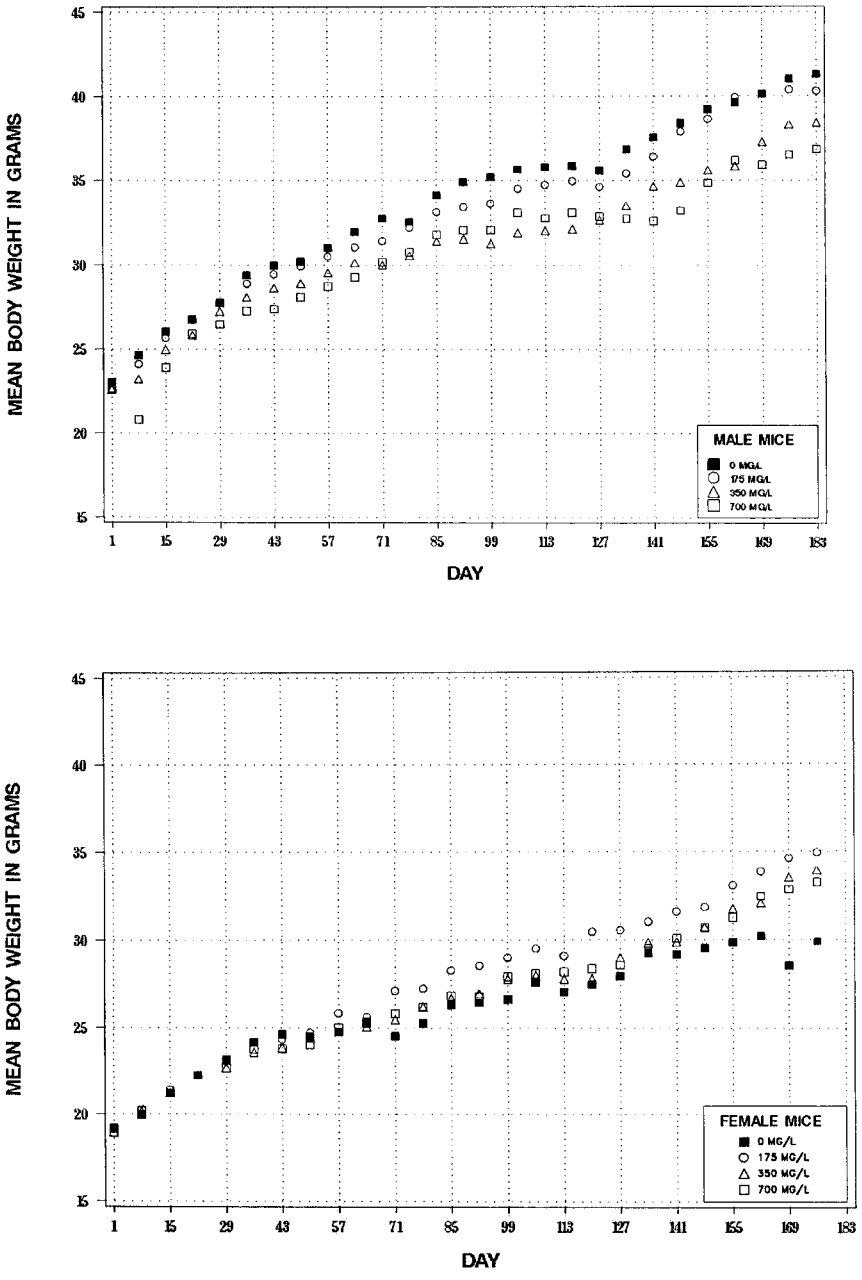


FIGURE 3
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Bromodichloromethane in Drinking Water for 26 Weeks

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables B1, B2, B3, and B4.

Liver: The incidences of hepatocyte fatty change in all exposed groups of females and hypertrophy in 350 and 700 mg/L females were significantly greater than those in the controls (Tables 11 and B4). The incidence of cytoplasmic vacuolization in 700 mg/L females was also significantly greater than that in the control group (Tables 11 and B4).

Liver lesions were generally of minimal severity and included hepatocyte fatty change, vacuolization, and hypertrophy. Fatty change and cytoplasmic vacuolization occurred together and were generally diffuse within the liver sections. Fatty change was characterized by the presence of variably sized, discrete, round (punch-hole) vacuoles within the cytoplasm. This change was considered to be consistent with intracytoplasmic lipid accumulation. Hepatocyte vacuolization was characterized by vacuoles that were not sharply defined and consisted of poorly demarcated clear spaces in the cytosol that separated irregular strands of eosinophilic cytoplasm. The vacuole area sometimes displayed light basophilic staining, a discoloration occasionally observed in controls. This change was considered to be consistent with hepatocellular glycogen accumulation. Hypertrophy was generally centrilobular and characterized by an increase in the size of the hepatocytes around the central vein. This was reflected in a decreased number of nuclei per unit of area in the affected areas.

Kidney: The incidences of renal tubule dilatation and renal tubule degeneration in all exposed groups of males, renal tubule hypertrophy in 350 and 700 mg/L males, and nephropathy in 700 mg/L males were significantly greater than those in the control group (Tables 11 and B2).

Kidney changes were generally of minimal severity and included nephropathy, renal tubule degeneration, renal tubule hypertrophy, renal tubular dilatation, and protein casts. Nephropathy consisted of a spectrum of changes that included small clusters of tubules with cytoplasmic basophilia (regeneration), tubular dilatation, proteinaceous casts, basement membrane thickening, and interstitial inflammation. Renal tubular degeneration consisted of vacuolization or flocculent cytoplasm in epithelial cells, necrosis of tubular epithelial cells, and faint tubular basophilia. The affected cells occasionally had large, bizarre nuclei, or were binucleated or multinucleated. Renal tubule hypertrophy consisted of tubules containing pale-stained hypertrophic epithelial cells that did not show other features suggestive of degeneration. Renal tubule dilatation was predominantly observed at the corticomedullary junction and consisted of enlarged tubular lumens lined by attenuated epithelium and filled with eosinophilic flocculent material (consistent with granular casts). Protein casts were evidenced as eosinophilic hyaline material filling the lumen of a tubule. On occasion, these casts could distend the tubule.

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Male and Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L		350 mg/L		700 mg/L	
Male								
Kidney[a]	15		15		15		15	
Nephropathy[b]	4	(1.0)[c]	3	(1.3)	4	(1.3)	11*	(1.3)
Renal Tubule, Degeneration	0		4*	(1.0)	4*	(1.0)	9*	(1.3)
Renal Tubule, Dilatation	4	(1.0)	11*	(1.2)	14**	(1.6)	15**	(1.7)
Renal Tubule, Hypertrophy	1	(1.0)	3	(1.0)	6*	(1.2)	11**	(1.0)
Protein Casts	1	(1.0)	2	(1.0)	1	(1.0)	1	(1.0)
Female								
Liver	15		15		15		15	
Hepatocyte, Fatty Change	0		4*	(1.0)	8**	(1.1)	10**	(1.5)
Hepatocyte, Hypertrophy	1	(2.0)	2	(2.5)	8**	(2.4)	12**	(2.8)
Hepatocyte, Vacuolization Cytoplasmic	2	(1.5)	5	(1.2)	4	(1.5)	8*	(1.6)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** ($P \leq 0.01$)
[a] Number of mice with tissue examined microscopically
[b] Number of mice with lesion
[c] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

42-Week Drinking Water Study in Tg.AC Hemizygous Mice

Survival

Estimates of 42-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 12. The survival of exposed males and females was similar to that of the control groups.

Body Weights, Water and Compound Consumption, Clinical Findings, and Organ Weights

Although mean body weights of exposed males and females were similar to those of the controls during most of the study, mean body weights of 350 and 700 mg/L males were less than those of the controls during the last 2 weeks of the study, and mean body weights of 350 mg/L females were greater than those of the controls during the last half of the study (Figure 4 and Tables 13 and 14). Due to poor palatability, water consumption declined with increasing exposure concentration (Tables J3 and J4). During the first 13 weeks of the study, water consumption by males averaged 5.0 g/day for the controls and 3.2, 2.8, and 2.5 g/day for the 175, 350, and 700 mg/L groups, respectively. The decrease was less marked during weeks 14 to 42, averaging 4.5 g/day for the controls and 3.2 g/day for the 700 mg/L exposed group. In females, the decrease in water consumption with increasing exposure concentration was less, averaging 6.1 g/day for the controls, and between 4.2 and 4.6 g/day for exposed groups during the first 13 weeks of the study. As in the males, the decrease was less during weeks 14 to 42, averaging 5.3 g/day in the controls and between 4.4 and 4.8 g/day in the exposed females. Drinking water concentrations of 175, 350, or 700 mg/L delivered average daily doses of approximately 18, 33, or 64 mg/kg to males and 28, 49, or 111 mg/kg to females. No clinical findings related to bromodichloromethane exposure were observed. Absolute right kidney weights of 350 and 700 mg/L males were significantly less than those of the control group (Table H4). The weights of these organs decreased with increasing dose, mirroring a similar pattern in overall body weight.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables B5, B6, B7, and B8.

Liver: The incidences of hepatocyte fatty change in all exposed groups of females were significantly greater than that in the controls (0 mg/L, 0/10; 175 mg/L, 6/10; 350 mg/L, 6/10; 700 mg/L, 6/10; Table B8). Hepatocyte fatty change was morphologically similar to that previously described in the 26-week drinking water study.

Kidney: The incidences of renal tubule dilatation (1/10, 8/10, 8/10, 10/10) in exposed groups of males and nephropathy (4/10, 7/10, 8/10, 9/10) in 700 mg/L males were significantly greater than those in the control group (Table B6). The kidney lesions were morphologically similar to those previously described in the 26-week drinking water study.

TABLE 12
Survival of Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
Animals initially in study	10	10	10	10
Moribund	4	0	0	1
Natural deaths	0	1	2	0
Animals surviving to study termination	6	9	8	9
Percent probability of survival at end of study[a]	60	90	80	90
Mean survival (days)[b]	260	280	279	294
Survival analysis[c]	P=0.263N	P=0.346N	P=0.610N	P=0.256N
Female				
Animals initially in study	10	10	10	10
Moribund	3	2	5	5
Natural deaths	2	0	1	1
Animals surviving to study termination	5	8	4	4
Percent probability of survival at end of study	50	80	40	40
Mean survival (days)	251	276	254	248
Survival analysis	P=0.504	P=0.332N	P=0.977	P=1.000

[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 exposure group is indicated by **N**.

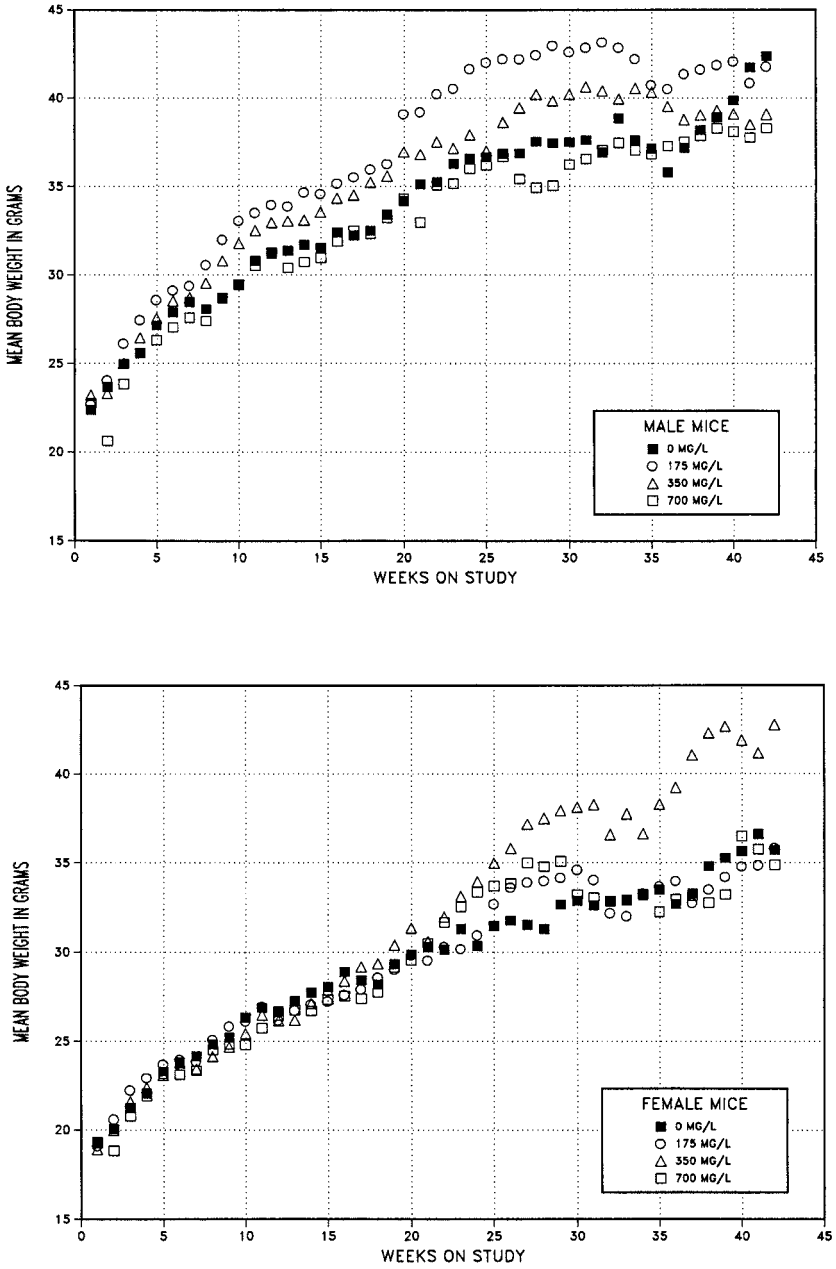


FIGURE 4
Growth Curves for Male and Female Tg.AC Hemizygous Mice Exposed to Bromodichloromethane in Drinking Water for 42 Weeks

TABLE 13
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.4	10	22.7	101	10	23.2	104	10	22.8	102	10
2	23.7	10	24.0	101	10	23.3	98	10	20.6	87	10
3	25.0	10	26.1	104	10	25.0	100	10	23.8	95	10
4	25.6	10	27.5	107	10	26.5	104	10	25.6	100	10
5	27.2	10	28.6	105	10	27.6	102	10	26.3	97	10
6	27.9	10	29.1	104	10	28.5	102	10	27.0	97	10
7	28.5	10	29.4	103	10	28.7	101	10	27.6	97	10
8	28.1	10	30.6	109	10	29.5	105	10	27.4	98	10
9	28.7	10	32.0	112	10	30.8	107	10	28.7	100	10
10	29.4	10	33.1	113	10	31.8	108	10	29.5	100	10
11	30.8	10	33.5	109	10	32.5	106	10	30.5	99	10
12	31.2	10	34.0	109	10	33.0	106	10	31.3	100	10
13	31.4	10	33.9	108	10	33.1	105	10	30.4	97	10
14	31.7	10	34.7	110	10	33.1	104	10	30.7	97	10
15	31.6	10	34.6	110	10	33.6	106	10	31.0	98	10
16	32.4	10	35.2	109	10	34.3	106	10	31.9	99	10
17	32.2	10	35.5	110	10	34.5	107	10	32.5	101	10
18	32.5	10	36.0	111	10	35.2	108	10	32.3	99	10
19	33.4	10	36.3	109	10	35.6	107	10	33.2	99	10
20	34.2	10	39.1	114	9	37.0	108	10	34.3	100	10
21	35.1	10	39.2	112	9	36.8	105	10	33.0	94	10
22	35.3	10	40.2	114	9	37.5	106	10	35.1	99	10
23	36.3	8	40.5	112	9	37.2	103	10	35.2	97	10
24	36.6	8	41.6	114	9	37.9	104	10	36.0	98	10
25	36.7	8	42.0	114	9	37.0	101	10	36.2	99	10
26	36.9	8	42.2	114	9	38.6	105	10	36.7	100	10
27	36.9	8	42.2	114	9	39.4	107	9	35.4	96	10
28	37.6	8	42.4	113	9	40.2	107	9	35.0	93	10
29	37.5	8	42.9	114	9	39.8	106	9	35.1	94	10
30	37.5	8	42.6	114	9	40.2	107	9	36.3	97	10
31	37.6	8	42.8	114	9	40.6	108	9	36.6	97	10
32	37.0	8	43.1	117	9	40.4	109	9	37.1	100	10
33	38.9	8	42.8	110	9	40.0	103	9	37.5	96	10
34	37.6	8	42.2	112	9	40.5	108	9	37.1	99	10
35	37.2	8	40.7	109	9	40.3	108	9	36.8	99	10
36	35.8	8	40.5	113	9	39.5	110	8	37.3	104	10
37	37.2	7	41.3	111	9	38.8	104	8	37.5	101	10
38	38.2	7	41.6	109	9	39.1	102	8	37.9	99	10
39	38.9	7	41.8	108	9	39.3	101	8	38.3	99	10
40	39.9	7	42.0	105	9	39.1	98	8	38.1	96	10
41	41.7	6	40.8	98	9	38.5	92	8	37.8	91	9
42	42.4	6	41.8	99	9	39.1	92	8	38.3	90	9
Mean for weeks											
1-13	27.7		29.6	107		28.7	104		27.0	98	
14-42	36.4		40.3	111		38.0	105		35.5	98	

TABLE 14
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.2	10	19.1	100	10	18.9	98	10	19.3	101	10
2	20.0	10	20.6	103	10	20.0	100	10	18.8	94	10
3	21.2	10	22.2	105	10	21.6	102	10	20.8	98	10
4	22.0	10	22.9	104	10	22.4	102	10	21.9	100	10
5	23.3	10	23.7	102	10	23.1	99	10	23.2	100	10
6	23.8	10	24.0	101	10	23.8	100	10	23.1	97	10
7	24.2	10	23.8	98	10	23.4	97	10	23.3	96	10
8	24.8	10	25.1	101	10	24.1	97	10	24.5	99	10
9	25.2	10	25.9	103	10	24.7	98	10	24.8	98	10
10	26.4	10	26.1	99	10	25.4	96	10	24.8	94	10
11	26.9	10	27.0	100	10	26.5	99	10	25.8	96	10
12	26.7	10	26.2	98	10	26.2	98	10	26.5	99	10
13	27.3	10	26.7	98	10	26.2	96	10	26.8	98	10
14	27.8	10	27.1	98	10	27.1	98	10	26.7	96	10
15	28.1	10	27.3	97	10	27.9	99	9	27.4	98	10
16	28.9	10	27.6	96	10	28.4	98	9	27.6	96	10
17	28.5	10	27.9	98	10	29.2	103	9	27.4	96	10
18	28.2	10	28.6	101	10	29.4	104	9	27.8	99	10
19	29.3	9	29.0	99	10	30.4	104	9	29.2	100	10
20	29.9	9	29.8	100	10	31.4	105	9	29.6	99	10
21	30.3	8	29.5	97	10	30.6	101	9	30.5	101	9
22	30.2	8	30.3	100	10	32.0	106	9	31.7	105	9
23	31.3	8	30.2	97	10	33.1	106	9	32.6	104	8
24	30.4	8	30.9	102	10	33.9	112	9	33.4	110	8
25	31.5	8	32.7	104	9	35.0	111	9	33.7	107	8
26	31.8	8	33.6	106	9	35.8	113	9	33.9	107	8
27	31.5	8	33.9	108	9	37.2	118	9	35.0	111	7
28	31.3	8	34.0	109	9	37.5	120	9	34.8	111	7
29	32.7	8	34.2	105	9	37.9	116	9	35.1	107	7
30	32.9	8	34.6	105	9	38.1	116	9	33.2	101	7
31	32.6	8	34.0	104	9	38.3	118	9	33.1	102	7
32	32.9	8	32.2	98	9	36.6	111	9	32.9	100	7
33	32.9	8	32.0	97	9	37.7	115	9	32.9	100	7
34	33.2	7	33.3	100	9	36.6	110	9	33.3	100	7
35	33.5	7	33.7	101	8	38.3	114	8	32.3	96	7
36	32.7	7	34.0	104	8	39.2	120	8	33.0	101	7
37	33.3	7	32.8	99	8	41.0	123	7	33.2	100	7
38	34.8	6	33.5	96	8	42.3	122	5	32.8	94	7
39	35.3	6	34.2	97	8	42.7	121	5	33.2	94	7
40	35.7	6	34.8	98	8	41.9	117	4	36.5	102	5
41	36.6	6	34.9	95	8	41.2	113	4	35.8	98	5
42	35.7	5	35.8	100	8	42.8	120	4	34.9	98	5
Mean for weeks											
1-13	23.9		24.1	101		23.6	99		23.4	98	
14-42	31.9		31.9	100		35.6	112		32.2	101	

26-Week Gavage Study in Tg.AC Hemizygous Mice

Dose Selection Rationale

The doses administered in this 26-week gavage study (25, 50, and 100 mg/kg) were based on findings from previous 2- and 13-week gavage studies of bromodichloromethane performed in B6C3F$_1$ mice and F344/N rats (NTP, 1987).

Positive Control Tg.AC Hemizygous Mice

TPA (1.25 µg) was dermally administered to groups of 15 males and 15 females three times weekly. Ninety-three percent of dosed males and females developed 20 skin papillomas. Two males developed fewer than 20 skin papillomas and survived to the end of the study; all other mice were terminated by week 18 (data not shown). This is consistent with historical rates found in other studies (Tennant *et al.,* 2001).

Survival

Estimates of 26-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 15. The survival of dosed males and females was similar to that of the vehicle control groups.

Table 15
Survival of Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Animals initially in study	15	15	15	15
Accidental death[a]	1	0	0	0
Moribund	1	1	3	0
Natural death	0	0	0	0
Animals surviving to study termination	13	14	12	15[b]
Percent probability of survival at end of study[c]	93	93	80	100
Mean survival (days)[d]	167	179	170	182
Survival analysis[e]	P=0.761N	P=1.000N	P=0.678	P=0.972N
Female				
Animals initially in study	15	15	15	15
Accidental deaths[a]	1	0	0	1
Moribund	1	0	0	1
Natural deaths	2	1	2	0
Animals surviving to study termination	11	14	13	13
Percent probability of survival at end of study	79	93	87	93
Mean survival (days)	170	180	174	170
Survival analysis	P=0.587N	P=0.565N	P=0.987N	P=0.616N

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

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of dosed males were similar to those of the vehicle controls (Tables 16 and 17 and Figure 5). In general, mean body weights of 25, 50, and 100 mg/kg females were greater than those of the vehicle controls after weeks 17, 23, and 17, respectively. No clinical findings were attributed to administration of bromodichloromethane. The relative liver weight of 100 mg/kg males was significantly greater than that of the vehicle control group (Table H5).

Hematology

The hematology data for Tg.AC hemizygous mice in the 26-week gavage study of bromodichloromethane are listed in Table G3. A decrease (approximately 40%) in white blood cell counts occurred in the 25 and 50 mg/kg male mice; this decrease was reflected in decreased lymphocyte counts. No leukocyte decreases occurred in the 100 mg/kg males or in any of the treated female groups, so the clinical and toxicological relevance of this finding was questionable. There were minimal (approximately 4%) increases in mean cell hemoglobin and mean cell volume values in 100 mg/kg male mice; again, toxicological significance of these changes was questionable. There were increased platelet counts in treated female mice. This change appears, however, to reflect a slightly lower than expected platelet count for the vehicle control female mice and would not appear to be clinically or toxicologically relevant. No changes occurred in other variables.

TABLE 16
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.3	15	24.1	99	15	23.9	98	15	24.0	99	15
2	24.7	15	25.2	102	15	25.1	102	15	25.0	101	15
3	26.0	15	26.2	101	15	26.2	101	15	26.2	101	15
4	27.0	15	27.2	101	15	27.5	102	15	27.4	102	15
5	27.5	15	28.0	102	15	27.8	101	15	27.9	102	15
6	28.1	15	28.5	101	15	28.8	103	15	28.5	101	15
7	28.8	14	28.5	99	15	28.8	100	15	29.3	102	15
8	29.0	14	29.4	101	15	29.1	100	15	29.1	100	15
9	30.1	14	30.0	100	15	29.9	99	15	30.3	101	15
10	30.3	14	30.2	100	15	29.7	98	15	30.3	100	15
11	30.3	14	30.8	102	15	29.8	98	15	30.8	102	15
12	31.2	14	31.4	101	15	30.9	99	15	30.8	99	15
13	31.6	14	31.9	101	15	31.6	100	15	31.1	98	15
14	31.5	14	31.2	99	15	30.9	98	15	31.5	100	15
15	32.2	13	32.2	100	15	31.2	97	15	31.5	98	15
16	32.4	13	32.6	101	15	31.1	96	15	32.1	99	15
17	32.3	13	33.2	103	15	30.8	95	15	32.5	101	15
18	33.2	13	33.4	101	15	32.7	99	13	32.3	97	15
19	33.9	13	33.9	100	15	33.2	98	13	33.2	98	15
20	34.4	13	34.0	99	15	33.0	96	13	33.2	97	15
21	33.9	13	33.6	99	15	32.6	96	13	33.1	98	15
22	34.7	13	34.5	99	14	34.1	98	12	33.6	97	15
23	34.5	13	34.8	101	14	34.1	99	12	32.8	95	15
24	35.3	13	35.4	100	14	35.2	100	12	33.1	94	15
25	34.5	13	35.3	102	14	33.9	98	12	32.9	95	15
26	35.2	13	35.8	102	14	33.8	96	12	33.5	95	15
Mean for weeks											
1-13	28.4		28.6	101		28.4	100		28.5	101	
14-26	33.7		33.8	100		32.8	97		32.7	97	

TABLE 17
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.5	15	20.6	101	15	20.3	99	15	20.1	98	15
2	20.5	15	20.9	102	15	20.9	102	15	20.7	101	15
3	21.6	15	21.4	99	15	21.7	101	15	21.7	101	15
4	22.8	15	23.0	101	15	22.7	100	15	22.8	100	15
5	23.0	15	23.4	102	15	23.5	102	15	23.7	103	15
6	23.1	15	23.6	102	15	23.8	103	15	24.2	105	15
7	23.7	14	23.9	101	15	24.0	101	15	24.2	102	14
8	23.7	14	24.4	103	15	24.4	103	15	25.0	106	14
9	24.4	14	24.4	100	15	24.6	101	15	24.8	102	14
10	24.7	14	25.3	102	15	25.3	102	15	26.1	106	14
11	24.8	14	25.5	103	15	25.6	103	15	26.6	107	14
12	25.1	14	25.3	101	15	25.7	102	15	26.2	104	14
13	25.1	14	26.6	106	15	25.9	103	15	26.5	106	14
14	25.6	14	26.6	104	15	26.1	102	15	26.7	104	14
15	25.8	14	26.5	103	15	26.2	102	15	27.0	105	14
16	25.7	14	26.9	105	15	26.3	102	14	26.7	104	14
17	25.7	14	27.0	105	15	27.4	107	14	27.0	105	14
18	26.0	14	27.8	107	15	28.0	108	14	28.0	108	14
19	26.5	14	28.0	106	15	28.2	106	13	28.1	106	14
20	26.7	14	28.6	107	15	27.5	103	13	28.6	107	13
21	26.7	14	28.6	107	15	27.9	105	13	28.4	106	13
22	26.8	14	28.6	107	14	27.5	103	13	29.0	108	13
23	27.4	14	29.5	108	14	28.2	103	13	28.6	104	13
24	27.4	12	30.6	112	14	29.5	108	13	29.6	108	13
25	27.1	11	29.6	109	14	29.0	107	13	28.6	106	13
26	27.2	11	30.2	111	14	28.7	106	13	28.6	105	13
Mean for weeks											
1-13	23.3		23.7	102		23.7	102		24.0	103	
14-26	26.5		28.3	107		27.7	105		28.1	106	

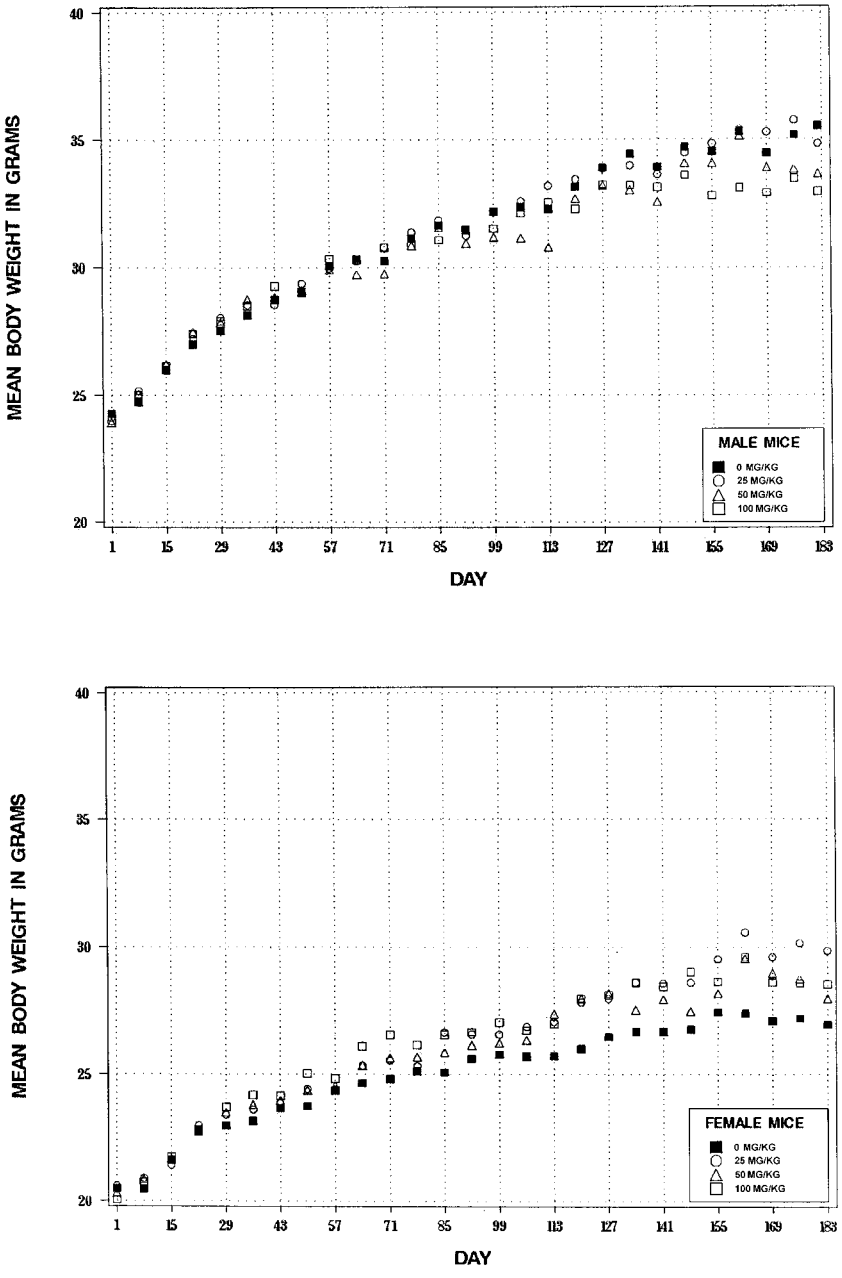


FIGURE 5
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Bromodichloromethane by Gavage for 26 Weeks

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the forestomach and nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables C1, C2, C3, and C4.

Forestomach: The incidence of multiple squamous cell papilloma in 100 mg/kg females was significantly greater than that in the vehicle controls (Tables 18 and C3). However, when single and multiple squamous cell papillomas were combined, there were no significant differences between the dosed and vehicle control groups. Squamous cell papillomas occurred in the mucosa of the forestomach. Typically, papillomas were exophytic lesions projecting from the mucosa of the forestomach and consisting of frond-like proliferations of squamous epithelium that radiated from a central stalk of stromal tissue that was continuous with the lamina propria.

Liver: The incidences of hepatocyte fatty change in all dosed groups of females and hepatocyte cytoplasmic vacuolization in 25 and 50 mg/kg females were significantly greater than those in the vehicle controls (Tables 18 and C4). These lesions were morphologically similar to those previously described in the 26-week drinking water study.

Kidney: The incidences of renal tubule degeneration in 100 mg/kg males and renal tubule hypertrophy in 100 mg/kg females were significantly greater than those in the vehicle controls (Tables 18, C2, and C4). The incidences of nephropathy, renal tubule dilatation, and renal tubule hypertrophy were not increased in males as they were in the drinking water study (Tables B2 and C2). These lesions were morphologically similar to those previously described in the 26-week drinking water study.

TABLE 18
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Kidney[a]	15	15	15	15
Renal Tubule, Degeneration[b]	0	0	0	4* (1.0)[c]
Female				
Forestomach	15	15	15	15
Squamous Cell Papilloma, Multiple	3	5	6	11**
Squamous Cell Papilloma (includes multiple)	6	8	10	11
Kidney	15	15	15	15
Renal Tubule, Hypertrophy	1 (1.0)	1 (1.0)	1 (1.0)	8** (1.0)
Liver	15	15	15	15
Hepatocyte, Fatty Change	0	5* (1.0)	8** (1.0)	7** (1.1)
Hepatocyte, Vacuolization Cytoplasmic	0	6** (1.0)	4* (1.3)	3 (1.0)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** ($P \leq 0.01$)
[a] Number of mice with tissue examined microscopically
[b] Number of mice with lesion
[c] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

41-Week Gavage Study in Tg.AC Hemizygous Mice

Survival

Estimates of 41-week survival probabilities for male and female Tg.AC hemizygous mice are shown in Table 19. The survival of dosed males and females was similar to that of the vehicle control groups.

Body Weights, Clinical Findings, and Organ Weights

Although mean body weights of dosed groups tended to be greater than those of the vehicle controls toward the end of the study, only 25 mg/kg males and 100 mg/kg females ended the study with mean body weights that were greater (Tables 20 and 21 and Figure 6). No clinical findings were attributed to administration of bromodichloromethane. Absolute and relative organ weights of dosed males and females were similar to those of the vehicle controls (Table H6).

Table 19
Survival of Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Animals initially in study	10	10	10	10
Accidental deaths[a]	1	3	0	0
Moribund	3	1	4	2
Animals surviving to study termination	6	6	6	8
Percent probability of survival at end of study[b]	69	86	60	80
Mean survival (days)[c]	240	195	248	257
Survival analysis[d]	P=0.964N	P=0.780N	P=1.000	P=0.974N
Female				
Animals initially in study	10	10	10	10
Moribund	1	0	0	2
Natural deaths	2	1	1	1
Animals surviving to study termination	7	9	9	7
Percent probability of survival at end of study	70	90	90	70
Mean survival (days)	248	287	272	263
Survival analysis	P=1.000	P=0.500N	P=0.578N	P=1.000N

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

TABLE 20
Mean Body Weights and Survival of Male Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	24.5	10	24.3	99	10	24.3	99	10	23.9	98	10
2	25.2	10	25.9	103	10	25.5	101	10	25.5	101	10
3	26.0	10	26.6	102	8	26.1	100	10	25.7	99	10
4	27.6	10	27.6	100	8	27.2	99	10	27.4	99	10
5	27.8	10	28.5	103	8	27.8	100	10	27.6	99	10
6	28.4	10	28.9	102	8	28.7	101	10	28.3	100	10
7	28.9	10	29.0	100	8	28.4	98	10	29.2	101	10
8	29.1	10	29.6	102	8	29.2	100	10	29.0	100	10
9	30.0	10	29.8	99	7	28.4	95	10	29.5	98	10
10	30.0	10	29.9	100	7	27.9	93	10	29.7	99	10
11	30.8	10	30.0	97	7	29.9	97	10	30.4	99	10
12	31.5	10	31.5	100	7	30.5	97	10	30.5	97	10
13	31.9	10	31.6	99	7	31.2	98	10	30.1	94	10
14	31.7	10	30.7	97	7	30.7	97	10	30.0	95	10
15	32.0	10	31.7	99	7	31.2	98	10	31.6	99	9
16	32.8	10	32.0	98	7	31.9	97	10	31.5	96	9
17	33.8	10	32.4	96	7	32.9	97	10	32.0	95	9
18	34.9	10	32.9	94	7	33.5	96	10	32.3	93	9
19	35.6	10	32.9	92	7	33.1	93	10	32.8	92	9
20	33.0	8	32.9	100	7	32.9	100	8	32.3	98	9
21	33.5	8	33.5	100	7	32.9	98	8	33.0	99	9
22	32.7	8	34.4	105	6	32.7	100	8	33.2	102	9
23	34.1	8	35.1	103	6	32.9	97	8	32.9	97	9
24	34.8	8	35.4	102	6	32.2	93	8	33.6	97	9
25	33.7	8	35.6	106	6	33.3	99	8	34.1	101	9
26	34.2	8	37.0	108	6	33.9	99	8	34.2	100	8
27	35.0	8	36.2	103	6	33.5	96	8	31.7	91	8
28	33.6	7	36.6	109	6	34.1	102	8	32.8	98	8
29	33.2	7	37.4	113	6	34.3	103	8	34.4	104	8
30	33.4	7	36.4	109	6	34.3	103	8	34.5	103	8
31	33.9	7	37.4	110	6	34.2	101	8	35.2	104	8
32	33.8	7	37.5	111	6	34.4	102	8	34.6	102	8
33	33.2	7	38.0	115	6	34.5	104	8	34.4	104	8
34	34.1	6	35.7	105	6	35.8	105	7	34.7	102	8
35	33.6	6	37.8	113	6	35.4	105	7	34.2	102	8
36	31.9	6	38.4	120	6	35.3	111	7	34.3	108	8
37	32.6	6	38.5	118	6	35.6	109	7	33.9	104	8
38	32.8	6	38.9	119	6	36.4	111	7	34.6	106	8
39	33.8	6	39.2	116	6	36.6	108	7	34.5	102	8
40	33.2	6	37.6	113	6	35.5	107	7	33.7	102	8
41	34.4	6	39.6	115	6	34.2	99	6	34.2	99	8
Mean for weeks											
1-13	28.6		28.7	100		28.1	98		28.2	99	
14-41	33.5		35.8	107		33.9	101		33.4	100	

TABLE 21
Mean Body Weights and Survival of Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	20.6	10	20.9	102	10	20.7	101	10	20.4	99	10
2	20.8	10	21.2	102	10	20.8	100	10	20.8	100	10
3	21.7	10	21.4	99	10	21.7	100	10	21.4	99	10
4	22.8	10	22.9	100	10	22.4	98	10	22.8	100	10
5	23.3	10	23.3	100	10	23.0	99	10	23.0	99	10
6	23.6	10	23.7	100	10	23.5	100	10	24.1	102	10
7	24.3	10	24.2	100	10	24.0	99	10	24.2	100	10
8	24.4	10	25.1	103	10	24.9	102	10	24.7	101	10
9	24.4	10	24.1	99	10	24.4	100	10	24.5	100	10
10	24.8	10	24.4	98	10	25.4	102	10	25.8	104	10
11	25.3	10	26.1	103	10	25.7	102	10	25.9	102	10
12	25.4	10	26.1	103	10	26.6	105	10	25.8	102	10
13	25.8	10	26.0	101	10	26.3	102	10	26.0	101	10
14	26.1	10	26.2	100	10	25.7	99	10	26.4	101	10
15	26.9	10	27.4	102	10	27.0	100	10	26.5	99	10
16	26.6	9	26.9	101	10	26.7	100	10	26.7	100	10
17	27.3	9	27.9	102	10	27.4	100	10	27.1	99	10
18	27.6	9	27.7	100	10	28.1	102	10	27.6	100	10
19	28.1	8	28.0	100	10	27.7	99	9	27.7	99	10
20	28.5	8	27.8	98	10	27.5	97	9	28.0	98	10
21	29.3	8	28.4	97	10	28.1	96	9	28.9	99	10
22	29.3	8	27.6	94	10	27.9	95	9	28.7	98	10
23	29.0	8	27.6	95	10	28.7	99	9	28.5	98	10
24	30.3	8	28.0	92	10	29.2	96	9	29.6	98	10
25	30.0	8	29.2	97	10	29.1	97	9	29.6	99	9
26	30.3	8	29.3	97	10	28.6	94	9	29.8	98	9
27	30.0	8	29.0	97	10	28.7	96	9	29.5	98	9
28	30.7	8	29.7	97	10	29.1	95	9	31.2	102	8
29	30.9	8	30.1	97	10	28.9	94	9	30.9	100	8
30	31.5	8	30.5	97	10	29.0	92	9	31.6	100	8
31	31.8	8	30.5	96	10	29.1	92	9	31.9	100	8
32	31.1	8	30.4	98	10	29.4	95	9	32.5	105	8
33	32.5	8	31.3	96	10	29.8	92	9	32.0	99	8
34	33.3	8	31.6	95	10	30.2	91	9	32.2	97	8
35	29.3	7	32.2	110	10	29.6	101	9	32.5	111	8
36	28.9	7	32.3	112	10	29.9	104	9	32.5	113	8
37	29.1	7	32.5	112	10	29.8	102	9	32.5	112	8
38	29.8	7	32.3	108	10	30.1	101	9	32.9	110	8
39	30.2	7	31.7	105	10	30.4	101	9	34.1	113	7
40	28.5	7	30.3	106	9	29.2	103	9	34.0	119	7
41	29.8	7	30.4	102	9	29.9	100	9	33.5	112	7
Mean for weeks											
1-13	23.6		23.8	101		23.8	101		23.8	101	
14-41	29.5		29.5	100		28.7	98		30.3	103	

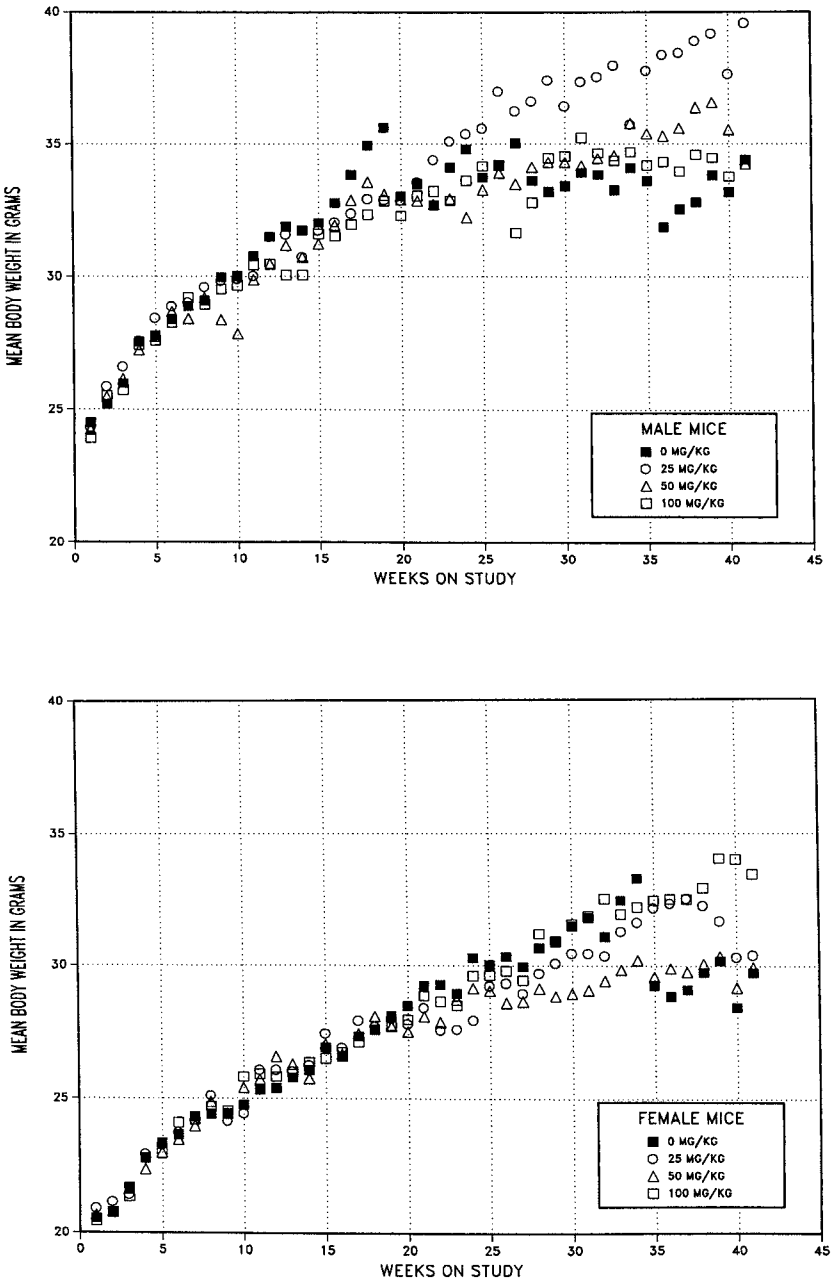


FIGURE 6
Growth Curves for Male and Female Tg.AC Hemizygous Mice Administered Bromodichloromethane by Gavage for 41 Weeks

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms of the forestomach and nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables C5, C6, C7, and C8.

Forestomach: The incidences of multiple squamous cell papilloma in dosed females were increased, and the difference was significant at 25 and 100 mg/kg (Tables 22 and C7). The combined incidence of single and multiple squamous cell papilloma was also significantly increased in 100 mg/kg females (Table 22). These lesions were morphologically similar to those previously described in the 26-week gavage study.

Liver: The incidences of hepatocyte cytoplasmic vacuolization were increased in all dosed females compared to the vehicle controls, but the difference was significant only in the 50 mg/kg group (Tables 22 and C8). The incidences of hepatocellular fatty change in 50 and 100 mg/kg females were significantly increased compared to that of the vehicle control group. These lesions were morphologically similar to those previously described in the 26-week drinking water study.

Kidney: In 100 mg/kg males, the incidence of renal tubule degeneration was significantly greater than that in the vehicle control group (Tables 22 and C6). Renal tubule degeneration was morphologically similar to that previously described in the 26-week drinking water study.

TABLE 22
Incidences of Selected Neoplasms and Nonneoplastic Lesions in Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Kidney[a]	10	10	10	10
Renal Tubule, Degeneration[b]	0	0	0	6** (1.7)[c]
Forestomach	10	10	10	10
Squamous Cell Papilloma, Multiple	6	5	5	5
Squamous Cell Papilloma (includes multiple)	8	6	9	6
Female				
Forestomach	10	10	10	10
Squamous Cell Papilloma, Multiple	1	6*	5	9**
Squamous Cell Papilloma (includes multiple)	4	7	8	10**
Liver	10	10	10	10
Hepatocyte, Fatty Change	0	2 (1.5)	8** (1.4)	5* (1.6)
Hepatocyte, Vacuolization Cytoplasmic	6 (1.3)	9 (1.3)	10* (1.1)	9 (1.7)

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** ($P \leq 0.01$)
[a] Number of mice with site examined microscopically
[b] Number of mice with lesion
[c] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

26-Week Drinking Water Study in p53 Haploinsufficient Mice

Dose Selection Rationale

The doses administered in this 26-week drinking water study (175, 350, and 700 mg/L) were the same as those used in a previous 2-year drinking water study of bromodichloromethane in male F344/N rats and female $B6C3F_1$ mice (NTP, 2006).

Survival

Estimates of 26-week survival probabilities for male and female p53 haploinsufficient mice are shown in Table 23. The survival of exposed males and females was similar to that of the control groups.

Body Weights, Water and Compound Consumption, Clinical Findings, and Organ Weights

Mean body weights of males exposed to 350 or 700 mg/L were less than those of the controls throughout most of the study (Table 24 and Figure 7). Mean body weights of 175, 350, and 700 mg/L females were less than control body weights after weeks 15, 23, and 18, respectively (Table 25 and Figure 7). Water consumption by exposed males and females declined with increasing exposure concentration at the beginning of the study (Tables J5 and J6). Water consumption by exposed females was similar to that by controls by the end of the study, but that by males remained low. Drinking water concentrations of 175, 350, and 700 mg/L delivered average daily doses of approximately 16, 31, or 65 mg/kg to males and 26, 50, or 100 mg/kg to females. There were no chemical-related clinical findings. The absolute heart weight in 700 mg/L males and absolute right kidney and liver weights in 350 and 700 mg/L males were significantly less than those of the control group (Table H7). The weights of these organs decreased with increasing dose, mirroring a similar pattern in overall body weight. The relative lung weight in 700 mg/L males, the relative right testis weight in 350 and 700 mg/L males, and the relative liver weight in 700 mg/L females were all greater than those of the control groups.

Hematology

The hematology data for p53 haploinsufficient mice in the 26-week drinking water study of bromodichloromethane are listed in Table G4. Very minimal decreases in hematocrit (3%) and hemoglobin concentration (4%) values occurred in the 700 mg/kg male mice. The significance, if any, was not known, and females were not affected.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the kidney and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables D1, D2, D3, and D4.

Kidney: The incidences of renal tubule dilatation in all exposed groups of males (0 mg/L, 0/15; 175 mg/L 5/15; 350 mg/L, 4/15; 700 mg/L, 6/15), renal tubule degeneration in 350 and 700 mg/L males (0/15, 0/15, 9/15, 12/15), and protein casts in 700 mg/L males (1/15, 5/15, 4/15, 7/15) were significantly greater than those in controls (Table D2). These lesions were morphologically similar to those previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

Liver: The incidence of fatty change in hepatocytes of 700 mg/L females was significantly greater than that in the control group (0/15, 1/15, 1/15, 10/15; Table D4). Hepatocyte fatty change was morphologically similar to that previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

TABLE 23
Survival of p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
Animals initially in study	15	15	15	15
Animals surviving to study termination	15	15	15	15
Percent probability of survival at end of study[a]	100	100	100	100
Mean survival (days)[b]	184	184	184	184
Survival analysis[c]	—[d]	—	—	—
Female				
Animals initially in study	15	15	15	15
Natural death	0	0	1	0
Animals surviving to study termination	15	15	14	15
Percent probability of survival at end of study	100	100	93	100
Mean survival (days)	185	185	175	185
Survival analysis	P=1.000	—	P=1.000	—

[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.
[d] No deaths occurred in this group; value of statistic cannot be calculated.

TABLE 24
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	22.8	15	23.0	101	15	22.9	100	15	22.5	99	15
2	24.2	15	23.7	98	15	21.8	90	15	20.1	83	15
3	25.6	15	25.0	98	15	24.2	95	15	22.0	86	15
4	26.7	15	26.2	98	15	25.2	94	15	24.1	90	15
5	27.9	15	26.9	96	15	26.4	95	15	24.8	89	15
6	28.3	15	28.1	99	15	26.9	95	15	24.8	88	15
7	30.2	15	28.5	94	15	27.4	91	15	25.2	83	15
8	31.1	15	30.3	97	15	28.3	91	15	26.0	84	15
9	33.0	15	32.1	97	15	29.7	90	15	27.2	82	15
10	34.7	15	33.5	97	15	30.7	89	15	27.9	80	15
11	36.1	15	35.2	98	15	32.1	89	15	27.9	77	15
12	37.0	15	36.3	98	15	33.1	90	15	29.0	78	15
13	38.4	15	37.3	97	15	33.3	87	15	29.6	77	15
14	38.9	15	38.3	99	15	34.2	88	15	30.1	77	15
15	39.8	15	38.9	98	15	35.2	88	15	30.8	77	15
16	40.8	15	39.2	96	15	36.1	89	15	31.2	77	15
17	42.0	15	40.6	97	15	37.1	88	15	32.1	76	15
18	42.9	15	40.8	95	15	37.5	87	15	33.0	77	15
19	43.4	15	42.0	97	15	37.6	87	15	33.3	77	15
20	43.8	15	43.0	98	15	38.8	89	15	33.9	77	15
21	45.1	15	43.7	97	15	39.4	87	15	34.7	77	15
22	45.9	15	44.2	96	15	39.6	86	15	34.8	76	15
23	46.4	15	45.0	97	15	41.0	88	15	35.1	76	15
24	47.1	15	45.6	97	15	41.1	87	15	36.9	78	15
25	47.4	15	45.2	95	15	41.5	88	15	36.0	76	15
26	47.8	15	46.3	97	15	42.7	89	15	36.5	76	15
Mean for weeks											
1-13	30.5		29.7	98		27.8	92		25.5	84	
14-26	43.9		42.5	97		38.6	88		33.7	77	

TABLE 25
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.9	15	18.9	100	15	18.8	100	15	18.6	98	15
2	19.6	15	19.6	100	15	19.3	99	15	19.1	97	15
3	20.8	15	20.2	97	15	20.0	96	15	20.0	96	15
4	21.8	15	21.4	98	15	21.4	98	15	21.1	97	15
5	22.1	15	21.9	99	15	21.9	99	15	22.0	100	15
6	22.0	15	21.9	100	15	22.4	102	14	22.3	101	15
7	22.3	15	22.1	99	15	21.8	98	14	21.9	98	15
8	23.3	15	22.5	97	15	23.2	100	14	22.7	97	15
9	23.6	15	23.0	98	15	23.5	100	14	22.4	95	15
10	24.6	15	23.7	96	15	24.3	99	14	24.1	98	15
11	24.7	15	24.4	99	15	24.6	100	14	24.1	98	15
12	25.3	15	24.5	97	15	25.2	100	14	24.6	97	15
13	26.5	15	25.2	95	15	25.8	97	14	24.9	94	15
14	27.1	15	26.0	96	15	27.0	100	14	25.9	96	15
15	27.9	15	26.4	95	15	27.3	98	14	26.3	94	15
16	29.0	15	26.4	91	15	27.8	96	14	26.9	93	15
17	28.7	15	26.8	93	15	28.3	99	14	28.4	99	15
18	29.1	15	27.6	95	15	28.7	99	14	27.6	95	15
19	29.8	15	28.1	94	15	29.2	98	14	27.5	92	15
20	30.6	15	29.1	95	15	29.4	96	14	28.3	93	15
21	31.5	15	29.7	94	15	30.4	97	14	29.2	93	15
22	32.7	15	30.8	94	15	30.9	95	14	30.0	92	15
23	34.0	15	32.2	95	15	32.9	97	14	30.4	89	15
24	35.3	15	32.1	91	15	33.2	94	14	30.8	87	15
25	35.6	15	32.1	90	15	32.6	92	14	31.3	88	15
26	36.4	15	33.2	91	15	34.0	93	14	31.7	87	15
Mean for weeks											
1-13	22.7		22.3	98		22.5	99		22.1	97	
14-26	31.4		29.3	93		30.1	96		28.8	92	

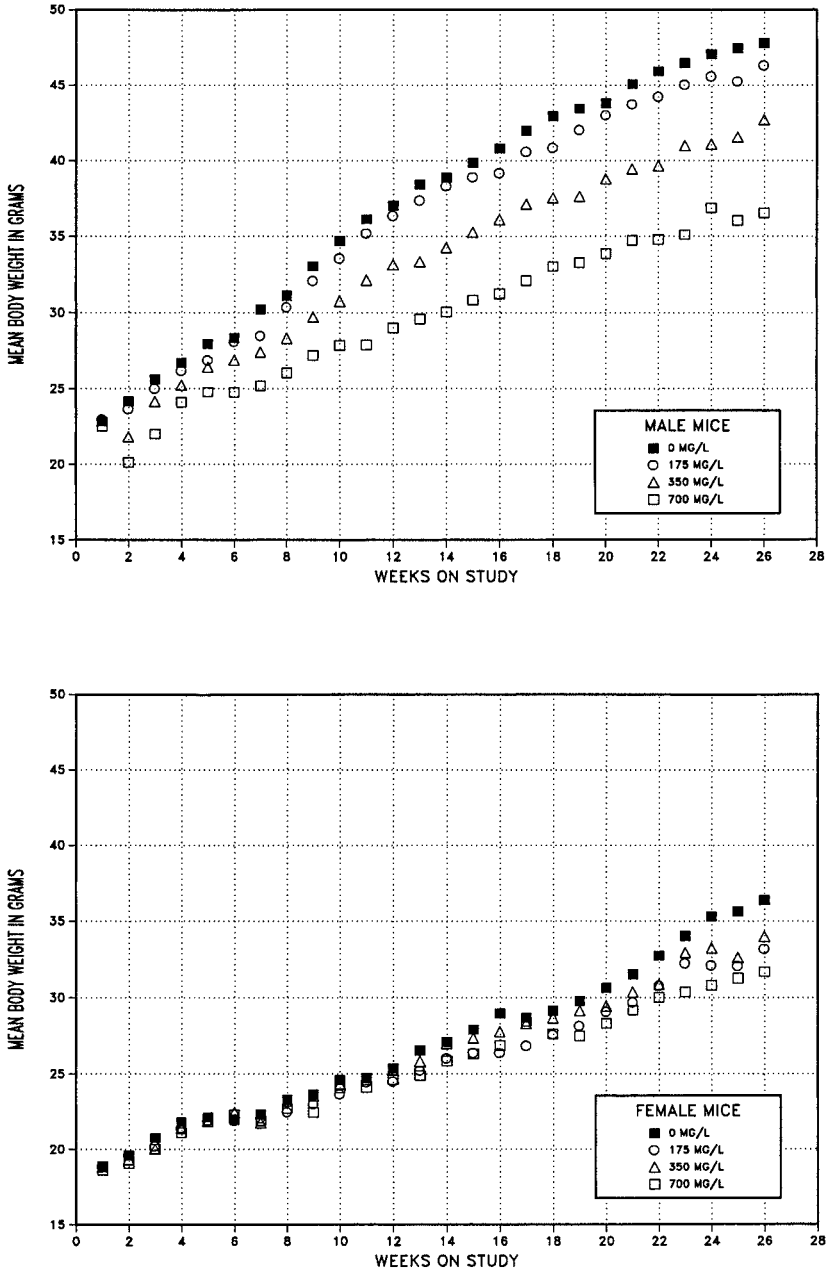


FIGURE 7
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Bromodichloromethane in Drinking Water for 26 Weeks

42-Week Drinking Water Study in p53 Haploinsufficient Mice

Survival

Estimates of 42-week survival probabilities for male and female p53 haploinsufficient mice are shown in Table 26. The survival of exposed males and females was similar to that of the control groups.

Body Weights, Water and Compound Consumption, Clinical Findings, and Organ Weights

The mean body weights of males exposed to 350 or 700 mg/L were less than those of the controls after week 8 and week 1, respectively (Figure 8 and Table 27). Mean body weights of females were generally similar to those of the controls but were less than controls in 700 mg/L females during the last 3 weeks of the study (Figure 8 and Table 28). Water consumption by exposed groups was less than that by controls at the beginning of the study (Tables J7 and J8). Water consumption by exposed males and females improved, but that by exposed males remained lower at the end of the study. Drinking water concentrations of 175, 350, and 700 mg/L delivered average daily doses of approximately 14, 30, or 55 mg/kg to males and 22, 43, or 98 mg/kg to females. There were no chemical-related clinical findings. Absolute right kidney weights in 350 and 700 mg/L males were significantly less than those of the control group (Table H8). The weights of these organs decreased with increasing dose, mirroring a similar pattern in overall body weight. The relative right testis weight in 700 mg/L males and the relative liver weight in 700 mg/L females were greater than those of the control groups.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables D5, D6, D7, and D8.

Kidney: The incidences of renal tubule degeneration in 350 and 700 mg/L males were significantly greater than that in the control group (0 mg/L, 0/10; 175 mg/L 0/10; 350 mg/L, 6/10; 700 mg/L, 10/10; Table D6). Renal tubule degeneration was morphologically similar to that previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

TABLE 26
Survival of p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
Animals initially in study	10	10	10	10
Moribund	1	0	0	3
Natural death	0	0	1	0
Animals surviving to study termination	9	10	9	7
Percent probability of survival at end of study[a]	90	100	90	70
Mean survival (days)[b]	289	296	294	258
Survival analysis[c]	P=0.147	P=1.000N	P=1.000N	P=0.540
Female				
Animals initially in study	10	10	10	10
Moribund	1	1	0	1
Natural death	0	0	0	1
Animals surviving to study termination	9	9	10	8
Percent probability of survival at end of study	90	90	100	80
Mean survival (days)	296	286	297	266
Survival analysis	P=0.681	P=1.000	P=1.000N	P=0.924

[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 lower mortality in an exposure group is indicated by **N**.

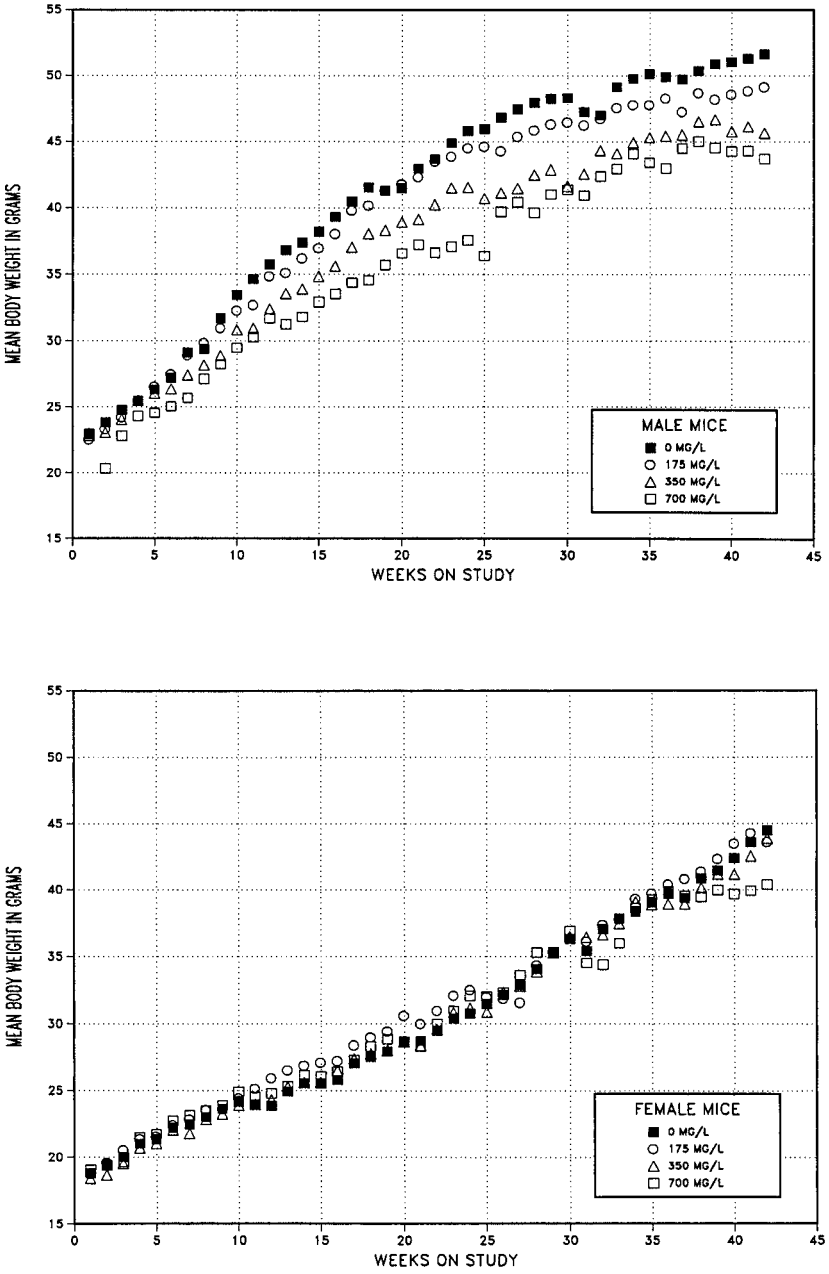


FIGURE 8
Growth Curves for Male and Female p53 Haploinsufficient Mice Exposed to Bromodichloromethane in Drinking Water for 42 Weeks

TABLE 27
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.0	10	22.5	98	10	23.0	100	10	22.8	99	10
2	23.8	10	23.3	98	10	23.0	97	10	20.3	85	10
3	24.8	10	24.2	98	10	24.1	97	10	22.8	92	10
4	25.5	10	25.4	100	10	25.5	100	10	24.3	95	10
5	26.3	10	26.5	101	10	26.0	99	10	24.6	94	10
6	27.2	10	27.5	101	10	26.4	97	10	25.0	92	10
7	29.1	10	28.9	99	10	27.4	94	10	25.7	88	10
8	29.4	10	29.8	101	10	28.2	96	10	27.1	92	10
9	31.7	10	31.0	98	10	28.9	91	10	28.2	89	10
10	33.5	10	32.3	96	10	30.8	92	10	29.5	88	10
11	34.6	10	32.7	95	10	30.9	89	10	30.3	88	10
12	35.7	10	34.8	98	10	32.4	91	10	31.7	89	10
13	36.8	10	35.1	95	10	33.5	91	10	31.3	85	10
14	37.4	10	36.2	97	10	33.9	91	10	31.8	85	10
15	38.2	10	37.0	97	10	34.8	91	10	32.9	86	9
16	39.3	10	38.0	97	10	35.6	91	10	33.5	85	9
17	40.5	10	39.8	98	10	37.0	91	10	34.4	85	9
18	41.5	10	40.2	97	10	38.0	92	10	34.5	83	9
19	41.3	10	41.3	100	10	38.3	93	10	35.7	86	9
20	41.5	10	41.8	101	10	38.9	94	10	36.6	88	9
21	43.0	10	42.3	98	10	39.1	91	10	37.2	87	9
22	43.7	10	43.5	100	10	40.2	92	10	36.6	84	9
23	44.9	10	43.9	98	10	41.5	92	10	37.1	83	9
24	45.8	10	44.5	97	10	41.6	91	10	37.6	82	9
25	46.0	10	44.6	97	10	40.7	89	10	36.4	79	9
26	46.8	10	44.3	95	10	41.1	88	10	39.7	85	8
27	47.4	10	45.4	96	10	41.5	88	10	40.4	85	8
28	47.9	10	45.8	96	10	42.5	89	10	39.6	83	8
29	48.2	10	46.3	96	10	42.9	89	10	41.0	85	8
30	48.3	10	46.4	96	10	41.6	86	10	41.4	86	8
31	47.2	10	46.2	98	10	42.6	90	10	40.9	87	8
32	47.0	10	46.7	99	10	44.3	94	10	42.4	90	8
33	49.1	9	47.5	97	10	44.1	90	10	43.0	88	8
34	49.8	9	47.7	96	10	44.9	90	10	44.1	89	8
35	50.1	9	47.7	95	10	45.3	90	10	43.4	87	8
36	49.9	9	48.3	97	10	45.4	91	10	43.0	86	8
37	49.7	9	47.2	95	10	45.5	92	10	44.5	90	7
38	50.3	9	48.7	97	10	46.5	92	10	45.0	90	7
39	50.9	9	48.2	95	10	46.7	92	10	44.5	87	7
40	51.0	9	48.5	95	10	45.8	90	10	44.3	87	7
41	51.3	9	48.8	95	10	46.1	90	9	44.3	86	7
42	51.6	9	49.1	95	10	45.6	88	9	43.7	85	7
Mean for weeks											
1-13	29.3		28.8	98		27.7	95		26.4	90	
14-42	46.2		44.7	97		41.8	91		39.6	86	

TABLE 28
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Weeks on Study	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	10	18.7	100	10	18.4	98	10	19.1	102	10
2	19.5	10	19.6	101	10	18.7	96	10	19.4	100	10
3	20.0	10	20.5	103	10	19.5	98	10	19.6	98	10
4	21.0	10	21.4	102	10	20.7	99	10	21.5	102	10
5	21.3	10	21.5	101	10	21.0	99	10	21.7	102	10
6	22.2	10	22.4	101	10	22.0	99	10	22.7	102	10
7	22.5	10	22.8	101	10	21.8	97	10	23.1	103	10
8	23.0	10	23.5	102	10	22.8	99	10	23.5	102	10
9	23.7	10	23.6	100	10	23.3	98	10	23.9	101	9
10	24.2	10	24.4	101	10	23.9	99	10	24.9	103	9
11	24.0	10	25.1	105	10	24.0	100	10	24.6	103	9
12	23.9	10	25.9	108	10	24.3	102	10	24.8	104	9
13	24.9	10	26.5	106	10	25.3	102	10	25.3	102	9
14	25.6	10	26.9	105	10	25.6	100	10	26.1	102	9
15	25.6	10	27.1	106	10	25.6	100	10	26.1	102	9
16	25.8	10	27.2	105	10	26.5	103	10	26.4	102	9
17	27.0	10	28.4	105	10	27.4	102	10	27.3	101	9
18	27.6	10	29.0	105	10	27.8	101	10	28.3	103	9
19	27.9	10	29.4	105	10	28.0	100	10	28.9	104	9
20	28.7	10	30.6	107	10	28.7	100	10	28.7	100	9
21	28.7	10	30.0	105	10	28.4	99	10	28.3	99	9
22	29.5	10	31.0	105	10	29.6	100	10	30.0	102	9
23	30.4	10	32.1	106	10	30.8	101	10	31.0	102	9
24	30.8	10	32.5	106	10	31.2	101	10	32.1	104	9
25	31.5	10	32.0	102	10	30.9	98	10	32.0	102	9
26	32.1	10	31.9	99	10	32.3	101	10	32.3	101	9
27	32.9	10	31.6	96	10	32.8	100	10	33.6	102	9
28	34.0	10	34.3	101	9	33.8	99	10	35.3	104	9
29	35.3	10	35.3	100	9	35.3	100	10	35.2	100	9
30	36.3	10	36.3	100	9	36.5	101	10	36.9	102	9
31	35.4	10	36.1	102	9	36.4	103	10	34.5	98	9
32	37.0	10	37.3	101	9	36.6	99	10	34.4	93	9
33	37.8	10	37.8	100	9	37.4	99	10	36.0	95	9
34	38.3	10	39.3	103	9	39.1	102	10	38.6	101	8
35	39.0	10	39.7	102	9	38.9	100	10	39.3	101	8
36	39.7	10	40.4	102	9	38.9	98	10	39.9	101	8
37	39.4	10	40.8	104	9	38.9	99	10	39.5	100	8
38	40.8	10	41.4	102	9	40.2	99	10	39.4	97	8
39	41.5	10	42.3	102	9	41.2	99	10	40.0	96	8
40	42.4	10	43.5	103	9	41.2	97	10	39.7	94	8
41	43.6	10	44.2	101	9	42.5	98	10	39.9	92	8
42	44.5	9	43.6	98	9	43.9	99	10	40.4	91	8
Mean for weeks											
1-13	22.2		22.8	102		22.0	99		22.6	102	
14-42	34.1		34.9	103		34.0	100		33.8	100	

26-Week Gavage Study in p53 Haploinsufficient Mice

Dose Selection Rationale

The doses administered in this 26-week gavage study (25, 50, and 100 mg/kg) were based on findings from previous 2- and 13-week gavage studies of bromodichloromethane in B6C3F$_1$ mice and F344/N rats (NTP, 1987).

Survival

Estimates of 26-week survival probabilities for male and female p53 haploinsufficient mice are shown in Table 29. The survival of dosed males and females was similar to that of the vehicle control groups.

Body Weights, Clinical Findings, and Organ Weights

The mean body weights of males administered 50 or 100 mg/kg were less than those of the vehicle controls after weeks 5 and 1, respectively (Table 30 and Figure 9). Mean body weights of 50 mg/kg females were less than those of the vehicle control group after week 12, but those of 25 and 100 mg/kg females were generally similar to those of the vehicle controls throughout the study (Table 31 and Figure 9). No clinical findings related to bromodichloromethane administration occurred. The absolute heart, right kidney, and right testis weights in 100 mg/kg males were significantly less than those in the vehicle controls, and the relative weights in 50 and 100 mg/kg males were significantly greater (Table H9). The relative lung and liver weights in 100 mg/kg males were also greater. The absolute and relative liver weights in 100 mg/kg females were significantly greater than those in the vehicle control group.

Hematology

The hematology data for p53 haploinsufficient mice in the 26-week gavage study of bromodichloromethane are listed in Table G5. There was a minimal decrease (5%) in erythrocyte count in 100 mg/kg male mice; the significance, if any, was not known, and females were not affected. Apparent dose-related increases in platelet counts occurred in 50 (2%) and 100 (7%) mg/kg male mice; however, the values were within what would be considered an acceptable reference limit; the relevance was not known, and females were unaffected.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables E1, E2, E3, and E4.

Liver: The incidence of fatty change in hepatocytes of 100 mg/kg females was significantly greater than that in the vehicle control group (vehicle control, 2/15; 25 mg/kg, 2/15; 50 mg/kg, 3/15; 100 mg/kg, 11/15; Table E4). Fatty change was morphologically similar to that previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

Kidney: In 100 mg/kg males, the incidence of renal tubule degeneration was significantly greater than that in the vehicle control group (0/15, 0/15, 0/15, 4/15; Table E2). Renal tubule degeneration was morphologically similar to that previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

TABLE 29
Survival of p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Animals initially in study	15	15	15	15
Animals surviving to study termination	15	15	15	15
Percent probability of survival at end of study[a]	100	100	100	100
Mean survival (days)[b]	183	183	183	183
Survival analysis[c]	—[d]	—	—	—
Female				
Animals initially in study	15	15	15	15
Accidental death[e]	0	0	1	0
Moribund	0	1	0	1
Animals surviving to study termination	15	14	14	14
Percent probability of survival at end of study	100	93	100	93
Mean survival (days)	184	178	172	176
Survival analysis	P=0.794	P=1.000	—	P=1.000

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns.
[d] No deaths occurred in this group; value of statistic cannot be calculated.
[e] Censored from survival analyses

TABLE 30
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.5	15	23.7	101	15	21.6	92	15	22.8	97	15
2	24.3	15	24.4	100	15	23.5	97	15	22.7	93	15
3	25.5	15	25.6	100	15	24.3	95	15	23.8	93	15
4	26.3	15	26.4	100	15	25.1	95	15	24.2	92	15
5	26.6	15	26.3	99	15	25.3	95	15	24.2	91	15
6	28.4	15	28.0	99	15	26.6	94	15	24.9	88	15
7	28.5	15	28.6	100	15	26.5	93	15	25.2	88	15
8	29.8	15	29.9	100	15	27.3	92	15	25.5	86	15
9	30.1	15	30.8	102	15	27.4	91	15	25.6	85	15
10	31.1	15	32.0	103	15	28.0	90	15	25.8	83	15
11	31.8	15	32.5	102	15	28.3	89	15	26.5	83	15
12	34.2	15	34.3	100	15	29.1	85	15	27.1	79	15
13	35.2	15	34.5	98	15	29.8	85	15	27.2	77	15
14	35.8	15	35.8	100	15	30.5	85	15	27.1	76	15
15	35.9	15	35.9	100	15	30.6	85	15	28.2	79	15
16	37.6	15	37.2	99	15	31.9	85	15	29.0	77	15
17	38.3	15	38.8	101	15	33.3	87	15	30.2	79	15
18	39.0	15	39.4	101	15	34.2	88	15	31.1	80	15
19	39.9	15	39.4	99	15	34.4	86	15	31.0	78	15
20	40.6	15	38.6	95	15	34.4	85	15	31.4	77	15
21	41.4	15	40.2	97	15	34.8	84	15	31.4	76	15
22	42.4	15	41.5	98	15	35.5	84	15	32.4	76	15
23	43.5	15	42.2	97	15	35.7	82	15	31.5	72	15
24	43.1	15	43.1	100	15	34.2	79	15	31.7	74	15
25	44.4	15	43.3	98	15	35.9	81	15	32.6	73	15
26	45.6	15	44.7	98	15	35.8	79	15	32.7	72	15
Mean for weeks											
1-13	28.9		29.0	100		26.4	92		25.0	87	
14-26	40.6		40.0	99		33.9	84		30.8	76	

TABLE 31
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.4	15	18.8	97	15	19.2	99	15	19.4	100	15
2	20.0	15	19.6	98	15	19.6	98	15	19.7	99	15
3	21.0	15	20.7	99	15	21.0	100	14	21.2	101	15
4	21.7	15	21.4	99	15	21.4	99	14	21.8	101	15
5	22.5	15	21.9	97	15	21.5	96	14	22.1	98	15
6	23.0	15	22.1	96	15	22.7	99	14	22.8	99	15
7	23.2	15	22.4	97	15	22.5	97	14	22.8	98	15
8	23.7	15	22.9	97	15	22.8	96	14	23.2	98	15
9	23.8	15	23.3	98	15	22.9	96	14	23.5	99	15
10	24.6	15	23.9	97	15	23.4	95	14	23.5	96	14
11	24.6	15	24.0	98	15	23.2	94	14	23.7	96	14
12	25.1	15	24.6	98	15	24.3	97	14	24.3	97	14
13	26.1	15	24.8	95	15	23.9	92	14	24.4	94	14
14	25.6	15	25.5	100	15	24.0	94	14	24.5	96	14
15	25.8	15	25.0	97	14	24.0	93	14	24.9	97	14
16	26.9	15	25.7	96	14	24.8	92	14	25.6	95	14
17	28.2	15	27.0	96	14	26.2	93	14	26.7	95	14
18	28.5	15	27.3	96	14	26.5	93	14	26.9	94	14
19	28.9	15	27.5	95	14	26.9	93	14	27.3	95	14
20	29.6	15	27.9	94	14	27.1	92	14	27.6	93	14
21	29.3	15	28.4	97	14	27.4	94	14	28.1	96	14
22	29.7	15	28.9	97	14	27.6	93	14	28.1	95	14
23	30.0	15	29.2	97	14	27.5	92	14	29.0	97	14
24	30.2	15	29.2	97	14	27.8	92	14	28.9	96	14
25	31.3	15	30.4	97	14	28.7	92	14	29.1	93	14
26	31.3	15	31.0	99	14	29.3	94	14	29.6	95	14
Mean for weeks											
1-13	23.0		22.3	97		22.2	97		22.5	98	
13-26	28.9		27.9	97		26.8	93		27.4	95	

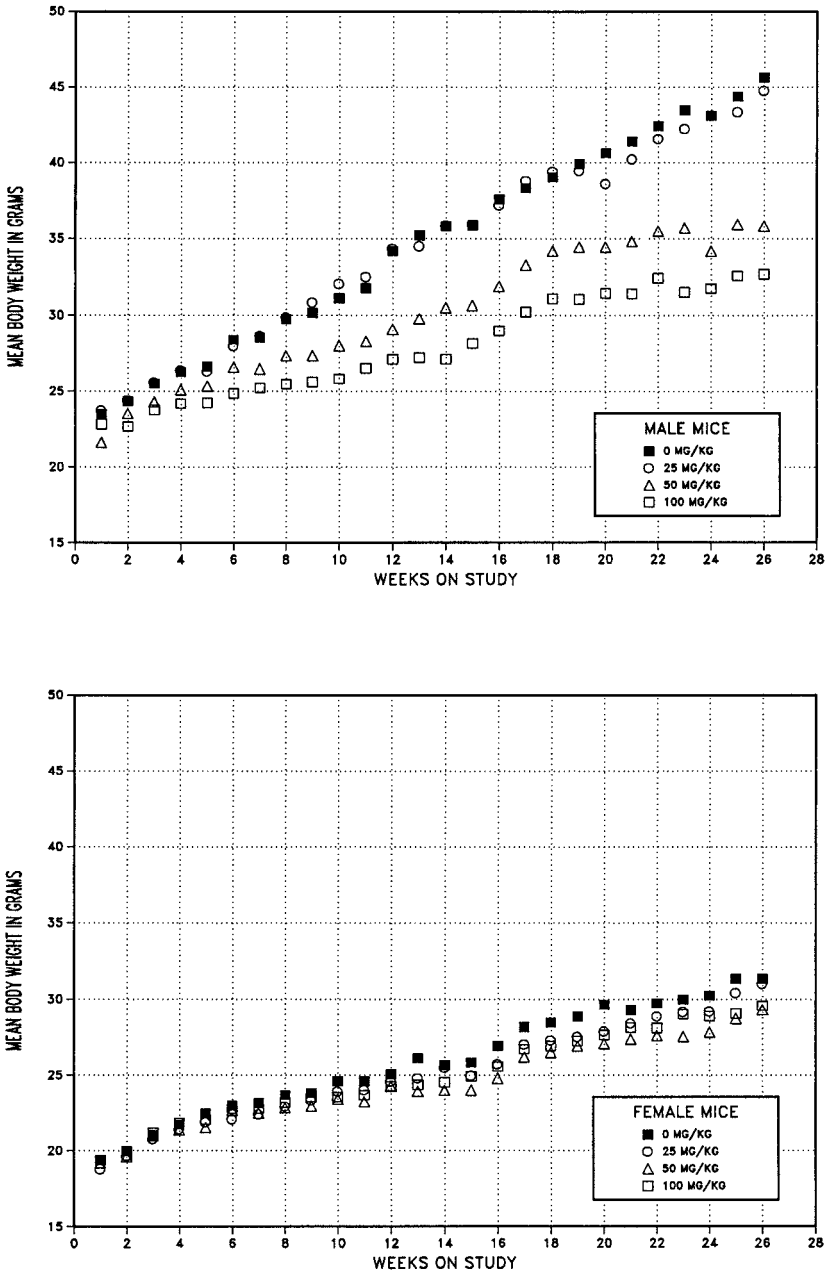


FIGURE 9
Growth Curves for Male and Female p53 Haploinsufficient Mice Administered Bromodichloromethane by Gavage for 26 Weeks

41-Week Gavage Study in p53 Haploinsufficient Mice

Survival

Estimates of 41-week survival probabilities for male and female p53 haploinsufficient mice are shown in Table 32. The survival of dosed males and females was similar to that of the vehicle control groups.

Body Weights, Clinical Findings, and Organ Weights

Mean body weights of 50 and 100 mg/kg males were less than those of the vehicle controls after week 4; mean body weights of 25, 50, and 100 mg/kg females were less after weeks 9, 14, and 24, respectively (Figure 10 and Tables 33 and 34). There were no clinical findings related to bromodichloromethane administration. The relative liver weights in 100 mg/kg males and females and in 25 and 50 mg/kg females were significantly greater than those of the vehicle controls, as was the relative right testis weight in 100 mg/kg males (Table H10). The absolute liver weight in 100 mg/kg females was increased with respect to the vehicle controls, and the absolute heart and right kidney weights in 100 mg/kg in males were decreased.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the liver and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Tables E5, E6, E7, and E8.

Liver: The incidences of hepatocyte fatty change in 100 mg/kg males and females were significantly greater than those in the vehicle controls (males: vehicle control, 6/10; 25 mg/kg, 6/10; 50 mg/kg, 5/10; 100 mg/kg, 10/10; females: 3/10, 3/10, 6/10, 9/10; Tables E6 and E8). These lesions were morphologically similar to those previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

Kidney: The incidences of renal tubule degeneration (0/10, 1/10, 0/10, 10/10) and nephropathy (4/10, 3/10, 4/10, 9/10) in 100 mg/kg males were significantly greater than those in the vehicle control group (Table E6). These lesions were morphologically similar to those previously described in the 26-week drinking water study in Tg.AC hemizygous mice.

TABLE 32
Survival of p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
Animals initially in study	10	10	10	10
Moribund	0	1	0	0
Animals surviving to study termination	10	9	10	10
Percent probability of survival at end of study[a]	100	90	100	100
Mean survival (days)[b]	288	267	288	288
Survival analysis[c]	P=1.000N	P=1.000	—[d]	—
Female				
Animals initially in study	10	10	10	10
Accidental death[e]	0	0	1	0
Moribund	0	1	1	1
Natural death	1	0	0	0
Animals surviving to study termination	9	9	8	9
Percent probability of survival at end of study	90	90	89	90
Mean survival (days)	286	288	257	286
Survival analysis	P=1.000	P=1.000N	P=1.000	P=1.000N

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, and terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by **N**.
[d] No deaths occurred in this group; value of statistic cannot be calculated.
[e] Censored from survival analyses

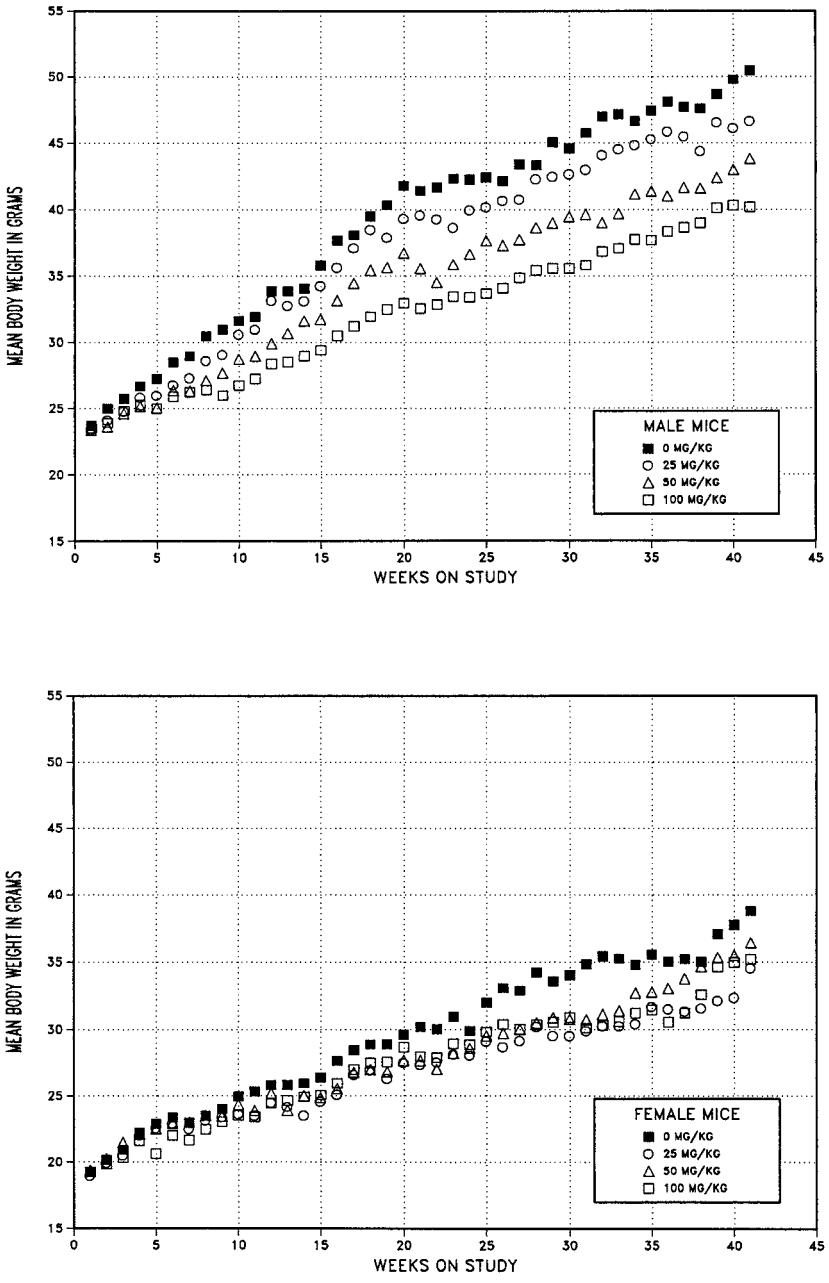


FIGURE 10
Growth Curves for Male and Female p53 Haploinsufficient Mice Administered Bromodichloromethane by Gavage for 41 Weeks

TABLE 33
Mean Body Weights and Survival of Male p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.7	10	23.4	99	10	23.4	99	10	23.5	99	10
2	25.0	10	24.1	96	10	23.6	94	10	23.9	96	10
3	25.7	10	24.8	97	10	24.6	96	10	24.8	97	10
4	26.7	10	25.8	97	10	25.2	94	10	25.3	95	10
5	27.3	10	26.0	95	10	25.1	92	10	25.0	92	10
6	28.5	10	26.7	94	10	26.4	93	10	25.9	91	10
7	29.0	10	27.3	94	10	26.3	91	10	26.2	90	10
8	30.5	10	28.6	94	10	27.1	89	10	26.4	87	10
9	31.0	10	29.1	94	10	27.7	89	10	26.0	84	10
10	31.7	10	30.6	97	10	28.7	91	10	26.8	85	10
11	31.9	10	31.0	97	10	29.0	91	10	27.2	85	10
12	33.9	10	33.1	98	9	29.9	88	10	28.4	84	10
13	33.8	10	32.8	97	9	30.7	91	10	28.5	84	10
14	34.1	10	33.1	97	9	31.6	93	10	29.0	85	10
15	35.8	10	34.2	96	9	31.8	89	10	29.4	82	10
16	37.7	10	35.6	94	9	33.2	88	10	30.5	81	10
17	38.1	10	37.1	97	9	34.4	90	10	31.2	82	10
18	39.5	10	38.5	98	9	35.4	90	10	32.0	81	10
19	40.3	10	37.9	94	9	35.6	88	10	32.5	81	10
20	41.8	10	39.3	94	9	36.7	88	10	33.0	79	10
21	41.4	10	39.6	96	9	35.6	86	10	32.6	79	10
22	41.7	10	39.2	94	9	34.5	83	10	32.9	79	10
23	42.3	10	38.6	91	9	35.9	85	10	33.5	79	10
24	42.3	10	39.9	94	9	36.7	87	10	33.4	79	10
25	42.4	10	40.1	95	9	37.7	89	10	33.7	80	10
26	42.2	10	40.6	96	9	37.3	88	10	34.1	81	10
27	43.4	10	40.7	94	9	37.7	87	10	34.9	80	10
28	43.3	10	42.3	98	9	38.7	89	10	35.4	82	10
29	45.1	10	42.5	94	9	39.0	87	10	35.6	79	10
30	44.6	10	42.6	96	9	39.5	89	10	35.6	80	10
31	45.7	10	43.0	94	9	39.6	87	10	35.8	78	10
32	47.0	10	44.1	94	9	39.0	83	10	36.9	79	10
33	47.1	10	44.5	95	9	39.7	84	10	37.1	79	10
34	46.6	10	44.8	96	9	41.2	88	10	37.8	81	10
35	47.4	10	45.3	96	9	41.4	87	10	37.7	80	10
36	48.1	10	45.8	95	9	41.0	85	10	38.4	80	10
37	47.7	10	45.4	95	9	41.7	87	10	38.7	81	10
38	47.6	10	44.4	93	9	41.6	87	10	39.0	82	10
39	48.7	10	46.5	96	9	42.4	87	10	40.1	82	10
40	49.8	10	46.1	93	9	43.0	86	10	40.3	81	10
41	50.5	10	46.6	92	9	43.8	87	10	40.2	80	10
Mean for weeks											
1-13	29.1		27.9	96		26.7	92		26.0	90	
14-41	43.7		41.4	95		38.1	87		35.0	80	

TABLE 34
Mean Body Weights and Survival of Female p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

Weeks on Study	Vehicle Control		25 mg/kg			50 mg/kg			100 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	19.3	10	18.9	98	10	19.4	101	10	19.3	100	10
2	20.1	10	19.9	99	10	20.2	101	10	19.8	99	10
3	20.9	10	20.5	98	10	21.5	103	9	20.3	97	10
4	22.2	10	22.0	99	10	22.1	100	9	21.6	97	10
5	22.9	10	22.5	98	10	22.5	98	9	20.6	90	10
6	23.4	10	22.9	98	10	23.0	98	9	22.0	94	10
7	23.0	10	22.5	98	10	23.0	100	9	21.6	94	10
8	23.5	10	23.1	98	10	23.5	100	9	22.5	96	10
9	24.0	10	23.4	98	10	23.4	98	9	23.0	96	10
10	25.0	10	23.6	94	10	24.3	97	9	23.5	94	10
11	25.3	10	23.5	93	10	23.9	95	9	23.4	93	10
12	25.8	10	24.5	95	10	25.2	98	9	24.4	95	10
13	25.8	10	24.1	93	10	23.9	93	9	24.7	96	10
14	25.9	10	23.5	91	10	25.0	97	9	24.9	96	10
15	26.4	10	24.6	93	10	24.8	94	9	25.0	95	10
16	27.6	10	25.1	91	10	25.5	92	9	25.9	94	10
17	28.5	10	26.6	93	10	26.8	94	9	27.0	95	10
18	28.9	10	26.9	93	10	27.0	93	9	27.5	95	10
19	28.9	10	26.3	91	10	26.8	93	9	27.5	95	10
20	29.6	10	27.5	93	10	27.7	94	9	28.7	97	10
21	30.2	10	27.3	90	10	27.7	92	9	28.0	93	10
22	30.0	10	27.5	92	10	27.0	90	9	27.9	93	10
23	31.0	10	28.1	91	10	28.2	91	9	28.9	93	10
24	29.9	10	28.0	94	10	28.6	96	9	28.9	97	10
25	32.0	10	29.1	91	10	29.5	92	9	29.8	93	10
26	33.1	10	28.7	87	10	29.7	90	9	30.4	92	10
27	32.9	10	29.1	88	10	30.0	91	9	30.0	91	10
28	34.2	10	30.2	88	10	30.5	89	9	30.4	89	10
29	33.6	10	29.5	88	10	30.9	92	9	30.6	91	10
30	34.0	10	29.5	87	10	30.7	90	9	30.9	91	10
31	34.8	10	29.9	86	10	30.7	88	9	30.1	87	10
32	35.4	10	30.2	85	10	31.1	88	9	30.3	86	10
33	35.3	10	30.2	86	10	31.4	89	9	30.6	87	10
34	34.8	10	30.4	87	10	32.7	94	9	31.2	90	10
35	35.5	10	31.7	89	10	32.8	92	9	31.5	89	10
36	35.0	10	31.5	90	10	33.0	94	9	30.5	87	10
37	35.2	10	31.3	89	10	33.8	96	8	31.2	89	10
38	35.0	9	31.6	90	10	34.7	99	8	32.6	93	10
39	37.1	9	32.1	87	10	35.3	95	8	34.6	93	9
40	37.8	9	32.4	86	10	35.5	94	8	35.0	93	9
41	38.8	9	34.5	89	9	36.4	94	8	35.2	91	9
Mean for weeks											
1-13	23.2		22.4	97		22.8	99		22.1	95	
14-41	32.6		29.0	89		30.1	93		29.8	92	

GENETIC TOXICOLOGY

Five independent peripheral blood micronucleus tests were conducted with combinations of two transgenic mouse strains and three routes of administration to assess the ability of bromodichloromethane to induce chromosomal damage in erythrocytes after 26 weeks of exposure. No consistent pattern of effects or clearly positive responses emerged from these studies. Equivocal responses were obtained in tests with male and female Tg.AC hemizygous mice exposed to concentrations of 175, 350, or 700 mg/L in drinking water (Table F1) and male Tg.AC hemizygous mice dermally exposed to 64, 128, or 256 mg/kg (Table F2). Results of the dermal study in female Tg.AC hemizygous mice were judged to be negative (Table F2). The equivocal responses in these tests were the result of either a significant increase in micronucleated normochromatic erythrocytes (NCEs) at a single exposed or dosed group only, in the absence of a significant trend, or a significant trend in the absence of a significant increase in micronucleated NCEs for any one exposed or dosed group. Results of the micronucleus tests in male and female Tg.AC hemizygous mice administered 25, 50, or 100 mg/kg by gavage were negative (Table F3).

In p53 haploinsufficient mice, concentrations of 175, 350, or 700 mg/L in drinking water yielded equivocal results in male mice, based on a significant increase in micronucleated NCEs only at 350 mg/L; the trend test was not significant (P=0.057; Table F4). Female p53 haploinsufficient mice exposed to bromodichloromethane via drinking water showed no increase in micronucleated NCEs (Table F4). Results of peripheral blood micronucleus tests in male and female p53 haploinsufficient mice administered 25, 50, or 100 mg/kg by gavage were negative (Table F5).

DISCUSSION AND CONCLUSIONS

Bromodichloromethane is a disinfection by-product of the chlorination of drinking water (Weisel *et al.,* 1999). Bromodichloromethane was nominated to the NTP by the United States Environmental Protection Agency (EPA) for toxicity and carcinogenicity studies in transgenic mice to provide additional information in support of EPA drinking water regulations (40 CFR Part 141). A second goal was to determine whether transgenic mouse models could prove effective in either hazard identification or in prioritizing which disinfection by-products warranted further research (Fawell *et al.,* 1997; Boorman *et al.,* 1999).

The combined use of Tg.AC hemizygous mice and p53 haploinsufficient mice has been suggested as an effective means of identifying chemical carcinogens and assessing potential risk (Tennant *et al.,* 1995). Tg.AC hemizygous mice were reported to respond to tumor promoters, mutagenic chemicals, and nonmutagenic chemicals, while p53 haploinsufficient mice responded to mutagens within 6 months, allowing the testing of more chemicals within a shorter period of time. The NTP evaluated drinking water and gavage routes of exposure both to evaluate the utility of the transgenic models and because inconsistent results had been reported between drinking water exposure and gavage administration for bromodichloromethane in 2-year rodent studies (IARC, 1999b). Dermal studies were also included for the Tg.AC hemizygous mice because it was reported that tumors usually occurred within 10 weeks of initiation of exposure (Tennant *et al.,* 1995). A visually observable and quantitative tumor response could allow evaluation of either individual chemicals or disinfection by-product mixtures as they might be found with different disinfection processes in a very rapid and cost-effective manner.

Tg.AC hemizygous mice were dermally administered 0, 64, 128, or 256 mg bromodichloromethane/kg body weight for 26 or 39 weeks. There were no neoplastic or nonneoplastic lesions associated with dermal administration in either males or females at 26 or 39 weeks. The highest dose for this dermal study was more than twice that achieved on a mg/kg basis in either the drinking water or gavage studies conducted at the same time in this strain. The dose was administered in 3.3 mL acetone/kg body weight resulting in dose concentrations of 19.4, 38.8, and 77.6 mg/mL or 19,400 to 77,600 mg/L. The maximum allowable exposure concentration for a total of four common trihalomethanes found in drinking water is 0.1 mg/L (40 CFR §141.12). Measured concentrations of bromodichloromethane in drinking water samples are usually less than 10 µg/L (Gibbons and Laha, 1999; Wright *et al.,* 2004).

In a study involving New Jersey municipal drinking water samples, the mean bromodichloromethane concentration was 5.7 µg/L with the maximum concentration of 48 µg/L (Weisel *et al.,* 1999). Nearly identical bromodichloromethane drinking water concentrations were found in samples from 109 New England towns (Wright *et al.,* 2004). The lowest concentration applied dermally in this study was more than 400,000 times the maximum bromodichloromethane concentrations reported in these studies (Weisel *et al.,* 1999; Wright *et al.,* 2004). One might conclude, based on this one dermal study, that bromodichloromethane is not toxic. Based on high cancer incidences reported in both rats and mice with bromodichloromethane gavage studies (NTP, 1987), one might alternatively conclude that dermal administration in Tg.AC hemizygous mice is a relatively insensitive model to predict toxicity or carcinogenicity of trihalomethanes.

Tg.AC hemizygous mice were exposed to drinking water containing 0, 175, 350, or 700 mg/L bromodichloromethane for 26 or 42 weeks. These exposure concentrations were the same as those used in the bromodichloromethane studies in male F344/N rats and female B6C3F$_1$ mice conducted by the NTP (2006). In the Tg.AC hemizygous mice, exposures to 0, 175, 350, or 700 mg/L corresponded to doses of 18 to 64 mg bromodichloromethane/kg body weight to males and between 28 and 130 mg/kg to females. The mean body weights of the 700 mg/L males and females tended to be less than controls at the end of the studies. Water consumption declined with increasing exposure

concentration, especially in males, suggesting that higher concentration exposures were not feasible. The incidences of hepatocyte fatty change were generally increased in exposed groups of females at 26 and 42 weeks. Nephropathy was observed in exposed groups of males, especially in the 700 mg/L group at 26 and 42 weeks. Renal tubule degeneration was also increased in male Tg.AC hemizygous mice exposed to bromodichloromethane in drinking water. This change was considered related to the bromodichloromethane exposure and was considered a less severe toxic change than nephropathy.

p53 Haploinsufficient mice were also exposed to drinking water containing 0, 175, 350, or 700 mg/L bromodichloromethane for 26 or 42 weeks. The highest exposures ranged from 55 to 65 mg bromodichloromethane/kg body weight to males and 98 to 100 mg/kg to females. The survival of exposed males and females was unaffected by bromodichloromethane exposure. The mean body weights of the 700 mg/L males and females were less than control mean body weights in both studies. Water consumption declined with increasing exposure concentration in males, but not females. The change was dramatic with 700 mg/L males drinking only 68% as much as the control group during the first 13 weeks of the study. They appeared to tolerate the bromodichloromethane somewhat better in the second half of the study, but water consumption by 700 mg/L males was only 74% to 76% that by controls at the end of the study. The incidences of renal tubule degeneration in 350 and 700 mg/L males were significantly greater than those in the control group at both 26 and 42 weeks. Whether the decreased water consumption contributed to the renal disease is not known. There was also a modest increase in nephropathy in the male mice. Incidences of fatty change in the liver were increased in females in the 26-week study and only marginally increased in the 42-week study. Increased incidences of fatty change in the liver were found in male and female rats and male mice in the NTP (1987) gavage study of bromodichloromethane, with only a marginal increase in female mice. In both the Tg.AC hemizygous and p53 haploinsufficient mice, fatty change was more pronounced in females. This may have been due to the fact that female mice tolerated the bromodichloromethane and drank more, resulting in doses nearly double those of male mice.

There were no increases in neoplasms at any site in male or female Tg.AC hemizygous or p53 haploinsufficient mice exposed to bromodichloromethane in drinking water. These results are consistent with the lack of neoplasms in other bromodichloromethane drinking water studies in female mice (George *et al.,* 2002; NTP, 2006). Thus, bromodichloromethane drinking water studies in two strains of transgenic mice dosed for up to 42 weeks and in $B6C3F_1$ mice dosed for up to 2 years provide no evidence of carcinogenicity.

The results for rats exposed to bromodichloromethane in the drinking water are less clear. George *et al.* (2002) observed increased liver neoplasms in male rats exposed to 3.9 mg bromodichloromethane/kg body weight per day in drinking water; however, as exposure levels increased, liver neoplasm incidences decreased. Rats exposed to 20.6 mg/kg had a marginal increase in liver neoplasms ($P \leq 0.1$), and those exposed to 36.3 mg/kg had liver neoplasm rates similar to the control group. This decrease in liver neoplasm rates with increasing dose cannot be explained by increased mortality, and high dose male rat weights were 96% that of the control group. In an NTP (2006) study, no increase in the incidences of neoplasms was found in male F344/N rats exposed to bromodichloromethane in drinking water at daily levels of 6, 12, and 25 mg bromodichloromethane/kg body weight. Thus, bromodichloromethane drinking water results in rats are generally negative except for a marginal increased incidence of liver neoplasms in one low dose group without increased incidences of neoplasms at higher doses.

Male and female Tg.AC hemizygous mice were administered 25, 50, or 100 mg/kg bromodichloromethane in corn oil by gavage for 26 or 41 weeks. The survival of dosed males and females was similar to that of the vehicle control groups in both studies. There was no effect of bromodichloromethane exposure on mean body weights in males, while the mean body weights of dosed females tended to be greater than those of vehicle controls. The incidences of multiple squamous cell papilloma of the forestomach in the 100 mg/kg females were significantly greater than those of the vehicle controls in both studies. However, the incidence of single forestomach papillomas in female mice generally decreased with increasing exposure concentration. It is perhaps noteworthy that the increased forestomach neoplasms were not seen in the males. There were no increases in papillomas in the integumentary system of males or females in this drinking water study. Further, there was no increase in papillomas at the site of application in dermal studies in Tg.AC hemizygous mice of either sex.

This suggests that there was no direct or systemic effect of bromodichloromethane on the squamous epithelial cells of the integumentary system. The incidences of hepatocyte fatty change were increased in dosed females compared to vehicle control groups in both studies. The incidences of renal tubule degeneration in 100 mg/kg males were significantly increased in both studies. The incidences of renal changes in male mice receiving approximately 30 or 60 mg/kg in drinking water were higher than those in mice receiving 50 or 100 mg/kg by gavage. This suggests that the decrease in water consumption associated with drinking water exposure may have contributed to the renal disease. The changes in the kidney in male mice and in the liver of female mice related to toxicity suggest that a toxic dose was reached in this gavage study.

Male and female p53 haploinsufficient mice were administered 0, 25, 50, or 100 mg bromodichloromethane/kg corn oil by gavage for 26 or 41 weeks. The survival of dosed males and females was similar to that of the vehicle control groups in both studies. The mean body weights of 50 and 100 mg/kg males were decreased as were those for females administered 50 mg/kg for 26 or 41 weeks. The incidences of fatty change in hepatocytes of 100 mg/kg females were significantly greater than those of the vehicle control groups for both studies. The incidences of renal tubule degeneration in 100 mg/kg males were significantly greater than those of the vehicle control groups for both studies. There were no increases in neoplasms at any site for male or female mice in either study.

In contrast to the 2-year studies where all four of the sex and species combinations evaluated had increased incidences of tumors with bromodichloromethane gavage exposure (NTP, 1987), only one of four of the transgenic models showed increased cancer rates. Only a marginal increase in multiple forestomach papillomas was observed in the female Tg.AC hemizygous mice. The lack of an increase in neoplasms at other sites and lack of an increase of neoplasms in the males is surprising. Tg.AC hemizygous mice are reported to be very sensitive to tumor induction (Tennant *et al.,* 1995). Male $B6C3F_1$ mice had increased incidences of kidney adenomas and adenocarcinomas when given 50 mg/kg by gavage for 2 years (NTP, 1987). In the same study, bromodichloromethane exposure caused an increase in both hepatocellular adenomas and hepatocellular carcinomas at 75 and 150 mg/kg. In the current study, doses of bromodichloromethane in the Tg.AC hemizygous mouse exceeded those that caused increased tumor incidences in the $B6C3F_1$ mouse.

Other chemicals that cause cancer in 2-year rodent studies have also failed to cause neoplasms in the Tg.AC hemizygous mouse model. *N*-methylolacrylamide exposure caused increased incidences of harderian gland, liver, and lung neoplasms in male and female $B6C3F_1$ mice as well as increased incidences of ovarian neoplasms in females in 2-year studies (NTP, 1989). *N*-methylolacrylamide failed to cause tumors in Tg.AC hemizygous mice when evaluated both by oral gavage and dermal application at the highest dose used in the 2-year study (Eastin, 1998). In a review of 38 chemicals evaluated by NIEHS/NTP in genetically altered mice, eleven (29%) of the chemicals that produced tumors in the 2-year assays were not detected as carcinogens in the transgenic mouse models (Bucher, 1998). A larger review found that the Tg.AC model has about a 77% accuracy for detecting potential carcinogens (Pritchard *et al.,* 2003).

The results in the p53 haploinsufficient mice are also somewhat surprising, as this strain is expected to respond to mutagenic chemicals. Bromodichloromethane has been shown to be mutagenic in some assays (McGregor *et al.*, 1988; DeMarini *et al.*, 1997; Landi *et al.*, 2003), although negative results have been reported in several other assays (NTP, 1987; Anderson *et al.*, 1990), perhaps as a result of inadequate exposure due to the volatility of the chemical. However, results of erythrocyte micronucleus assays reported in the current study also provide no clear indication of mutagenicity following subchronic exposure of mice to bromodichloromethane. In the current study, p53 haploinsufficient mice administered bromodichloromethane by gavage failed to produce increased incidences of neoplasms at any site.

One goal of the current studies was to determine whether genetically modified mice would prove useful as a rapid screen for disinfection by-products found in drinking water. However, these studies, which use several routes of exposure, provide no evidence that two common strains of transgenic mice are a sensitive or rapid means of assessing potential toxicity of trihalomethanes in the drinking water. The utility of these strains for assessing

other classes of disinfection by-products is under study. Another goal of the current studies was to attempt to replicate the previously observed disparity in neoplasm incidences between bromodichloromethane given by gavage and bromodichloromethane given in drinking water. Bromodichloromethane has been shown to cause colon and kidney neoplasms in rats and kidney (males) and liver (females) neoplasms in mice in 2-year gavage studies, but failed to cause neoplasms in male rats or female mice in a 2-year drinking water study (NTP, 1987, 2006). In the present study, bromodichloromethane failed to cause neoplasms in p53 haploinsufficient or male Tg.AC hemizygous mice by either route. The modest increase in multiple stomach papillomas in female Tg.AC hemizygous mice in the gavage studies but not in the drinking water studies is consistent with the route differences found in the previous 2-year studies. However, the lack of concordance between neoplasm sites in the 2-year studies (colon, liver, kidney) and the current studies suggests that the Tg.AC hemizygous mouse is perhaps not the best model for pursuing which route of bromodichloromethane exposure may be most predictive for humans. This remains the crucial question for bromodichloromethane because the role of trihalomethane exposure in the drinking water and the potential risk for colon cancer in humans is still subject to debate (Lawrence *et al.,* 1984; Young *et al.,* 1987; King *et al.,* 2000).

In conclusion, though bromodichloromethane is a known rodent carcinogen, no neoplastic lesions observed in p53 haploinsufficient mice administered bromodichloromethane by gavage or in drinking water were attributable to chemical exposure. In Tg.AC hemizygous mice administered bromodichloromethane dermally, by gavage, or in drinking water, a marginal increase in forestomach papillomas was found only in the female mice dosed by gavage. These studies suggest that these transgenic mouse models are not a sensitive and rapid means of assessing potential toxicity and carcinogenicity of trihalomethanes as they occur in the drinking water.

CONCLUSIONS

Under the conditions of these drinking water studies, there was *no evidence of carcinogenic activity** of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 175, 350, or 700 mg/L for 26 or 42 weeks.

Under the conditions of these gavage studies, there was *no evidence of carcinogenic activity** of bromodichloromethane in male or female p53 haploinsufficient mice exposed to 25, 50, or 100 mg/kg body weight 5 days per week for 26 or 41 weeks.

In both the drinking water and the gavage studies in p53 haploinsufficient mice, there were increased incidences of renal tubule degeneration in male mice and fatty change of the hepatocyte in female mice exposed to bromodichloromethane.

No treatment-related neoplasms or nonneoplastic lesions were seen in male or female Tg.AC hemizygous mice exposed dermally to 64, 128, or 256 mg bromodichloromethane/kg body weight 5 days per week for 26 or 39 weeks.

No treatment-related neoplasms were seen in male or female Tg.AC hemizygous mice exposed by drinking water to 175, 350, or 700 mg bromodichloromethane/L for 26 or 42 weeks.

No treatment-related neoplasms were seen in male Tg.AC hemizygous mice exposed by gavage to 25, 50, or 100 mg bromodichloromethane/kg body weight 5 days per week for 26 or 41 weeks. An increased incidence of multiple forestomach papillomas was seen in female Tg.AC hemizygous mice exposed to bromodichloromethane by gavage for 26 or 41 weeks.

In the drinking water and gavage studies in Tg.AC hemizygous mice, there were increased incidences of nephropathy and/or renal tubule degeneration in male mice and fatty change and/or cytoplasmic vacuolization of the hepatocyte in female mice exposed to bromodichloromethane.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 13. A summary of the Technical Reports Review Subcommittee comments and the public the discussion on this Report appears on page 15.

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APPENDIX A
SUMMARY OF LESIONS
IN Tg.AC HEMIZYGOUS MICE
IN THE DERMAL STUDIES
OF BROMODICHLOROMETHANE

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	1		
Natural deaths				2
Survivors				
Terminal sacrifice	13	14	15	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands		(1)		
Duct, carcinoma		1 (100%)		
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	4 (27%)	4 (27%)	4 (27%)
Squamous cell papilloma, multiple	1 (7%)		2 (13%)	2 (13%)
Tooth	(3)	(3)		
Odontogenic tumor	3 (100%)	3 (100%)		
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Squamous cell papilloma		3 (20%)	2 (13%)	3 (20%)
Squamous cell papilloma, multiple	1 (7%)		1 (7%)	1 (7%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma	1 (7%)			
Squamous cell carcinoma, metastatic, salivary glands		1 (7%)		
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)			

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE A1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	9	8	8
Total primary neoplasms	10	11	9	10
Total animals with benign neoplasms	5	7	8	8
Total benign neoplasms	6	7	9	10
Total animals with malignant neoplasms	1	1		
Total malignant neoplasms	1	1		
Total animals with metastatic neoplasms		1		
Total metastatic neoplasms		1		
Total animals with uncertain neoplasms-benign or malignant	3	3		
Total uncertain neoplasms	3	3		

[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
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	1		
Natural deaths				2
Survivors				
Terminal sacrifice	13	14	15	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	1 (7%)	1 (7%)		1 (7%)
Inflammation	8 (53%)	8 (53%)	5 (33%)	6 (40%)
Necrosis	1 (7%)	1 (7%)	1 (7%)	2 (13%)
Hepatocyte, fatty change	1 (7%)			
Hepatocyte, vacuolization cytoplasmic	4 (27%)	2 (13%)	5 (33%)	4 (27%)
Mesentery			(1)	
Fat, necrosis			1 (100%)	
Stomach, forestomach	(15)	(15)	(15)	(15)
Hyperkeratosis				1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hyperplasia	1 (7%)			
Hypertrophy	9 (60%)	9 (60%)	6 (40%)	8 (53%)
Vacuolization cytoplasmic				1 (7%)
Subcapsular, hyperplasia	1 (7%)	1 (7%)		2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)
Cyst	1 (7%)		2 (13%)	
Genital System				
Testes	(15)	(15)	(15)	(15)
Germinal epithelium, degeneration	3 (20%)	1 (7%)	2 (13%)	1 (7%)
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(14)	(14)
Atrophy				1 (7%)
Infiltration cellular, plasma cell	1 (7%)			
Lymph node, mesenteric	(14)	(15)	(15)	(15)
Atrophy		1 (7%)		
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	1 (7%)	2 (13%)	1 (7%)	1 (7%)
Lymphoid follicle, atrophy				1 (7%)
Lymphoid follicle, hyperplasia				1 (7%)
Thymus	(15)	(15)	(15)	(15)
Atrophy	1 (7%)	1 (7%)		2 (13%)

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

TABLE A2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Epidermis, hyperplasia, focal	1 (7%)			
Subcutaneous tissue, inflammation		1 (7%)		
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation		1 (7%)	1 (7%)	
Thrombosis			1 (7%)	
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	3 (20%)	1 (7%)		2 (13%)
Cyst	1 (7%)			1 (7%)
Hydronephrosis	1 (7%)			1 (7%)
Inflammation				1 (7%)
Nephropathy	7 (47%)	6 (40%)	7 (47%)	6 (40%)
Artery, inflammation	1 (7%)		1 (7%)	
Renal tubule, dilatation	1 (7%)			1 (7%)
Renal tubule, hypertrophy				1 (7%)
Renal tubule, necrosis				1 (7%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	3	1	4
Natural deaths	2	2	2	1
Survivors				
Terminal sacrifice	11	10	12	10
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands	(1)			
Duct, carcinoma	1 (100%)			
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma		3 (20%)	2 (13%)	4 (27%)
Squamous cell papilloma, multiple	2 (13%)	1 (7%)	3 (20%)	
Tooth	(2)	(3)	(2)	(1)
Odontogenic tumor	2 (100%)	3 (100%)	2 (100%)	1 (100%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Adrenal medulla	(15)	(15)	(15)	(15)
Pituitary gland	(15)	(15)	(15)	(15)
Genital System				
Ovary	(15)	(14)	(15)	(15)
Uterus	(15)	(15)	(15)	(15)
Vagina			(1)	
Squamous cell papilloma			1 (100%)	
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(15)
Spleen	(15)	(15)	(15)	(15)
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Squamous cell papilloma	1 (7%)	4 (27%)	2 (13%)	1 (7%)
Skin, site of application, squamous cell papilloma		1 (7%)		
Special Senses System				
Zymbal's gland				(1)
Squamous cell papilloma				1 (100%)

TABLE A3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic		1 (7%)		1 (7%)
Leukemia granulocytic		1 (7%)		
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	6	11	8	6
Total primary neoplasms	6	14	10	8
Total animals with benign neoplasms	3	8	7	5
Total benign neoplasms	3	9	8	6
Total animals with malignant neoplasms	1	2		1
Total malignant neoplasms	1	2		1
Total animals with uncertain neoplasms-benign or malignant	2	3	2	1
Total uncertain neoplasms	2	3	2	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 A4
Summary of the Incidence Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	3	1	4
Natural deaths	2	2	2	1
Survivors				
Terminal sacrifice	11	10	12	10
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	1 (7%)	1 (7%)	1 (7%)	3 (20%)
Inflammation	14 (93%)	11 (73%)	14 (93%)	11 (73%)
Necrosis	3 (20%)	2 (13%)	4 (27%)	2 (13%)
Hepatocyte, vacuolization cytoplasmic	3 (20%)	7 (47%)	5 (33%)	5 (33%)
Mesentery	(1)			
Necrosis	1 (100%)			
Stomach, forestomach	(15)	(15)	(15)	(15)
Inflammation			1 (7%)	
Epithelium, hyperkeratosis	1 (7%)			
Epithelium, hyperplasia	1 (7%)			
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Subcapsular, hyperplasia	8 (53%)	8 (53%)	11 (73%)	5 (33%)
Pituitary gland	(15)	(15)	(15)	(15)
Cyst	4 (27%)	1 (7%)	1 (7%)	2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)
Cyst	2 (13%)	1 (7%)	1 (7%)	1 (7%)
Genital System				
Ovary	(15)	(14)	(15)	(15)
Cyst	1 (7%)			2 (13%)
Uterus	(15)	(15)	(15)	(15)
Inflammation, suppurative	2 (13%)		1 (7%)	
Endometrium, hyperplasia, cystic	7 (47%)	6 (40%)	10 (67%)	6 (40%)
Hematopoietic System				
Lymph node	(1)			
Mediastinal, hyperplasia	1 (100%)			
Lymph node, mandibular	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation				1 (7%)
Infiltration cellular, plasma cell		1 (7%)	1 (7%)	1 (7%)
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	2 (13%)	1 (7%)	1 (7%)	4 (27%)
Thymus	(15)	(15)	(15)	(14)
Atrophy		3 (20%)	1 (7%)	

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

TABLE A4
Summary of the Incidence Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Integumentary System				
Skin	(15)	(15)	(15)	(15)
Epidermis, hyperplasia, focal			1 (7%)	
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation	1 (7%)			
Alveolar epithelium, hyperplasia	1 (7%)			
Artery, inflammation		1 (7%)		
Special Senses System				
Eye		(1)		(1)
Retina, degeneration		1 (100%)		1 (100%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	6 (40%)	5 (33%)	5 (33%)	4 (27%)
Cyst	2 (13%)	2 (13%)		1 (7%)
Hydronephrosis			1 (7%)	
Mineralization		1 (7%)		
Nephropathy			2 (13%)	3 (20%)
Artery, inflammation		1 (7%)		1 (7%)
Renal tubule, hypertrophy		1 (7%)	1 (7%)	4 (27%)
Renal tubule, necrosis	1 (7%)			

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System

TABLE A5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	3		1	1
Natural deaths	1	2		1
Survivors				
Terminal sacrifice	6	8	9	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Salivary glands	(1)			
Duct, carcinoma	1 (100%)			
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	4 (40%)	3 (30%)	3 (30%)
Squamous cell papilloma, multiple	1 (10%)	2 (20%)	2 (20%)	3 (30%)
Tooth	(3)	(1)	(2)	(2)
Odontogenic tumor	3 (100%)	1 (100%)	2 (100%)	2 (100%)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Squamous cell papilloma	4 (40%)	1 (10%)		1 (10%)
Squamous cell papilloma, multiple	2 (20%)	4 (40%)	7 (70%)	2 (20%)
Site of application, squamous cell papilloma			1 (10%)	
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma		2 (20%)		
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic		1 (10%)		

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE A5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	7	8	9	9
Total primary neoplasms	13	15	15	11
Total animals with benign neoplasms	6	7	9	8
Total benign neoplasms	9	13	13	9
Total animals with malignant neoplasms	1	1		
Total malignant neoplasms	1	1		
Total animals with uncertain neoplasms-benign or malignant	3	1	2	2
Total uncertain neoplasms	3	1	2	2

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

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	3		1	1
Natural deaths	1	2		1
Survivors				
Terminal sacrifice	6	8	9	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	3 (30%)	1 (10%)	1 (10%)	1 (10%)
Inflammation	6 (60%)	3 (30%)	6 (60%)	3 (30%)
Necrosis	1 (10%)	1 (10%)	1 (10%)	1 (10%)
Hepatocyte, fatty change		1 (10%)		
Hepatocyte, vacuolization cytoplasmic	6 (60%)	7 (70%)	8 (80%)	7 (70%)
Mesentery			(1)	
Fat, necrosis			1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis				1 (10%)
Epithelium, hyperplasia	2 (20%)			
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	5 (50%)	3 (30%)	4 (40%)	4 (40%)
Subcapsular, hyperplasia			1 (10%)	1 (10%)
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	1 (10%)	2 (20%)	1 (10%)	2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Cyst				3 (30%)
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Inflammation			1 (10%)	
Testes	(10)	(10)	(10)	(10)
Germinal epithelium, degeneration	1 (10%)	2 (20%)		1 (10%)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia		1 (10%)	1 (10%)	
Infiltration cellular, plasma cell	2 (20%)			
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	4 (40%)	2 (20%)	3 (30%)	1 (10%)
Pigmentation				1 (10%)
Lymphoid follicle, atrophy				1 (10%)
Thymus	(9)	(10)	(9)	(9)
Atrophy	1 (11%)		2 (22%)	1 (11%)
Cyst	4 (44%)	2 (20%)	1 (11%)	3 (33%)

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

TABLE A6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Hyperplasia		1 (10%)		1 (10%)
Inflammation				1 (10%)
Inflammation, focal	1 (10%)	2 (20%)	1 (10%)	
Ulcer				1 (10%)
Control epidermis, hyperplasia				1 (10%)
Dermis skin, site of application, inflammation				1 (10%)
Epidermis, hyperplasia, focal	1 (10%)	1 (10%)		
Epidermis, skin, site of application, hyperplasia			1 (10%)	1 (10%)
Epidermis, skin, site of application, hyperplasia, focal	1 (10%)			
Epidermis, skin, site of application, inflammation, focal	1 (10%)			
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation	1 (10%)		1 (10%)	
Alveolar epithelium, hyperplasia			1 (10%)	
Perivascular, inflammation	1 (10%)		1 (10%)	1 (10%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein	1 (10%)	2 (20%)	4 (40%)	2 (20%)
Cyst	1 (10%)	1 (10%)	2 (20%)	2 (20%)
Hydronephrosis		2 (20%)		
Nephropathy	6 (60%)	5 (50%)	3 (30%)	6 (60%)
Artery, inflammation			1 (10%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE A7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	4	4	3	4
Natural deaths	1	2		1
Survivors				
Terminal sacrifice	5	4	7	5
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Leiomyosarcoma, metastatic, vagina	1 (10%)			
Mesentery				(1)
Salivary glands			(1)	
Duct, carcinoma			1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma		2 (20%)	3 (30%)	2 (20%)
Squamous cell papilloma, multiple	1 (10%)	2 (20%)	2 (20%)	2 (20%)
Tooth	(4)	(4)	(4)	(3)
Odontogenic tumor	4 (100%)	3 (75%)	3 (75%)	3 (100%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Leiomyosarcoma, metastatic, vagina	1 (10%)			
Uterus	(10)	(10)	(10)	(10)
Vagina	(2)	(1)	(2)	(4)
Leiomyosarcoma	1 (50%)			
Squamous cell papilloma	1 (50%)	1 (100%)	2 (100%)	2 (50%)
Hematopoietic System				
Lymph node	(1)	(1)		(1)
Lymph node, mandibular	(10)	(10)	(10)	(9)
Lymph node, mesenteric	(9)	(9)	(10)	(10)
Leiomyosarcoma, metastatic, vagina	1 (11%)			
Spleen	(10)	(10)	(10)	(10)
Thymus	(10)	(10)	(10)	(10)
Integumentary System				
Mammary gland	(10)	(10)	(10)	(10)
Skin	(10)	(10)	(10)	(10)
Squamous cell carcinoma	1 (10%)			
Squamous cell papilloma	4 (40%)	3 (30%)	5 (50%)	1 (10%)
Squamous cell papilloma, multiple	2 (20%)		2 (20%)	
Vulva, squamous cell papilloma				1 (10%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma			1 (10%)	1 (10%)

TABLE A7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic	2 (20%)	3 (30%)		2 (20%)
Lymphoma malignant				1 (10%)
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	8	8	9	9
Total primary neoplasms	16	14	19	15
Total animals with benign neoplasms	6	5	8	4
Total benign neoplasms	8	8	15	9
Total animals with malignant neoplasms	4	3	1	3
Total malignant neoplasms	4	3	1	3
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	3			
Total animals with uncertain neoplasms-benign or malignant	4	3	3	3
Total uncertain neoplasms	4	3	3	3

[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 A8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	4	4	3	4
Natural deaths	1	2		1
Survivors				
Terminal sacrifice	5	4	7	5
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	4 (40%)	2 (20%)	1 (10%)	4 (40%)
Inflammation	7 (70%)	6 (60%)	9 (90%)	5 (50%)
Necrosis		1 (10%)	1 (10%)	
Hepatocyte, vacuolization cytoplasmic	4 (40%)	4 (40%)	7 (70%)	6 (60%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Epithelium, hyperplasia	1 (10%)			1 (10%)
Tooth	(4)	(4)	(4)	(3)
Peridontal tissue, hyperplasia		1 (25%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation			1 (10%)	1 (10%)
Subcapsular, hyperplasia	7 (70%)	5 (50%)	5 (50%)	7 (70%)
Parathyroid gland	(1)			
Cyst	1 (100%)			
Pituitary gland	(10)	(10)	(10)	(10)
Cyst		1 (10%)	1 (10%)	1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	3 (30%)	1 (10%)	1 (10%)
Inflammation				1 (10%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Atrophy	1 (10%)		3 (30%)	1 (10%)
Cyst	1 (10%)	1 (10%)	2 (20%)	1 (10%)
Inflammation		1 (10%)		
Inflammation, suppurative	1 (10%)			
Oviduct			(1)	
Inflammation			1 (100%)	
Uterus	(10)	(10)	(10)	(10)
Hydrometra			1 (10%)	
Inflammation		1 (10%)		
Endometrium, hyperplasia, cystic	5 (50%)	4 (40%)	5 (50%)	7 (70%)

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

TABLE A8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(9)
Hyperplasia	3 (30%)		1 (10%)	1 (11%)
Infiltration cellular, plasma cell		1 (10%)		
Lymph node, mesenteric	(9)	(9)	(10)	(10)
Hyperplasia	1 (11%)			
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	3 (30%)	2 (20%)	1 (10%)	2 (20%)
Lymphoid follicle, atrophy		1 (10%)		
Thymus	(10)	(10)	(10)	(10)
Atrophy	2 (20%)	6 (60%)	2 (20%)	2 (20%)
Cyst	3 (30%)	2 (20%)	2 (20%)	3 (30%)
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Hyperplasia			2 (20%)	1 (10%)
Inflammation, focal	2 (20%)	1 (10%)		1 (10%)
Control epidermis, hyperplasia	2 (20%)			1 (10%)
Epidermis, hyperplasia, focal	1 (10%)	1 (10%)	1 (10%)	1 (10%)
Epidermis, skin, site of application, hyperplasia		1 (10%)		1 (10%)
Epidermis, skin, site of application, inflammation		1 (10%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation	1 (10%)			
Alveolar epithelium, hyperplasia			2 (20%)	
Perivascular, infiltration cellular, mononuclear cell	1 (10%)			
Perivascular, inflammation				1 (10%)
Special Senses System				
Eye				(1)
Retina, degeneration				1 (100%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Accumulation, hyaline droplet	1 (10%)	1 (10%)		
Casts protein	2 (20%)	1 (10%)	1 (10%)	1 (10%)
Cyst	1 (10%)			2 (20%)
Hydronephrosis	1 (10%)	1 (10%)		
Inflammation		1 (10%)		
Mineralization			1 (10%)	
Nephropathy	2 (20%)	1 (10%)	4 (40%)	1 (10%)
Artery, inflammation	1 (10%)			
Glomerulus, inflammation, membranoproliferative	1 (10%)	3 (30%)		2 (20%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System

APPENDIX B
SUMMARY OF LESIONS IN Tg.AC HEMIZYGOUS MICE IN THE DRINKING WATER STUDIES OF BROMODICHLOROMETHANE

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	3	3	1
Survivors				
Terminal sacrifice	13	12	12	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands	(1)	(2)		(1)
Duct, carcinoma		2 (100%)		1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	3 (20%)	5 (33%)	3 (20%)
Squamous cell papilloma, multiple	2 (13%)	2 (13%)	1 (7%)	1 (7%)
Tooth	(4)	(4)	(3)	(2)
Odontogenic tumor	4 (100%)	4 (100%)	3 (100%)	2 (100%)
Integumentary System				
Skin	(6)	(2)	(2)	(1)
Squamous cell papilloma	2 (33%)	2 (100%)	1 (50%)	
Squamous cell papilloma, multiple	4 (67%)			1 (100%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Carcinoma, metastatic, salivary glands		1 (7%)		
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic		1 (7%)		

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE B1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	11	9	7
Total primary neoplasms	15	14	10	8
Total animals with benign neoplasms	9	6	7	5
Total benign neoplasms	11	7	7	5
Total animals with malignant neoplasms		3		1
Total malignant neoplasms		3		1
Total animals with metastatic neoplasms		1		
Total metastatic neoplasms		1		
Total animals with uncertain neoplasms-benign or malignant	4	4	3	2
Total uncertain neoplasms	4	4	3	2

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

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	2	3	3	1
Survivors				
Terminal sacrifice	13	12	12	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Esophagus	(1)			
Hyperkeratosis	1 (100%)			
Intestine small, duodenum	(15)	(15)	(15)	(15)
Erosion				1 (7%)
Liver	(15)	(15)	(15)	(15)
Cyst				1 (7%)
Hematopoietic cell proliferation	1 (7%)	1 (7%)		
Inflammation	7 (47%)	6 (40%)	7 (47%)	5 (33%)
Necrosis	3 (20%)	1 (7%)	3 (20%)	3 (20%)
Hepatocyte, vacuolization cytoplasmic	6 (40%)	4 (27%)	3 (20%)	3 (20%)
Salivary glands	(1)	(2)		(1)
Inflammation	1 (100%)			
Inflammation, chronic active		1 (50%)		
Stomach, forestomach	(15)	(15)	(15)	(15)
Epithelium, hyperplasia				1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hyperplasia	1 (7%)			
Hypertrophy	6 (40%)	7 (47%)	7 (47%)	9 (60%)
Subcapsular, hyperplasia		1 (7%)	1 (7%)	1 (7%)
Pituitary gland	(15)	(15)	(15)	(15)
Cyst		1 (7%)		
Inflammation, suppurative	1 (7%)			
Necrosis	1 (7%)			
Thyroid gland	(15)	(15)	(15)	(15)
Cyst	2 (13%)	1 (7%)		1 (7%)
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Inflammation			1 (7%)	
Preputial gland	(3)			(1)
Inflammation	2 (67%)			1 (100%)
Duct, ectasia	3 (100%)			1 (100%)
Testes	(15)	(15)	(15)	(15)
Hypoplasia		1 (7%)		
Germinal epithelium, degeneration	3 (20%)	2 (13%)	3 (20%)	3 (20%)

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

TABLE B2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(14)	(13)
Hyperplasia		1 (7%)		
Infiltration cellular, plasma cell		1 (7%)	1 (7%)	
Inflammation	1 (7%)			
Necrosis		1 (7%)		
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	4 (27%)	2 (13%)		
Lymphoid follicle, atrophy	1 (7%)			1 (7%)
Thymus	(15)	(14)	(15)	(15)
Atrophy		1 (7%)	1 (7%)	1 (7%)
Integumentary System				
Skin	(6)	(2)	(2)	(1)
Epidermis, hyperplasia, focal			1 (50%)	
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation	1 (7%)		1 (7%)	1 (7%)
Thrombosis			1 (7%)	1 (7%)
Alveolar epithelium, hyperplasia	1 (7%)	2 (13%)		
Special Senses System				
Eye		(1)		
Retina, degeneration		1 (100%)		
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	1 (7%)	2 (13%)	1 (7%)	1 (7%)
Cyst		2 (13%)	3 (20%)	
Mineralization				2 (13%)
Nephropathy	4 (27%)	3 (20%)	4 (27%)	11 (73%)
Renal tubule, degeneration		4 (27%)	4 (27%)	9 (60%)
Renal tubule, dilatation	4 (27%)	11 (73%)	14 (93%)	15 (100%)
Renal tubule, hypertrophy	1 (7%)	3 (20%)	6 (40%)	11 (73%)
Renal tubule, necrosis				1 (7%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		3	1
Natural deaths	4	2	1	1
Survivors				
Terminal sacrifice	10	13	11	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands			(1)	(1)
Duct, carcinoma				1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	2 (13%)	2 (13%)	4 (27%)
Squamous cell papilloma, multiple	1 (7%)	2 (13%)	2 (13%)	2 (13%)
Tooth	(3)	(2)	(4)	(2)
Odontogenic tumor	3 (100%)	2 (100%)	3 (75%)	2 (100%)
Odontogenic tumor, multiple			1 (25%)	
Genital System				
Vagina	(2)	(2)	(1)	
Squamous cell papilloma	2 (100%)	2 (100%)		
Integumentary System				
Skin		(1)	(6)	(3)
Squamous cell papilloma		1 (100%)	4 (67%)	1 (33%)
Squamous cell papilloma, multiple			1 (17%)	2 (67%)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	2 (13%)			

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE B3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	9	10	9
Total primary neoplasms	11	9	13	12
Total animals with benign neoplasms	6	7	7	8
Total benign neoplasms	6	7	9	9
Total animals with malignant neoplasms	2			1
Total malignant neoplasms	2			1
Total animals with uncertain neoplasms-benign or malignant	3	2	4	2
Total uncertain neoplasms	3	2	4	2

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

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Moribund	1		3	1
Natural deaths	4	2	1	1
Survivors				
Terminal sacrifice	10	13	11	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Esophagus	(1)			
Hyperkeratosis	1 (100%)			
Liver	(15)	(15)	(15)	(15)
Basophilic focus			1 (7%)	
Hematopoietic cell proliferation	1 (7%)	1 (7%)	3 (20%)	1 (7%)
Inflammation	11 (73%)	11 (73%)	13 (87%)	14 (93%)
Mitotic alteration				1 (7%)
Necrosis		2 (13%)		1 (7%)
Hepatocyte, fatty change		4 (27%)	8 (53%)	10 (67%)
Hepatocyte, hypertrophy	1 (7%)	2 (13%)	8 (53%)	12 (80%)
Hepatocyte, vacuolization cytoplasmic	2 (13%)	5 (33%)	4 (27%)	8 (53%)
Salivary glands			(1)	(1)
Inflammation			1 (100%)	
Stomach, forestomach	(15)	(15)	(15)	(15)
Hyperkeratosis		1 (7%)		1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			1 (7%)	1 (7%)
Hypertrophy			1 (7%)	
Subcapsular, hyperplasia	8 (53%)	8 (53%)	7 (47%)	10 (67%)
Pituitary gland	(15)	(15)	(15)	(15)
Cyst	1 (7%)	1 (7%)		1 (7%)
Hypertrophy				1 (7%)
Thyroid gland	(15)	(14)	(15)	(15)
Cyst	1 (7%)	1 (7%)	2 (13%)	1 (7%)
Inflammation				1 (7%)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Cyst		2 (13%)	1 (7%)	
Oviduct	(1)			
Inflammation, suppurative	1 (100%)			
Uterus	(15)	(15)	(15)	(15)
Hydrometra		1 (7%)		
Inflammation, suppurative	1 (7%)			
Endometrium, hyperplasia, cystic	9 (60%)	8 (53%)	9 (60%)	8 (53%)

[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 Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(15)
Hyperplasia		1 (7%)	1 (7%)	
Infiltration cellular, plasma cell	1 (7%)			1 (7%)
Infiltration cellular, polymorphonuclear	1 (7%)	1 (7%)		
Spleen	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Hematopoietic cell proliferation	2 (13%)	3 (20%)	3 (20%)	1 (7%)
Lymphoid follicle, atrophy	1 (7%)		1 (7%)	
Red pulp, atrophy				1 (7%)
Thymus	(14)	(15)	(15)	(14)
Atrophy	2 (14%)	1 (7%)	1 (7%)	1 (7%)
Integumentary System				
Skin		(1)	(6)	(3)
Epidermis, hyperplasia, focal			1 (17%)	
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Infiltration cellular, histiocyte				1 (7%)
Inflammation			1 (7%)	1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein		1 (7%)	3 (20%)	2 (13%)
Cyst	2 (13%)	3 (20%)	1 (7%)	2 (13%)
Inflammation		1 (7%)		
Nephropathy	1 (7%)		1 (7%)	4 (27%)
Renal tubule, hypertrophy	1 (7%)	2 (13%)	5 (33%)	2 (13%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE B5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	4			1
Natural deaths		1	2	
Survivors				
Terminal sacrifice	6	9	8	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine large, rectum		(1)		
Anus, squamous cell papilloma		1 (100%)		
Liver	(10)	(10)	(10)	(10)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	4 (40%)	4 (40%)	4 (40%)	2 (20%)
Squamous cell papilloma, multiple	3 (30%)	2 (20%)	3 (30%)	4 (40%)
Tooth	(3)	(2)	(3)	(2)
Odontogenic tumor	3 (100%)	2 (100%)	3 (100%)	2 (100%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Adenoma				1 (10%)
Adrenal medulla	(10)	(10)	(10)	(10)
Pituitary gland	(10)	(10)	(10)	(10)
Integumentary System				
Skin	(8)	(8)	(8)	(8)
Squamous cell papilloma		1 (13%)		3 (38%)
Squamous cell papilloma, multiple	8 (100%)	5 (63%)	8 (100%)	5 (63%)
Subcutaneous tissue, sarcoma		1 (13%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma		2 (20%)	1 (10%)	1 (10%)
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic	1 (10%)		1 (10%)	1 (10%)

TABLE B5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	10	10	10
Total primary neoplasms	19	18	20	19
Total animals with benign neoplasms	8	9	9	9
Total benign neoplasms	15	15	16	16
Total animals with malignant neoplasms	1	1	1	1
Total malignant neoplasms	1	1	1	1
Total animals with uncertain neoplasms-benign or malignant	3	2	3	2
Total uncertain neoplasms	3	2	3	2

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

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	4			1
Natural deaths		1	2	
Survivors				
Terminal sacrifice	6	9	8	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Esophagus			(1)	
Inflammation			1 (100%)	
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)	1 (10%)	1 (10%)
Inflammation	5 (50%)	4 (40%)	8 (80%)	7 (70%)
Necrosis			1 (10%)	1 (10%)
Hepatocyte, fatty change		3 (30%)	2 (20%)	
Hepatocyte, vacuolization cytoplasmic	7 (70%)	9 (90%)	8 (80%)	8 (80%)
Mesentery	(1)		(1)	
Fat, necrosis	1 (100%)		1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis		1 (10%)		
Epithelium, hyperplasia	1 (10%)			
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	5 (50%)	4 (40%)	4 (40%)	4 (40%)
Subcapsular, hyperplasia	1 (10%)	1 (10%)	2 (20%)	
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	3 (30%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	2 (20%)	1 (10%)	2 (20%)
Follicle, cyst		1 (10%)		1 (10%)
Genital System				
Preputial gland	(1)			
Duct, ectasia	1 (100%)			
Testes	(10)	(10)	(10)	(10)
Mineralization	2 (20%)			
Germinal epithelium, degeneration	3 (30%)		1 (10%)	1 (10%)

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

TABLE B6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Angiectasis			1 (10%)	
Hyperplasia			1 (10%)	
Infiltration cellular, plasma cell	1 (10%)	1 (10%)	1 (10%)	2 (20%)
Spleen	(10)	(10)	(10)	(10)
Atrophy		1 (10%)		
Hematopoietic cell proliferation		1 (10%)	1 (10%)	1 (10%)
Thymus	(9)	(9)	(10)	(10)
Atrophy	1 (11%)	1 (11%)	1 (10%)	
Cyst	2 (22%)	3 (33%)	4 (40%)	5 (50%)
Integumentary System				
Skin	(8)	(8)	(8)	(8)
Hyperplasia		2 (25%)	1 (13%)	1 (13%)
Inflammation	1 (13%)			
Control epidermis, hyperplasia	2 (25%)			
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	1 (10%)			
Inflammation		2 (20%)	1 (10%)	
Alveolar epithelium, hyperplasia	1 (10%)			1 (10%)
Perivascular, inflammation			1 (10%)	3 (30%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein	2 (20%)		1 (10%)	4 (40%)
Cyst	3 (30%)	1 (10%)	1 (10%)	
Hydronephrosis	1 (10%)		2 (20%)	
Infiltration cellular, plasma cell			1 (10%)	
Mineralization				2 (20%)
Nephropathy	4 (40%)	7 (70%)	8 (80%)	9 (90%)
Artery, inflammation		1 (10%)		
Glomerulus, inflammation, membranoproliferative				1 (10%)
Renal tubule, degeneration			2 (20%)	6 (60%)
Renal tubule, dilatation	1 (10%)	8 (80%)	8 (80%)	10 (100%)
Renal tubule, hypertrophy				1 (10%)
Renal tubule, vacuolization cytoplasmic	1 (10%)			

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

TABLE B7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	3	2	5	5
Natural deaths	2		1	1
Survivors				
Terminal sacrifice	5	8	4	4
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Salivary glands	(1)			
Duct, carcinoma	1 (100%)			
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	1 (10%)	3 (30%)	4 (40%)
Squamous cell papilloma, multiple	3 (30%)	2 (20%)	3 (30%)	1 (10%)
Tooth	(3)	(3)	(3)	(2)
Odontogenic tumor	3 (100%)	3 (100%)	3 (100%)	2 (100%)
Genital System				
Ovary	(10)	(10)	(10)	(10)
Uterus	(10)	(10)	(10)	(10)
Leiomyosarcoma			1 (10%)	
Sarcoma stromal		1 (10%)		
Integumentary System				
Skin	(8)	(8)	(8)	(6)
Squamous cell papilloma	2 (25%)	1 (13%)	3 (38%)	1 (17%)
Squamous cell papilloma, multiple	4 (50%)	6 (75%)	4 (50%)	4 (67%)
Subcutaneous tissue, lipoma		1 (13%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma			1 (10%)	
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic	1 (10%)			1 (10%)

TABLE B7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	9	10	10	9
Total primary neoplasms	16	15	18	13
Total animals with benign neoplasms	7	8	8	7
Total benign neoplasms	11	11	14	10
Total animals with malignant neoplasms	2	1	1	1
Total malignant neoplasms	2	1	1	1
Total animals with uncertain neoplasms-benign or malignant	3	3	3	2
Total uncertain neoplasms	3	3	3	2

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

TABLE B8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	3	2	5	5
Natural deaths	2		1	1
Survivors				
Terminal sacrifice	5	8	4	4
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine large, colon	(10)	(10)	(10)	(10)
Necrosis`	1 (10%)			
Epithelium, regeneration	1 (10%)			
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)	3 (30%)	1 (10%)	1 (10%)
Infiltration cellular	2 (20%)			
Inflammation	5 (50%)	10 (100%)	8 (80%)	7 (70%)
Necrosis	2 (20%)		2 (20%)	
Hepatocyte, fatty change		6 (60%)	6 (60%)	6 (60%)
Hepatocyte, vacuolization cytoplasmic	5 (50%)	8 (80%)	8 (80%)	8 (80%)
Mesentery	(1)	(1)		(1)
Inflammation, suppurative	1 (100%)			
Artery, inflammation		1 (100%)		1 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis			1 (10%)	
Epithelium, hyperplasia	3 (30%)		1 (10%)	1 (10%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)			
Subcapsular, hyperplasia	6 (60%)	5 (50%)	7 (70%)	8 (80%)
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	3 (30%)	1 (10%)	2 (20%)
Hyperplasia	1 (10%)			
Thyroid gland	(10)	(10)	(10)	(10)
Cyst		1 (10%)	1 (10%)	
Inflammation	2 (20%)	1 (10%)	1 (10%)	
General Body System				
Peritoneum	(1)			
Inflammation	1 (100%)			

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

TABLE B8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Genital System				
Ovary	(10)	(10)	(10)	(10)
Atrophy	2 (20%)	2 (20%)	1 (10%)	2 (20%)
Cyst	1 (10%)	1 (10%)	1 (10%)	
Inflammation		2 (20%)		
Inflammation, suppurative	2 (20%)			
Oviduct	(1)			
Inflammation	1 (100%)			
Uterus	(10)	(10)	(10)	(10)
Inflammation	1 (10%)		1 (10%)	
Endometrium, hyperplasia, cystic	7 (70%)	9 (90%)	6 (60%)	6 (60%)
Hematopoietic System				
Lymph node	(2)			
Mediastinal, infiltration cellular, plasma cell	1 (50%)			
Renal, hyperplasia	1 (50%)			
Lymph node, mandibular	(10)	(10)	(10)	(10)
Atrophy			1 (10%)	
Hyperplasia		1 (10%)	2 (20%)	
Infiltration cellular, plasma cell	1 (10%)	1 (10%)		2 (20%)
Lymph node, mesenteric	(10)	(10)	(9)	(10)
Hematopoietic cell proliferation	1 (10%)			
Spleen	(10)	(10)	(10)	(10)
Atrophy			1 (10%)	1 (10%)
Hematopoietic cell proliferation	3 (30%)	3 (30%)		2 (20%)
Thymus	(10)	(10)	(10)	(10)
Atrophy	4 (40%)	2 (20%)	2 (20%)	2 (20%)
Cyst	3 (30%)	3 (30%)	3 (30%)	4 (40%)
Integumentary System				
Skin	(8)	(8)	(8)	(6)
Abscess		1 (13%)		
Hyperkeratosis				1 (17%)
Hyperplasia, focal				1 (17%)
Ulcer				1 (17%)
Control epidermis, hyperplasia		1 (13%)		
Epidermis, hyperplasia, focal				1 (17%)
Subcutaneous tissue, inflammation		1 (13%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation			2 (20%)	
Alveolar epithelium, hyperplasia	1 (10%)		2 (20%)	1 (10%)
Perivascular, inflammation		1 (10%)		

TABLE B8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein		1 (10%)		2 (20%)
Cyst		1 (10%)	5 (50%)	2 (20%)
Hydronephrosis	2 (20%)			
Infarct			1 (10%)	
Inflammation	1 (10%)			
Mineralization				1 (10%)
Nephropathy	4 (40%)	2 (20%)	4 (40%)	2 (20%)
Glomerulus, inflammation, membranoproliferative	1 (10%)			1 (10%)
Renal tubule, accumulation, hyaline droplet	1 (10%)			
Renal tubule, hypertrophy			2 (20%)	1 (10%)

Systems Examined with No Lesions Observed
Cardiovascular System
Musculoskeletal System
Nervous System
Special Senses System

APPENDIX C
SUMMARY OF LESIONS IN Tg.AC HEMIZYGOUS MICE IN THE GAVAGE STUDIES OF BROMODICHLOROMETHANE

TABLE C1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental death	1			
Moribund	1	1	3	
Survivors				
Died last week of study				1
Terminal sacrifice	13	14	12	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	4 (27%)	2 (13%)	2 (13%)	5 (33%)
Squamous cell papilloma, multiple	5 (33%)	3 (20%)	9 (60%)	7 (47%)
Tooth	(3)	(2)	(3)	(3)
Odontogenic tumor	2 (67%)	2 (100%)	3 (100%)	2 (67%)
Sarcoma	1 (33%)			
Integumentary System				
Skin	(1)	(1)	(1)	(5)
Squamous cell papilloma		1 (100%)		4 (80%)
Squamous cell papilloma, multiple	1 (100%)			1 (20%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar adenoma			1 (7%)	1 (7%)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	1 (7%)			1 (7%)
Lymphoma malignant		1 (7%)		

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

TABLE C1
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	11	6	13	14
Total primary neoplasms	14	9	15	21
Total animals with benign neoplasms	9	5	11	13
Total benign neoplasms	10	6	12	18
Total animals with malignant neoplasms	2	1		1
Total malignant neoplasms	2	1		1
Total animals with uncertain neoplasms-benign or malignant	2	2	3	2
Total uncertain neoplasms	2	2	3	2

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

TABLE C2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental death	1			
Moribund	1	1	3	
Survivors				
Died last week of study				1
Terminal sacrifice	13	14	12	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Esophagus	(1)			
Inflammation	1 (100%)			
Liver	(15)	(15)	(15)	(15)
Cyst				1 (7%)
Hematopoietic cell proliferation		1 (7%)		
Inflammation	6 (40%)	7 (47%)	9 (60%)	8 (53%)
Necrosis	1 (7%)		1 (7%)	
Hepatocyte, fatty change	1 (7%)			
Hepatocyte, vacuolization cytoplasmic	3 (20%)	1 (7%)	3 (20%)	3 (20%)
Mesentery	(1)		(1)	
Fat, necrosis	1 (100%)		1 (100%)	
Stomach, forestomach	(15)	(15)	(15)	(15)
Hyperkeratosis	1 (7%)			
Tooth	(3)	(2)	(3)	(3)
Inflammation				1 (33%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hypertrophy	6 (40%)	8 (53%)	5 (33%)	7 (47%)
Subcapsular, hyperplasia		2 (13%)		
Pituitary gland	(15)	(15)	(15)	(15)
Cyst	2 (13%)	2 (13%)	1 (7%)	1 (7%)
Thyroid gland	(15)	(15)	(15)	(15)
Cyst	2 (13%)	2 (13%)		1 (7%)
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Inflammation			1 (7%)	
Preputial gland			(2)	
Cyst			1 (50%)	
Duct, ectasia			1 (50%)	
Testes	(15)	(15)	(15)	(15)
Germinal epithelium, degeneration	1 (7%)	4 (27%)	1 (7%)	

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

TABLE C2
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Lymph node, mandibular	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Hyperplasia	1 (7%)			2 (13%)
Infiltration cellular, plasma cell		2 (13%)		
Lymph node, mesenteric	(15)	(14)	(15)	(15)
Atrophy	1 (7%)			1 (7%)
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation	1 (7%)	1 (7%)	2 (13%)	
Lymphoid follicle, atrophy	1 (7%)		1 (7%)	
Thymus	(14)	(15)	(15)	(15)
Atrophy	2 (14%)	1 (7%)	3 (20%)	2 (13%)
Cyst	7 (50%)	5 (33%)	4 (27%)	8 (53%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Edema		1 (7%)		
Inflammation				1 (7%)
Alveolar epithelium, hyperplasia		1 (7%)		
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	3 (20%)			6 (40%)
Cyst		1 (7%)		
Hydronephrosis		1 (7%)		
Nephropathy	2 (13%)	8 (53%)	3 (20%)	4 (27%)
Artery, inflammation		1 (7%)		
Renal tubule, degeneration				4 (27%)
Renal tubule, dilatation	2 (13%)	1 (7%)	5 (33%)	4 (27%)
Renal tubule, hypertrophy			4 (27%)	1 (7%)
Renal tubule, necrosis				1 (7%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE C3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental deaths	1			1
Moribund	1			1
Natural deaths	2	1	2	
Survivors				
Terminal sacrifice	11	14	13	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Salivary glands	(1)			(1)
Duct, carcinoma	1 (100%)			1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Squamous cell papilloma	3 (20%)	3 (20%)	4 (27%)	
Squamous cell papilloma, multiple	3 (20%)	5 (33%)	6 (40%)	11 (73%)
Tooth	(4)		(3)	(2)
Odontogenic tumor	4 (100%)		3 (100%)	2 (100%)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Uterus	(15)	(15)	(15)	(15)
Polyp stromal		1 (7%)		
Integumentary System				
Skin	(1)	(3)	(6)	(2)
Squamous cell papilloma	1 (100%)	1 (33%)	5 (83%)	2 (100%)
Squamous cell papilloma, multiple		2 (67%)		
Special Senses System				
Zymbal's gland			(1)	
Adenoma			1 (100%)	
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Leukemia erythrocytic	2 (13%)	2 (13%)	1 (7%)	

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Urinary System

TABLE C3
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	10	12	13
Total primary neoplasms	14	14	20	16
Total animals with benign neoplasms	7	9	12	12
Total benign neoplasms	7	12	16	13
Total animals with malignant neoplasms	3	2	1	1
Total malignant neoplasms	3	2	1	1
Total animals with uncertain neoplasms-benign or malignant	4		3	2
Total uncertain neoplasms	4		3	2

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

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental deaths	1			1
Moribund	1			1
Natural deaths	2	1	2	
Survivors				
Terminal sacrifice	11	14	13	13
Animals examined microscopically	15	15	15	15
Alimentary System				
Esophagus	(1)			(1)
Perforation	1 (100%)			1 (100%)
Liver	(15)	(15)	(15)	(15)
Basophilic focus	1 (7%)			
Cyst			1 (7%)	
Hematopoietic cell proliferation	1 (7%)		1 (7%)	1 (7%)
Hemorrhage		1 (7%)		
Hepatodiaphragmatic nodule		1 (7%)		
Inflammation	11 (73%)	13 (87%)	13 (87%)	13 (87%)
Necrosis	1 (7%)		4 (27%)	1 (7%)
Hepatocyte, fatty change		5 (33%)	8 (53%)	7 (47%)
Hepatocyte, vacuolization cytoplasmic		6 (40%)	4 (27%)	3 (20%)
Salivary glands	(1)			(1)
Atrophy				1 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Hyperkeratosis	1 (7%)			
Epithelium, hyperplasia		1 (7%)		
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Mineralization		1 (7%)		
Subcapsular, hyperplasia	7 (47%)	10 (67%)	8 (53%)	6 (40%)
Parathyroid gland		(1)		
Cyst		1 (100%)		
Pituitary gland	(15)	(15)	(15)	(15)
Angiectasis			1 (7%)	
Cyst	1 (7%)		1 (7%)	2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)
Cyst	2 (13%)	2 (13%)	3 (20%)	1 (7%)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Cyst	4 (27%)	2 (13%)	4 (27%)	4 (27%)
Uterus	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Inflammation		1 (7%)		
Endometrium, hyperplasia, cystic	12 (80%)	9 (60%)	12 (80%)	13 (87%)

[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 Female Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Lymph node, mandibular	(14)	(15)	(15)	(15)
Hyperplasia				1 (7%)
Infiltration cellular, plasma cell	1 (7%)			1 (7%)
Lymph node, mesenteric	(14)	(15)	(15)	(15)
Atrophy		1 (7%)		
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			2 (13%)	1 (7%)
Lymphoid follicle, atrophy	3 (20%)			1 (7%)
Thymus	(13)	(15)	(15)	(15)
Atrophy	2 (15%)	3 (20%)	1 (7%)	3 (20%)
Cyst	2 (15%)	5 (33%)	5 (33%)	2 (13%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation			2 (13%)	
Thrombosis				1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	4 (27%)	5 (33%)	5 (33%)	4 (27%)
Cyst			1 (7%)	2 (13%)
Mineralization				1 (7%)
Nephropathy	1 (7%)	3 (20%)	3 (20%)	1 (7%)
Renal tubule, degeneration			1 (7%)	
Renal tubule, dilatation	1 (7%)			
Renal tubule, hypertrophy	1 (7%)	1 (7%)	1 (7%)	8 (53%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE C5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Accidental deaths	1	3		
Moribund	3	1	4	2
Survivors				
Terminal sacrifice	6	6	6	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine large, rectum	(1)	(1)	(1)	(1)
Anus, squamous cell papilloma				1 (100%)
Liver	(10)	(10)	(10)	(10)
Salivary glands	(2)		(2)	(1)
Duct, carcinoma	2 (100%)		2 (100%)	1 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	2 (20%)	1 (10%)	4 (40%)	1 (10%)
Squamous cell papilloma, multiple	6 (60%)	5 (50%)	5 (50%)	5 (50%)
Tooth	(4)	(2)	(4)	(4)
Odontogenic tumor	4 (100%)	2 (100%)	4 (100%)	4 (100%)
Integumentary System				
Skin	(5)	(6)	(5)	(7)
Squamous cell papilloma	1 (20%)	2 (33%)		4 (57%)
Squamous cell papilloma, multiple	4 (80%)	4 (67%)	4 (80%)	2 (29%)
Special Senses System				
Harderian gland			(1)	
Adenoma			1 (100%)	
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic			1 (10%)	

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Urinary System

TABLE C5
Summary of the Incidence of Neoplasms in Male Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	7	10	10
Total primary neoplasms	19	14	21	18
Total animals with benign neoplasms	8	6	10	7
Total benign neoplasms	13	12	14	13
Total animals with malignant neoplasms	2		3	1
Total malignant neoplasms	2		3	1
Total animals with uncertain neoplasms-benign or malignant	4	2	4	4
Total uncertain neoplasms	4	2	4	4

[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 C6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Accidental deaths	1	3		
Moribund	3	1	4	2
Survivors				
Terminal sacrifice	6	6	6	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)		1 (10%)	1 (10%)
Inflammation	7 (70%)	6 (60%)	8 (80%)	5 (50%)
Necrosis		2 (20%)		
Hepatocyte, fatty change			2 (20%)	
Hepatocyte, vacuolization cytoplasmic	6 (60%)	3 (30%)	7 (70%)	7 (70%)
Hepatocyte, vacuolization cytoplasmic, diffuse		2 (20%)		
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis	2 (20%)		3 (30%)	2 (20%)
Epithelium, hyperplasia	2 (20%)		3 (30%)	2 (20%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	7 (70%)	3 (30%)	6 (60%)	7 (70%)
Subcapsular, hyperplasia		1 (10%)	2 (20%)	
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	1 (10%)	1 (10%)		1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)		1 (10%)	1 (10%)
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			1 (10%)
Preputial gland			(1)	
Inflammation, suppurative			1 (100%)	
Testes	(10)	(10)	(10)	(10)
Germinal epithelium, degeneration	2 (20%)	1 (10%)		1 (10%)

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

TABLE C6
Summary of the Incidence of Nonneoplastic Lesions in Male Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Lymph node, mandibular	(10)	(9)	(9)	(10)
Hematopoietic cell proliferation	1 (10%)			
Hyperplasia	1 (10%)	1 (11%)	1 (11%)	1 (10%)
Infiltration cellular, plasma cell	1 (10%)		2 (22%)	1 (10%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Hyperplasia	1 (10%)			
Spleen	(10)	(10)	(10)	(10)
Atrophy		1 (10%)		
Hematopoietic cell proliferation	4 (40%)		2 (20%)	1 (10%)
Thymus	(9)	(10)	(10)	(10)
Atrophy	3 (33%)	3 (30%)	3 (30%)	2 (20%)
Cyst	4 (44%)	2 (20%)	2 (20%)	3 (30%)
Integumentary System				
Skin	(5)	(6)	(5)	(7)
Hyperplasia		1 (17%)	2 (40%)	4 (57%)
Inflammation				1 (14%)
Subcutaneous tissue, fibrosis				1 (14%)
Nervous System				
Peripheral nerve			(1)	
Degeneration			1 (100%)	
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation	1 (10%)	1 (10%)	2 (20%)	
Alveolar epithelium, hyperplasia				1 (10%)
Perivascular, inflammation		1 (10%)	1 (10%)	
Pleura		(3)		
Inflammation, suppurative		3 (100%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein	3 (30%)	1 (10%)		1 (10%)
Cyst		1 (10%)	2 (20%)	
Nephropathy	3 (30%)	4 (40%)	6 (60%)	6 (60%)
Glomerulus, inflammation, membranoproliferative			1 (10%)	
Renal tubule, degeneration				6 (60%)
Renal tubule, dilatation		2 (20%)	2 (20%)	4 (40%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Special Senses System

TABLE C7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1			2
Natural deaths	2	1	1	1
Survivors				
Terminal sacrifice	7	9	9	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Salivary glands				(3)
Duct, carcinoma				3 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Squamous cell papilloma	3 (30%)	1 (10%)	3 (30%)	1 (10%)
Squamous cell papilloma, multiple	1 (10%)	6 (60%)	5 (50%)	9 (90%)
Tooth	(4)	(3)	(3)	(2)
Odontogenic tumor	4 (100%)	3 (100%)	3 (100%)	2 (100%)
Hematopoietic System				
Lymph node				(1)
Mediastinal, squamous cell carcinoma, metastatic, skin				1 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(9)
Squamous cell carcinoma, metastatic, skin				1 (11%)
Spleen	(10)	(10)	(10)	(10)
Integumentary System				
Skin	(6)	(5)	(7)	(7)
Squamous cell carcinoma				1 (14%)
Squamous cell papilloma	3 (50%)	2 (40%)	2 (29%)	3 (43%)
Squamous cell papilloma, multiple	3 (50%)	2 (40%)	4 (57%)	4 (57%)
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia erythrocytic		1 (10%)		

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE C7
Summary of the Incidence of Neoplasms in Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	10	7	9	10
Total primary neoplasms	14	15	17	23
Total animals with benign neoplasms	8	7	9	10
Total benign neoplasms	10	11	14	17
Total animals with malignant neoplasms		1		4
Total malignant neoplasms		1		4
Total animals with metastatic neoplasms				1
Total metastatic neoplasms				2
Total animals with uncertain neoplasms-benign or malignant	4	3	3	2
Total uncertain neoplasms	4	3	3	2

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

TABLE C8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1			2
Natural deaths	2	1	1	1
Survivors				
Terminal sacrifice	7	9	9	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Gallbladder		(1)		
Inflammation, chronic active		1 (100%)		
Intestine large, colon	(10)	(10)	(10)	(10)
Inflammation	1 (10%)			
Intestine large, cecum	(10)	(10)	(10)	(10)
Edema	1 (10%)			
Inflammation	1 (10%)			
Intestine small, ileum	(10)	(10)	(10)	(10)
Ulcer	1 (10%)			
Liver	(10)	(10)	(10)	(10)
Cytomegaly				1 (10%)
Hematopoietic cell proliferation	1 (10%)	2 (20%)		3 (30%)
Inflammation	8 (80%)	9 (90%)	10 (100%)	6 (60%)
Necrosis	2 (20%)	2 (20%)	1 (10%)	1 (10%)
Hepatocyte, fatty change		2 (20%)	8 (80%)	5 (50%)
Hepatocyte, vacuolization cytoplasmic	6 (60%)	9 (90%)	10 (100%)	9 (90%)
Mesentery	(1)			
Hemorrhage	1 (100%)			
Artery, inflammation	1 (100%)			
Artery, thrombosis	1 (100%)			
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis	1 (10%)	2 (20%)	4 (40%)	4 (40%)
Epithelium, hyperplasia	1 (10%)	2 (20%)	4 (40%)	4 (40%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)			1 (10%)
Subcapsular, hyperplasia	5 (50%)	6 (60%)	4 (40%)	7 (70%)
Islets, pancreatic				(1)
Hyperplasia				1 (100%)
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	1 (10%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Cyst	2 (20%)	5 (50%)	2 (20%)	2 (20%)

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

TABLE C8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Genital System				
Ovary	(10)	(10)	(10)	(10)
Amyloid deposition				1 (10%)
Atrophy		1 (10%)		
Cyst	1 (10%)		3 (30%)	1 (10%)
Inflammation, suppurative			1 (10%)	
Oviduct				(1)
Inflammation, suppurative				1 (100%)
Uterus	(10)	(10)	(10)	(10)
Inflammation		1 (10%)		1 (10%)
Inflammation, granulomatous				1 (10%)
Endometrium, hyperplasia, cystic	7 (70%)	10 (100%)	9 (90%)	8 (80%)
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(9)
Hyperplasia			1 (10%)	
Infiltration cellular, plasma cell	2 (20%)	1 (10%)		2 (22%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Atrophy		1 (10%)		1 (10%)
Ectasia	1 (10%)			
Spleen	(10)	(10)	(10)	(10)
Atrophy	2 (20%)			1 (10%)
Hematopoietic cell proliferation	1 (10%)	2 (20%)		5 (50%)
Thymus	(10)	(10)	(10)	(9)
Atrophy	2 (20%)	1 (10%)		4 (44%)
Cyst	3 (30%)	2 (20%)	5 (50%)	3 (33%)
Integumentary System				
Mammary gland				(1)
Inflammation, granulomatous				1 (100%)
Skin	(6)	(5)	(7)	(7)
Hyperplasia	1 (17%)	3 (60%)		2 (29%)
Epidermis, hyperplasia, focal		1 (20%)	1 (14%)	
Subcutaneous tissue, hemorrhage			1 (14%)	
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Inflammation				1 (10%)
Perivascular, inflammation	2 (20%)			1 (10%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Amyloid deposition			1 (10%)	
Casts protein	1 (10%)	2 (20%)	6 (60%)	
Cyst			1 (10%)	
Infiltration cellular, lymphocyte		1 (10%)		
Nephropathy	3 (30%)	4 (40%)		5 (50%)
Renal tubule, hypertrophy				1 (10%)

TABLE C8
Summary of the Incidence of Nonneoplastic Lesions in Female Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Special Senses System				

APPENDIX D
SUMMARY OF LESIONS IN p53 HAPLOINSUFFICIENT MICE IN THE DRINKING WATER STUDIES OF BROMODICHLOROMETHANE

TABLE D1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Hepatocellular adenoma	1 (7%)			
Endocrine System				
Thyroid gland	(15)	(14)	(15)	(15)
Follicular cell, carcinoma	1 (7%)			
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Genital System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[b]	2			
Total primary neoplasms	2			
Total animals with benign neoplasms	1			
Total benign neoplasms	1			
Total animals with malignant neoplasms	1			
Total malignant neoplasms	1			

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

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Inflammation	12 (80%)	14 (93%)	13 (87%)	13 (87%)
Hepatocyte, fatty change	4 (27%)	5 (33%)	1 (7%)	
Hepatocyte, vacuolization cytoplasmic	15 (100%)	15 (100%)	13 (87%)	11 (73%)
Mesentery			(1)	
Fat, necrosis			1 (100%)	
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hyperplasia		2 (13%)		
Hypertrophy		2 (13%)		2 (13%)
Subcapsular, hyperplasia	2 (13%)	3 (20%)	1 (7%)	3 (20%)
Pituitary gland	(15)	(15)	(15)	(15)
Cyst	3 (20%)	1 (7%)	1 (7%)	4 (27%)
Thyroid gland	(15)	(14)	(15)	(15)
Cyst	1 (7%)			
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Granuloma sperm		1 (7%)		1 (7%)
Inflammation			1 (7%)	
Testes	(15)	(15)	(15)	(15)
Mineralization		1 (7%)	1 (7%)	2 (13%)
Germinal epithelium, degeneration		2 (13%)		1 (7%)
Hematopoietic System				
Thymus	(15)	(15)	(15)	(15)
Cyst	9 (60%)	12 (80%)	6 (40%)	9 (60%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Fibrosis		1 (7%)		
Infiltration cellular, histiocyte		1 (7%)		
Alveolar epithelium, hyperplasia		1 (7%)		
Special Senses System				
Eye		(1)		
Cataract		1 (100%)		

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

TABLE D2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	1 (7%)	5 (33%)	4 (27%)	7 (47%)
Cyst				1 (7%)
Mineralization				1 (7%)
Nephropathy	7 (47%)	3 (20%)	11 (73%)	13 (87%)
Cortex, crystals				1 (7%)
Renal tubule, degeneration			9 (60%)	12 (80%)
Renal tubule, dilatation		5 (33%)	4 (27%)	6 (40%)
Renal tubule, vacuolization cytoplasmic	14 (93%)	1 (7%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System

TABLE D3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early death				
Natural death			1	
Survivors				
Terminal sacrifice	15	15	14	15
Animals examined microscopically	15	15	15	15
Genital System				
Uterus	(15)	(15)	(15)	(15)
Polyp stromal		2 (13%)		1 (7%)
Polyp stromal, multiple	1 (7%)			
Systems Examined with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Hematopoietic System				
Integumentary System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[b]	1	2		1
Total primary neoplasms	1	2		1
Total animals with benign neoplasms	1	2		1
Total benign neoplasms	1	2		1

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

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	15	15	15	15
Early death				
Natural death			1	
Survivors				
Terminal sacrifice	15	15	14	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Infiltration cellular, lymphocyte	1 (7%)	4 (27%)	4 (27%)	1 (7%)
Inflammation	13 (87%)	13 (87%)	12 (80%)	12 (80%)
Necrosis		2 (13%)	2 (13%)	1 (7%)
Hepatocyte, fatty change		1 (7%)	1 (7%)	10 (67%)
Hepatocyte, vacuolization cytoplasmic	11 (73%)	10 (67%)	7 (47%)	11 (73%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Subcapsular, hyperplasia	13 (87%)	12 (80%)	14 (93%)	14 (93%)
Parathyroid gland		(1)		
Cyst		1 (100%)		
Pituitary gland	(15)	(15)	(15)	(15)
Cyst			1 (7%)	2 (13%)
Thyroid gland	(15)	(15)	(15)	(14)
Cyst		1 (7%)		
C-cell, hyperplasia		1 (7%)		
Genital System				
Ovary	(15)	(15)	(15)	(15)
Angiectasis				1 (7%)
Cyst	2 (13%)	1 (7%)	2 (13%)	5 (33%)
Uterus	(15)	(15)	(15)	(15)
Inflammation	1 (7%)		1 (7%)	1 (7%)
Endometrium, hyperplasia, cystic	8 (53%)	7 (47%)	9 (60%)	8 (53%)
Hematopoietic System				
Spleen	(15)	(15)	(15)	(15)
Lymphoid follicle, atrophy			1 (7%)	
Lymphoid follicle, hyperplasia				1 (7%)
Thymus	(15)	(15)	(15)	(15)
Atrophy			1 (7%)	
Cyst	6 (40%)	11 (73%)	9 (60%)	11 (73%)

[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 p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Congestion			1 (7%)	
Infiltration cellular, lymphocyte	1 (7%)	1 (7%)	1 (7%)	
Inflammation				1 (7%)
Thrombosis			1 (7%)	
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	3 (20%)	10 (67%)	7 (47%)	6 (40%)
Nephropathy	2 (13%)	2 (13%)	2 (13%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE D5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1			3
Natural death			1	
Survivors				
Terminal sacrifice	9	10	9	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine small, duodenum	(10)	(10)	(10)	(10)
Intestine small, jejunum	(10)	(10)	(9)	(10)
Intestine small, ileum	(10)	(10)	(10)	(10)
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma		1 (10%)	1 (10%)	
Mesentery			(1)	
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Prostate				(1)
Sarcoma				1 (100%)
Musculoskeletal System				
Skeletal muscle			(1)	
Rhabdomyosarcoma			1 (100%)	
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar carcinoma				1 (10%)
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Lymphoma malignant	1 (10%)		2 (20%)	

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Integumentary System
Nervous System
Special Senses System
Urinary System

TABLE D5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	1	1	4	2
Total primary neoplasms	1	1	4	2
Total animals with benign neoplasms		1	1	
Total benign neoplasms		1	1	
Total animals with malignant neoplasms	1		3	2
Total malignant neoplasms	1		3	2

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

TABLE D6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1			3
Natural death			1	
Survivors				
Terminal sacrifice	9	10	9	7
Animals examined microscopically	10	10	10	10
Alimentary System				
Esophagus				(1)
Muscularis, degeneration				1 (100%)
Liver	(10)	(10)	(10)	(10)
Atypia cellular				1 (10%)
Clear cell focus		1 (10%)		
Hematopoietic cell proliferation				1 (10%)
Infiltration cellular, lymphocyte	1 (10%)	2 (20%)		
Inflammation	9 (90%)	5 (50%)	7 (70%)	6 (60%)
Necrosis				2 (20%)
Hepatocyte, fatty change	8 (80%)	4 (40%)	3 (30%)	4 (40%)
Hepatocyte, vacuolization cytoplasmic	9 (90%)	10 (100%)	10 (100%)	9 (90%)
Salivary glands				(2)
Inflammation				2 (100%)
Stomach, forestomach	(10)	(10)	(10)	(10)
Inflammation				1 (10%)
Epithelium, hyperplasia	1 (10%)	1 (10%)	1 (10%)	1 (10%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hyperplasia		1 (10%)		
Hypertrophy	2 (20%)	4 (40%)	2 (20%)	
Subcapsular, hyperplasia	2 (20%)	2 (20%)	3 (30%)	2 (20%)
Islets, pancreatic		(1)		
Hyperplasia		1 (100%)		
Parathyroid gland		(1)		
Cyst		1 (100%)		
Pituitary gland	(10)	(9)	(10)	(10)
Cyst	4 (40%)	3 (33%)	3 (30%)	3 (30%)
Genital System				
Testes	(10)	(10)	(10)	(10)
Mineralization		1 (10%)		
Germinal epithelium, degeneration	2 (20%)	2 (20%)	1 (10%)	3 (30%)

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

TABLE D6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Atrophy				1 (10%)
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			2 (20%)
Spleen	(10)	(10)	(10)	(10)
Atrophy				3 (30%)
Hematopoietic cell proliferation	1 (10%)			
Thymus	(10)	(10)	(10)	(10)
Atrophy			1 (10%)	3 (30%)
Cyst	8 (80%)	7 (70%)	3 (30%)	4 (40%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar epithelium, hyperplasia			1 (10%)	
Special Senses System				
Harderian gland				(1)
Inflammation				1 (100%)
Lacrimal gland	(1)			(1)
Inflammation	1 (100%)			1 (100%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein			1 (10%)	
Hydronephrosis			1 (10%)	1 (10%)
Infiltration cellular, lymphocyte	1 (10%)			
Mineralization			1 (10%)	1 (10%)
Nephropathy	5 (50%)	4 (40%)	9 (90%)	8 (80%)
Renal tubule, degeneration			6 (60%)	10 (100%)
Renal tubule, dilatation	1 (10%)		3 (30%)	2 (20%)
Renal tubule, necrosis				1 (10%)
Renal tubule, vacuolization cytoplasmic	10 (100%)	5 (50%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System

TABLE D7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1	1		1
Natural death				1
Survivors				
Terminal sacrifice	9	9	10	8
Animals examined microscopically	10	10	10	10
Genital System				
Uterus	(10)	(10)	(10)	(10)
Polyp stromal	2 (20%)			
Hematopoietic System				
Lymph node	(1)	(2)		
Carcinoma, metastatic, skin	1 (100%)			
Mediastinal, osteosarcoma, metastatic, bone		1 (50%)		
Renal, carcinoma, metastatic, skin	1 (100%)			
Integumentary System				
Mammary gland				(1)
Carcinoma				1 (100%)
Skin	(1)			
Carcinoma	1 (100%)			
Musculoskeletal System				
Bone		(2)		
Vertebra, osteosarcoma		2 (100%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Carcinoma, metastatic, skin	1 (10%)			
Osteosarcoma, metastatic, bone		1 (10%)		
Osteosarcoma, metastatic, uncertain primary site	1 (10%)			
Pleura		(1)		
Osteosarcoma, metastatic, bone		1 (100%)		

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Nervous System
Special Senses System
Urinary System

TABLE D7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[b]	3	2		1
Total primary neoplasms	3	2		1
Total animals with benign neoplasms	2			
Total benign neoplasms	2			
Total animals with malignant neoplasms	1	2		1
Total malignant neoplasms	1	2		1
Total animals with metastatic neoplasms	2	1		
Total metastatic neoplasms	4	3		
Total animals with malignant neoplasms of uncertain primary site	1			

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

TABLE D8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Moribund	1	1		1
Natural death				1
Survivors				
Terminal sacrifice	9	9	10	8
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)	1 (10%)		1 (10%)
Infiltration cellular		1 (10%)		
Infiltration cellular, lymphocyte	2 (20%)	4 (40%)	4 (40%)	2 (20%)
Inflammation	8 (80%)	7 (70%)	8 (80%)	7 (70%)
Necrosis	3 (30%)	2 (20%)	1 (10%)	1 (10%)
Hepatocyte, fatty change	2 (20%)	2 (20%)	3 (30%)	6 (60%)
Hepatocyte, vacuolization cytoplasmic	9 (90%)	9 (90%)	10 (100%)	8 (80%)
Serosa, inflammation				1 (10%)
Mesentery		(1)		(1)
Inflammation				1 (100%)
Fat, necrosis		1 (100%)		
Salivary glands			(1)	
Atrophy			1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis		1 (10%)		2 (20%)
Inflammation			1 (10%)	
Epithelium, hyperplasia		2 (20%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Subcapsular, hyperplasia	9 (90%)	10 (100%)	10 (100%)	8 (80%)
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	1 (10%)			
Hyperplasia				1 (10%)
Pars intermedia, hyperplasia			1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Inflammation			1 (10%)	
Genital System				
Ovary	(10)	(9)	(10)	(10)
Angiectasis		1 (11%)		
Atrophy				1 (10%)
Cyst	3 (30%)	2 (22%)	1 (10%)	2 (20%)
Uterus	(10)	(10)	(10)	(10)
Inflammation	2 (20%)	1 (10%)	2 (20%)	1 (10%)
Endometrium, hyperplasia, cystic	8 (80%)	8 (80%)	8 (80%)	6 (60%)

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

TABLE D8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Hematopoietic System				
Lymph node	(1)	(2)		
Mediastinal, infiltration cellular, plasma cell		1 (50%)		
Lymph node, mandibular	(10)	(10)	(10)	(9)
Atrophy	1 (10%)			
Hematopoietic cell proliferation		1 (10%)		
Hyperplasia, atypical			1 (10%)	
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			
Hematopoietic cell proliferation	1 (10%)	1 (10%)		
Spleen	(10)	(10)	(10)	(10)
Atrophy				1 (10%)
Hematopoietic cell proliferation	2 (20%)	1 (10%)		2 (20%)
Thymus	(10)	(9)	(10)	(10)
Atrophy	2 (20%)	1 (11%)		2 (20%)
Cyst	7 (70%)	6 (67%)	8 (80%)	8 (80%)
Integumentary System				
Skin	(1)			
Ulcer	1 (100%)			
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage		1 (10%)		
Infiltration cellular, lymphocyte	1 (10%)			1 (10%)
Infiltration cellular, histiocyte		1 (10%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Accumulation, hyaline droplet				1 (10%)
Casts protein	8 (80%)	1 (10%)	5 (50%)	3 (30%)
Infiltration cellular, lymphocyte	1 (10%)	4 (40%)		1 (10%)
Mineralization				1 (10%)
Nephropathy	3 (30%)	1 (10%)	8 (80%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Special Senses System

APPENDIX E
SUMMARY OF LESIONS IN p53 HAPLOINSUFFICIENT MICE IN THE GAVAGE STUDIES OF BROMODICHLOROMETHANE

TABLE E1
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Histiocytic sarcoma				1 (7%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Alveolar/bronchiolar carcinoma				1 (7%)
Systemic Lesions				
Multiple organs[b]	(15)	(15)	(15)	(15)
Histiocytic sarcoma				1 (7%)

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System
Urinary System

Neoplasm Summary	
Total animals with primary neoplasms[c]	2
Total primary neoplasms	2
Total animals with malignant neoplasms	2
Total malignant neoplasms	2

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

TABLE E2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Survivors				
Terminal sacrifice	15	15	15	15
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Infiltration cellular, lymphocyte			2 (13%)	
Inflammation	13 (87%)	14 (93%)	13 (87%)	15 (100%)
Necrosis				1 (7%)
Hepatocyte, fatty change	10 (67%)	9 (60%)	4 (27%)	11 (73%)
Hepatocyte, vacuolization cytoplasmic	15 (100%)	15 (100%)	13 (87%)	15 (100%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Hyperkeratosis				1 (7%)
Inflammation		1 (7%)	1 (7%)	1 (7%)
Epithelium, hyperplasia		2 (13%)		
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Hypertrophy	6 (40%)	2 (13%)		
Subcapsular, hyperplasia	2 (13%)	6 (40%)	6 (40%)	3 (20%)
Pituitary gland	(14)	(15)	(15)	(15)
Cyst	1 (7%)	4 (27%)		2 (13%)
Thyroid gland	(15)	(15)	(15)	(15)
Inflammation	1 (7%)			
Genital System				
Epididymis	(15)	(15)	(15)	(15)
Granuloma sperm	2 (13%)			
Inflammation	1 (7%)			1 (7%)
Testes	(15)	(15)	(15)	(15)
Inflammation, granulomatous		1 (7%)		
Mineralization		1 (7%)		
Germinal epithelium, degeneration	3 (20%)	2 (13%)	1 (7%)	1 (7%)
Hematopoietic System				
Spleen	(15)	(15)	(15)	(15)
Lymphoid follicle, hyperplasia				1 (7%)
Thymus	(15)	(15)	(15)	(15)
Cyst	11 (73%)	9 (60%)	9 (60%)	11 (73%)
Nervous System				
Brain				(1)
Hydrocephalus				1 (100%)

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

TABLE E2
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Inflammation				1 (7%)
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	2 (13%)		5 (33%)	2 (13%)
Hydronephrosis		1 (7%)		
Nephropathy	8 (53%)	9 (60%)	8 (53%)	8 (53%)
Renal tubule, degeneration				4 (27%)
Renal tubule, vacuolization cytoplasmic	12 (80%)	5 (33%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Special Senses System

TABLE E3
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental death			1	
Moribund		1		1
Survivors				
Terminal sacrifice	15	14	14	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Liver	(15)	(15)	(15)	(15)
Osteosarcoma, metastatic, uncertain primary site				1 (7%)
Hematopoietic System				
Lymph node				(2)
Lumbar, osteosarcoma, metastatic, uncertain primary site				1 (50%)
Integumentary System				
Skin				(2)
Subcutaneous tissue, osteosarcoma				1 (50%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Osteosarcoma, metastatic, uncertain primary site				1 (7%)
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Musculoskeletal System				
Nervous System				
Special Senses System				
Urinary System				
Neoplasm Summary				
Total animals with primary neoplasms[b]				1
Total primary neoplasms				1
Total animals with malignant neoplasms				1
Total malignant neoplasms				1
Total animals with metastatic neoplasms				1
Total metastatic neoplasms				3
Total animals with malignant neoplasms uncertain primary site				1

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

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	15	15	15	15
Early deaths				
Accidental death			1	
Moribund		1		1
Survivors				
Terminal sacrifice	15	14	14	14
Animals examined microscopically	15	15	15	15
Alimentary System				
Esophagus			(1)	
Hyperkeratosis			1 (100%)	
Liver	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation				2 (13%)
Infiltration cellular, lymphocyte	5 (33%)	4 (27%)	3 (20%)	
Inflammation	14 (93%)	15 (100%)	14 (93%)	14 (93%)
Hepatocyte, fatty change	2 (13%)	2 (13%)	3 (20%)	11 (73%)
Hepatocyte, vacuolization cytoplasmic	10 (67%)	9 (60%)	10 (67%)	13 (87%)
Stomach, forestomach	(15)	(15)	(15)	(15)
Inflammation			1 (7%)	1 (7%)
Epithelium, hyperplasia				1 (7%)
Endocrine System				
Adrenal cortex	(15)	(15)	(15)	(15)
Subcapsular, hyperplasia	14 (93%)	15 (100%)	12 (80%)	14 (93%)
Parathyroid gland			(2)	
Cyst			2 (100%)	
Pituitary gland	(15)	(15)	(15)	(15)
Cyst			1 (7%)	
Hyperplasia		1 (7%)		
Thyroid gland	(15)	(15)	(15)	(15)
Ectopic thymus	1 (7%)			1 (7%)
Inflammation	3 (20%)	1 (7%)	1 (7%)	
General Body System				
Peritoneum				(1)
Inflammation				1 (100%)
Genital System				
Ovary	(15)	(15)	(15)	(15)
Cyst	2 (13%)	1 (7%)	2 (13%)	2 (13%)
Uterus	(15)	(15)	(15)	(15)
Inflammation	2 (13%)	2 (13%)		
Necrosis				1 (7%)
Thrombosis				1 (7%)
Endometrium, hyperplasia, cystic	9 (60%)	9 (60%)	12 (80%)	10 (67%)

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

TABLE E4
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Lymph node				(2)
Mediastinal, atrophy				1 (50%)
Lymph node, mandibular	(15)	(15)	(15)	(15)
Atrophy				1 (7%)
Lymph node, mesenteric	(15)	(15)	(15)	(14)
Atrophy				1 (7%)
Spleen	(15)	(15)	(15)	(15)
Hematopoietic cell proliferation			2 (13%)	2 (13%)
Thymus	(15)	(15)	(15)	(15)
Atrophy				2 (13%)
Cyst	6 (40%)	5 (33%)	9 (60%)	7 (47%)
Hyperplasia, atypical				1 (7%)
Necrosis			1 (7%)	
Integumentary System				
Skin				(2)
Subcutaneous tissue, edema				1 (50%)
Nervous System				
Brain				(1)
Hydrocephalus				1 (100%)
Respiratory System				
Lung	(15)	(15)	(15)	(15)
Infiltration cellular, lymphocyte	3 (20%)		2 (13%)	1 (7%)
Infiltration cellular, histiocyte	1 (7%)			1 (7%)
Inflammation	1 (7%)			
Pleura			(1)	
Inflammation, suppurative			1 (100%)	
Urinary System				
Kidney	(15)	(15)	(15)	(15)
Casts protein	12 (80%)	11 (73%)	7 (47%)	7 (47%)
Hydronephrosis				1 (7%)
Inflammation		1 (7%)		
Nephropathy	1 (7%)	3 (20%)	1 (7%)	

Systems Examined with No Lesions Observed
Cardiovascular System
Musculoskeletal System
Special Senses System

TABLE E5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early death				
Moribund		1		
Survivors				
Terminal sacrifice	10	9	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Intestine small, duodenum	(10)	(10)	(10)	(9)
Intestine small, jejunum	(10)	(10)	(10)	(9)
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma	2 (20%)			
Mesentery	(1)	(1)		
Fibrosarcoma		1 (100%)		
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Adenoma				1 (10%)
Integumentary System				
Skin				(1)
Squamous cell papilloma				1 (100%)
Musculoskeletal System				
Skeletal muscle		(1)		
Sarcoma		1 (100%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Alveolar/bronchiolar adenoma				1 (10%)
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Lymphoma malignant	1 (10%)		1 (10%)	1 (10%)

Systems Examined with No Neoplasms Observed
Cardiovascular System
General Body System
Genital System
Hematopoietic System
Nervous System
Special Senses System
Urinary System

TABLE E5
Summary of the Incidence of Neoplasms in Male p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	3	2	1	4
Total primary neoplasms	3	2	1	4
Total animals with benign neoplasms	2			3
Total benign neoplasms	2			3
Total animals with malignant neoplasms	1	2	1	1
Total malignant neoplasms	1	2	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 E6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early death				
Moribund		1		
Survivors				
Terminal sacrifice	10	9	10	10
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)		
Infiltration cellular, lymphocyte	2 (20%)	3 (30%)	1 (10%)	3 (30%)
Inflammation	7 (70%)	9 (90%)	9 (90%)	9 (90%)
Necrosis				1 (10%)
Hepatocyte, fatty change	6 (60%)	6 (60%)	5 (50%)	10 (100%)
Hepatocyte, vacuolization cytoplasmic	10 (100%)	9 (90%)	10 (100%)	10 (100%)
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Hypertrophy	3 (30%)	2 (20%)	2 (20%)	1 (10%)
Subcapsular, hyperplasia	2 (20%)		1 (10%)	2 (20%)
Parathyroid gland	(1)			
Cyst	1 (100%)			
Pituitary gland	(10)	(10)	(10)	(10)
Cyst	4 (40%)	2 (20%)	3 (30%)	1 (10%)
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)	1 (10%)		
Inflammation			1 (10%)	
Testes	(10)	(10)	(10)	(10)
Mineralization		2 (20%)	1 (10%)	
Germinal epithelium, degeneration	2 (20%)	5 (50%)	2 (20%)	2 (20%)
Hematopoietic System				
Lymph node, mandibular	(9)	(10)	(10)	(10)
Infiltration cellular, polymorphonuclear				1 (10%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation		1 (10%)		
Thymus	(9)	(10)	(9)	(10)
Cyst	6 (67%)	7 (70%)	6 (67%)	4 (40%)
Epithelial cell, hyperplasia		1 (10%)		
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)	1 (10%)		
Alveolar epithelium, hyperplasia			1 (10%)	

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

TABLE E6
Summary of the Incidence of Nonneoplastic Lesions in Male p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Casts protein	1 (10%)	2 (20%)		
Cyst	1 (10%)			
Mineralization				1 (10%)
Nephropathy	4 (40%)	3 (30%)	4 (40%)	9 (90%)
Renal tubule, degeneration		1 (10%)		10 (100%)
Renal tubule, dilatation		1 (10%)		
Renal tubule, vacuolization cytoplasmic	8 (80%)	5 (50%)	1 (10%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

TABLE E7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Accidental death			1	
Moribund		1	1	1
Natural death	1			
Survivors				
Terminal sacrifice	9	9	8	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Mesentery			(1)	
Histiocytic sarcoma			1 (100%)	
Pancreas			(1)	
Histiocytic sarcoma			1 (100%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Pituitary gland	(10)	(10)	(10)	(10)
Adenoma		1 (10%)		
Genital System				
Ovary	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Uterus	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Hematopoietic System				
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Spleen	(10)	(10)	(10)	(10)
Musculoskeletal System				
Bone	(1)	(2)		(1)
Osteoma		1 (50%)		
Osteosarcoma	1 (100%)	1 (50%)		1 (100%)
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Osteosarcoma, metastatic, bone	1 (10%)			
Alveolar epithelium, adenoma			1 (10%)	

TABLE E7
Summary of the Incidence of Neoplasms in Female p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Histiocytic sarcoma			1 (10%)	
Lymphoma malignant		1 (10%)	2 (20%)	
Systems Examined with No Neoplasms Observed				
Cardiovascular System				
General Body System				
Integumentary System				
Nervous System				
Special Senses System				
Neoplasm Summary				
Total animals with primary neoplasms[c]	1	4	4	1
Total primary neoplasms	1	4	4	1
Total animals with benign neoplasms		2	1	
Total benign neoplasms		2	1	
Total animals with malignant neoplasms	1	2	3	1
Total malignant neoplasms	1	2	3	1
Total animals with metastatic neoplasms	1			
Total metastatic neoplasms	1			

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

TABLE E8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Disposition Summary				
Animals initially in study	10	10	10	10
Early deaths				
Accidental death			1	
Moribund		1	1	1
Natural death	1			
Survivors				
Terminal sacrifice	9	9	8	9
Animals examined microscopically	10	10	10	10
Alimentary System				
Esophagus			(1)	
Perforation			1 (100%)	
Liver	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	3 (30%)	2 (20%)	3 (30%)	
Inflammation	6 (60%)	10 (100%)	7 (70%)	8 (80%)
Necrosis		2 (20%)	1 (10%)	
Hepatocyte, fatty change	3 (30%)	3 (30%)	6 (60%)	9 (90%)
Hepatocyte, vacuolization cytoplasmic	9 (90%)	10 (100%)	9 (90%)	10 (100%)
Mesentery			(1)	
Inflammation, suppurative			1 (100%)	
Stomach, forestomach	(10)	(10)	(10)	(10)
Hyperkeratosis			1 (10%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Subcapsular, hyperplasia	10 (100%)	9 (90%)	9 (90%)	10 (100%)
Parathyroid gland	(1)			
Cyst	1 (100%)			
Pituitary gland	(10)	(10)	(10)	(10)
Cyst			1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
Inflammation	1 (10%)			
Genital System				
Ovary	(10)	(10)	(10)	(10)
Atrophy	1 (10%)	1 (10%)		
Cyst			3 (30%)	1 (10%)
Oviduct	(2)		(1)	
Infiltration cellular, lymphocyte	2 (100%)		1 (100%)	
Uterus	(10)	(10)	(10)	(10)
Inflammation	2 (20%)	2 (20%)	1 (10%)	1 (10%)
Endometrium, hyperplasia, cystic	9 (90%)	8 (80%)	8 (80%)	10 (100%)

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

TABLE E8
Summary of the Incidence of Nonneoplastic Lesions in Female p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Hematopoietic System				
Lymph node, mandibular	(10)	(10)	(10)	(10)
Hyperplasia		1 (10%)		
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			1 (10%)
Spleen	(10)	(10)	(10)	(10)
Atrophy	1 (10%)			
Hematopoietic cell proliferation	1 (10%)	1 (10%)	1 (10%)	
Thymus	(9)	(10)	(10)	(10)
Atrophy	1 (11%)	2 (20%)	2 (20%)	1 (10%)
Cyst	6 (67%)	8 (80%)	8 (80%)	8 (80%)
Necrosis			1 (10%)	
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Infiltration cellular, lymphocyte	1 (10%)	2 (20%)	1 (10%)	
Alveolar epithelium, hyperplasia		1 (10%)		
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Accumulation, hyaline droplet			1 (10%)	
Casts protein	7 (70%)	4 (40%)	6 (60%)	6 (60%)
Mineralization	1 (10%)			
Nephropathy	2 (20%)	3 (30%)	1 (10%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Integumentary System
Musculoskeletal System
Nervous System
Special Senses System

APPENDIX F
GENETIC TOXICOLOGY

TABLE F1
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Administration of Bromodichloromethane in Drinking Water for 26 Weeks[a]

Compound	Dose (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Male					
Water[d]		13	1.19 ± 0.27		2.9 ± 0.1
Bromodichloromethane	175	12	0.92 ± 0.14	0.8280	3.6 ± 0.3
	350	12	1.83 ± 0.28	0.0321	3.4 ± 0.2
	700	14	1.79 ± 0.35	0.0375	3.0 ± 0.2
			P=0.008[e]		
Female					
Water		10	0.65 ± 0.18		3.5 ± 0.3
Bromodichloromethane	175	13	1.04 ± 0.22	0.0806	3.4 ± 0.2
	350	11	1.41 ± 0.15	0.0082	3.7 ± 0.3
	700	13	0.88 ± 0.20	0.1862	3.3 ± 0.3
			P=0.314		

[a] Study was performed at SITEK Research Laboratories. 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≤0.008 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE F2
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Dermal Administration of Bromodichloromethane for 26 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Male					
Acetone[d]		13	1.04 ± 0.24		3.0 ± 0.2
Bromodichloromethane	64	14	1.39 ± 0.21	0.1195	3.0 ± 0.2
	128	15	1.40 ± 0.18	0.1119	3.4 ± 0.2
	256	13	1.81 ± 0.21	0.0100	3.2 ± 0.2
			P=0.012[e]		
Female					
Acetone		11	0.77 ± 0.16		3.5 ± 0.2
Bromodichloromethane	64	10	1.25 ± 0.19	0.0611	3.5 ± 0.3
	128	12	1.25 ± 0.21	0.0547	3.6 ± 0.3
	256	10	1.25 ± 0.24	0.0611	3.3 ± 0.2
			P=0.103		

[a] Study was performed at SITEK Research Laboratories. 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 vehicle control group; significant at P≤0.008 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE F3
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Tg.AC Hemizygous Mice Following Treatment with Bromodichloromethane by Gavage for 26 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]		13	1.00 ± 0.21		2.9 ± 0.2
Bromodichloromethane	25	14	1.25 ± 0.14	0.1938	2.9 ± 0.2
	50	12	1.54 ± 0.23	0.0440	3.0 ± 0.2
	100	14	1.29 ± 0.22	0.1636	2.7 ± 0.2
			P=0.188[e]		
Female					
Corn oil		11	1.05 ± 0.24		3.6 ± 0.2
Bromodichloromethane	25	14	1.00 ± 0.19	0.5628	3.8 ± 0.6
	50	13	0.96 ± 0.24	0.6140	3.1 ± 0.2
	100	13	0.96 ± 0.20	0.6140	3.5 ± 0.3
			P=0.613		

[a] Study was performed at SITEK Research Laboratories. 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 vehicle control group; significant at P≤0.008 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE F4
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of p53 Haploinsufficient Mice Following Administration of Bromodichloromethane in Drinking Water for 26 Weeks[a]

Compound	Dose (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Male					
Water[d]		15	1.13 ± 0.19		2.9 ± 0.2
Bromodichloromethane	175	14	1.36 ± 0.21	0.2222	2.7 ± 0.1
	350	15	2.23 ± 0.25	0.0005	2.8 ± 0.1
	700	15	1.57 ± 0.21	0.0742	3.0 ± 0.2
			P=0.057[e]		
Female					
Water		15	0.97 ± 0.19		3.0 ± 0.2
Bromodichloromethane	175	15	1.00 ± 0.17	0.4482	2.9 ± 0.1
	350	14	1.29 ± 0.19	0.1256	2.9 ± 0.2
	700	15	1.33 ± 0.14	0.0926	3.1 ± 0.1
			P=0.064		

[a] Study was performed at SITEK Research Laboratories. 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≤0.008 (ILS, 1990)
[d] Untreated control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

TABLE F5
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of p53 Haploinsufficient Mice Following Treatment with Bromodichloromethane by Gavage for 26 Weeks[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	Pairwise P Value[c]	PCEs[b] (%)
Male					
Corn oil[d]		15	1.67 ± 0.21		2.8 ± 0.2
Bromodichloromethane	25	15	1.67 ± 0.17	0.5000	2.6 ± 0.2
	50	15	1.47 ± 0.17	0.7322	2.6 ± 0.2
	100	15	1.60 ± 0.24	0.5801	2.6 ± 0.1
			P=0.615[e]		
Female					
Corn oil		15	1.07 ± 0.14		3.1 ± 0.2
Bromodichloromethane	25	14	0.86 ± 0.18	0.7916	2.9 ± 0.2
	50	14	1.11 ± 0.18	0.4412	3.0 ± 0.2
	100	14	1.25 ± 0.19	0.2580	2.9 ± 0.2
			P=0.161		

[a] Study was performed at SITEK Research Laboratories. 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 vehicle control group; significant at P≤0.008 (ILS, 1990)
[d] Vehicle control
[e] Significance of micronucleated NCEs/1,000 NCEs tested by one-tailed trend test, significant at P≤0.025 (ILS, 1990)

APPENDIX G
HEMATOLOGY RESULTS

TABLE G1
Hematology Data for Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Male				
n	13	14	15	13
Automated hematocrit (%)	44.2 ± 0.6	43.7 ± 0.7	43.5 ± 0.4	43.7 ± 0.4
Hemoglobin (g/dL)	14.2 ± 0.2	14.1 ± 0.2	14.0 ± 0.1	14.0 ± 0.1
Erythrocytes (10^6/μL)	10.20 ± 0.24	10.04 ± 0.21	9.96 ± 0.12	10.01 ± 0.16
Reticulocytes (10^6/μL)	0.15 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Mean cell volume (fL)	43.5 ± 0.6	43.6 ± 0.5	43.7 ± 0.3	43.7 ± 0.5
Mean cell hemoglobin (pg)	14.0 ± 0.2	14.1 ± 0.2	14.1 ± 0.1	14.0 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.1	32.2 ± 0.1	32.2 ± 0.1	32.0 ± 0.1
Platelets (10^3/μL)	1,011.2 ± 28.2	1,082.4 ± 83.5	984.9 ± 20.7	1,021.6 ± 37.8
Leukocytes (10^3/μL)	4.35 ± 0.61	3.89 ± 0.37	4.53 ± 0.40	4.07 ± 0.34
Segmented neutrophils (10^3/μL)	0.93 ± 0.18	0.94 ± 0.10	0.98 ± 0.13	0.75 ± 0.10
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	3.37 ± 0.43	2.91 ± 0.32	3.51 ± 0.31	3.27 ± 0.29
Monocytes (10^3/μL)	0.04 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.02 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.01 ± 0.00	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01
Female				
n	11	10	12	10
Automated hematocrit (%)	44.3 ± 0.6	43.8 ± 0.3	43.8 ± 0.6	45.0 ± 0.7
Hemoglobin (g/dL)	14.6 ± 0.2	14.3 ± 0.1	14.3 ± 0.3	14.6 ± 0.2
Erythrocytes (10^6/μL)	9.90 ± 0.19	9.73 ± 0.11	9.82 ± 0.17	10.32 ± 0.24
Reticulocytes (10^6/μL)	0.17 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.20 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.9 ± 0.4	45.0 ± 0.3	44.6 ± 0.4	43.7 ± 0.6
Mean cell hemoglobin (pg)	14.8 ± 0.2	14.7 ± 0.1	14.6 ± 0.2	14.2 ± 0.2
Mean cell hemoglobin concentration (g/dL)	33.0 ± 0.2	32.6 ± 0.1	32.7 ± 0.2	32.4 ± 0.1*
Platelets (10^3/μL)	896.9 ± 53.5	937.3 ± 106.8	837.3 ± 34.1	924.2 ± 127.7
Leukocytes (10^3/μL)	4.29 ± 0.35	4.92 ± 0.56	4.19 ± 0.26	5.46 ± 0.77
Segmented neutrophils (10^3/μL)	0.86 ± 0.20	1.19 ± 0.32	0.89 ± 0.17	1.26 ± 0.42
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05
Lymphocytes (10^3/μL)	3.39 ± 0.20	3.66 ± 0.28	3.24 ± 0.18	4.08 ± 0.37
Monocytes (10^3/μL)	0.01 ± 0.01	0.04 ± 0.02	0.02 ± 0.01	0.05 ± 0.02
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.03 ± 0.01

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

TABLE G2
Hematology Data for Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	12	12	12	14
Automated hematocrit (%)	41.9 ± 0.3	42.6 ± 0.8	43.0 ± 0.3	42.8 ± 0.4
Hemoglobin (g/dL)	13.6 ± 0.1	13.8 ± 0.3	14.0 ± 0.1	14.0 ± 0.2
Erythrocytes (10^6/µL)	9.46 ± 0.12	9.60 ± 0.19	9.57 ± 0.09	9.55 ± 0.14
Reticulocytes (10^6/µL)	0.19 ± 0.01	0.24 ± 0.02	0.023 ± 0.02*	0.26 ± 0.02**
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.3 ± 0.4	44.4 ± 0.3	45.0 ± 0.3	44.8 ± 0.2
Mean cell hemoglobin (pg)	14.4 ± 0.1	14.4 ± 0.1	14.6 ± 0.1	14.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.6 ± 0.1	32.4 ± 0.1	32.5 ± 0.1	32.8 ± 0.2
Platelets (10^3/µL)	1,201.8 ± 26.3	1,159.0 ± 55.1	1,112.5 ± 33.7*	1,050.4 ± 24.2**
Leukocytes (10^3/µL)	5.17 ± 0.28	5.29 ± 0.53	4.24 ± 0.52	4.16 ± 0.37
Segmented neutrophils (10^3/µL)	1.38 ± 0.12	1.62 ± 0.36	0.92 ± 0.19**	1.16 ± 0.27*
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	3.69 ± 0.28	3.57 ± 0.26	3.26 ± 0.32	2.92 ± 0.20
Monocytes (10^3/µL)	0.04 ± 0.01	0.04 ± 0.02	0.04 ± 0.02	0.02 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.06 ± 0.01	0.07 ± 0.02	0.03 ± 0.02*	0.05 ± 0.02
Female				
n	10	13	11	13
Automated hematocrit (%)	43.5 ± 0.6	42.9 ± 0.6	45.2 ± 1.1	43.4 ± 0.6
Hemoglobin (g/dL)	14.3 ± 0.2	14.1 ± 0.2	14.8 ± 0.4	14.2 ± 0.2
Erythrocytes (10^6/µL)	9.55 ± 0.18	9.31 ± 0.11	9.99 ± 0.29	9.52 ± 0.16
Reticulocytes (10^6/µL)	0.19 ± 0.02	0.17 ± 0.01	0.22 ± 0.02	0.20 ± 0.02
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.6 ± 0.4	46.1 ± 0.2	45.3 ± 0.5	45.6 ± 0.4
Mean cell hemoglobin (pg)	15.0 ± 0.1	15.2 ± 0.1	14.9 ± 0.2	14.9 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.9 ± 0.1	32.8 ± 0.1	32.7 ± 0.1
Platelets (10^3/µL)	906.4 ± 30.3	961.4 ± 66.2	991.5 ± 95.9	961.1 ± 26.8
Leukocytes (10^3/µL)	3.69 ± 0.37	5.25 ± 0.75	4.23 ± 0.41	4.30 ± 0.40
Segmented neutrophils (10^3/µL)	0.87 ± 0.14	1.51 ± 0.74	0.83 ± 0.19	0.97 ± 0.36
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.77 ± 0.30	3.66 ± 0.20*	3.33 ± 0.31	3.25 ± 0.20
Monocytes (10^3/µL)	0.01 ± 0.01	0.05 ± 0.03	0.02 ± 0.01	0.03 ± 0.03
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.04 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.05 ± 0.01

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

TABLE G3
Hematology Data for Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	13	14	12	14
Automated hematocrit (%)	43.9 ± 0.7	45.2 ± 0.6	42.9 ± 0.9	43.4 ± 0.8
Hemoglobin (g/dL)	14.1 ± 0.3	14.5 ± 0.2	13.8 ± 0.3	14.0 ± 0.3
Erythrocytes (10^6/μL)	9.87 ± 0.22	9.95 ± 0.12	9.51 ± 0.29	9.41 ± 0.19
Reticulocytes (10^6/μL)	0.16 ± 0.02	0.17 ± 0.01	0.15 ± 0.01	0.015 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	44.6 ± 0.4	45.4 ± 0.4	45.3 ± 0.5	46.1 ± 0.3**
Mean cell hemoglobin (pg)	14.4 ± 0.1	14.6 ± 0.1	14.6 ± 0.2	14.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.2 ± 0.1	32.1 ± 0.1	32.2 ± 0.1	32.3 ± 0.1
Platelets (10^3/μL)	1,034.8 ± 35.2	990.4 ± 31.7	1,011.3 ± 31.4	1,053.4 ± 45.7
Leukocytes (10^3/μL)	2.43 ± 0.35	1.45 ± 0.30*	1.35 ± 0.90	2.56 ± 0.33
Segmented neutrophils (10^3/μL)	0.60 ± 0.18	0.35 ± 0.17	0.21 ± 0.03	0.60 ± 0.22
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	1.81 ± 0.27	1.09 ± 0.14*	1.12 ± 0.07	1.93 ± 0.22
Monocytes (10^3/μL)	0.00 ± 0.00	0.01 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.01
Female				
n	10	13	12	13
Automated hematocrit (%)	45.4 ± 0.5	44.6 ± 1.0	44.0 ± 0.5	42.9 ± 0.8
Hemoglobin (g/dL)	14.8 ± 0.2	14.6 ± 0.3	14.5 ± 0.2	14.0 ± 0.3
Erythrocytes (10^6/μL)	9.86 ± 0.14	9.74 ± 0.28	9.57 ± 0.12	9.35 ± 0.24
Reticulocytes (10^6/μL)	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	46.1 ± 0.3	46.0 ± 0.3	46.0 ± 0.2	46.0 ± 0.5
Mean cell hemoglobin (pg)	15.0 ± 0.1	15.1 ± 0.2	15.1 ± 0.1	15.1 ± 0.2
Mean cell hemoglobin concentration (g/dL)	32.7 ± 0.1	32.8 ± 0.1	32.9 ± 0.1	32.8 ± 0.1
Platelets (10^3/μL)	734.3 ± 58.7	859.1 ± 36.9*	894.7 ± 67.1*	1,015.8 ± 80.2**
Leukocytes (10^3/μL)	3.12 ± 0.26	3.50 ± 0.27	3.74 ± 0.24	4.36 ± 0.45
Segmented neutrophils (10^3/μL)	0.43 ± 0.13	0.35 ± 0.05	0.41 ± 0.08	0.56 ± 0.13
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	2.66 ± 0.20	3.13 ± 0.23	3.28 ± 0.23	3.75 ± 0.38*
Monocytes (10^3/μL)	0.01 ± 0.01	0.00 ± 0.00	0.02 ± 0.01	0.03 ± 0.02
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.02 ± 0.01

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

TABLE G4
Hematology Data for p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	15	14	15	15
Automated hematocrit (%)	47.7 ± 0.3	47.5 ± 0.3	47.5 ± 0.3	46.3 ± 0.5**
Hemoglobin (g/dL)	15.5 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.0 ± 0.2**
Erythrocytes (10^6/μL)	10.60 ± 0.09	10.49 ± 0.07	10.59 ± 0.09	10.43 ± 0.08
Reticulocytes (10^6/μL)	0.09 ± 0.01	0.09 ± 0.013	0.10 ± 0.02	0.14 ± 0.02
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.1 ± 0.2	45.3 ± 0.1	44.9 ± 0.3	44.3 ± 0.2
Mean cell hemoglobin (pg)	14.6 ± 0.1	14.7 ± 0.0	14.4 ± 0.1	14.3 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.1	32.4 ± 0.1	32.2 ± 0.1*	32.4 ± 0.1
Platelets (10^3/μL)	911.9 ± 14.6	906.6 ± 15.3	898.7 ± 19.9	998.3 ± 44.1
Leukocytes (10^3/μL)	5.23 ± 0.49	5.07 ± 0.40	5.01 ± 0.33	5.03 ± 0.52
Segmented neutrophils (10^3/μL)	0.91 ± 0.08	0.87 ± 0.08	0.81 ± 0.06	0.89 ± 0.11
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	4.23 ± 0.42	4.10 ± 0.35	4.13 ± 0.34	4.04 ± 0.43
Monocytes (10^3/μL)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.01 ± 0.01
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.06 ± 0.01	0.08 ± 0.02	0.05 ± 0.01	0.09 ± 0.02
Female				
n	15	15	14	15
Automated hematocrit (%)	46.4 ± 0.4	46.6 ± 0.3	46.0 ± 0.3	45.9 ± 0.3
Hemoglobin (g/dL)	15.1 ± 0.1	15.3 ± 0.1	15.1 ± 0.1	15.1 ± 0.1
Erythrocytes (10^6/μL)	10.30 ± 0.10	10.30 ± 0.08	10.20 ± 0.08	10.07 ± 0.08
Reticulocytes (10^6/μL)	0.09 ± 0.01	0.12 ± 0.02	0.10 ± 0.01	0.09 ± 0.01
Nucleated erythrocytes (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.1 ± 0.2	45.3 ± 0.2	45.2 ± 0.1	45.6 ± 0.2
Mean cell hemoglobin (pg)	14.7 ± 0.1	14.9 ± 0.1	14.8 ± 0.1	15.0 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	32.6 ± 0.1	32.8 ± 0.1	32.8 ± 0.1	32.9 ± 0.1
Platelets (10^3/μL)	826.5 ± 23.7	819.3 ± 26.5	855.0 ± 27.5	912.8 ± 46.4
Leukocytes (10^3/μL)	3.79 ± 0.40	3.53 ± 0.21	4.29 ± 0.34	4.42 ± 0.41
Segmented neutrophils (10^3/μL)	0.46 ± 0.05	0.52 ± 0.05	0.50 ± 0.06	0.57 ± 0.06
Bands (10^3/μL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/μL)	3.27 ± 0.34	2.96 ± 0.18	3.75 ± 0.29	3.83 ± 0.36
Monocytes (10^3/μL)	0.00 ± 0.00	0.01 ± 0.00	0.01 ± 0.01	0.00 ± 0.00
Basophils (10^3/μL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/μL)	0.05 ± 0.02	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.01

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

TABLE G5
Hematology Data for p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	15	15	15	15
Automated hematocrit (%)	48.2 ± 0.6	47.7 ± 0.4	46.9 ± 0.4	46.5 ± 0.5
Hemoglobin (g/dL)	15.5 ± 0.2	15.3 ± 0.2	15.1 ± 0.1	14.9 ± 0.2
Erythrocytes (10^6/µL)	10.69 ± 0.13	10.51 ± 0.12	10.39 ± 0.08	10.19 ± 0.11**
Reticulocytes (10^6/µL)	0.12 ± 12.1	0.12 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.1 ± 0.2	45.5 ± 0.2	45.2 ± 0.2	45.6 ± 0.2
Mean cell hemoglobin (pg)	14.5 ± 0.1	14.6 ± 0.1	14.6 ± 0.1	14.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.1	32.1 ± 0.1	32.3 ± 0.1	32.1 ± 0.1
Platelets (10^3/µL)	983.1 ± 77.3	936.5 ± 35.8	1,001.0 ± 30.0*	1,055.4 ± 19.9**
Leukocytes (10^3/µL)	5.15 ± 0.31	6.31 ± 0.41*	5.60 ± 0.21	6.09 ± 0.41
Segmented neutrophils (10^3/µL)	0.45 ± 0.05	0.48 ± 0.05	0.46 ± 0.05	0.52 ± 0.05
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	4.64 ± 0.30	5.76 ± 0.38*	5.08 ± 0.22	5.51 ± 0.38
Monocytes (10^3/µL)	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.01
Female				
n	15	14	14	13
Automated hematocrit (%)	46.9 ± 0.5	46.7 ± 0.5	46.6 ± 0.3	46.1 ± 0.7
Hemoglobin (g/dL)	15.2 ± 0.2	15.2 ± 0.2	15.2 ± 0.1	15.0 ± 0.2
Erythrocytes (10^6/µL)	10.42 ± 0.13	10.29 ± 0.12	10.16 ± 0.10	10.09 ± 0.16
Reticulocytes (10^6/µL)	0.19 ± 0.01	0.18 ± 0.01	0.19 ± 0.01	0.18 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	45.0 ± 0.2	45.4 ± 0.2	45.8 ± 0.3**	45.8 ± 0.2**
Mean cell hemoglobin (pg)	14.6 ± 0.1	14.8 ± 0.1	15.0 ± 0.1**	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.5 ± 0.1	32.6 ± 0.2	32.6 ± 0.1	32.5 ± 0.2
Platelets (10^3/µL)	1,007.9 ± 91.9	947.4 ± 64.7	978.8 ± 55.3	998.3 ± 40.0
Leukocytes (10^3/µL)	3.15 ± 0.33	3.56 ± 0.14	3.19 ± 0.23	3.74 ± 0.20*
Segmented neutrophils (10^3/µL)	0.31 ± 0.04	0.37 ± 0.03	0.36 ± 0.03	0.40 ± 0.03
Bands (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Lymphocytes (10^3/µL)	2.80 ± 0.29	3.14 ± 0.14	2.80 ± 0.21	3.32 ± 0.18*
Monocytes (10^3/µL)	0.01 ± 0.00	0.01 ± 0.01	0.01 ± 0.00	0.01 ± 0.01
Basophils (10^3/µL)	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Eosinophils (10^3/µL)	0.04 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01

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

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

TABLE H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 26-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Male				
n	13	14	15	13
Necropsy body wt.	35.0 ± 1.4	34.6 ± 1.0	36.1 ± 0.9	33.8 ± 0.8
Heart				
Absolute	0.181 ± 0.005	0.185 ± 0.004	0.180 ± 0.005	0.173 ± 0.004
Relative	5.232 ± 0.159	5.388 ± 0.165	5.006 ± 0.128	5.143 ± 0.126
R. Kidney				
Absolute	0.294 ± 0.005	0.313 ± 0.008	0.305 ± 0.008	0.288 ± 0.009
Relative	8.555 ± 0.320	9.070 ± 0.185	8.542 ± 0.328	8.533 ± 0.211
Liver				
Absolute	1.630 ± 0.053	1.654 ± 0.052	1.722 ± 0.044	1.674 ± 0.057
Relative	46.914 ± 0.955	47.877 ± 1.004	47.791 ± 0.639	49.579 ± 1.318
Lung				
Absolute	0.283 ± 0.013	0.286 ± 0.014	0.297 ± 0.010	0.261 ± 0.014
Relative	8.234 ± 0.476	8.281 ± 0.346	8.286 ± 0.354	7.786 ± 0.467
R. Testis				
Absolute	0.084 ± 0.003	0.089 ± 0.002	0.087 ± 0.002	0.087 ± 0.002
Relative	2.455 ± 0.140	2.589 ± 0.096	2.433 ± 0.081	2.579 ± 0.085
Thymus				
Absolute	0.034 ± 0.003	0.038 ± 0.003	0.043 ± 0.004	0.034 ± 0.003
Relative	0.954 ± 0.068	1.095 ± 0.071	1.173 ± 0.078	1.004 ± 0.077
Female				
n	11	10	12	10
Necropsy body wt.	28.6 ± 0.9	28.8 ± 1.4	29.2 ± 1.0	29.3 ± 1.0
Heart				
Absolute	0.150 ± 0.004	0.152 ± 0.005	0.149 ± 0.004	0.148 ± 0.007
Relative	5.263 ± 0.107	5.334 ± 0.152	5.142 ± 0.153	5.043 ± 0.188
R. Kidney				
Absolute	0.218 ± 0.007	0.221 ± 0.008	0.219 ± 0.007	0.213 ± 0.006
Relative	7.649 ± 0.207	7.730 ± 0.144	7.527 ± 0.220	7.292 ± 0.190
Liver				
Absolute	1.460 ± 0.052	1.495 ± 0.058	1.509 ± 0.048	1.486 ± 0.030
Relative	51.084 ± 0.923	52.269 ± 1.205	51.858 ± 1.101	50.946 ± 0.938
Lung				
Absolute	0.266 ± 0.014	0.233 ± 0.007	0.262 ± 0.012	0.258 ± 0.017
Relative	9.334 ± 0.471	8.283 ± 0.454	9.108 ± 0.565	8.835 ± 0.503
Thymus				
Absolute	0.036 ± 0.002	0.039 ± 0.003	0.037 ± 0.003	0.035 ± 0.002
Relative	1.242 ± 0.057	1.341 ± 0.071	1.271 ± 0.071	1.179 ± 0.064

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

TABLE H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 39-Week Dermal Study of Bromodichloromethane[a]

	Vehicle Control	64 mg/kg	128 mg/kg	256 mg/kg
Male				
n	6	8	9	8
Necropsy body wt.	38.3 ± 2.5	38.8 ± 2.4	37.6 ± 1.9	36.9 ± 2.0
Heart				
Absolute	0.208 ± 0.011	0.201 ± 0.009	0.202 ± 0.009	0.223 ± 0.025
Relative	5.527 ± 0.376	5.223 ± 0.179	5.443 ± 0.254	6.008 ± 0.510
R. Kidney				
Absolute	0.356 ± 0.014	0.350 ± 0.016	0.346 ± 0.014	0.316 ± 0.007
Relative	9.421 ± 0.461	9.159 ± 0.454	9.251 ± 0.251	8.697 ± 0.381
Liver				
Absolute	2.065 ± 0.104	1.998 ± 0.114	1.987 ± 0.122	1.982 ± 0.126
Relative	54.519 ± 2.390	51.657 ± 0.965	52.660 ± 1.965	53.636 ± 1.006
Lung				
Absolute	0.245 ± 0.007	0.239 ± 0.011	0.254 ± 0.010	0.295 ± 0.027
Relative	6.543 ± 0.437	6.283 ± 0.389	6.822 ± 0.268	8.104 ± 0.795
R. Testis				
Absolute	0.082 ± 0.004	0.090 ± 0.003	0.092 ± 0.002	0.084 ± 0.005
Relative	2.175 ± 0.153	2.380 ± 0.178	2.511 ± 0.162	2.312 ± 0.181
Thymus				
Absolute	0.037 ± 0.007	0.035 ± 0.004	0.034 ± 0.006	0.039 ± 0.005
Relative	0.925 ± 0.121	0.912 ± 0.080	0.907 ± 0.159	1.071 ± 0.127
Female				
n	5	4	7	5
Necropsy body wt.	28.7 ± 1.8	28.1 ± 1.7	33.5 ± 3.0	28.9 ± 0.7
Heart				
Absolute	0.143 ± 0.006	0.146 ± 0.005	0.168 ± 0.008*	0.171 ± 0.009*
Relative	5.010 ± 0.100	5.230 ± 0.288	5.168 ± 0.365	5.926 ± 0.287
R. Kidney				
Absolute	0.259 ± 0.014	0.247 ± 0.010	0.249 ± 0.011	0.222 ± 0.007
Relative	9.075 ± 0.441	8.865 ± 0.502	7.674 ± 0.518*	7.691 ± 0.160
Liver				
Absolute	1.706 ± 0.138	1.613 ± 0.029	1.833 ± 0.133	1.604 ± 0.043
Relative	59.551 ± 3.727	58.115 ± 3.602	55.566 ± 2.783	55.541 ± 1.445
Lung				
Absolute	0.220 ± 0.008	0.233 ± 0.010	0.226 ± 0.006	0.281 ± 0.025*
Relative	7.794 ± 0.590	8.346 ± 0.187	7.124 ± 0.715	9.787 ± 0.965
Thymus				
Absolute	0.025 ± 0.003	0.039 ± 0.007	0.042 ± 0.005*	0.031 ± 0.002
Relative	0.857 ± 0.080	1.359 ± 0.194*	1.233 ± 0.082*	1.078 ± 0.083

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

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	13	12	12	14
Necropsy body wt.	41.3 ± 1.3	40.3 ± 1.6	38.4 ± 0.9	36.9 ± 1.0*
Heart				
Absolute	0.188 ± 0.004	0.175 ± 0.005*	0.171 ± 0.004**	0.162 ± 0.004**
Relative	4.588 ± 0.155	4.373 ± 0.110	4.458 ± 0.076	4.403 ± 0.102
R. Kidney				
Absolute	0.322 ± 0.009	0.293 ± 0.008**	0.281 ± 0.007**	0.280 ± 0.005**
Relative	7.822 ± 0.158	7.318 ± 0.160	7.317 ± 0.126	7.676 ± 0.295
Liver				
Absolute	1.904 ± 0.080	1.869 ± 0.090	1.800 ± 0.053	1.726 ± 0.055
Relative	45.942 ± 1.006	46.259 ± 0.790	46.829 ± 0.895	46.817 ± 0.739
Lung				
Absolute	0.236 ± 0.007	0.226 ± 0.008	0.250 ± 0.015	0.226 ± 0.009
Relative	5.773 ± 0.246	5.687 ± 0.246	6.555 ± 0.437	6.212 ± 0.316
R. Testis				
Absolute	0.094 ± 0.002	0.085 ± 0.003	0.090 ± 0.004	0.084 ± 0.004
Relative	2.286 ± 0.066	2.155 ± 0.149	2.353 ± 0.114	2.311 ± 0.130
Thymus				
Absolute	0.045 ± 0.003	0.053 ± 0.007	0.053 ± 0.005	0.046 ± 0.004
Relative	1.088 ± 0.053	1.298 ± 0.142	1.357 ± 0.105	1.244 ± 0.112
Female				
n	10	13	11	13
Necropsy body wt.	30.0 ± 1.4	35.0 ± 1.5	33.6 ± 2.4	33.2 ± 1.8
Heart				
Absolute	0.151 ± 0.006	0.159 ± 0.005	0.160 ± 0.006	0.150 ± 0.005
Relative	5.111 ± 0.226	4.605 ± 0.165	4.900 ± 0.219	4.595 ± 0.163
R. Kidney				
Absolute	0.223 ± 0.009	0.244 ± 0.011	0.232 ± 0.007	0.229 ± 0.007
Relative	7.505 ± 0.311	7.228 ± 0.678	7.149 ± 0.399	7.020 ± 0.272
Liver				
Absolute	1.506 ± 0.049	1.775 ± 0.041	1.760 ± 0.145	1.824 ± 0.116
Relative	50.533 ± 0.925	51.695 ± 2.470	52.343 ± 1.508	54.879 ± 1.933
Lung				
Absolute	0.241 ± 0.014	0.232 ± 0.009	0.250 ± 0.013	0.236 ± 0.006
Relative	8.168 ± 0.557	6.756 ± 0.355	7.840 ± 0.692	7.326 ± 0.427
Thymus				
Absolute	0.035 ± 0.003	0.039 ± 0.003	0.041 ± 0.004	0.040 ± 0.003
Relative	1.162 ± 0.066	1.114 ± 0.058	1.217 ± 0.081	1.216 ± 0.072

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

TABLE H4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	6	9	8	9
Necropsy body wt.	43.0 ± 1.5	42.0 ± 2.2	38.9 ± 1.4	38.9 ± 2.2
Heart				
Absolute	0.189 ± 0.007	0.179 ± 0.007	0.189 ± 0.008	0.184 ± 0.006
Relative	4.393 ± 0.106	4.299 ± 0.157	4.907 ± 0.268	4.817 ± 0.256
R. Kidney				
Absolute	0.364 ± 0.012	0.330 ± 0.014	0.308 ± 0.010**	0.285 ± 0.008**
Relative	8.507 ± 0.428	7.922 ± 0.268	7.995 ± 0.432	7.480 ± 0.375
Liver				
Absolute	2.112 ± 0.112	2.049 ± 0.110	2.011 ± 0.079	1.991 ± 0.136
Relative	49.019 ± 1.501	48.869 ± 1.258	51.928 ± 1.920	51.252 ± 2.147
Lung				
Absolute	0.227 ± 0.005	0.240 ± 0.013	0.267 ± 0.025	0.238 ± 0.013
Relative	5.310 ± 0.252	5.788 ± 0.291	6.983 ± 0.770	6.273 ± 0.499
R. Testis				
Absolute	0.082 ± 0.005	0.089 ± 0.003	0.090 ± 0.003	0.089 ± 0.003
Relative	1.916 ± 0.145	2.159 ± 0.142	2.331 ± 0.123	2.351 ± 0.196
Thymus				
Absolute	0.036 ± 0.006	0.040 ± 0.005	0.040 ± 0.005	0.040 ± 0.005
Relative	0.853 ± 0.150	0.965 ± 0.112	1.017 ± 0.105	1.028 ± 0.113
Female				
n	5	8	4	4
Necropsy body wt.	35.7 ± 1.7	36.3 ± 3.2	42.6 ± 4.6	39.8 ± 3.6
Heart				
Absolute	0.168 ± 0.001	0.171 ± 0.015	0.170 ± 0.012	0.178 ± 0.011
Relative	4.763 ± 0.239	4.800 ± 0.322	4.020 ± 0.154	4.568 ± 0.477
R. Kidney				
Absolute	0.260 ± 0.016	0.252 ± 0.008	0.262 ± 0.031	0.242 ± 0.011
Relative	7.300 ± 0.332	7.264 ± 0.572	6.149 ± 0.361	6.189 ± 0.514
Liver				
Absolute	1.882 ± 0.057	1.915 ± 0.113	2.390 ± 0.266	2.274 ± 0.106
Relative	53.151 ± 2.437	54.010 ± 2.515	56.105 ± 1.791	58.085 ± 3.803
Lung				
Absolute	0.229 ± 0.013	0.251 ± 0.031	0.233 ± 0.015	0.239 ± 0.016
Relative	6.549 ± 0.710	7.121 ± 0.826	5.563 ± 0.404	6.276 ± 1.058
Thymus				
Absolute	0.034 ± 0.004	0.037 ± 0.004	0.045 ± 0.009	0.041 ± 0.008
Relative	0.982 ± 0.139	1.008 ± 0.064	1.040 ± 0.097	1.006 ± 0.162

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

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

TABLE H5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	13	14	12	14
Necropsy body wt.	35.5 ± 1.5	34.8 ± 0.9	33.7 ± 1.1	33.0 ± 1.0
Heart				
Absolute	0.185 ± 0.008	0.185 ± 0.004	0.178 ± 0.006	0.185 ± 0.006
Relative	5.258 ± 0.205	5.383 ± 0.260	5.308 ± 0.168	5.659 ± 0.199
R. Kidney				
Absolute	0.295 ± 0.012	0.300 ± 0.008	0.291 ± 0.009	0.287 ± 0.005
Relative	8.344 ± 0.179	8.663 ± 0.281	8.682 ± 0.201	8.779 ± 0.261
Liver				
Absolute	1.667 ± 0.073	1.581 ± 0.038	1.626 ± 0.044	1.740 ± 0.071
Relative	47.118 ± 1.314	45.579 ± 1.001	48.515 ± 1.062	52.608 ± 0.955**
Lung				
Absolute	0.308 ± 0.019	0.331 ± 0.015	0.274 ± 0.012	0.283 ± 0.015
Relative	8.782 ± 0.565	9.591 ± 0.540	8.191 ± 0.390	8.605 ± 0.389
R. Testis				
Absolute	0.089 ± 0.002	0.083 ± 0.006	0.091 ± 0.003	0.088 ± 0.003
Relative	2.556 ± 0.125	2.397 ± 0.172	2.712 ± 0.099	2.690 ± 0.108
Thymus				
Absolute	0.035 ± 0.003	0.030 ± 0.003	0.031 ± 0.003	0.034 ± 0.003
Relative	0.967 ± 0.076	0.838 ± 0.060	0.890 ± 0.074	1.014 ± 0.069
Female				
n	11	14	13	13
Necropsy body wt.	27.0 ± 0.4	29.8 ± 1.3	28.0 ± 1.1	28.6 ± 1.2
Heart				
Absolute	0.147 ± 0.004	0.143 ± 0.003	0.148 ± 0.004	0.143 ± 0.003
Relative	5.464 ± 0.171	4.894 ± 0.201	5.363 ± 0.221	5.083 ± 0.161
R. Kidney				
Absolute	0.205 ± 0.004	0.211 ± 0.005	0.203 ± 0.006	0.216 ± 0.005
Relative	7.634 ± 0.208	7.216 ± 0.292	7.316 ± 0.173	7.671 ± 0.265
Liver				
Absolute	1.432 ± 0.052	1.544 ± 0.053[b]	1.490 ± 0.059	1.586 ± 0.059
Relative	53.104 ± 1.706	52.597 ± 1.223[b]	53.284 ± 0.719	55.706 ± 0.944
Lung				
Absolute	0.305 ± 0.012	0.294 ± 0.012	0.301 ± 0.015	0.293 ± 0.011
Relative	11.366 ± 0.547	10.023 ± 0.512	10.986 ± 0.765	10.414 ± 0.459
Thymus				
Absolute	0.029 ± 0.002	0.034 ± 0.002	0.037 ± 0.002	0.034 ± 0.003
Relative	1.087 ± 0.076	1.160 ± 0.077	1.330 ± 0.066	1.166 ± 0.078

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

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

[b] n=13

TABLE H6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Tg.AC Hemizygous Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	6	6	6	8
Necropsy body wt.	34.1 ± 2.1	38.7 ± 1.8	34.3 ± 2.1	33.4 ± 0.8
Heart				
Absolute	0.181 ± 0.016	0.185 ± 0.007	0.181 ± 0.009	0.179 ± 0.009
Relative	5.293 ± 0.245	4.804 ± 0.123	5.289 ± 0.109	5.418 ± 0.374
R. Kidney				
Absolute	0.295 ± 0.012	0.331 ± 0.021	0.290 ± 0.013	0.291 ± 0.005
Relative	8.718 ± 0.333	8.564 ± 0.455	8.498 ± 0.337	8.734 ± 0.150
Liver				
Absolute	1.851 ± 0.159	1.969 ± 0.099	1.993 ± 0.072	1.753 ± 0.047
Relative	53.920 ± 1.792	50.887 ± 1.117	58.687 ± 2.523	52.662 ± 1.567
Lung				
Absolute	0.273 ± 0.026	0.225 ± 0.012	0.238 ± 0.021	0.233 ± 0.014
Relative	8.041 ± 0.704	5.904 ± 0.477	7.160 ± 0.977	6.998 ± 0.430
R. Testis				
Absolute	0.085 ± 0.005	0.085 ± 0.003	0.085 ± 0.003	0.087 ± 0.003
Relative	2.545 ± 0.206	2.220 ± 0.119	2.509 ± 0.161	2.617 ± 0.102
Thymus				
Absolute	0.024 ± 0.001	0.032 ± 0.006	0.025 ± 0.004	0.026 ± 0.003
Relative	0.718 ± 0.061	0.799 ± 0.120	0.697 ± 0.094	0.756 ± 0.071
Female				
n	7	9	9	7
Necropsy body wt.	28.9 ± 1.3	30.5 ± 1.4	29.2 ± 0.9	33.0 ± 3.0
Heart				
Absolute	0.147 ± 0.007	0.136 ± 0.005	0.142 ± 0.005	0.157 ± 0.012
Relative	5.127 ± 0.276	4.481 ± 0.113	4.857 ± 0.116	4.843 ± 0.305
R. Kidney				
Absolute	0.229 ± 0.008	0.228 ± 0.010	0.222 ± 0.005	0.227 ± 0.009
Relative	7.953 ± 0.144	7.523 ± 0.275	7.665 ± 0.338	7.094 ± 0.412
Liver				
Absolute	1.541 ± 0.101	1.724 ± 0.140	1.696 ± 0.047	1.885 ± 0.145
Relative	53.132 ± 1.712	56.274 ± 3.140	58.106 ± 0.836	57.858 ± 2.881
Lung				
Absolute	0.224 ± 0.015	0.253 ± 0.017	0.221 ± 0.014	0.246 ± 0.023
Relative	7.875 ± 0.769	8.416 ± 0.676	7.609 ± 0.496	7.707 ± 0.830
Thymus				
Absolute	0.029 ± 0.002	0.028 ± 0.003	0.028 ± 0.003	0.038 ± 0.004
Relative	1.018 ± 0.055	0.927 ± 0.077	0.954 ± 0.096	1.151 ± 0.085

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

TABLE H7
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	15	15	15	15
Necropsy body wt.	48.5 ± 1.1	47.2 ± 1.0	43.6 ± 0.9**	37.4 ± 1.0**
Heart				
Absolute	0.219 ± 0.013	0.204 ± 0.008	0.197 ± 0.007	0.171 ± 0.004**
Relative	4.503 ± 0.198	4.332 ± 0.154	4.528 ± 0.132	4.593 ± 0.092
R. Kidney				
Absolute	0.302 ± 0.015	0.278 ± 0.005	0.254 ± 0.007**	0.225 ± 0.006**
Relative	6.234 ± 0.278	5.898 ± 0.102	5.839 ± 0.112	6.089 ± 0.242
Liver				
Absolute	2.796 ± 0.177	2.753 ± 0.165	2.277 ± 0.113*	2.025 ± 0.061**
Relative	57.157 ± 2.619	57.815 ± 2.444	51.939 ± 1.763	54.284 ± 0.907
Lung				
Absolute	0.253 ± 0.013	0.245 ± 0.009	0.247 ± 0.011	0.271 ± 0.011
Relative	5.238 ± 0.276	5.228 ± 0.218	5.710 ± 0.282	7.264 ± 0.261**
R. Testis				
Absolute	0.111 ± 0.002	0.111 ± 0.002	0.113 ± 0.002	0.110 ± 0.002
Relative	2.299 ± 0.032	2.374 ± 0.062	2.599 ± 0.040**	2.977 ± 0.080**
Thymus				
Absolute	0.071 ± 0.005	0.068 ± 0.002	0.068 ± 0.004	0.059 ± 0.004
Relative	1.462 ± 0.087	1.442 ± 0.050	1.553 ± 0.086	1.571 ± 0.090
Female				
n	15	15	14	15
Necropsy body wt.	36.8 ± 2.3	33.8 ± 1.1	35.1 ± 2.1	32.9 ± 1.7
Heart				
Absolute	0.172 ± 0.007	0.159 ± 0.005	0.167 ± 0.007	0.164 ± 0.007
Relative	4.825 ± 0.229	4.753 ± 0.185	4.894 ± 0.218	5.076 ± 0.216
R. Kidney				
Absolute	0.193 ± 0.005	0.190 ± 0.003	0.192 ± 0.006	0.193 ± 0.004
Relative	5.447 ± 0.255	5.685 ± 0.159	5.611 ± 0.209	6.007 ± 0.214
Liver				
Absolute	1.606 ± 0.082	1.496 ± 0.038	1.624 ± 0.081	1.687 ± 0.077
Relative	44.170 ± 0.966	44.590 ± 1.049	46.722 ± 0.856	51.562 ± 0.874**
Lung				
Absolute	0.232 ± 0.010	0.233 ± 0.010	0.249 ± 0.010	0.243 ± 0.007
Relative	6.603 ± 0.450	7.036 ± 0.412	7.301 ± 0.352	7.659 ± 0.415
Thymus				
Absolute	0.065 ± 0.004	0.062 ± 0.004	0.068 ± 0.003	0.063 ± 0.005
Relative	1.790 ± 0.094	1.853 ± 0.118	1.990 ± 0.103	1.895 ± 0.082

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

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

TABLE H8
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane[a]

	0 mg/L	175 mg/L	350 mg/L	700 mg/L
Male				
n	9	10	9	7
Necropsy body wt.	51.9 ± 0.8	48.9 ± 2.0	46.8 ± 1.7	43.5 ± 2.6**
Heart				
Absolute	0.224 ± 0.009	0.230 ± 0.008	0.214 ± 0.010	0.203 ± 0.011
Relative	4.313 ± 0.164	4.727 ± 0.124	4.575 ± 0.093	4.681 ± 0.082
R. Kidney				
Absolute	0.337 ± 0.013	0.330 ± 0.013	0.281 ± 0.017*	0.251 ± 0.017**
Relative	6.500 ± 0.240	6.816 ± 0.273	5.992 ± 0.257	5.833 ± 0.391
Liver				
Absolute	3.380 ± 0.173	3.359 ± 0.278	2.816 ± 0.197	2.670 ± 0.224
Relative	65.087 ± 2.942	67.531 ± 3.669	59.640 ± 2.393	61.124 ± 2.834
Lung				
Absolute	0.228 ± 0.008	0.222 ± 0.006	0.226 ± 0.008	0.345 ± 0.097
Relative	4.400 ± 0.186	4.619 ± 0.249	4.871 ± 0.252	8.894 ± 3.386
R. Testis				
Absolute	0.117 ± 0.002	0.116 ± 0.003	0.116 ± 0.004	0.112 ± 0.004
Relative	2.265 ± 0.038	2.915 ± 0.137	2.478 ± 0.061	2.623 ± 0.165*
Thymus				
Absolute	0.051 ± 0.004	0.052 ± 0.003	0.048 ± 0.006	0.049 ± 0.005
Relative	0.984 ± 0.066	1.069 ± 0.060	1.008 ± 0.109	1.129 ± 0.075
Female				
n	9	9	10	8
Necropsy body wt.	45.8 ± 2.0	42.5 ± 2.1	43.0 ± 1.7	41.1 ± 2.0
Heart				
Absolute	0.205 ± 0.012	0.179 ± 0.006	0.203 ± 0.008	0.199 ± 0.009
Relative	4.476 ± 0.185	4.274 ± 0.206	4.784 ± 0.286	4.876 ± 0.168
R. Kidney				
Absolute	0.254 ± 0.006	0.235 ± 0.007	0.237 ± 0.007	0.237 ± 0.006
Relative	5.610 ± 0.218	5.593 ± 0.214	5.565 ± 0.201	5.851 ± 0.252
Liver				
Absolute	2.021 ± 0.086	1.719 ± 0.106	1.884 ± 0.064	2.104 ± 0.109
Relative	44.213 ± 1.005	40.410 ± 1.539	44.190 ± 1.610	51.522 ± 2.221*
Lung				
Absolute	0.243 ± 0.014	0.280 ± 0.051	0.235 ± 0.014	0.228 ± 0.006
Relative	5.322 ± 0.202	6.686 ± 1.256	5.612 ± 0.541	5.623 ± 0.274
Thymus				
Absolute	0.050 ± 0.002	0.056 ± 0.008	0.053 ± 0.004	0.047 ± 0.002
Relative	1.103 ± 0.065	1.344 ± 0.200	1.244 ± 0.092	1.170 ± 0.063

* 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 as grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE H9
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 26-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	15	15	15	15
Necropsy body wt.	44.8 ± 1.3	44.7 ± 1.6	36.8 ± 1.2**	33.0 ± 1.5**
Heart				
Absolute	0.195 ± 0.008	0.192 ± 0.007	0.188 ± 0.007	0.162 ± 0.007**
Relative	4.361 ± 0.136	4.356 ± 0.186	5.173 ± 0.237**	4.946 ± 0.148**
R. Kidney				
Absolute	0.253 ± 0.010	0.250 ± 0.007	0.232 ± 0.006	0.222 ± 0.011*
Relative	5.654 ± 0.154	5.687 ± 0.231	6.350 ± 0.104**	6.745 ± 0.190**
Liver				
Absolute	1.923 ± 0.099	2.073 ± 0.144	1.682 ± 0.057	1.738 ± 0.111
Relative	42.739 ± 1.224	45.844 ± 1.987	45.957 ± 1.085	52.180 ± 0.987**
Lung				
Absolute	0.291 ± 0.014	0.270 ± 0.011	0.270 ± 0.013	0.251 ± 0.013
Relative	6.522 ± 0.299	6.225 ± 0.432	7.384 ± 0.324	7.730 ± 0.413*
R. Testis				
Absolute	0.115 ± 0.002	0.109 ± 0.002	0.110 ± 0.002	0.106 ± 0.003**
Relative	2.593 ± 0.066	2.471 ± 0.075	3.027 ± 0.077**	3.249 ± 0.098**
Thymus				
Absolute	0.059 ± 0.002	0.061 ± 0.004	0.050 ± 0.002*	0.046 ± 0.004**
Relative	1.330 ± 0.044	1.372 ± 0.069	1.355 ± 0.060	1.381 ± 0.084
Female				
n	15	14	14	14
Necropsy body wt.	31.4 ± 1.5	30.8 ± 1.2	29.2 ± 1.2	30.3 ± 1.1
Heart				
Absolute	0.161 ± 0.005	0.149 ± 0.004	0.161 ± 0.008	0.165 ± 0.009
Relative	5.294 ± 0.291	4.906 ± 0.150	5.603 ± 0.314	5.530 ± 0.343
R. Kidney				
Absolute	0.191 ± 0.005	0.185 ± 0.005	0.184 ± 0.006	0.185 ± 0.007
Relative	6.209 ± 0.195	6.057 ± 0.145	6.398 ± 0.293	6.158 ± 0.224
Liver				
Absolute	1.390 ± 0.054	1.416 ± 0.039	1.451 ± 0.069	1.755 ± 0.088**
Relative	44.780 ± 1.294	46.313 ± 0.869	49.536 ± 0.826**	57.653 ± 1.080**
Lung				
Absolute	0.287 ± 0.012	0.266 ± 0.013	0.259 ± 0.011	0.259 ± 0.011
Relative	9.230 ± 0.316	8.720 ± 0.422	8.963 ± 0.401	8.726 ± 0.519
Thymus				
Absolute	0.057 ± 0.003	0.053 ± 0.003	0.054 ± 0.003	0.048 ± 0.003
Relative	1.844 ± 0.079	1.763 ± 0.110	1.871 ± 0.092	1.606 ± 0.109

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

TABLE H10
Organ Weights and Organ-Weight-to-Body-Weight Ratios for p53 Haploinsufficient Mice in the 41-Week Gavage Study of Bromodichloromethane[a]

	Vehicle Control	25 mg/kg	50 mg/kg	100 mg/kg
Male				
n	10	9	10	10
Necropsy body wt.	49.8 ± 1.5	47.1 ± 1.7	44.3 ± 2.4*	38.2 ± 1.8**
Heart				
Absolute	0.197 ± 0.008	0.187 ± 0.006	0.184 ± 0.006	0.174 ± 0.007*
Relative	3.973 ± 0.162	3.999 ± 0.126	4.256 ± 0.254	4.609 ± 0.219
R. Kidney				
Absolute	0.288 ± 0.014	0.278 ± 0.012	0.266 ± 0.010	0.246 ± 0.008*
Relative	5.828 ± 0.324	5.912 ± 0.196	6.094 ± 0.204	6.562 ± 0.403
Liver				
Absolute	2.242 ± 0.171	2.183 ± 0.146	2.204 ± 0.154	2.453 ± 0.279
Relative	44.629 ± 2.472	46.056 ± 1.699	49.417 ± 1.265	66.126 ± 10.219**
Lung				
Absolute	0.228 ± 0.012	0.239 ± 0.013	0.200 ± 0.015	0.226 ± 0.014
Relative	4.608 ± 0.268	5.127 ± 0.348	4.674 ± 0.470	6.086 ± 0.566
R. Testis				
Absolute	0.112 ± 0.003	0.105 ± 0.003	0.107 ± 0.004	0.103 ± 0.003
Relative	2.274 ± 0.106	2.245 ± 0.106	2.463 ± 0.104	2.760 ± 0.144**
Thymus				
Absolute	0.058 ± 0.005	0.056 ± 0.002	0.057 ± 0.005	0.048 ± 0.004
Relative	1.163 ± 0.085	1.207 ± 0.067	1.338 ± 0.156	1.282 ± 0.151
Female				
n	9	9	8	9
Necropsy body wt.	38.8 ± 3.2	34.0 ± 2.8	35.0 ± 1.9	34.9 ± 2.9
Heart				
Absolute	0.162 ± 0.008	0.163 ± 0.005	0.166 ± 0.005	0.161 ± 0.007
Relative	4.326 ± 0.253	4.985 ± 0.354	4.828 ± 0.279	4.777 ± 0.318
R. Kidney				
Absolute	0.222 ± 0.011	0.217 ± 0.005	0.213 ± 0.006	0.196 ± 0.011
Relative	5.888 ± 0.308	6.641 ± 0.450	6.180 ± 0.298	5.749 ± 0.331
Liver				
Absolute	1.656 ± 0.088	1.644 ± 0.087	1.755 ± 0.096	2.106 ± 0.170*
Relative	43.721 ± 1.991	49.240 ± 1.661*	50.371 ± 1.614**	60.433 ± 1.162**
Lung				
Absolute	0.217 ± 0.012	0.213 ± 0.006	0.224 ± 0.011	0.217 ± 0.008
Relative	5.825 ± 0.453	6.604 ± 0.540	6.568 ± 0.561	6.522 ± 0.561
Thymus				
Absolute	0.055 ± 0..005	0.048 ± 0.003	0.053 ± 0.005	0.044 ± 0.003
Relative	1.442 ± 0.136	1.434 ± 0.118	1.523 ± 0.155	1.311 ± 0.113

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

APPENDIX I
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization

Bromodichloromethane

A single lot of bromodichloromethane (14522LS) was obtained from Aldrich Chemical Co. (Milwaukee, WI) for use in the 26-, 39-, 41-, and 42-week studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Battelle Memorial Institute (Columbus, OH), and the study laboratory, Battelle Columbus Operations (Columbus, OH). Reports on analyses performed in support of the bromodichloromethane studies are on file at the National Institute of Environmental Health Sciences.

Lot 14522LS, a clear, colorless liquid, was identified as bromodichloromethane by the analytical chemistry laboratory and the study laboratory using infrared (IR) spectroscopy. All spectra were consistent with the structure of bromodichloromethane, a literature spectra (*Aldrich,* 1985) of bromodichloromethane, and with the spectrum of a previously analyzed lot of bromodichloromethane. The infrared spectrum is presented in Figure I1.

The purity of lot 14522LS was determined by the analytical chemistry laboratory using gas chromatography (GC) by system A (Table I1) and by the study laboratory using GC by system B. GC by system A indicated one major peak and three impurity peaks with a combined peak area of 1.9% relative to the major peak area. GC by system B indicated a purity of 98.4% relative to a frozen reference standard of the same lot. The overall purity of lot 14522LS was determined to be 98% or greater.

Stability studies of another lot of bulk chemical (02107TG) were performed by the analytical chemistry laboratory using GC by system C. GC by system C indicated that bromodichloromethane was stable as a bulk chemical for 15 days when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at less than or equal to –20° C, protected from light, in heat-sealed glass ampules with potassium carbonate stabilizer. Stability of lot 14522LS was monitored by the study laboratory during the studies using GC by system B. No degradation of the bulk chemical was detected.

12-*O*-Tetradecanoylphorbol-13-acetate (TPA)

12-*O*-tetradecanoylphorbol-13-acetate was obtained from Sigma-Aldrich Chemical Company (St. Louis, MO) in one lot (48H1178) that was used in the 26-week studies in Tg.AC hemizygous mice. Identity and purity analyses were performed by Research Triangle Institute (RTI; Research Triangle Park, NC).

Lot 48H1178, a white crystalline powder, was identified as 12-*O*-tetradecanoylphorbol-13-acetate using IR and proton nuclear magnetic resonance (NMR) spectrometry. All spectra were consistent with the structure of 12-*O*-tetradecanoylphorbol-13-acetate.

The purity of lot 48H1178 was determined by RTI using high performance liquid chromatography (HPLC). HPLC analysis was performed with a Dupont Zorbax Rx C8 column (25 cm × 4.6 mm; Agilent Technologies, Palo Alto, CA), photodiode array detection monitored at 232 nm, and an isocratic mobile phase of water:acetonitrile (10:90) with a flow rate of 1.0 mL/minute. Analysis indicated one major peak and one impurity peak with an area equal to approximately 0.11% of the total integrated peak area. The overall purity of lot 48H1178 was determined to be greater than 99%.

Acetone

USP-grade acetone was obtained from Spectrum Chemicals and Laboratory Products (Gardena, CA) in three lots (NV0163, OG0513, OX0312) that were used during the 26- and 39-week dermal studies. Identity and purity analyses were performed by the study laboratory.

The identity of each lot was determined by IR spectroscopy; all spectra were consistent with a literature spectrum (*Aldrich,* 1985). The purity of all lots was determined using GC by system D. These analyses did not indicate any impurities with relative peak areas greater than 0.1% of the major peak area. The overall purity of all lots used was determined to be greater than 99%. No degradation of the acetone was detected.

Corn Oil

USP-grade corn oil was obtained from Spectrum Chemicals and Laboratory Products in six lots (OT0213, OU0101, OV0137, OH0409, PN0012, PO0173) that were used during the 26- and 41-week gavage studies. The study laboratory analyzed peroxide levels in bulk corn oil upon receipt and at least monthly thereafter using potentiometric titration. Potentiometric titration monitored via a double platinum sheet electrode demonstrated peroxide concentrations below the acceptable limit of 3 mEq/kg.

Preparation and Analysis of Dose Formulations

Dermal Studies

The dose formulations were prepared approximately every 4 weeks by mixing bromodichloromethane with USP-grade acetone to give the required concentration (Table I2). The dose formulations were stored at room temperature in amber glass bottles with Teflon®-lined lids for up to 39 days. A positive control dose formulation of TPA was prepared twice during the studies by adding the appropriate amount of TPA to acetone; the formulations were stored at approximately 5° C in amber glass bottles for up to 6 months.

Stability studies of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 µg/mL dose formulations were performed by the study laboratory using GC by systems similar to system B (Table I1). Stability was confirmed for dose formulations stored in amber glass bottles with Teflon®-lined lids for up to 39 days at room temperature.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC by systems similar to system B. During the 26- and 39-week studies, dose formulations were analyzed four times (Table I3). All 12 dose formulations for Tg.AC hemizygous mice were within 10% of the target concentration. Animal room samples of these dose formulations were also analyzed; all nine animal room samples were within 10% of the target concentration.

Drinking Water Studies

The dose formulations were prepared every 1 to 3 weeks by mixing bromodichloromethane with tap water (Table I2). Formulations were stored in glass bottles with Teflon®-lined lids at 5° C for up to 35 days. Positive control dose formulations of TPA were prepared and stored as described for the dermal studies.

Stability studies of 0.75, 0.9, 0.8, 1.0, 1.05, and 1.2 µg/mL dose formulations were performed by the study laboratory using GC by system E (Table I1). Stability was confirmed for at least 35 days for dose formulations stored in amber glass bottles at 5° C.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC by system E (Table I1). During the 26- and 42-week studies, dose formulations were analyzed four times. All 12 of the dose formulations for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target

concentration (Table I4). Animal room samples of these dose formulations were also analyzed; three of nine Tg.AC hemizygous mouse animal room samples and none of the nine p53 haploinsufficient mouse animal room samples were within 10% of the target concentration. These low results were attributed to the volatility and hydrophobic nature of bromodichloromethane.

Gavage Studies

The dose formulations were prepared approximately every 4 weeks by mixing bromodichloromethane with USP-grade corn oil to give the required concentrations (Table I2). Dose formulations were stored in amber glass bottles with Teflon®-lined lids and refrigerated for up to 35 days. The positive control dose formulations of TPA were prepared and stored as described for the dermal studies.

Stability studies of 0.4, 0.8, 1.6, 3.2, 6.4, and 12.8 µg/mL dose formulations were performed by the study laboratory using GC by systems similar to system B (Table I1). Stability was confirmed for at least 21 days for dose formulations stored in glass bottles protected from light at room temperature.

Periodic analyses of the dose formulations of bromodichloromethane were conducted by the study laboratory using GC by systems similar to system B. During the 26- and 41-week studies dose formulations were analyzed five times. All 12 dose formulations used in the studies for Tg.AC hemizygous and p53 haploinsufficient mice were within 10% of the target concentration (Table I5). Animal room samples of these dose formulations were also analyzed; eight of nine animal room samples were within 10% of the target concentration.

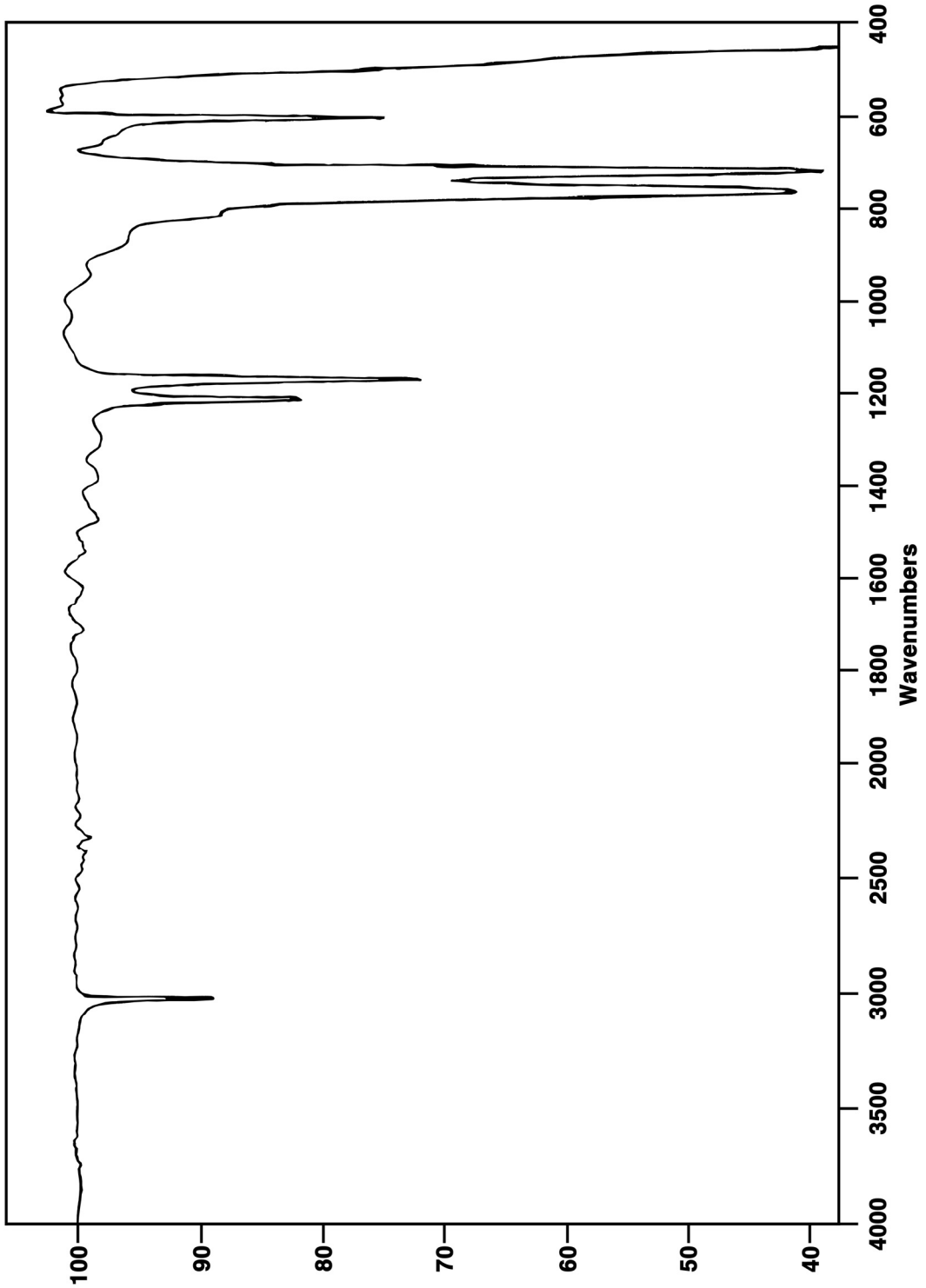

FIGURE I1
Infrared Absorption Spectrum of Bromodichloromethane

TABLE I1
Gas Chromatography Systems Used in the Dermal, Drinking Water, and Gavage Studies of Bromodichloromethane

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	Supelco Vocol 30 m × 0.25 mm, 1.5-µm film thickness (Supelco, Inc., Bellefonte, PA)	Helium at 3.5 mL/minute	40° C for 4 minutes, then 6° C/minute to 210° C, held for 2 minutes
System B			
Electron capture	Supelco Vocol 30 m × 0.25 mm, 1.5-µm film thickness (Supelco, Inc.)	Helium at 4 mL/minute	55° C for 7 minutes, then 5° C/minute to 100° C, then 30° C/minute to 150° C, held for 3 minutes
System C			
Electron capture	Supelco Vocol 30 m × 0.25 mm, 3.0-µm film thickness (Supelco, Inc.)	Helium at 8 mL/minute	40° C to 120° C at 10° C/minute, then 49° C/minute to 169° C, held for 1 minute
System D			
Flame ionization	20% SP-2401/0.1% Carbowax 1500 on 100/120 Supelcoport, 2.4 m × 2 mm (Supelco, Inc.)	Helium at 30 mL/minute	40° C for 4 minutes, then 10° C/minute to 170° C
System E			
Flame ionization	1% SP-1000.on 60/80 Carbopack B, 2.4 m × 2.0 mm (Supelco, Inc.)	Helium at 10 mL/minute	150° C isothermal

TABLE I2
Preparation and Storage of Dose Formulations in the Dermal, Drinking Water, and Gavage Studies of Bromodichloromethane

Dermal Studies	Drinking Water Studies	Gavage Studies
Preparation		
Bromodichloromethane: The required amount of bromodichloromethane was added to a specified initial volume of USP-grade acetone in a graduated mixing cylinder. The cylinder was sealed, shaken vigorously, and inverted at least 10 times then diluted to volume with USP-grade acetone and mixed as before. Dose formulations were prepared approximately every 4 weeks.	Bromodichloromethane: A specified amount of bromodichloromethane was added to 16 L of tap water in a 20 L glass mixing bottle and sealed with a Teflon®-lined screw cap. The bottle was sealed, shaken vigorously, and rolled on a bottle roller until the chemical was dissolved. Dose formulations were prepared every 1 to 3 weeks.	Bromodichloromethane: The required amount of bromodichloromethane was added to 600 mL of USP-grade corn oil in a calibrated glass mixing bottle, diluted to volume with USP-grade corn oil, sealed, shaken, and inverted at least 10 times. A stir bar was added and the formulation was stirred for at least 2 hours. Dose formulations were prepared approximately every 4 weeks.
TPA: A 12.5 µg/mL formulation was prepared by diluting the appropriate amount of TPA in acetone. Formulations were prepared twice during the studies.	TPA: A 12.5 µg/mL formulation was prepared by diluting the appropriate amount of TPA in acetone. Formulations were prepared twice during the studies.	TPA: A 12.5 µg/mL formulation was prepared by diluting the appropriate amount of TPA in acetone. Formulations were prepared twice during the studies.
Chemical Lot Number		
Bromodichloromethane: 14522LS TPA: 48H1178	Bromodichloromethane: 14522LS TPA: 48H1178	Bromodichloromethane: 14522LS TPA: 48H1178
Maximum Storage Time		
Bromodichloromethane: 39 days TPA: 6 months	Bromodichloromethane: 35 days TPA: 6 months	Bromodichloromethane: 35 days TPA: 6 months
Storage Conditions		
Bromodichloromethane: Formulations were transferred to 15-mL amber glass bottles, sealed with Teflon®-lined lids, and stored at room temperature.	Bromodichloromethane: Formulations remained in the glass containers in which they were prepared; the lids were sealed with Teflon®-lined screw caps and the containers were stored refrigerated at approximately 5° C.	Bromodichloromethane: Formulations were transferred to 15-mL amber glass bottles, sealed with Teflon®-lined lids, and stored refrigerated at approximately 5° C.
TPA: Stored in amber glass bottles sealed with Teflon®-lined lids and refrigerated at approximately 5° C.	TPA: Stored in amber glass bottles, sealed with Teflon®-lined lids, and refrigerated at approximately 5° C.	TPA: Stored in amber glass bottles sealed with Teflon®-lined lids and refrigerated at approximately 5° C.
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)	Battelle Columbus Operations (Columbus, OH)

TABLE I3
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous Mice in the 26- and 39-Week Dermal Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
August 11, 1999	August 11, 1999	19.4	19.15	–1
		38.8	39.38	+1
		77.6	77.03	–1
	September 16, 1999[b]	19.4	20.55	+6
		38.8	41.27	+6
		77.6	82.46	+6
November 2, 1999	November 3-4, 1999	19.4	19.30	–1
		38.8	39.48	+2
		77.6	77.50	0
	December 10, 1999[b]	19.4	19.48	0
		38.8	41.11	+6
		77.6	80.79	+4
January 25, 2000	January 26, 2000	19.4	20.26	+4
		38.8	38.49	–1
		77.6	75.98	–2
April 18, 2000	April 19, 2000	19.4	19.23	–1
		38.8	38.00	–2
		77.6	73.77	–5
	May 23-24, 2000[b]	19.4	20.40	+5
		38.8	40.18	+4
		77.6	79.10	+2

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

TABLE I4
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous and p53 Haploinsufficient Mice in the 26- and 42-Week Drinking Water Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
August 23, 1999	August 26, 1999	175	174.6	0
		175	171.7	–2
		175	174.4	0
		350	339.7	–3
		350	353.1	+1
		350	336.3	–4
		700	639.1	–9
		700	668.1	–5
		700	704.3	+1
November 9, 1999	November 10-11, 1999	175	170.3	–3
		175	166.3	–5
		350	327.7	–6
		350	329.2	–6
		700	686.3	–2
		700	631.4	–10
February 1, 2000	February 2-3, 2000	175	170.8	–2
		350	354.0	+1
		700	649.7	–7
May 3, 2000	May 8, 2000	175	188.2	+8
		350	378.2	+8
		700	751.8	+7
Animal Room Samples for Tg.AC Hemizygous Mice				
August 23, 1999	September 20-21, 1999	175	121.0	–31
		350	264.8	–24
		700	637.5	–9
November 9, 1999	December 6-7, 1999	175	136.6	–22
		350	372.3	+6
		700	662.7	–5
April 11, 2000	May 31, 2000	175	145.9	–17
		350	239.7	–32
		700	465.7	–33
Animal Room Samples for p53 Haploinsufficient Mice				
August 23, 1999	September 20, 1999	175	133.3	–24
		350	252.6	–28
		700	575.4	–18
November 9, 1999	December 6-7, 1999	175	139.9	–20
		350	247.3	–29
		700	619.7	–11
April 11, 2000	May 31, 2000	175	144.4	–17
		350	254.2	–27
		700	534.5	–24

[a] Results of duplicate analyses

TABLE I5
Results of Analyses of Dose Formulations Administered to Tg.AC Hemizygous and p53 Haploinsufficient Mice in the 26- and 41-Week Gavage Studies of Bromodichloromethane

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
September 8, 1999	September 9, 1999	2.5	2.418	–3
		5.0	4.977	0
		10.0	10.17	+2
	October 14-15, 1999	2.5	2.461	–2
		5.0	4.829	–3
		10.0	9.492	–5
December 1, 1999	December 3, 1999	2.5	2.374	–5
		5.0	4.851	–3
		10.0	9.420	–6
	January 5, 2000	2.5	2.259	–10
		5.0	4.684	–6
		10.0	9.280	–7
February 22, 2000	February 23, 2000	2.5	2.362	–6
		5.0	4.806	–4
		10.0	8.855	–11[b]
February 24, 2000	February 25, 2000	10.0	9.835	–2
May 16, 2000	May 16-17, 2000	2.5	2.390	–4
		5.0	4.777	–4
		10.0	9.444	–6
	June 22, 2000	2.5	2.372	–5
		5.0	4.682	–6
		10.0	8.845	–12

[a] Results of duplicate analyses.
[b] Not used in study.

APPENDIX J
WATER AND COMPOUND CONSUMPTION IN THE DRINKING WATER STUDIES OF BROMODICHLOROMETHANE

TABLE J1
Water and Compound Consumption by Male Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Week	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	7.7	23.1	2.1	22.6	16	1.4	22.6	21	1.1	22.7	34
2	4.6	24.6	2.7	24.1	20	2.6	23.2	39	1.7	20.8	57
3	5.9	26.1	3.1	25.7	21	2.8	25.0	40	2.1	23.9	61
4	4.4	26.8	3.1	26.7	20	2.9	25.8	39	2.4	25.9	65
5	4.8	27.8	3.2	27.7	20	2.8	27.3	36	2.4	26.5	63
6	5.3	29.4	3.2	28.9	19	2.8	28.1	35	2.4	27.3	61
7	4.8	30.0	3.6	29.4	22	3.0	28.6	36	2.9	27.4	74
8	5.0	30.2	3.8	29.9	22	3.4	28.9	41	2.6	28.1	65
9	4.7	31.0	4.0	30.5	23	3.4	29.5	40	2.7	28.7	65
10	4.9	32.0	3.7	31.1	21	3.0	30.1	35	2.7	29.3	65
11	5.2	32.8	3.8	31.4	21	3.2	30.0	37	2.6	30.2	61
12	6.0	32.6	4.3	32.2	23	3.2	30.6	36	2.6	30.7	60
13	4.7	34.2	4.2	33.1	22	3.3	31.4	37	2.7	31.8	60
14	5.3	34.9	4.0	33.5	21	3.1	31.6	34	2.7	32.1	59
15	5.2	35.2	4.1	33.6	21	3.6	31.3	41	2.8	32.1	62
16	4.6	35.7	3.7	34.5	19	3.1	31.9	34	2.8	33.1	59
17	4.6	35.8	4.1	34.7	21	3.5	32.0	39	3.1	32.8	66
18	5.3	35.9	4.3	35.0	22	3.6	32.1	39	3.2	33.1	68
19	5.0	35.6	4.0	34.6	20	3.6	32.7	38	2.8	32.9	60
20	5.0	36.9	3.9	35.4	19	3.4	33.5	35	3.1	32.7	65
21	5.3	37.6	4.1	36.4	20	3.7	34.7	38	3.1	32.6	66
22	4.9	38.4	3.9	37.9	18	3.4	34.9	34	3.0	33.2	64
23	4.3	39.2	3.8	38.7	17	3.5	35.6	34	2.9	34.9	59
24	4.6	39.6	4.1	39.9	18	3.6	35.9	35	2.9	36.2	55
25	4.8	40.2	3.9	40.2	17	3.6	37.3	34	2.9	35.9	56
26	4.1	41.0	3.7	40.4	16	3.6	38.3	33	2.9	36.5	55
Mean for Weeks											
1-13	5.2	29.3	3.4	28.7	21	2.9	27.8	36	2.4	27.2	61
14-26	4.8	37.4	4.0	36.5	19	3.5	34.0	36	2.9	33.7	61

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

TABLE J2
Water and Compound Consumption by Female Tg.AC Hemizygous Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1			2.7	19.1	24	1.5	19.0	28	1.5	18.9	55
2	6.0	20.0	3.4	20.3	29	3.1	20.2	54	3.6	20.2	126
3	6.6	21.2	4.1	21.4	33	4.3	21.3	70	5.0	21.4	163
4	6.o	22.3	4.0	22.3	32	4.6	22.3	72	4.3	22.3	135
5	4.9	23.2	4.2	23.1	32	4.1	22.7	64	4.1	22.7	128
6	6.6	24.2	4.3	24.1	31	4.6	23.6	68	4.8	23.8	140
7	6.6	24.6	4.6	24.3	33	4.8	23.9	71	4.6	23.8	135
8			4.8	24.7	34	5.0	24.4	71	4.9	24.0	142
9	5.8	24.8	5.1	25.8	35	4.9	24.8	70	4.8	25.0	136
10	5.8	25.4	5.3	25.6	36	5.0	25.1	69	5.2	25.3	145
11	5.1	24.5	4.9	27.1	32	4.8	25.5	65	5.3	25.8	144
12	6.3	25.3	4.9	27.2	32	4.9	26.2	65	5.4	26.2	145
13	4.8	26.3	5.1	28.2	32	4.7	26.7	62	5.3	26.8	138
14	6.4	26.4	5.2	28.5	32	4.8	26.9	62	5.0	26.8	131
15	5.6	26.6	5.5	29.0	33	4.9	27.8	61	5.4	27.9	136
16	4.9	27.6	5.9	29.5	35	4.9	28.0	61	5.8	28.1	144
17	5.3	27.0	5.6	29.1	34	4.8	27.8	61	5.4	28.2	135
18	6.2	27.5	6.0	30.4	34	4.7	27.8	60	5.5	28.4	137
19	5.5	27.9	5.5	30.5	32	4.9	29.0	59	5.5	28.6	135
20	5.3	29.3	5.0	31.0	28	4.7	29.8	55	5.1	29.5	120
21	5.7	29.2	4.8	31.6	26	5.1	29.9	60	5.3	30.1	124
22	4.8	29.5	5.0	31.9	27	4.8	30.7	54	5.0	30.7	115
23	4.8	29.9	5.1	33.1	27	4.4	31.8	49	5.4	31.2	120
24	4.8	30.2	5.3	33.9	28	5.1	32.1	55	5.2	32.5	112
25	5.0	28.5	5.0	34.6	25	4.9	33.5	51	5.3	32.9	113
26	4.9	29.9	5.2	34.9	26	5.0	33.9	52	5.2	33.2	109
Mean for Weeks											
1-13	5.9	23.8	4.4	24.1	32	4.3	23.5	64	4.5	23.6	133
14-26	5.3	28.4	5.3	31.4	30	4.8	29.9	57	5.3	29.8	126

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

TABLE J3
Water and Compound Consumption by Male Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Week	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	8.7	22.4	2.4	22.7	18	1.0	23.2	15	0.8	22.8	24
2	5.5	23.7	2.9	24.0	21	2.5	23.3	37	1.9	20.6	66
3	7.4	25.0	2.9	26.1	19	2.8	25.0	39	2.0	23.8	60
4	5.7	25.6	3.3	27.5	21	2.9	26.5	38	2.6	25.6	71
5	5.0	27.2	3.1	28.6	19	2.7	27.6	35	2.5	26.3	68
6	4.7	27.9	3.1	29.1	19	2.8	28.5	34	2.6	27.0	67
7	4.2	28.5	3.2	29.4	19	2.9	28.7	36	2.4	27.6	61
8	4.1	28.1	3.6	30.6	20	3.1	29.5	37	2.7	27.4	69
9	3.0	28.7	3.9	32.0	21	3.1	30.8	35	3.0	28.7	72
10	4.6	29.4	3.4	33.1	18	3.1	31.8	34	2.9	29.5	68
11	4.3	30.8	3.6	33.5	19	3.1	32.5	34	2.7	30.5	63
12	4.2	31.2	3.4	34.0	18	3.3	33.0	35	3.1	31.3	70
13	4.3	31.4	3.4	33.9	17	3.3	33.1	35	3.0	30.4	68
14	3.4	31.7	3.8	34.7	19	3.1	33.1	33	3.2	30.7	74
15	4.0	31.6	4.2	34.6	21	3.5	33.6	37	3.1	31.0	70
16	4.8	32.4	4.0	35.2	20	3.2	34.3	33	3.3	31.9	72
17	4.7	32.2	4.2	35.5	20	3.3	34.5	33	3.6	32.5	78
18	5.1	32.5	3.8	36.0	19	3.6	35.2	35	3.4	32.3	73
19	4.5	33.4	3.3	36.3	16	3.3	35.6	32	3.3	33.2	69
20	5.4	34.2	3.5	39.1	16	3.3	37.0	31	2.9	34.3	60
21	4.3	35.1	3.5	39.2	16	3.4	36.8	32	2.8	33.0	60
22	4.9	35.3	3.8	40.2	16	3.7	37.5	34	3.1	35.1	62
23	5.5	36.3	3.8	40.5	16	3.8	37.2	35	3.0	35.2	59
24	5.2	36.6	3.7	41.6	16	3.5	37.9	32	2.9	36.0	56
25	3.7	36.7	3.5	42.0	15	3.5	37.0	33	3.2	36.2	61
26	5.0	36.9	3.8	42.2	16	3.2	38.6	29	3.1	36.7	58
27	4.1	36.9	3.6	42.2	15	3.5	39.4	31	3.2	35.4	63
28	4.4	37.6	3.8	42.4	16	3.4	40.2	30	3.5	35.0	70
29	5.4	37.5	3.7	42.9	15	3.6	39.8	31	3.5	35.1	69
30	5.0	37.5	4.1	42.6	17	4.0	40.2	35	3.6	36.3	69
31	5.2	37.6	4.0	42.8	16	3.7	40.6	32	3.0	36.6	57
32	3.7	37.0	3.9	43.1	16	3.6	40.4	31	3.2	37.1	61
33	5.0	38.9	4.0	42.8	16	3.5	40.0	30	3.2	37.5	59
34	3.9	37.6	3.6	42.2	15	3.2	40.5	28	3.1	37.1	58
35	4.2	37.2	3.9	40.7	17	3.4	40.3	29	3.2	36.8	61
36	4.0	35.8	3.9	40.5	17	3.3	39.5	29	3.6	37.3	67
37	4.5	37.2	3.8	41.3	16	3.6	38.8	32	3.4	37.5	63
38	4.7	38.2	3.9	41.6	16	3.2	39.1	28	3.4	37.9	62
39	4.1	38.9	3.8	41.8	16	3.1	39.3	27	3.0	38.3	56
40	4.2	39.9	3.8	42.0	16	3.4	39.1	30	2.9	38.1	53
41	4.1	41.7	4.0	40.8	17	3.4	38.5	31	3.4	37.8	63
42	4.0	42.4	4.1	41.8	17	3.2	39.1	29	3.2	38.3	58
Mean for Weeks											
1-13	5.0	27.7	3.2	29.6	19	2.8	28.7	34	2.5	27.0	64
14-42	4.5	36.4	3.8	40.3	17	3.4	38.0	32	3.2	35.5	64

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

TABLE J4
Water and Compound Consumption by Female Tg.AC Hemizygous Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1			2.3	19.1	21	1.4	18.9	26	0.9	19.3	32
2	6.6	20.0	3.8	20.6	32	3.3	20.0	58	2.6	18.8	98
3	8.9	21.2	4.5	22.2	35	4.5	21.6	73	3.6	20.8	121
4	6.0	22.0	4.5	22.9	34	4.1	22.4	64	4.3	21.9	137
5	4.5	23.3	4.4	23.7	33	4.2	23.1	64	4.0	23.2	122
6			4.3	24.0	32	4.6	23.8	67	4.7	23.1	142
7	6.2	24.2	4.6	23.8	34	4.5	23.4	68	4.4	23.3	132
8	6.8	24.8	5.0	25.1	35	5.0	24.1	72	5.2	24.5	149
9	5.4	25.2	5.4	25.9	37	4.0	24.7	57	5.2	24.8	147
10	5.3	26.4	5.4	26.1	36	5.0	25.4	69	5.1	24.8	143
11	6.8	26.9	5.0	27.0	33	4.8	26.5	63	6.1	25.8	166
12	6.0	26.7	5.5	26.2	36	4.6	26.2	61	5.7	26.5	152
13	5.0	27.3	5.1	26.7	34	4.1	26.2	55	5.2	26.8	136
14	4.8	27.8	4.7	27.1	30	4.4	27.1	57	5.1	26.7	134
15	5.1	28.1	4.7	27.3	30	4.9	27.9	61	5.1	27.4	130
16	4.1	28.9	4.6	27.6	29	4.3	28.4	54	4.8	27.6	122
17	5.7	28.5	4.9	27.9	30	4.8	29.2	57	4.8	27.4	123
18	5.1	28.2	5.0	28.6	30	5.0	29.4	60	5.1	27.8	128
19	4.3	29.3	4.8	29.0	29	5.1	30.4	58	4.4	29.2	105
20	4.8	29.9	4.7	29.8	28	4.1	31.4	45	4.6	29.6	109
21	5.2	30.3	4.8	29.5	29	4.9	30.6	56	4.8	30.5	111
22	5.5	30.2	4.9	30.3	28	4.9	32.0	54	4.6	31.7	102
23	5.8	31.3	4.6	30.2	27	4.7	33.1	49	4.2	32.6	91
24	6.2	30.4	4.3	30.9	24	4.3	33.9	45	4.8	33.4	101
25	5.1	31.5	4.8	32.7	26	4.5	35.0	45	4.1	33.7	86
26	5.6	31.8	4.5	33.6	24	4.4	35.8	43	4.5	33.9	93
27	4.9	31.5	4.7	33.9	24	4.0	37.2	38	4.6	35.0	93
28	5.3	31.3	4.3	34.0	22	3.5	37.5	33	4.6	34.8	93
29	5.4	32.7	4.6	34.2	24	3.6	37.9	34	4.8	35.1	95
30	5.5	32.9	4.3	34.6	22	4.1	38.1	38	4.2	33.2	89
31	5.2	32.6	4.6	34.0	23	4.0	38.3	37	3.9	33.1	83
32	4.5	32.9	4.7	32.2	26	3.6	36.6	34	4.9	32.9	105
33	4.5	32.9	5.3	32.0	29	3.7	37.7	34	4.4	32.9	93
34	5.9	33.2	4.9	33.3	26	3.8	36.6	37	4.4	33.3	93
35	4.9	33.5	4.8	33.7	25	4.2	38.3	39	5.4	32.3	117
36	5.5	32.7	5.3	34.0	27	4.6	39.2	41	5.1	33.0	108
37	5.0	33.3	5.2	32.8	28	4.5	41.0	38	4.8	33.2	101
38	5.6	34.8	5.3	33.5	28	4.5	42.3	38	5.0	32.8	107
39	6.1	35.3	5.1	34.2	26	4.9	42.7	40	4.4	33.2	93
40	5.6	35.7	5.0	34.8	25	4.7	41.9	39	4.1	36.5	79
41	6.1	36.6	5.0	34.9	25	4.9	41.2	42	4.5	35.8	88
42	6.4	35.7	4.8	35.8	24	4.7	42.8	38	5.4	34.9	108
Mean for Weeks											
1-13	6.1	24.4	4.6	24.1	33	4.2	23.6	61	4.4	23.3	129
14-42	5.3	31.8	4.8	31.9	26	4.4	35.6	44	4.7	32.2	103

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

TABLE J5
Water and Compound Consumption by Male p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

Week	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	3.7	22.8	2.2	23.0	17	1.4	22.9	21	1.2	22.5	37
2	3.7	24.2	2.9	23.7	21	2.5	21.8	41	2.2	20.1	76
3	3.6	25.6	3.1	25.0	22	2.5	24.2	36	2.5	22.0	81
4	3.4	26.7	2.9	26.2	20	2.5	25.2	35	2.3	24.1	68
5	3.6	27.9	3.0	26.9	20	2.7	26.4	36	2.4	24.8	69
6	3.3	28.3	2.9	28.1	18	2.7	26.9	35	2.4	24.8	69
7	3.7	30.2	3.1	28.5	19	2.8	27.4	35	2.7	25.2	74
8	4.0	31.1	3.3	30.3	19	2.9	28.3	36	2.6	26.0	71
9	3.7	33.0	3.1	32.1	17	2.8	29.7	33	2.6	27.2	67
10	4.0	34.7	3.2	33.5	17	3.0	30.7	34	2.8	27.9	70
11	3.7	36.1	3.2	35.2	16	2.9	32.1	32	3.1	27.9	77
12	3.7	37.0	3.1	36.3	15	2.7	33.1	28	2.9	29.0	70
13	3.9	38.4	3.2	37.3	15	2.9	33.3	30	2.9	29.6	68
14	4.0	38.9	3.2	38.3	15	2.9	34.2	30	2.8	30.1	65
15	4.0	39.8	3.2	38.9	14	2.9	35.2	29	2.8	30.8	64
16	3.8	40.8	3.2	39.2	14	2.7	36.1	27	2.8	31.2	62
17	4.1	42.0	3.3	40.6	14	3.0	37.1	28	2.8	32.1	62
18	4.0	42.9	3.4	40.8	15	3.1	37.5	29	2.9	33.0	62
19	3.9	43.4	3.1	42.0	13	3.0	37.6	28	2.9	33.3	61
20	3.9	43.8	3.2	43.0	13	3.1	38.8	28	2.8	33.9	58
21	4.1	45.1	3.2	43.7	13	3.1	39.4	28	3.0	34.7	61
22	4.0	45.9	3.4	44.2	13	3.6	39.6	31	3.0	34.8	61
23	4.0	46.4	3.2	45.0	12	3.0	41.0	26	3.0	35.1	61
24	4.0	47.1	2.9	45.6	11	2.9	41.1	25	2.8	36.9	53
25	3.9	47.4	3.3	45.2	13	3.0	41.5	26	3.1	36.0	61
26	4.1	47.8	3.3	46.3	12	3.1	42.7	26	3.1	36.5	59
Mean for Weeks											
1-13	3.7	30.5	3.0	29.7	18	2.6	27.9	33	2.5	25.5	69
14-26	4.0	43.9	3.2	42.5	13	3.0	38.6	28	2.9	33.7	61

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

TABLE J6
Water and Compound Consumption by Female p53 Haploinsufficient Mice in the 26-Week Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	4.1	18.9	2.3	18.9	22	1.6	18.8	30	1.4	18.6	54
2	4.1	19.6	2.9	19.6	26	2.8	19.3	51	2.7	19.1	98
3	4.3	20.8	3.6	20.2	31	3.2	20.0	56	3.1	20.0	107
4	3.8	21.8	3.5	21.4	28	3.6	21.4	59	3.3	21.1	110
5	4.1	22.1	3.5	21.9	28	3.7	21.9	59	3.8	22.0	120
6	4.1	22.0	3.6	21.9	29	3.7	22.4	58	3.7	22.3	117
7	4.2	22.3	4.0	22.1	31	4.0	21.8	65	3.9	21.9	126
8	4.1	23.3	3.8	22.5	29	3.9	23.2	59	3.6	22.7	112
9	4.2	23.6	3.7	23.0	28	3.7	23.5	55	3.9	22.4	121
10	4.1	24.6	3.8	23.7	28	3.8	24.3	55	3.8	24.1	112
11	4.1	24.7	3.8	24.4	27	3.8	24.6	54	3.6	24.1	105
12	4.2	25.3	3.8	24.5	27	3.9	25.2	54	3.7	24.6	104
13	3.9	26.5	3.7	25.2	26	3.7	25.8	50	3.6	24.9	101
14	4.1	27.1	3.8	26.0	25	3.8	27.0	50	3.5	25.9	96
15	4.1	27.9	3.8	26.4	25	3.8	27.3	48	3.7	26.3	98
16	4.2	29.0	4.0	26.4	27	3.9	27.8	50	3.7	26.9	97
17	4.3	28.7	4.0	26.8	26	4.1	28.3	51	3.9	28.4	96
18	4.4	29.1	4.2	27.6	27	4.1	28.7	50	4.0	27.6	100
19	4.3	29.8	4.0	28.1	25	4.0	29.2	48	3.9	27.5	99
20	4.3	30.6	4.1	29.1	25	3.9	29.4	46	4.0	28.3	98
21	4.3	31.5	3.9	29.7	23	4.0	30.4	46	3.9	29.2	95
22	4.4	32.7	4.2	30.8	24	4.3	30.9	49	4.0	30.0	92
23	4.3	34.0	3.9	32.2	21	3.8	32.9	41	4.0	30.4	92
24	4.1	35.3	3.5	32.1	19	3.4	33.2	36	3.7	30.8	83
25	4.3	35.6	4.3	32.1	23	4.1	32.6	44	3.9	31.3	88
26	4.3	36.4	4.1	33.2	21	3.9	34.0	40	3.9	31.7	85
Mean for Weeks											
1-13	4.1	22.7	3.5	22.2	28	3.5	22.5	54	3.4	22.1	107
14-26	4.3	31.4	4.0	29.3	24	3.9	30.1	46	3.8	28.8	94

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

TABLE J7
Water and Compound Consumption by Male p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

Week	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	3.5	23.0	1.8	22.5	14	2.2	23.0	34	0.9	22.8	28
2	3.5	23.8	2.8	23.3	21	2.7	23.0	40	2.2	20.3	74
3	3.2	24.8	2.7	24.2	20	2.6	24.1	38	2.2	22.8	67
4	3.3	25.5	2.8	25.4	20	2.7	25.5	38	2.3	24.3	66
5	3.3	26.3	2.9	26.5	19	2.7	26.0	36	2.2	24.6	64
6	2.3	27.2	2.1	27.5	14	2.0	26.4	26	1.7	25.0	48
7	3.4	29.1	3.0	28.9	18	2.8	27.4	35	2.6	25.7	72
8	3.7	29.4	3.2	29.8	19	3.2	28.2	39	2.7	27.1	70
9	3.6	31.7	3.0	31.0	17	3.0	28.9	37	2.5	28.2	63
10	3.7	33.5	3.2	32.3	17	3.1	30.8	35	2.8	29.5	66
11	3.7	34.6	3.1	32.7	17	3.1	30.9	35	2.6	30.3	59
12	3.6	35.7	2.9	34.8	15	3.1	32.4	33	2.6	31.7	57
13	3.6	36.8	3.3	35.1	16	3.0	33.5	32	2.7	31.3	60
14	3.7	37.4	3.0	36.2	15	3.0	33.9	31	2.7	31.8	59
15	3.9	38.2	3.0	37.0	14	3.1	34.8	31	2.6	32.9	56
16	4.0	39.3	3.0	38.0	14	3.2	35.6	31	2.7	33.5	57
17	4.0	40.5	3.3	39.8	15	3.3	37.0	31	3.0	34.4	61
18	4.0	41.5	3.1	40.2	13	3.3	38.0	31	3.3	34.5	67
19	4.0	41.3	3.2	41.3	14	3.2	38.3	30	2.8	35.7	56
20	4.3	41.5	3.2	41.8	13	3.2	38.9	28	2.9	36.6	56
21	4.0	43.0	3.1	42.3	13	3.2	39.1	29	2.7	37.2	51
22	4.4	43.7	3.1	43.5	13	3.3	40.2	29	2.9	36.6	56
23	4.3	44.9	3.1	43.9	12	3.1	41.5	26	3.1	37.1	58
24	4.1	45.8	2.8	44.5	11	3.1	41.6	26	2.6	37.6	49
25	4.0	46.0	3.1	44.6	12	3.4	40.7	29	2.9	36.4	56
26	4.1	46.8	3.2	44.3	13	3.2	41.1	27	2.8	39.7	50
27	4.4	47.4	3.2	45.4	12	3.4	41.5	29	3.0	40.4	53
28	4.2	47.9	3.2	45.8	12	3.4	42.5	28	3.2	39.6	57
29	4.3	48.2	3.1	46.3	12	3.4	42.9	28	3.4	41.0	58
30	3.9	48.3	3.0	46.4	11	3.2	41.6	26	2.8	41.4	47
31	4.7	47.2	3.4	46.2	13	3.4	42.6	28	3.1	40.9	52
32	4.4	47.0	3.2	46.7	12	3.4	44.3	26	2.9	42.4	47
33	5.0	49.1	3.4	47.5	12	3.4	44.1	27	2.9	43.0	48
34	4.6	49.8	3.4	47.7	13	3.4	44.9	27	3.2	44.1	50
35	4.6	50.1	3.3	47.7	12	3.5	45.3	27	3.0	43.4	49
36	4.8	49.9	3.5	48.3	13	3.7	45.4	29	2.9	43.0	48
37	4.9	49.7	3.2	47.2	12	3.5	45.5	27	2.9	44.5	45
38	4.9	50.3	3.5	48.7	12	3.7	46.5	28	3.1	45.0	48
39	4.7	50.9	3.6	48.2	13	3.6	46.7	27	3.4	44.5	53
40	4.6	51.0	3.3	48.5	12	3.5	45.8	27	3.4	44.3	53
41	4.7	51.3	3.5	48.8	12	3.5	46.1	27	3.5	44.3	55
42	4.6	51.6	3.3	49.1	12	3.6	45.6	28	3.4	43.7	54
Mean for Weeks											
1-13	3.4	29.3	2.8	28.8	17	2.8	27.7	35	2.3	26.4	61
14-42	4.4	46.2	3.2	44.7	13	3.3	41.8	28	3.0	39.6	53

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

TABLE J8
Water and Compound Consumption by Female p53 Haploinsufficient Mice in the 42-Week Drinking Water Study of Bromodichloromethane

	0 mg/L		175 mg/L			350 mg/L			700 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Water (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
1	3.6	18.8	1.9	18.7	18	1.4	18.4	26	1.8	19.1	66
2	3.7	19.5	2.8	19.6	25	3.2	18.7	59	3.0	19.4	108
3	3.7	20.0	3.1	20.5	26	3.1	19.5	55	3.1	19.6	109
4	3.6	21.0	3.2	21.4	26	3.4	20.7	57	3.3	21.5	108
5	4.0	21.3	3.6	21.5	29	3.8	21.0	63	3.7	21.7	120
6	2.9	22.2	2.3	22.4	18	2.7	22.0	43	2.9	22.7	89
7	4.1	22.5	3.7	22.8	29	3.8	21.8	61	4.3	23.1	130
8	4.2	23.0	3.8	23.5	28	3.8	22.8	58	4.0	23.5	118
9	3.8	23.7	3.9	23.6	29	3.7	23.3	56	4.1	23.9	121
10	4.0	24.2	4.0	24.4	28	3.7	23.9	54	4.1	24.9	116
11	4.2	24.0	3.7	25.1	26	3.5	24.0	52	4.0	24.6	113
12	4.4	23.9	3.7	25.9	25	3.7	24.3	54	4.3	24.8	123
13	4.3	24.9	3.7	26.5	25	3.6	25.3	50	4.2	25.3	115
14	5.0	25.6	3.8	26.9	25	3.8	25.6	52	4.3	26.1	116
15	4.3	25.6	3.8	27.1	24	3.7	25.6	51	4.1	26.1	111
16	4.6	25.8	3.8	27.2	24	3.8	26.5	50	4.3	26.4	114
17	4.7	27.0	4.2	28.4	26	3.8	27.4	48	4.5	27.3	116
18	4.7	27.6	3.8	29.0	23	3.8	27.8	48	4.4	28.3	109
19	4.9	27.9	4.1	29.4	24	3.7	28.0	46	4.4	28.9	107
20	4.5	28.7	4.1	30.6	23	3.7	28.7	45	4.4	28.7	107
21	4.4	28.7	4.0	30.0	23	3.8	28.4	47	4.5	28.3	112
22	4.4	29.5	4.2	31.0	24	3.9	29.6	46	4.4	30.0	104
23	4.5	30.4	3.9	32.1	21	3.7	30.8	42	4.4	31.0	100
24	4.0	30.8	3.4	32.5	18	3.7	31.2	42	4.1	32.1	90
25	4.3	31.5	4.0	32.0	22	3.8	30.9	43	4.5	32.0	99
26	4.3	32.1	3.8	31.9	21	3.6	32.3	39	4.2	32.3	91
27	4.8	32.9	4.0	31.6	22	3.7	32.8	40	4.4	33.6	92
28	4.5	34.0	3.9	34.3	20	3.7	33.8	38	4.3	35.3	85
29	4.6	35.3	4.1	35.3	20	3.7	35.3	37	4.3	35.2	86
30	4.5	36.3	3.8	36.3	18	3.5	36.5	34	3.8	36.9	72
31	4.5	35.4	4.1	36.1	20	3.7	36.4	36	4.2	34.5	86
32	4.8	37.0	4.0	37.3	19	3.5	36.6	33	4.6	34.4	95
33	4.8	37.8	4.0	37.8	18	3.7	37.4	35	5.0	36.0	96
34	5.1	38.3	4.1	39.3	18	3.7	39.1	33	4.5	38.6	82
35	5.3	39.0	4.0	39.7	18	3.7	38.9	33	4.4	39.3	78
36	5.0	39.7	3.9	40.4	17	3.8	38.9	34	4.3	39.9	76
37	5.3	39.4	4.0	40.8	17	3.7	38.9	34	4.2	39.5	74
38	5.6	40.8	4.1	41.4	17	3.7	40.2	32	4.2	39.4	75
39	5.0	41.5	3.8	42.3	16	3.8	41.2	32	4.2	40.0	73
40	4.8	42.4	3.6	43.5	15	3.6	41.2	30	4.2	39.7	73
41	5.1	43.6	3.9	44.2	16	3.9	42.5	32	4.6	39.9	80
42	4.4	44.5	3.6	43.6	15	3.7	43.9	29	4.3	40.4	74
Mean for Weeks											
1-13	3.9	22.2	3.3	22.8	25	3.3	22.0	53	3.6	22.6	110
14-42	4.7	34.1	3.9	34.9	20	3.7	34.0	39	4.4	33.8	92

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

National Toxicology Program
National Institute of Environmental Health Sciences
National Institutes of Health
P.O. Box 12233, MD K2-05
Durham, NC 27709
Tel: 984-287-3211
ntpwebrequest@niehs.nih.gov

https://ntp.niehs.nih.gov

ISSN 1556-5246