NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF DIBROMOACETONITRILE

(CAS NO. 3252-43-5)

IN F344/N RATS AND B6C3F1 MICE

(DRINKING WATER STUDIES)

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709

June 2010

NTP TR 544

NIH Publication No. 10-5886

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.

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SUMMARY

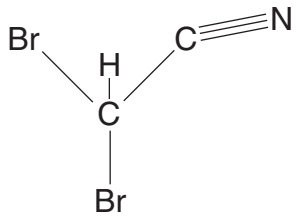
Background
Dibromoacetonitrile occurs in chlorinated drinking water that contains bromine and interacts with organic matter. We studied the effects of dibromoacetonitrile in drinking water on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods
We gave drinking water containing 50, 100, or 200 milligrams of dibromoacetonitrile per liter of water to groups of 50 male and female rats and mice for two years. Control animals received the same tap water with no chemical added. At the end of the study, tissues from more than 40 sites were examined for every animal.

Results
Survival was similar for animals receiving dibromoacetonitrile and the controls. Male and female rats receiving dibromoacetonitrile had increased rates of squamous cell adenomas or carcinomas of the mouth (oral mucosa or tongue). Two male rats receiving 200 mg/L had rare adenomas of the glandular stomach. There were a few instances of skin tumors in female rats receiving 100 or 200 mg/L. Male and female mice exposed to dibromoacetonitrile had increased rates of squamous cell papillomas of the forestomach. There were also slight increases in the occurrence of liver tumors in male mice.

Conclusions
We conclude that dibromoacetonitrile in the drinking water caused cancer of the oral cavity in male and female rats and of the glandular stomach in male rats. We conclude that dibromoacetonitrile caused cancer of the forestomach in male and female mice. Tumors of the skin in female rats and of the liver in male mice may also have been related to exposure to dibromoacetonitrile.

ABSTRACT



DIBROMOACETONITRILE

CAS No. 3252-43-5

Chemical Formula: C_2HBr_2N Molecular Weight: 198.84

Synonym: 2,2-dibromoacetonitrile

Dibromoacetonitrile is formed as a result of the reaction of chlorine oxidizing compounds (e.g., chlorine gas, hypochlorous acid, and hypochlorite) with natural organic matter, particularly nitrogen-containing organic compounds, in water containing bromine; it is also a by-product of ozone disinfection. Dibromoacetonitrile is not produced on a large industrial scale. Thus, chlorinated drinking water is the primary source of human exposure to dibromoacetonitrile. The United States Environmental Protection Agency nominated dibromoacetonitrile for carcinogenicity studies as part of an interagency initiative to characterize the potential chronic toxicity and carcinogenicity of exposure to various water disinfection by-products in drinking water. Dibromoacetonitrile was selected as a representative of the family of haloacetonitriles. The drinking water route mimics the major pathway of human exposure to dibromoacetonitrile. Male and female F344/N rats and B6C3F1 mice were exposed to dibromoacetonitrile (98.6% pure) in drinking water for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Escherichia coli*, *Drosophila melanogaster*, and mouse peripheral blood erythrocytes.

2-Week Study in Rats

Groups of five male and five female rats were exposed to drinking water containing 0, 12.5, 25, 50, 100, or 200 mg/L dibromoacetonitrile for 15 days (equivalent to average daily doses of approximately 2, 3, 7, 12, or 18 mg dibromoacetonitrile/kg body weight for males and 2, 4, 7, 12, or 19 mg/kg for females). All rats survived to the end of the study. The mean body weights of 200 mg/L males were significantly less than those of the control group. Water consumption decreased in an exposure-related manner. Atrophy of the testicular germinal epithelium occurred in two males exposed to 200 mg/L.

2-Week Study in Mice

Groups of five male and five female mice were exposed to drinking water containing 0, 12.5, 25, 50, 100, or 200 mg/L dibromoacetonitrile for 15 days (equivalent to average daily doses of approximately 2, 4, 8, 15, or 21 mg/kg for males and 2, 3, 10, 14, or 22 mg/kg for females). All mice survived to the end of the study.

Mean body weights of all exposed groups were similar to those of the controls. Water consumption by mice exposed to 200 mg/L was less than that by the controls. The liver weights of females exposed to 50, 100, or 200 mg/L were significantly decreased. No lesions were attributed to exposure to dibromoacetonitrile.

3-Month Study in Rats

Groups of 10 male and 10 female rats were exposed to drinking water containing 0, 12.5, 25, 50, 100, or 200 mg/L dibromoacetonitrile for 3 months (equivalent to average daily doses of approximately 1, 2, 3, 6, or 11 mg/kg for males and 1, 2, 4, 7, or 13 mg/kg for females). All rats survived to the end of the study. The mean body weights of 200 mg/L females were significantly less than those of the control group. Water consumption by 200 mg/L rats was less than that by the control groups. No histopathologic lesions were attributed to exposure to dibromoacetonitrile.

3-Month Study in Mice

Groups of 10 male and 10 female mice were exposed to drinking water containing 0, 12.5, 25, 50, 100, or 200 mg/L dibromoacetonitrile for 3 months (equivalent to average daily doses of approximately 2, 3, 6, 11, or 18 mg/kg for males and females). All mice survived to the end of the study. The mean body weights of exposed groups were similar to those of the controls. Water consumption by 200 mg/L females was less than that by the controls. No histopathologic lesions were attributed to exposure to dibromoacetonitrile.

2-Year Study in Rats

Groups of 50 male and 50 female rats were exposed to drinking water containing 0, 50, 100, or 200 mg/L dibromoacetonitrile for 105 to 106 weeks (equivalent to average daily doses of approximately 2, 4, or 7 mg/kg for males and 2, 4, or 8 mg/kg for females). Survival of all exposed groups of rats was similar to that of the controls. Mean body weights of 200 mg/L males were approximately 7% less than those of the controls during the second year of the study. Water consumption by the 100 and 200 mg/L groups was generally less than that by the controls throughout the study.

The combined incidence of squamous cell papilloma or carcinoma of the oral mucosa or tongue was significantly increased in 200 mg/L males. The incidence of squamous cell papilloma in the oral mucosa or tongue was increased in 100 mg/L females, but not significantly. Oral cavity neoplasms are uncommon in untreated F344/N rats, occurring at a mean incidence of 1% or less in males and females. The incidence of squamous epithelial hyperplasia of the tongue was increased in 200 mg/L females. The latter lesion is considered to be part of the continuum of proliferative changes in oral cavity neoplasia.

Glandular stomach adenomas were observed in two 200 mg/L males. This is a rare neoplasm that has not been seen in nearly 2,000 historical control male F344/N rats. The incidence of glandular ectasia of the glandular stomach in 200 mg/L females was significantly increased. The incidences of epithelial hyperkeratosis of the esophagus were significantly increased in 100 and 200 mg/L males and females.

The incidences of squamous cell papilloma or keratoacanthoma (combined) and squamous cell papilloma, keratoacanthoma, basal cell adenoma, or basal cell carcinoma (combined) of the skin occurred with a positive trend in female rats.

2-Year Study in Mice

Groups of 50 male and 50 female mice were exposed to drinking water containing 0, 50, 100, or 200 mg/L dibromoacetonitrile/L for 105 to 106 weeks (equivalent to average daily doses of approximately 4, 7, or 13 mg/kg for males and 3, 6, or 11 mg/kg for females). Survival of female mice exposed to 100 or 200 mg/L was significantly greater than that of the controls. Mean body weights of 200 mg/L males and females were less than those of the controls throughout most of the study. Water consumption by exposed groups was also less than that of controls throughout most of the study.

The incidence of squamous cell papilloma or carcinoma (combined) of the forestomach was significantly increased in 200 mg/L males. The incidence of squamous cell papilloma of the forestomach was significantly increased in 200 mg/L females. Squamous

cell neoplasms of the forestomach are uncommon in control male and female B6C3F1 mice, occurring at a mean incidence of about 1% to 2%. The incidences of epithelial hyperplasia of the forestomach were slightly increased in 50 and 200 mg/L males. The latter lesion is considered to be part of the continuum of proliferative changes in forestomach neoplasia.

The incidence of hepatoblastoma was significantly increased in 50 mg/L males; the incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were significantly increased in 50 and 100 mg/L males.

Genetic Toxicology

Dibromoacetonitrile was tested in five independent bacterial mutagenicity assays, each using multiple tester strains of *S. typhimurium* or *E. coli*, and mutagenic responses were seen in all five assays, primarily in *S. typhimurium* strain TA100 in the presence of exogenous metabolic activation (S9), but sometimes also in TA97, TA1535, and *E. coli* Wp2*uvrA*/pKM101 with S9. Induced hamster liver S9 enzymes were most effective in generating the mutagenic metabolite, although some very weak activity was also seen with induced rat liver S9 at some of the laboratories.

In vivo, dibromoacetonitrile did not induce sex-linked recessive lethal mutations in germ cells of male *D. melanogaster* exposed by feeding or by injection, and no increases in the frequencies of micronucleated erythrocytes were observed in peripheral blood of male or female mice administered dibromoacetonitrile for 3 months in drinking water.

Conclusions

Under the conditions of these 2-year drinking water studies there was *clear evidence of carcinogenic activity** of dibromoacetonitrile in male rats based on increased incidences of squamous cell papillomas or carcinomas of the oral cavity; adenomas in the glandular stomach of male rats were also considered to be exposure-related. There was *some evidence of carcinogenic activity* of dibromoacetonitrile in female rats based on an increased incidence of squamous cell papillomas of the oral cavity; increased incidences of basal cell or squamous cell neoplasms of the skin in female rats may have been related to dibromoacetonitrile exposure. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male mice based on increased incidences of squamous cell papillomas or carcinomas of the forestomach. Increased incidences of neoplasms in the liver of male mice may have been related to dibromoacetonitrile exposure. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in female mice based on increased incidences of squamous cell papilloma of the forestomach.

Exposure to dibromoacetonitrile for 2 years caused increased incidences of epithelial hyperkeratosis in the esophagus of male and female rats, glandular ectasia of the glandular stomach and squamous epithelial hyperplasia of the tongue in female rats, and squamous epithelial hyperplasia of the forestomach in male mice.

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

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

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Concentrations in drinking water	0, 50, 100, or 200 mg/L	0, 50, 100, or 200 mg/L	0, 50, 100, or 200 mg/L	0, 50, 100, or 200 mg/L
Body weights	200 mg/L group 7% less than control group after 1 year	Exposed groups similar to control group	200 mg/L group 7% less than control group after 8 weeks	200 mg/L group 7% less than control group after 8 weeks and 14% less after 1 year
Survival rates	31/50, 33/50, 25/50, 35/50	29/50, 35/50, 29/50, 31/50	40/50, 40/50, 35/50, 42/50	36/50, 36/50, 43/50, 47/50
Nonneoplastic effects	Esophagus: epithelial hyperkeratosis (6/48, 8/50, 34/50, 46/50)	Esophagus: epithelial hyperkeratosis (10/49, 8/50, 28/50, 48/50) Glandular Stomach: glandular ectasia (10/50, 4/50, 10/50, 26/50); Tongue: squamous epithelial hyperplasia (1/50, 1/50, 2/50, 6/50)	Forestomach: squamous epithelial hyperplasia (1/50, 4/50, 1/50, 6/50)	None
Neoplastic effects	Oral Cavity (oral mucosa or tongue): squamous cell papilloma or squamous cell carcinoma (0/50, 0/50, 2/50, 5/50) Glandular Stomach: adenoma (0/50, 0/50, 0/50, 2/50)	Oral Cavity (oral mucosa or tongue): squamous cell papilloma (1/50, 0/50, 4/50, 0/50)	Forestomach: squamous cell papilloma or squamous cell carcinoma (0/50, 1/50, 0/50, 5/50)	Forestomach: squamous cell papilloma (1/50, 0/50, 5/50, 14/50)
Equivocal findings	None	Skin: squamous cell papilloma, keratoacanthoma, basal cell adenoma, or basal cell carcinoma (0/50, 0/50, 3/50, 4/50)	Liver: hepatoblastoma (1/50, 7/50, 3/50, 2/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (37/50, 46/50, 43/50, 42/50)	None
Level of evidence of carcinogenic activity	Clear evidence	Some evidence	Clear evidence	Clear evidence
Genetic toxicology				
Salmonella typhimurium and *Escherichia coli* gene mutations:	Positive in *E. coli* WP *uvrA*/pKM101 with S9; weakly positive in strains TA97, TA100, and TA1535, with S9; negative in TA98 and TA1537 with and without S9			
Sex-linked recessive lethal mutations *Drosophila melanogaster:*	No induction of sex-linked recessive lethal mutations			
Micronucleated erythrocytes Mouse peripheral blood *in vivo:*	Negative			

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

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

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

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

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

Dr. R.L. Melnick, NIEHS, described the toxicology and carcinogenesis studies of dibromoacetonitrile by reviewing the occurrence of dibromoacetonitrile as a drinking water disinfection by-product and describing the toxic effects of dibromoacetonitrile in short-term studies and the neoplastic and nonneoplastic lesions observed in the 2-year studies. The proposed conclusions were *clear evidence of carcinogenic activity* of dibromoacetonitrile in male F344/N rats, *some evidence of carcinogenic activity* of dibromoacetonitrile in female F344/N rats, and *clear evidence of carcinogenic activity* of dibromoacetonitrile in male and female B6C3F1 mice.

Dr. Novak, the first principal reviewer, agreed with the proposed conclusions. He suggested that the lack of an exposure concentration-related effect added to the complexity of interpretation and inquired whether comparisons with human exposure levels could be drawn.

Dr. Cattley, the second principal reviewer, recommended that the forestomach tumor response be considered separately for male and female mice, as no carcinomas were seen in the latter.

Dr. Soper, the third principal reviewer, agreed with the proposed conclusions.

Dr. Melnick replied that higher concentrations than occur in drinking water were used in the 2-year studies because small groups of animals are used to detect hazards for many millions of people exposed for longer periods, including sensitive subpopulations. He noted that dibromoacetonitrile is just one of several hundred by-products of drinking water disinfection occurring in the public water supply. Dr. Melnick suggested revising the Technical Report discussion section to include documentation of the known progression to carcinoma of forestomach squamous cell papilloma.

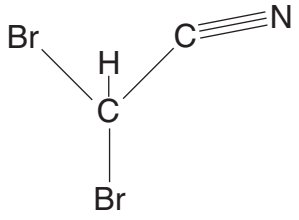
Dr. Cattley pointed out that carcinoma was seen in only one sex in the current study.

Dr. G.P. Flake, NIEHS, cited several comprehensive surveys in the pathology literature providing evidence of progression of squamous papilloma to carcinoma in the oral cavity and esophagus, in addition to the forestomach.

Dr. Pino noted that the testicular atrophy observed in the 2-week studies did not occur in the longer term studies and suggested that the kidney nonneoplastic lesions in the 2-year study be included in the document summary.

Dr. Mirsalis moved, and Dr. Novak seconded, that the conclusions be revised to reflect that the female mouse conclusions be based on papilloma, while the male mouse conclusions be based on papilloma and carcinoma combined. Dr. Pino asked that the kidney nonneoplastic lesions for rats be included in the summary conclusion. Dr. Melnick replied this was a modest increase in a lesion with a high background rate. The motion to approve the revised conclusions passed with seven yes votes and one no vote (Dr. Pino).

INTRODUCTION



DIBROMOACETONITRILE

CAS No. 3252-43-5

Chemical Formula: C_2HBr_2N Molecular Weight: 198.84

Synonym: 2,2-dibromoacetonitrile

Chemical and Physical Properties

Dibromoacetonitrile exists as a colorless to pale-yellow liquid with an organohalide odor. It has a boiling point of 67° to 69° C, a density of 2.296 (Lide, 2000), and a log octanol/water partition coefficient of 0.42 (IARC, 1991).

Production, Use, and Human Exposure

Although halogenated acetonitriles, including dibromoacetonitrile, are not produced on a large industrial scale, several chemicals in this family have been detected in chlorinated drinking water (IARC, 1991). Dibromoacetonitrile is formed as a result of the reaction of chlorine disinfection compounds (e.g., chlorine gas, hypochlorous acid, and hypochlorite) with natural organic matter, particularly nitrogen-containing organic compounds, in water containing bromine; it is also a by-product of ozone disinfection (Huang *et al*., 2003). Thus, chlorinated drinking water is the primary source of human exposure to dibromoacetonitrile. Factors that affect the formation of disinfection by-products produced in drinking water supplies include the nature and concentration of organic precursor compounds, water temperature, pH, the type of disinfectant, the disinfectant dose and contact time, and bromide ion concentration (Huang *et al.*, 2003, 2004; Liang and Singer, 2003). Bromide in the source water may be oxidized by chlorinated oxidizing compounds or by ozone to hypobromous acid-hypobromite ion. Hypobromite may be further oxidized by ozone to bromate, while hypobromous acid can react with organic matter to form brominated organic by-products. The hypobromous acid-hypobromite ion equilibrium is pH-dependent. Further chlorination of halogenated acetonitriles results in the formation of halogenated acetic acids (Ueno *et al*., 1996).

Dihaloacetonitriles (dibromoacetonitrile, dichloroacetonitrile, and bromochloroacetonitrile) were detected in chlorinated drinking water supplies in southern Florida at total concentrations up to 42 µg/L (Trehy and Bieber, 1981). In a nationwide study of the occurrence of disinfection by-products in drinking water in the United States from 1988 to 1989, the median concentrations (µg/L) of halogenated acetonitriles in samples obtained from 35 water utilities were: dibromoacetonitrile, 0.48 to 0.54; dichloroacetonitrile, 1.1 to 1.2; and bromochloroacetonitrile, 0.50 to 0.70; however, levels of dibromoacetonitrile as high as 11 µg/L were reported (Krasner *et al.*, 1989).

While levels of trihalomethanes and haloacetic acids in drinking water are regulated by the United States

Environmental Protection Agency under the disinfection by-product rule (USEPA, 1998), no drinking water standard has been established for exposure to halogenated acetonitriles. The World Health Organization (2006) has published a drinking water guideline value of 70 µg/L for dibromoacetonitrile.

Absorption, Distribution, Metabolism, and Excretion

Eight percent of a single oral dose of dibromoacetonitrile (0.75 mmol/kg, 149 mg/kg) in male Sprague-Dawley rats was excreted in urine within 24 hours as thiocyanate, the product of cyanide reaction with thiosulfate catalyzed by rhodanese, suggesting that haloacetonitriles are metabolized to hydroxyacetonitriles by direct displacement of a halide ion by a hydroxyl group or by CYP450-mediated oxidation (Pereira *et al*., 1984); subsequent release of cyanide or halide ion might result in the formation of formylhalide or cyanoformaldehyde. Chloroacetonitrile is also metabolized to cyanide in gastric mucosal cell suspensions (Ahmed *et al*., 1999).

Additional pathways of haloacetonitrile metabolism have been suggested based on *in vitro* studies. For example, dichloroacetonitrile was oxidized with cyanide release in a system that generates hydroxyl free radical (a Fenton-like reaction involving ferrous salts and hydrogen peroxide); the oxidation of dichloroacetonitrile was sensitive to hydrogen peroxide scavengers (e.g., catalase), iron chelator (desferrioxamine), or free radical scavengers (e.g., mannitol) (Mohamadin, 2001). Dibromoacetonitrile was also oxidized by a hydroxyl radical generated *in vitro* in a hypoxanthine/xanthine oxidase/iron system resulting in the release of cyanide (Mohamadin and Abdel-Naim, 2003). Thus, oxidative activation of haloacetonitriles may occur via a reactive oxygen-mediated mechanism (Mohamadin, 2001).

Disposition studies in F344/N rats and B6C3F1 mice were conducted after oral administration of 0.2, 2.0, or 20 mg [2-^{14}C]-dibromoacetonitrile/kg body weight or intravenous administration of 2.0 mg [2-^{14}C]-dibromoacetonitrile/kg body weight (NTP, 2002). Dibromoacetonitrile was well absorbed in both species. Approximately 60% of the oral dose was excreted in the urine (none as unmetabolized dibromoacetonitrile) within 24 hours after dosing in rats and by 72 hours after dosing in mice; 8% to 17% was excreted in feces, and 10% to 13% was exhaled as $^{14}CO_2$. At 72 hours postdosing, 5% to 6% of the oral dose was recovered in tissues of rats and approximately 2% to 3% in tissues of mice. At 72 hours after intravenous administration, less radioactivity (3%) was recovered in the feces of rats, and retention of radiolabel in tissues was three to four times greater (19% in rats and 10% in mice) than after oral administration in either species. Most of the radiolabel remaining in the stomach and liver at the later time points after oral administration was not extractable with organic solvents, suggesting covalent binding in these tissues. Parent compound accounted for less than 6% of circulating radiolabel in rats and was not detected in mouse blood; at 24 hours postdosing in rats, 50% to 80% of radioactivity in blood was not extractable into acetone. The major metabolite extracted with acetone and methanol from rat stomach or from rat or mouse liver was monoglutathionyl acetonitrile. Rat and mouse urinary metabolite profiles were unaffected by incubation with glucuronidase or sulfatase, but were altered by acylase; the major urinary metabolite identified from rats was acetonitrile mercapturate, and those from mice were acetonitrile mercaptoacetate, acetonitrile mercapturate, and cysteinyl acetonitrile. Because the molar ratio of glutathione consumed to dibromoacetonitrile undergoing reaction *in vitro* is 2.5 to 2.7 (Lin and Guion, 1989) and because bromoacetonitrile was detected in reaction media containing dibromoacetonitrile and glutathione, the formation of monoglutathionyl conjugate derivatives was suggested to occur by glutathione-dependent reduction of dibromoacetonitrile to bromoacetonitrile followed by reaction of bromoacetonitrile with another glutathione (NTP, 2002).

In a disposition study of orally administered [2-^{14}C] dichloroacetonitrile (0.2 to 15 mg/kg) in male F344/N rats and B6C3F1 mice, 35% to 43% of the radiolabel was excreted in urine, 31% to 37% was exhaled as carbon dioxide, and 8% to 13% was excreted in feces; 9% to 17% of the administered dose was retained in tissues at 1 to 2 days postdosing (Roby *et al*., 1986). Dichloroacetonitrile is metabolized to a greater extent to carbon dioxide than is dibromoacetonitrile (Roby *et al*., 1986, NTP, 2002). At 12 hours after intravenous administration of [2-^{14}C]-chloroacetonitrile in male Sprague-Dawley rats, 51% of the radioactivity was excreted in urine (none as parent compound), 2.7% was excreted in feces, and 11% was exhaled as carbon dioxide (Ahmed *et al*., 1991a). These values are similar to the elimination pattern of intravenously administered dibromoacetonitrile in male F344/N rats (NTP, 2002).

Results from *in vitro* studies indicate that dibromoacetonitrile reacts directly with glutathione, but not with lysine, to form an intermediate that can alkylate histidine; reacts with rat cecal contents to form polar products; and reacts rapidly with rat blood to form polar metabolites and a large nonextractable fraction that may represent covalent protein adducts (NTP, 2002).

TOXICITY

Experimental Animals

The acute oral LD_{50} of dibromoacetonitrile is 245 mg/kg in male Sprague-Dawley rats and 361 mg/kg in females; in CD-1 ICR mice, the oral LD_{50} is 289 mg/kg in males and 303 mg/kg in females (Hayes *et al*., 1986). In follow-up toxicity studies, dibromoacetonitrile was administered by gavage in corn oil to male and female Sprague-Dawley rats for 14 days at doses of 23, 45, 90, or 180 mg/kg and for 90 days at doses of 6, 23, or 45 mg/kg (Hayes *et al*., 1986). Dose-dependent decreases in body weight gain and increased mortality were observed in male and female rats in the 90 and 180 mg/kg groups in the 14-day study. In the 90-day study, increased mortality in male and female rats and decreased body weight gain in males were observed in the 45 mg/kg group. No consistent adverse effects were observed at necropsy or in serum chemistry, hematology, or urinary parameters.

In a 13-week drinking water study, male and female Sprague-Dawley rats were exposed to dibromoacetonitrile at concentrations of 0, 0.1, 1, 10, and 100 ppm (mg/L) (Poon *et al*., 2003). Although water consumption was reduced by 25% to 32% in the 100 ppm groups, average body weight gains were not significantly different from those of controls. Treatment-related effects occurred predominantly in the highest dose group and included increased kidney weights in males and females; increased liver peroxisomal enzyme activities; and minimal changes in the thyroid gland (reduced follicle size with papillary epithelial proliferation), bone marrow (increased myeloid to erythroid ratio), and kidney (cytoplasmic inclusions in proximal tubules). In addition, dibromoacetonitrile was shown to be a potent inhibitor of aldehyde dehydrogenase activity in rat liver homogenates, but did not decrease the activity of this enzyme in the liver of rats exposed to dibromoacetonitrile (up to 100 ppm) for 13 weeks.

Dibromoacetonitrile is an inhibitor of rat liver microsomal dimethylnitrosamine-demethylase activity *in vitro* (Pereira *et al*., 1984); however, no decrease in enzyme activity was detected in microsomes isolated from the liver of male Sprague-Dawley rats given 0.75 mmol dibromoacetonitrile/kg body weight (149 mg/kg) by gavage in tricaprylin. Dibromoacetonitrile and other haloacetonitriles are also potent *in vitro* inhibitors of hepatic glutathione-*S*-transferase activity (Ahmed *et al*., 1989). In male Sprague-Dawley rats, administration by gavage at doses of 25 to 100 mg dibromoacetonitrile/kg body weight caused reductions in glutathione levels and glutathione-*S*-transferase activities in the liver and the glandular stomach (without affecting kidney or blood glutathione levels) within 30 minutes to 2 hours after treatment (Ahmed *et al*., 1991b). Similarly, a single oral dose of chloroacetonitrile caused glutathione depletion, as well as DNA fragmentation and elevation in levels of 8-hydroxydeoxyguanosine, a marker of oxidative DNA damage, in the gastric mucosa of Sprague-Dawley rats (Ahmed *et al*., 1999). In addition to reduction in gastric glutathione content and inhibition of glutathione-*S*-transferase activity, single oral doses of dibromoacetonitrile (30, 60, or 120 mg/kg) inhibited gastric superoxide dismutase and catalase activities and induced increases in lipid peroxidation products (thiobarbituric acid reactive substances) in Swiss albino male mice (Abdel-Wahab *et al*., 2002); these changes were largely prevented by pretreatment of mice with melatonin. These results indicate that glutathione may protect gastric tissue from dibromoacetonitrile-induced oxidative damage.

Exposure of male and female F344 rats to dibromoacetonitrile in drinking water at concentrations of 0, 45, 108, or 270 mg/L for 6 months did not produce any detectable exposure-related neurobehavioral (functional observational battery and motor activity) or neuropathological changes (Moser *et al.*, 2007).

Dibromoacetonitrile was the most cytotoxic chemical among seven haloacetonitriles tested in Chinese hamster ovary cells (Muellner *et al.*, 2007). The assay measured the reduction in cell density at the end of a 72-hour incubation (approximately three cell divisions) with 10 concentrations of each chemical. As a class, haloacetonitriles were more cytotoxic than haloacetic acids.

Humans

There were no data available on the toxicity of dibromoacetonitrile in humans.

Reproductive and Developmental Toxicity

Experimental Animals

Haloacetonitriles were examined for reproductive and developmental toxicity in pregnant Long-Evans rats dosed between days 7 and 21 of gestation (Smith *et al*., 1987). The dose used in this study (0.25 mmol dibromoacetonitrile/kg body weight, equivalent to 50 mg/kg) caused reduction in maternal weight gain; a similar effect was observed with chloroacetonitrile, dichloroacetonitrile, and trichloroacetonitrile, but not with bromochloroacetonitrile, at doses of 0.36 to 0.72 mmol/kg (equivalent to 43 to 55 mg/kg). Dichloroacetonitrile and trichloroacetonitrile produced reductions in the percentage of dams delivering viable litters and in the mean number of live pups per litter, while all of the tested haloacetonitriles caused reductions in pup birth weights. Only with trichloroacetonitrile-treated dams was pup growth reduced at postnatal day 42. In follow-up studies, pregnant Long-Evans rats were exposed on gestation days 6 to 18 to dichloroacetonitrile at doses ranging from 5 to 45 mg/kg (Smith *et al*., 1989) or to trichloroacetonitrile at doses ranging from 1 to 55 mg/kg (Smith *et al*., 1988). Exposure to dichloroacetonitrile caused a significant reduction in maternal weight gain (45 mg/kg group), increases in resorptions of entire litters (7.5 mg/kg and higher doses) and post implantation loss (25 and 45 mg/kg groups), and dose-related increases in soft tissue malformations (predominantly in the cardiovascular and urogenital systems) and skeletal malformations (Smith *et al*., 1989). Exposure to trichloroacetonitrile caused a dose-related reduction in maternal weight gain; however, among dams bearing live fetuses, reduction in gestational weight gain was detected only in the 55 mg/kg group. Exposure to trichloroacetonitrile also caused dose-related increases in resorptions of entire litters, postimplantation loss, and soft tissue malformations (predominantly in the cardiovascular and urogenital systems) (Smith *et al*., 1988).

Dibromoacetonitrile toxicity was assessed using the NTP's short-term (approximately 35-day) reproductive and developmental toxicity screening protocol designed to identify which physiologic systems (development; female and/or male reproduction; various somatic organs and/or processes) are most sensitive to a chemical's toxicity. Dibromoacetonitrile was administered in drinking water at concentrations of 0, 15, 50, and 150 ppm (mg/L) for 20 to 30 days (NTP, 1997). Animals at the highest dose had decreased water consumption, but no adverse reproductive or developmental effects were noted in this study.

Intraperitoneal injection of 50 mg dibromoacetonitrile/kg body weight in male albino mice significantly decreased sperm count, sperm motility, and testicular glutathione levels, increased testicular malonyldialdehyde content, and induced seminiferous tubule degeneration at 3 hours after treatment (Abdel-Wahab, 2003). Because prior treatment with the antioxidant tertiary butylhydroquinone diminished these changes, the author suggested that dibromoacetonitrile could induce oxidative stress in the mouse testis resulting in reproductive impairment.

In the hydra assay, in which agents are tested for toxicity in adult organisms and developing embryos, neither dibromoacetonitrile nor trichloroacetonitrile selectively interfered with development (Fu *et al*., 1990).

Humans

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

Carcinogenicity

Experimental Animals

In an initiation-promotion study, six topical applications of dibromoacetonitrile (at doses ranging from 200 to 800 mg/kg body weight) to the shaved back of Sencar mice over a 2-week period followed by three times weekly application of 12-*O*-tetradecanoylphorbol-13-acetate produced increases in the incidences of squamous cell papillomas and carcinomas and the number of tumors per animal (Bull *et al*., 1985). Of five haloacetonitriles tested in this initiation-promotion protocol, dibromoacetonitrile was the most potent tumor initiator. Skin tumors were not seen in dibromoacetonitrile-initiated animals that were not administered 12-*O*-tetradecanoylphorbol-13-acetate. In a separate study, six oral

doses of dibromoacetonitrile (50 mg/kg over a 2-week period) were given to female Sencar mice followed by 12-*O*-tetradecanoylphorbol-13-acetate promotion; no increases in time to first tumor or incidence of skin tumors were observed. Neither dibromoacetonitrile, chloroacetonitrile, dichloroacetonitrile, nor trichloroacetonitrile initiated γ-glutamyltranspeptidase foci in a tumor initiating assay in which partially hepatectomized rats were given a single dose of the haloacetonitrile and exposed beginning 1 week later to sodium phenobarbital in the drinking water for 8 weeks (Lin *et al*., 1986).

Humans

No studies have been reported on the carcinogenicity of dibromoacetonitrile *per se*; however, several studies have examined cancer risks associated with exposure to water disinfection by-products. A meta-analysis of epidemiology studies published before 1989 on cancer and chlorination by-products in drinking water yielded a relative risk estimate of 1.21 (95% CI: 1.09 to 1.34) for bladder cancer and 1.38 (95% CI: 1.01 to 1.87) for rectal cancer (Morris *et al*., 1992). A population-based case-control study in Colorado also found an association between prolonged exposures to chlorinated surface water and increased bladder cancer risk in men and women for both smokers and nonsmokers (McGeehin *et al*., 1993). An elevation in brain cancer risk was also associated with exposure to chlorinated surface water (Cantor *et al*., 1999).

Genetic Toxicity

Dibromoacetonitrile induced primary DNA damage in *Escherichia coli* strain PQ37 (Le Curieux *et al.*, 1995) and was shown to be a weak mutagen in *Salmonella typhimurium* strains TA100 and TA1535 when tested in the presence of induced Syrian hamster liver S9 enzymes at concentrations that ranged up to 333 µg/plate; equivocal responses were observed in these strains when testing occurred in the presence of rat liver S9 (Mortelmans *et al.*, 1986). Results of a second study, in which the mutagenicity of several halogenated acetonitriles was evaluated, showed no increase in gene mutations in *S. typhimurium* strains TA98, TA100, TA1535, TA1537, or TA1538 exposed to dibromoacetonitrile with and without S9 (Bull *et al.*, 1985). In contrast, monochloroacetonitrile, dichloroacetonitrile, trichloroacetonitrile, monobromoacetonitrile, and bromochloroacetonitrile were reported to be mutagenic in *S. typhimurium* (Bull *et al*., 1985; Le Curieux *et al.*, 1995). Dibromoacetonitrile induced mitotic recombination in the yeast, *Saccharomyces cerevisiae* (Zimmerman and Mohr, 1992). In another comparative assessment of the mutagenicity of halogenated acetonitriles, dibromoacetonitrile was shown to induce a dose-related increase in DNA damage in HeLa S3 cells measured by single cell gel electrophoresis (comet assay) (Muller-Pillet *et al.*, 2000). These authors further reported that DNA damage induced by halogenated acetonitriles increased with increasing number of halogenated atoms, and, in this test system, brominated acetonitriles were more potent than chlorinated compounds. Dibromoacetonitrile induced sister chromatid exchanges in cultured Chinese hamster ovary cells, as did chloroacetonitrile, dichloroacetonitrile, trichloroacetonitrile, and bromochloroacetonitrile (Bull *et al*., 1985). These authors also reported that potency was directly related to the number of halogenated atoms present, and that bromine-substituted compounds produced stronger responses than the chlorinated compounds. Table 1 summarizes genotoxicity studies of haloacetonitriles.

No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered dibromoacetonitrile by feeding or injection (Valencia *et al*., 1985). And no induction of aneuploidy was observed in oocytes of female *Drosophila melanogaster* administered dibromoacetonitrile by inhalation (Osgood and Sterling, 1991). Dichloroacetonitrile did, however, induce significant increases in aneuploid oocytes of exposed *Drosophila melanogaster* (Osgood and Sterling, 1991). Dibromoacetonitrile was reported to be negative in the mouse bone marrow micronucleus assay, indicating no potential for induction of chromosomal alterations in this test system (Bull *et al*., 1985). But increased frequencies of micronucleated erythrocytes were reported in newt (*Pleurodeles waltl*) larvae exposed to dibromoacetonitrile in water for 12 days (Le Curieux *et al.*, 1995).

Study Rationale

Long-term studies of dibromoacetonitrile in rats and mice were nominated to the NTP by the United States Environmental Protection Agency as part of an interagency initiative to characterize the potential chronic toxicity and carcinogenicity of exposure to various water disinfection by-products in drinking water. Dibromoacetonitrile was selected as a representative of the family of haloacetonitriles. Brominated acetonitriles were considered to be potentially more active than chlorinated acetonitriles. The drinking water route mimics the major pathway of human exposure to dibromoacetonitrile.

Table 1
Genotoxicity Studies of Haloacetonitriles

Assay	Results[a]					
	CAN	DCAN	TCAN	BAN	BCAN	DBAN
Mutagenicity in *Salmonella typhimurium* TA98, TA100, or TA1535 (Bull *et al.,* 1985)	–	+	–	nt	+*	–
Mutagenicity in *S. typhimurium* TA100 or TA1535 (Mortelmans *et al.,* 1986)	–	+	+w	–	nt	+w
Mutagenicity in *S. typhimurium* TA100 (Le Curieux *et al.,* 1995)	+	+*	+	+	+	–
Mutagenicity in *S. typhimurium* TA100 (Muller-Pillet *et al.,* 2000)	+	+	+*	–	nt	–
Sister chromatid exchange in Chinese hamster ovary cells (Bull *et al.,* 1985)	+	+	+	nt	+	+*
DNA strand breaks in human lymphoblastic cells (Daniel *et al.,* 1986)	+	+	+*	nt	+	+
DNA single strand breaks in HeLa cells (Muller-Pillet *et al.,* 2000)	+	+	+	+	nt	+*
DNA strand breaks in Chinese hamster ovary cells (Muellner *et al.,* 2007)	+	+	+	+*	+	+*
SOS chromotest in *Escherichia coli* (Le Curieux *et al.,* 1995)	–	+	–	–	+*	+
Sex-linked recessive lethal mutations in *Drosophila melanogaster* (Valencia *et al.,* 1985)	nt	+	nt	nt	nt	–
Aneuploidy in *Drosophila melanogaster* oocytes (Osgood and Sterling, 1991)	nt	+	nt	nt	nt	–
Micronuclei in bone marrow erythrocytes of CD-1 mice (Bull *et al.,* 1985)	–	–	–	nt	–	–
Newt micronucleus test (Le Curieux *et al.,* 1995)	+	+	+	+*	+	+
Binding to 4-(*p*-nitrobenzyl)pyridine (Daniel *et al.,* 1986)	+	+	+	nt	+	+*

[a] + = positive; +w = weakly positive; – = negative; nt = not tested; * = the most active in assays where there was more than one positive, that is CAN=chloroacetonitrile; DCAN=dichloroacetonitrile; TCAN=trichloroacetonitrile; BAN=bromoacetonitrile; BCAN=bromochloroacetonitrile; DBAN=dibromoacetonitrile

MATERIALS AND METHODS

Procurement and Characterization of Dibromoacetonitrile

Dibromoacetonitrile was obtained from Oakwood Products, Inc. (West Columbia, SC), in one lot (S11C), which was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) and by the study laboratory at Southern Research Institute (Birmingham, AL) (Appendix J). Karl Fischer titration and elemental analysis were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the dibromoacetonitrile studies are on file at the National Institute of Environmental Health Sciences.

Lot S11C, a pale yellow to amber liquid, was identified as dibromoacetonitrile by the study laboratory using infrared and proton nuclear magnetic resonance (NMR) spectroscopy and by the analytical chemistry laboratory using infrared and proton and carbon-13 NMR spectroscopy. The purity of lot S11C was determined by elemental analysis and gas chromatography by the analytical chemistry laboratory; the moisture content was determined by Karl Fischer titration.

Karl Fischer titration indicated less than 0.01% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for dibromoacetonitrile. Prior to the 2-week studies, gas chromatography indicated one major peak and four impurities with peak areas greater than or equal to 0.1% of the total peak area; the impurities had a combined area of 1.4%. The overall purity was determined to be 98.6%.

The bulk chemical was stored at room temperature in glass carboys, protected from light from February 15, 2000, until July 29, 2000, when storage was changed to 5° C to ensure stability. Periodic reanalyses of the bulk chemical were performed by the study laboratory. No degradation of the bulk chemical occurred.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice during the 2-week studies, four times during the 3-month studies and approximately every 2 weeks throughout the 2-year studies. The dose formulations were prepared by mixing dibromoacetonitrile with tap water. Formulations were stable for at least 40 days when stored at 5° C in glass bottles sealed with Teflon®-lined lids and protected from light.

Periodic analyses of the dose formulations of dibromoacetonitrile were conducted by the study laboratory using gas chromatography. During the 2-week studies, the dose formulations were analyzed twice; eight of 10 dose formulations for rats and mice were within 10% of the target concentrations. For the 3-month studies, dose formulations were analyzed at the beginning, middle, and end of the studies; all 20 dose formulations analyzed for rats and mice were within 10% of the target concentrations. For the 2-year study, dose formulations were analyzed at least every 6 months; all 65 dose formulations analyzed for rats and mice were within 10% of the target concentrations. Animal room samples were also analyzed; dose formulations were generally within 20% of target concentrations except during the 2-week studies in which results within 30% of the target concentrations were attributed to poor recovery with the extraction method.

2-Week Studies

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4 to 5 weeks old. Animals were quarantined for 12 (rats) or 13 (mice) days and were 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. Groups of five male and five female rats and mice were exposed to 0, 12.5, 25, 50, 100, or 200 mg dibromoacetonitrile/L in the drinking water for 15 days. Feed and water were

available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded daily for rats and mice. Water consumption was recorded weekly. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on all animals. The heart, right kidney, liver, lung, right testis, and thymus were weighed. Histopathologic examinations were performed on the kidney, liver, lung, and testis of 0 and 200 mg/L rats and mice; these tissues were examined to a no-effect level in the remaining exposure groups.

Liver samples for glutathione-*S*-transferase analyses were rinsed in ice-cold saline and frozen in liquid nitrogen. Partially thawed liver samples were homogenized in a 1.15% potassium chloride aqueous solution and centrifuged. Calcium chloride was added to a concentration of 8.0 mM, and the sample was centrifuged to precipitate the microsomes. Glutathione-*S*-transferase activity was measured by determining the rate of conjugation of 1-chloro-2,4-dinitrobenzene with glutathione (Habig *et al*., 1974). Cytosolic protein levels were determined by a modification of the Lowry *et al*. (1951) method and used to normalize enzymatic activity.

3-Month Studies

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

Groups of 10 male and 10 female rats and mice were exposed to 0, 12.5, 25, 50, 100, or 200 mg dibromoacetonitrile/L in the drinking water for 3 months; groups of 10 male and 10 female clinical pathology study rats and mice were exposed to the same concentrations for 4 weeks. Feed and water were available *ad libitum*. Rats and female mice were housed five per cage, and male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. Water consumption was measured weekly for core study animals. Core study animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 2.

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 3 and 21 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats) analyses. Animals were anesthetized with a mixture of CO_2/O_2. The parameters measured are listed in Table 2. Blood samples for hematology were placed in tubes containing EDTA. Erythrocyte, platelet, and leukocyte counts; hematocrit value; hemoglobin concentration; and mean cell volume, hemoglobin, and hemoglobin concentration were measured using a Technicon H-1™ automated hematology analyzer (Bayer Healthcare, LLC, Tarrytown, NY) and reagents from Bayer, Inc. (Tustin, CA), R&D Systems, Inc. (Minneapolis, MN), or Fisher Scientific (Norcross, GA). Reticulocyte counts were determined using a Coulter Model XL Flow Cytometer (Coulter Corp., Miami, FL) with reagents supplied by the manufacturer or Molecular Probes (Eugene, OR). Blood smears were prepared within approximately 2 hours of sample collection to evaluate platelet and erythrocyte morphologies by light microscopy. Samples for clinical chemistry analyses were placed in tubes with no anticoagulant and analyzed using a Boehringer Mannheim/Hitachi 911 automated analyzer (Boehringer Mannheim, Indianapolis, IN) with reagents supplied by the manufacturer.

At the end of the 3-month studies, samples were collected for sperm count and motility and vaginal cytology evaluations on rats and mice exposed to 0, 50, 100, or 200 mg/L. The parameters evaluated are listed in Table 2. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk

(rats) or modified Tyrode's buffer (mice) was applied to slides and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study rats and mice. The heart, right kidney, liver, lung, right testis, and thymus of core study animals were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, trimmed, processed, and embedded in paraffin, sectioned to a thickness of 5 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on 0 and 200 mg/L rats and mice. Table 2 lists the tissues and organs examined.

2-Year Studies

Study Design

Groups of 50 male and 50 female rats and mice were exposed to 0, 50, 100, or 200 mg dibromoacetonitrile/L in the drinking water for 105 to 106 weeks.

Source and Specification of Animals

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 14 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were approximately 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix M).

Animal Maintenance

Male rats were housed up to three per cage; female rats and mice were housed five per cage, and male mice were housed individually. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix L.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings were recorded at 4-week intervals beginning at week 5. Water consumption was recorded weekly for the first 13 weeks and every 4 weeks thereafter. Body weights were recorded on day 1, weekly for the first 13 weeks, at 4-week intervals thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 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 2.

Because the standard evaluation of the kidney revealed slightly increased incidences of renal tubule adenoma in male mice, additional step sections of male kidney were prepared from the remaining formalin-fixed tissues. For the extended evaluation, kidneys were step-sectioned at 1 mm intervals, and four additional sections were obtained from each kidney (Eustis *et al*., 1994).

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy; the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which

included the esophagus, kidney, liver, forestomach, and glandular stomach, thyroid gland, and tongue of male and female rats; pituitary gland and mammary gland of female rats; forestomach of male and female mice; kidney and liver of male mice; and spleen of female mice. Selected lesions potentially related to treatment were also reviewed.

Normally, only lesions that are observed grossly on the tongue are evaluated histologically. Because hyperplasia and neoplasia of the tongue were noted in some exposed rats in the 2-year study, the tongues of all rats in which no lesions had been observed grossly were retrieved from the stored residual fixed tissues at the NTP archives and examined grossly and microscopically. No additional gross lesions were observed. The oral mucosa of the palate and gingiva present on slides of the three standard sections of nasal cavity was also reexamined microscopically in all rats.

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

Table 2
Experimental Design and Materials and Methods in the Drinking Water Studies of Dibromoacetonitrile

2-Week Studies	3-Month Studies	2-Year Studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species		
F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice	F344/N rats B6C3F1 mice
Animal Source		
Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)	Taconic Farms, Inc. (Germantown, NY)
Time Held Before Studies		
Rats: 12 days Mice: 13 days	Rats: 13 (males) or 14 (females) days Mice: 11 (females) or 12 (males) days	14 days
Average Age When Studies Began		
5 to 6 weeks	5 to 7 weeks	Rats: 6 to 7 weeks Mice: 5 to 7 weeks
Date of First Exposure		
Rats: September 25, 2000 Mice: September 26, 2000	Rats: December 19 (males) or 20 (females), 2000 Mice: December 17 (females) or 18 (males), 2000	Rats: September 5, 2001 Mice: August 22, 2001
Duration of Exposure		
15 days	Core Study: 14 weeks Clinical Pathology Study: 4 weeks	105 to 106 weeks
Date of Last Exposure		
Rats: October 9, 2000 Mice: October 10, 2000	Rats: March 21 (males) or 22 (females), 2001 (core study); January 14 (males) or 15 (females), 2001 (clinical pathology study) Mice: March 19 (females) or 20 (males), 2001; January 12 (females) or 13 (males), 2001 (special study)	Rats: September 3-10, 2003 Mice: August 20-28, 2003
Necropsy Dates		
Rats: October 9, 2000 Mice: October 10, 2000	Rats: March 21 (males) or 22 (females), 2001 (core study); January 14 (males) or 15 (females), 2001 (clinical pathology study) Mice: March 19 (females) or 20 (males), 2001 (core study); January 12 (females) or 13 (males), 2001 (special study)	Rats: September 3-10, 2003 Mice: August 20-28, 2003
Average Age at Necropsy		
7 to 8 weeks	18 to 20 weeks	Rats: 110 to 112 weeks Mice: 109 to 112 weeks
Size of Study Groups		
5 males and 5 females	Core: 10 males and 10 females Clinical Pathology Study: 10 male and 10 female rats	50 males and 50 females

Table 2
Experimental Design and Materials and Methods in the Drinking Water Studies of Dibromoacetonitrile

2-Week Studies	3-Month Studies	2-Year Studies
Method of Distribution Animals were distributed randomly into groups of approximately equal initial mean body weights.	Same as 2-week studies	Same as 2-week studies
Animals per Cage Rats: 5 Mice: 1 (males); 5 (females)	Rats: 5 Mice: 1 (males); 5 (females)	Rats: 3 (males); 5 (females) Mice: 1 (males); 5 (females)
Method of Animal Identification Tail tattoo	Tail tattoo	Tail tattoo
Diet Irradiated NTP-2000 wafer rodent feed (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 2-week studies	Same as 2-week studies
Water Tap water (Birmingham, AL, municipal water supply) provided in amber glass bottles (Wheaton, Millville, NJ) with Teflon®-lined caps and stainless steel sipper tubes (Allentown Caging, Allentown, NJ), available *ad libitum*.	Same as 2-week studies	Same as 2-week studies
Cages Solid bottom polycarbonate (Lab Products, Inc., Maywood, NJ) changed once (male mice) or twice weekly	Same as 2-week studies	Same as 2-week studies
Bedding Heat-treated irradiated hardwood chips (P.J. Murphy Forest Products, Inc., Montville, NJ); changed with cage once (male mice) or twice weekly	Same as 2-week studies	Same as 2-week studies
Cage Filters Reemay® spun-bonded polyester (Andico, Birmingham, AL) changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Racks Stainless steel (Lab Products, Inc., Maywood, NJ) changed every 2 weeks	Same as 2-week studies	Same as 2-week studies
Animal Room Environment Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour	Temperature: 72° ± 3° F Relative humidity: 50% ± 15% Room fluorescent light: 12 hours/day Room air changes: 10/hour
Exposure Concentrations 0, 12.5, 25, 50, 100, and 200 mg/L in drinking water	0, 12.5, 25, 50, 100, and 200 mg/L in drinking water	0, 50, 100, and 200 mg/L in drinking water

Table 2
Experimental Design and Materials and Methods in the Drinking Water Studies of Dibromoacetonitrile

2-Week Studies	3-Month Studies	2-Year Studies
Type and Frequency of Observation		
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily; water consumption was recorded weekly.	Observed twice daily; core study animals were weighed initially, weekly, and at the end of the studies; clinical findings and water consumption were recorded weekly.	Observed twice daily; animals were weighed initially, weekly for the first 13 weeks, at 4-week intervals thereafter, and at the end of the studies; clinical findings were recorded at 4-week intervals, beginning week 5. Water consumption was recorded weekly for the first 13 weeks, and every 4 weeks thereafter.
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy		
Necropsies were performed on all animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all core study animals. Organs weighed were the heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on all animals.
Clinical Pathology		
None	Blood was collected from the retroorbital sinus of clinical pathology study rats on days 3 and 21 and from core study rats and mice at the end of the studies for hematology (rats and mice) and clinical chemistry (rats). ***Hematology:*** hematocrit; hemoglobin; hemoglobin concentration; erythrocyte, nucleated erythrocyte, reticuloctye, and platelet counts; erythrocyte morphology; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and bile acids	None
Histopathology		
Microscopic examinations were performed on control and 200 mg/L animals. In addition to gross lesions and tissue masses, the following tissues were examined: right kidney, liver, lung, and right testis.	Complete histopathologic examinations were performed on control and 200 mg/L core study animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.	Complete histopathologic examinations were performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, brain, clitoral gland, esophagus, eye, gallbladder (mice), harderian gland, heart and aorta, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, oral mucosa (rats), ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, tongue (rats), trachea, urinary bladder, and uterus.

Table 2
Experimental Design and Materials and Methods in the Drinking Water Studies of Dibromoacetonitrile

2-Week Studies	3-Month Studies	2-Year Studies
Sperm Motility and Vaginal Cytology		
None	At the end of the studies, sperm samples were collected from core study male animals in the 0, 50, 100, and 200 mg/L groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from female animals in the 0, 50, 100, and 200 mg/L groups for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.	None
Liver Enzyme Assay		
At the end of the studies, the liver was collected from all study animals, and a standard section for histology was taken from the left lateral lobe. The remainder of the left lateral lobe was saved for analysis of glutathione-*S*-transferase activity.	None	None

Statistical Methods

Survival Analyses

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

Calculation of Incidence

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

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power.

This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier *et al.,* 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, glutathione-*S*-transferase activity, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis. Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across exposure concentrations. Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each exposure group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among exposure groups and between the control group and each exposed group was tested using chi-square statistics.

Historical Control Data

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

icity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

Quality Assurance Methods

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

Genetic Toxicology

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

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

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

The predictivity for carcinogenicity of a positive response in acute *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *et al.,* 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt *et al.,* 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of *in vivo* genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the *Salmonella* assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).

RESULTS

Rats

2-Week Study

All rats survived to the end of the study (Table 3). The final mean body weight and body weight gain of 200 mg/L males were significantly less than those of the control group. Exposure-related decreases in water consumption were less pronounced during the second week of the study. Average compound consumption values for males in the 12.5, 25, 50, 100, and 200 mg/L groups were 2, 3, 7, 12, and 18 mg dibromoacetonitrile/kg body weight per day, respectively. Average compound consumption values for females in the 12.5, 25, 50, 100, and 200 mg/L groups were 2, 4, 7, 12, and 19 mg/kg per day, respectively. There were no clinical findings related to dibromoacetonitrile exposure. The exposure concentrations selected for this study were based on previous studies (NTP, 1997) that showed reduced water consumption and decreased body weight gain in rats administered drinking water containing 200 mg/L of dibromoacetonitrile.

Table 3
Survival, Body Weights, and Water Consumption of Rats in the 2-Week Drinking Water Study of Dibromoacetonitrile

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	93 ± 3	154 ± 2	61 ± 2		14.6	15.0
12.5	5/5	95 ± 3	161 ± 6	66 ± 4	105	15.2	15.6
25	5/5	95 ± 4	161 ± 6	66 ± 3	104	14.7	15.0
50	5/5	91 ± 3	156 ± 4	65 ± 2	101	13.7	14.6
100	5/5	96 ± 4	152 ± 3	56 ± 3	99	11.5	13.3
200	5/5	94 ± 3	128 ± 3**	34 ± 2**	83	7.3	10.4
Female							
0	5/5	83 ± 3	121 ± 1	38 ± 2		14.0	14.1
12.5	5/5	83 ± 3	116 ± 1	33 ± 3	95	12.8	13.4
25	5/5	86 ± 2	125 ± 3	40 ± 1	103	13.1	13.7
50	5/5	82 ± 2	121 ± 5	39 ± 3	99	11.4	12.2
100	5/5	84 ± 3	120 ± 3	37 ± 2	99	9.9	11.4
200	5/5	84 ± 3	118 ± 3	34 ± 1	97	6.5	10.6

** Significantly different ($P \leq 0.01$) from the control group by Williams' test
[a] Number of animals surviving at 2 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Water consumption is expressed as grams per animal per day.

There were no significant differences between exposed and control groups in the mean liver glutathione-*S*-transferase activity in either males or females (Table G1).

At necropsy, the right testis of one 200 mg/L male was noted to be small. Decreases in absolute and relative right testes weights were observed in this group, although the differences were not statistically significant. The reduction in testicular weight was consistent with the microscopic finding of atrophy of the testicular germinal epithelium in two 200 mg/L males. The atrophy was characterized by moderate or marked hypospermia (depletion of normal mature spermatids from the seminiferous tubules). No chemical-related lesions of the liver, kidney, or lungs were observed.

Exposure Concentration Selection Rationale: Because there was no significant toxicity and body weight reduction was considered related to decreased water consumption, the exposure concentrations selected for the 3-month drinking water study of dibromoacetonitrile in rats were the same as those used for the 2-week study (12.5, 25, 50, 100, and 200 mg/L). Because water consumption was improved during the second week of this study, this effect was not expected to be a significant concern for the 3-month drinking water study.

3-Month Study

All rats survived to the end of the study (Table 4). Final mean body weights of 200 mg/L males and females were 94% of the respective control groups. The final mean body weight and body weight gain of 200 mg/L females were significantly less than those of the control group. Water consumption by 200 mg/L rats was less than that by the control groups. Average compound consumption values for males in the 12.5, 25, 50, 100, and 200 mg/L groups were 1, 2, 3, 6, and 11 mg/kg per day, respectively. Average compound consumption values for females in the 12.5, 25, 50, 100, and 200 mg/L groups were 1, 2, 4, 7, and 13 mg/kg per day, respectively. There were no clinical findings related to dibromoacetonitrile exposure.

On day 3, there was evidence of a mild erythrocytosis, characterized by increased hematocrit values, hemoglobin concentrations, and erythrocyte counts in male and female rats in the 100 and/or 200 mg/L groups (Table F1). These findings were consistent with a transient erythrocytosis related to the decreased water consumption during week 1 and subsequent dehydration (Kaneko, 1989). The dehydration was supported by increased serum urea nitrogen (males only), albumin, and total protein concentrations in the 100 and 200 mg/L groups. Generally, the only known cause of increased serum albumin concentration is dehydration (Kaneko, 1989). Other minor changes in hematology and clinical chemistry variables were within physiological normal levels, did not demonstrate an exposure relationship, and were not considered biologically important or toxicologically relevant.

Table 4
Survival, Body Weights, and Water Consumption of Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	107 ± 1	326 ± 6	219 ± 6		14.2	13.5
12.5	10/10	108 ± 1	325 ± 6	218 ± 6	100	14.8	14.1
25	10/10	109 ± 2	342 ± 5	233 ± 4	105	14.9	15.0
50	10/10	107 ± 1	320 ± 8	213 ± 8	98	12.7	12.2
100	10/10	108 ± 1	320 ± 9	212 ± 8	98	10.9	12.7
200	10/10	107 ± 1	306 ± 5	199 ± 5	94	8.4	11.5
Female							
0	10/10	97 ± 1	190 ± 3	93 ± 3		11.1	10.1
12.5	10/10	97 ± 1	190 ± 2	92 ± 1	100	12.1	11.4
25	10/10	97 ± 2	187 ± 4	89 ± 4	98	11.4	10.7
50	10/10	97 ± 2	188 ± 2	92 ± 1	99	11.7	9.5
100	10/10	97 ± 1	189 ± 2	92 ± 2	99	9.3	9.2
200	10/10	98 ± 1	179 ± 3*	81 ± 3**	94	7.2	7.8

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

Significant increases occurred in the relative kidney weights of 200 mg/L males and 100 and 200 mg/L females (Table H2). A significant increase also occurred in the relative liver weight of 200 mg/L males.

There were no biologically significant changes in reproductive organ weights in male or female rats or in estrous cyclicity in female rats at any dose (Tables I1 and I2). The epididymal sperm motility was 9% lower in 200 mg/L males compared to controls.

No gross or microscopic lesions were observed that were considered to be due to dibromoacetonitrile exposure.

Exposure Concentration Selection Rationale: Based on the absence of significant toxicity in the 3-month study, the exposure concentrations selected for the 2-year drinking water study of dibromoacetonitrile in rats were 50, 100, and 200 mg/L. Higher concentrations could not be used because of the decreased water consumption and the moderate body weight effect at 200 mg/L.

2-Year Study

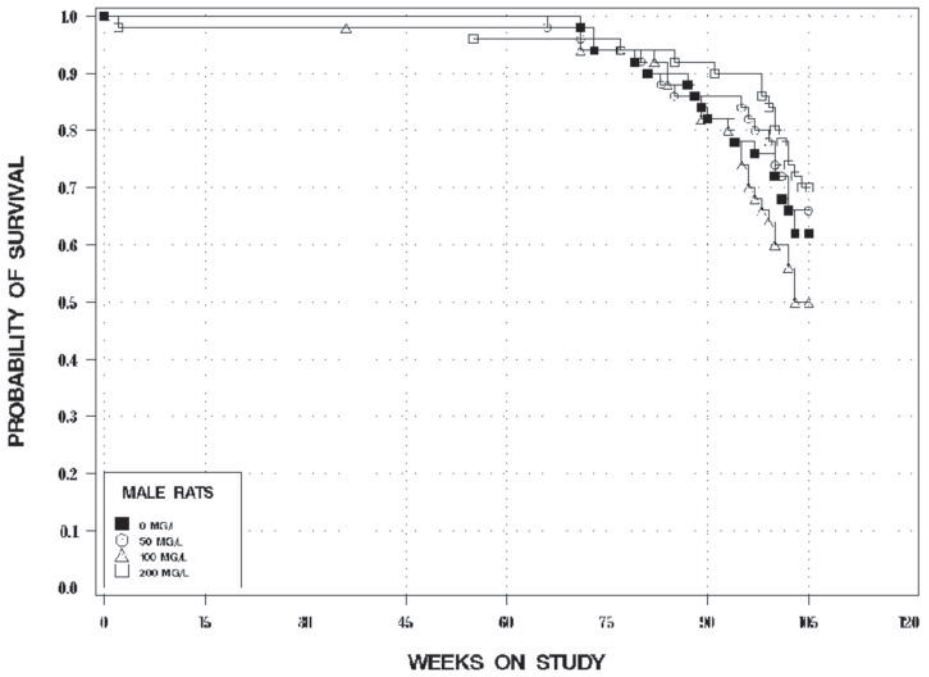
Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 5 and in the Kaplan-Meier survival curves (Figure 1). Survival of all exposed groups of rats was similar to that of the controls.

Table 5
Survival of Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Male				
Animals initially in study	50	50	50	50
Moribund	12	11	22	10
Natural deaths	7	6	3	5
Animals surviving to study termination	31	33	25	35
Percent probability of survival at end of study[a]	62	66	50	70
Mean survival (days)[b]	692	696	681	694
Survival analysis[c]	P=0.537N	P=0.824N	P=0.325	P=0.452N
Female				
Animals initially in study	50	50	50	50
Moribund	9	11	16	12
Natural deaths	12	4	5	7
Animals surviving to study termination	29	35	29	31
Percent probability of survival at end of study	58	70	58	62
Mean survival (days)	686	705	701	696
Survival analysis	P=0.935N	P=0.252N	P=0.930N	P=0.692N

[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**.



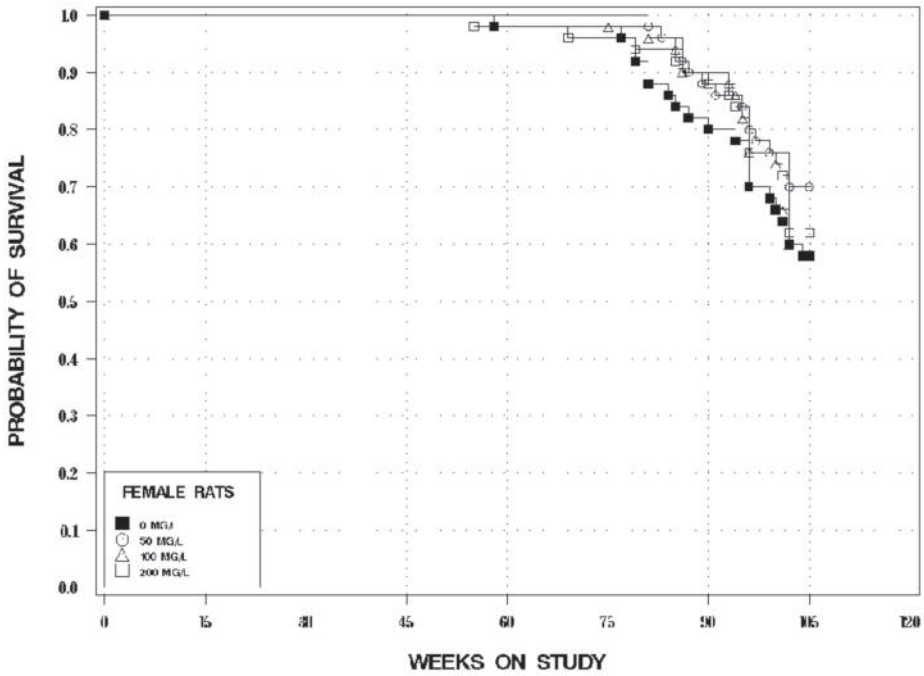


Figure 1
Kaplan-Meier Survival Curves for Male and Female Rats Exposed to Dibromoacetonitrile in Drinking Water for 2 Years

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 100 and 200 mg/L males and 200 mg/L females were slightly lower than those of the controls (Tables 6 and 7; Figure 2). Mean body weights of 200 mg/L males were approximately 7% less than those of the controls during the second year of the study. Water consumption by the 100 and 200 mg/L groups was generally less than that by the controls throughout the study. Drinking water concentrations of 50, 100, and 200 mg/L resulted in average daily doses of 2, 4, and 7 mg/kg per day to males and 2, 4, and 8 mg/kg per day to females (Tables K1 and K2). There were no clinical findings related to dibromoacetonitrile exposure.

Table 6
Mean Body Weights and Survival of Male Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

Days on Study	0 mg/L		50 mg/L			100 mg/L			200 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	94	50	94	100	50	94	100	50	94	100	50
10	132	50	133	101	50	128	97	50	122	93	50
17	164	50	166	102	50	162	99	50	156	95	49
24	193	50	196	101	50	191	99	50	185	96	49
31	217	50	220	101	50	214	99	50	208	96	49
38	239	50	241	101	50	235	98	50	231	96	49
45	258	50	259	100	50	250	97	50	246	95	49
52	270	50	272	101	50	264	98	50	262	97	49
59	285	50	286	100	50	277	97	50	276	97	49
66	300	50	300	100	50	290	97	50	289	96	49
73	311	50	311	100	50	299	96	50	299	96	49
80	319	50	322	101	50	309	97	50	308	96	49
87	327	50	330	101	50	315	96	50	312	95	49
115	352	50	357	101	50	339	96	50	337	96	49
150	384	50	386	101	50	371	97	50	364	95	49
171	400	50	399	100	50	385	96	50	380	95	49
199	413	50	413	100	50	397	96	50	391	95	49
234	437	50	434	99	50	420	96	50	412	95	49
255	441	50	439	100	50	425	96	49	415	94	49
283	453	50	451	100	50	435	96	49	428	95	49
311	465	50	461	99	50	445	96	49	436	94	49
339	472	50	468	99	50	451	96	49	441	94	49
367	480	50	473	99	50	457	95	49	448	93	49
395	489	50	483	99	50	466	95	49	459	94	48
423	487	50	481	99	50	467	96	49	454	93	48
451	495	50	489	99	50	474	96	49	462	94	48
479	500	50	492	98	49	476	95	49	464	93	48
507	503	48	495	98	48	484	96	47	466	93	48
535	500	47	491	98	47	478	96	47	464	93	48
563	508	45	494	97	45	476	94	47	464	91	47
591	504	45	495	98	44	481	95	44	466	92	47
619	499	42	495	99	43	474	95	42	457	92	46
647	497	41	496	100	43	472	95	40	462	93	45
675	502	39	492	98	40	473	94	34	457	91	45
703	488	36	491	101	37	466	96	30	457	94	40
Mean for weeks											
1-13	239		241	101		233	98		230	96	
14-52	424		423	100		408	96		400	95	
53-101	496		490	99		473	95		460	93	

Table 7
Mean Body Weights and Survival of Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

Days on Study	0 mg/L		50 mg/L			100 mg/L			200 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	86	50	87	101	50	86	100	50	87	100	50
14	119	50	121	102	50	117	99	50	114	96	50
21	133	50	135	101	50	133	99	50	128	96	50
28	142	50	144	102	50	141	100	50	138	97	50
35	152	50	153	101	50	150	99	50	146	96	50
42	160	50	161	101	50	157	98	50	155	97	50
49	167	50	168	101	50	164	99	50	161	96	50
56	171	50	173	101	50	170	99	50	165	96	50
63	175	50	176	101	50	174	99	50	168	96	50
70	181	50	182	101	50	178	98	50	173	96	50
77	184	50	186	101	50	183	99	50	176	96	50
84	184	50	187	102	50	182	99	50	177	96	50
91	192	50	193	100	50	187	97	50	184	96	50
119	199	50	202	102	50	200	101	50	191	96	50
154	210	50	210	100	50	208	99	50	203	97	50
175	216	50	216	100	50	213	99	50	206	95	50
203	223	50	224	100	50	222	99	50	215	96	50
238	233	50	235	101	50	231	99	50	223	95	50
259	239	50	242	102	50	237	99	50	228	95	50
287	247	50	249	101	50	243	98	50	238	96	50
315	256	50	257	100	50	252	98	50	244	95	50
343	263	50	266	101	50	258	98	50	248	94	50
371	271	50	272	100	50	266	98	50	259	96	50
399	276	50	282	102	50	275	100	50	267	97	49
427	288	49	290	101	50	281	98	50	273	95	49
455	298	49	301	101	50	293	99	50	282	95	49
483	306	49	311	102	50	301	98	50	290	95	48
511	310	49	319	103	50	308	99	50	298	96	48
539	311	48	320	103	50	310	100	49	296	95	48
567	319	44	327	103	49	319	100	48	302	95	47
595	325	42	329	101	48	324	100	47	307	95	46
623	326	41	335	103	44	325	100	45	307	94	45
651	328	40	337	103	43	327	100	44	311	95	43
679	335	35	343	102	39	337	101	38	318	95	38
707	338	32	347	103	38	340	101	33	317	94	36
Mean for weeks											
1-13	157		159	101		156	99		152	96	
14-52	232		233	101		229	99		222	95	
53-101	310		316	102		308	99		294	95	

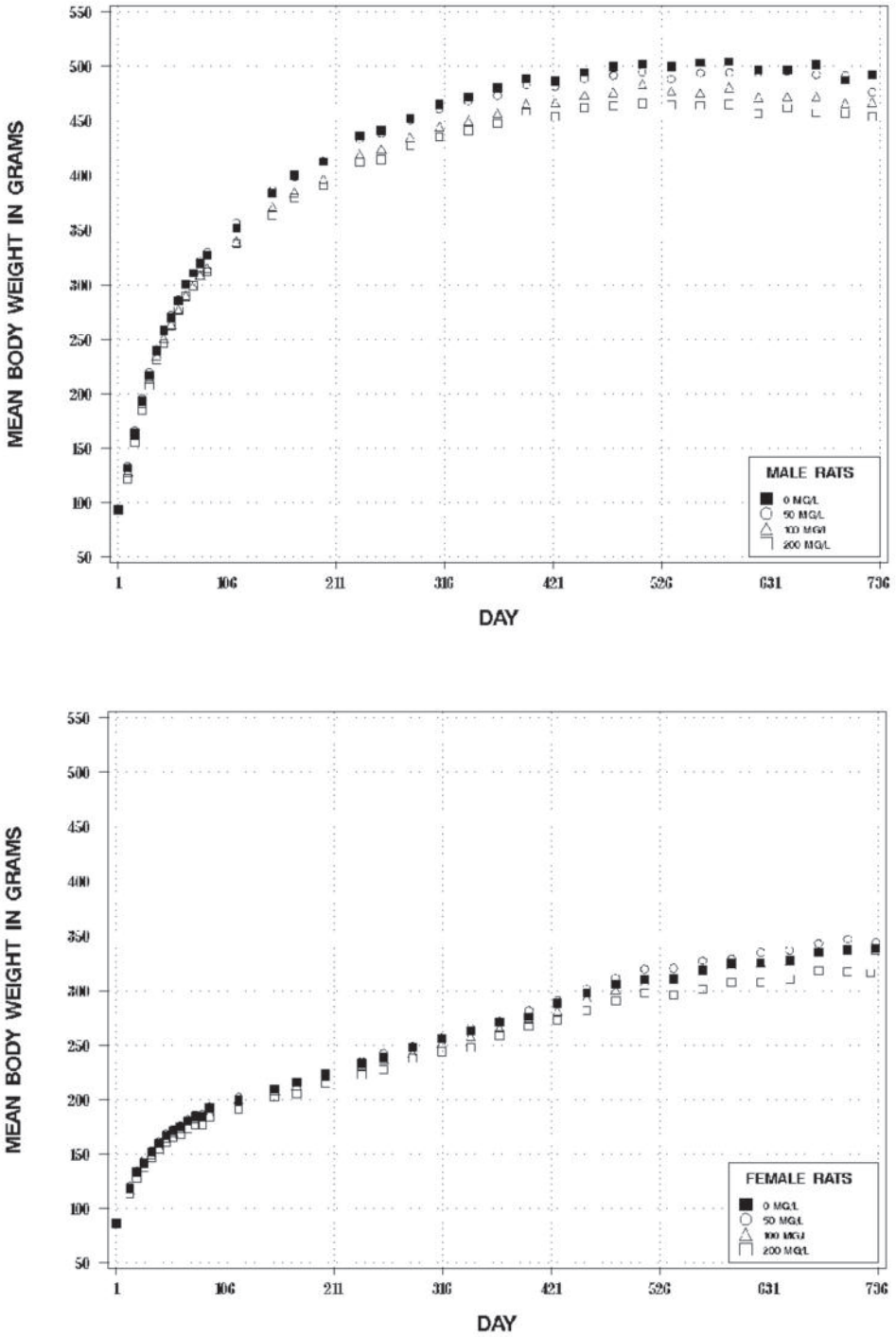


Figure 2
Growth Curves for Male and Female Rats Exposed to Dibromoacetonitrile in Drinking Water for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the oral cavity (oral mucosa and tongue), glandular stomach, esophagus, skin, and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical control incidences for the the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Oral cavity (oral mucosa and tongue): The combined incidences of squamous cell papilloma or carcinoma of the oral mucosa (buccal, gingival, hard palatine) or tongue in males occurred with a positive trend. The incidence in the 200 mg/L group was significantly increased and the incidences in the 100 and 200 mg/L groups exceeded the historical control ranges for drinking water studies and for all study routes (Tables 8, A1, A2, and A3). Squamous cell carcinomas were present on the oral mucosa in two 200 mg/L males and on the tongue in one 100 mg/L male and one 200 mg/L male. There was a positive trend in the incidence of squamous cell carcinoma of the oral mucosa or tongue, and the incidence in 200 mg/L males exceeded the historical control ranges for drinking water studies and for all study routes. Squamous cell papillomas of the oral cavity were present in four 100 mg/L females, one with the papilloma on the oral mucosa and three with the papilloma on the tongue (Tables 8, B1, B2, and B3). Although this incidence was not statistically significant, it did exceed the historical control range for both drinking water studies (0% to 2%) and for all study routes (0% to 4%). Microscopically, squamous cell papillomas were characterized by multiple papillary, often branching, projections, composed of thick, hyperplastic epithelium with a connective tissue core that radiated from a central stalk (Plate 1). The squamous cell carcinomas were invasive lesions consisting of cords and islands of pleomorphic squamous epithelial cells extending into the tissues underlying the surface epithelium. Two of the carcinomas were deeply invasive. One moderately well differentiated carcinoma of the tongue invaded deeply into the muscular portion of the tongue (Plate 2), and one large, poorly differentiated carcinoma of the palatine oral mucosa invaded through the lamina propria into underlying bone and blood vessels (Plates 3 and 4).

Six incidences of squamous epithelial hyperplasia of the tongue were observed in 200 mg/L females as compared to one in the control group (Plate 5); a slight increase in severity of the hyperplasia was noted in 100 and 200 mg/L females (Tables 8 and B4). Epithelial hyperplasia of the tongue was also noted in a few 100 and 200 mg/L males (Tables 8 and A4). Oral mucosal hyperplasia was present in one 50 mg/L and two 200 mg/L females.

Glandular stomach: Adenomas were present in the glandular stomach of two 200 mg/L males (Tables 9 and A1). This is a rare neoplasm that has not been seen in 1,199 (all routes) historical control animals. Microscopically, the adenomas were located within the submucosa and consisted of variably sized, often dilated, glands lined by relatively normal appearing cuboidal to tall columnar epithelial cells (Plate 6). Location of the adenomas within the submucosa was unusual since gastric adenomas usually appear as polypoid nodules or plaques of the mucosa (Brown and Hardisty, 1990).

Glandular hyperplasia was present in 100 and 200 mg/L males and 200 mg/L females (Tables 9, A4, and B4). The hyperplasia was graded as minimal to mild. Hyperplastic lesions were generally located within the deeper mucosa or submucosa and ranged from very minimal lesions affecting individual glands to more severe lesions composed of nodular aggregates of glands (Plate 7). The minimal lesions consisted of a few scattered glands lined by increased numbers of densely packed epithelial cells that appeared to form multiple layers and increased the diameter of the affected glands up to two or three times the normal size. Mitotic figures were sometimes seen within affected glands. More severe lesions consisted of nodular clusters of glands, some of which had variably dilated lumens lined by low cuboidal epithelial cells, as compared with more columnar epithelium seen in normal glands.

The incidence of glandular ectasia in the 200 mg/L females was significantly increased, and an increase in the severity of ectasia was noted in the 200 mg/L males (Tables 9, A4, and B4). Ectasia was characterized by dilatation of mucosal glands, usually deep in the mucosa. Since cystic dilation of gastric glands is a common background lesion in rats older than 15 months (Brown and Hardisty, 1990), ectasia was only diagnosed when the number of dilated glands exceeded the normal background.

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions in the Oral Cavity of Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Male				
Number Examined Microscopically	50	50	50	50
Oral Mucosa				
Squamous Cell Papilloma[a,b]	0	0	1	1
Squamous Cell Carcinoma[c]	0	0	0	2
Squamous Cell Papilloma or Squamous Cell Carcinoma (combined)	0	0	1	3
Tongue				
Epithelium, Hyperplasia	0	0	1 (2.0)[d]	2 (1.0)
Squamous Cell Papilloma[e]	0	0	1	1
Squamous Cell Carcinoma[f]	0	0	1	1
Oral Mucosa or Tongue				
Squamous Cell Papilloma[g]	0	0	1	2
Squamous Cell Carcinoma[c]				
Overall rate[h]	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate[i]	0.0%	0.0%	2.4%	6.6%
Terminal rate[j]	0/31 (0%)	0/33 (0%)	0/25 (0%)	2/35 (6%)
First incidence (days)	—[k]	—	709	704
Poly-3 test[l]	P=0.021	—[m]	P=0.493	P=0.126
Squamous Cell Papilloma or Squamous Cell Carcinoma (combined)[n]				
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	0.0%	4.7%	10.9%
Terminal rate	0/31 (0%)	0/33 (0%)	1/25 (4%)	3/35 (9%)
First incidence (days)	—	—	709	636
Poly-3 test	P=0.003	—	P=0.230	P=0.035
Female				
Number Examined Microscopically	50	50	50	50
Oral Mucosa				
Epithelium, Hyperplasia	0	1 (3.0)	0	2 (2.0)
Squamous Cell Papilloma[o]	0	0	1	0
Tongue				
Epithelium, Hyperplasia	1 (1.0)	1 (1.0)	2 (1.5)	6 (1.2)
Squamous Cell Papilloma[p]	1	0	3	0

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions in the Oral Cavity of Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Female (continued)				
Number Examined Microscopically	50	50	50	50
Oral Mucosa or Tongue				
Squamous Cell Papilloma[q]				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	8.8%	0.0%
Terminal rate	1/29 (3%)	0/35 (0%)	2/29 (7%)	0/31 (0%)
First incidence (days)	729 (T)	—	596	—
Poly-3 test	P=0.526N	P=0.487N	P=0.197	P=0.492N

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 0/300; all routes 3/1,199 (0.1% ± 0.4%), range 0%-2%
[c] Historical incidence for drinking water studies: 0/300; all routes 3/1,199 (0.3% ± 0.7%), range 0%-2%
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Historical incidence for drinking water studies: 1/300 (0.3% ± 0.8%), range 0%-2; all routes 4/1,199 (0.3% ± 0.8%), range 0%-2%
[f] Historical incidence for drinking water studies: 0/300; all routes 0/1,199
[g] Historical incidence for drinking water studies: 1/300 (0.3% ± 08%), range 0%-2%; all routes 5/1,199 (0.4% ± 0.8%), range 0%-2%
[h] Number of animals with neoplasm/number of animals necropsied
[i] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[j] Observed incidence at terminal kill
[k] Not applicable; no neoplasms in animal group
[l] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in an exposure group is indicated by **N**.
[m] Value of statistic cannot be computed
[n] Historical incidence for drinking water studies: 1/300 (0.3% ± 0.8%), range 0%-2%; all routes 8/1,199 (0.7% ± 1.0%), range 0%-2%
[o] Historical incidence for drinking water studies: 1/250 (0.4% ± 0.9%), range 0%-2%; all routes 1/1,100 (0.1% ± 0.4%), range 0%-2%
[p] Historical incidence for drinking water studies: 2/250 (0.8% ± 1.1%), range 0%-2%; all routes 5/1,100 (0.5% ± 1.1%), range 0%-4%
[q] Historical incidence for drinking water studies: 3/250 (1.2% ± 1.1%), range 0%-2%; all routes 6/1,100 (0.6% ± 1.1%), range 0%-4%

Table 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Glandular Stomach in Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Male				
Number Necropsied	50	50	50	50
Glands, Hyperplasia[a]	0	0	2 (1.0)[b]	2 (2.0)
Glands, Ectasia	4 (1.0)	3 (1.0)	3 (1.0)	10 (1.9)
Adenoma[c]				
Overall rate[d]	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Adjusted rate[e]	0.0%	0.0%	0.0%	4.4%
Terminal rate	0/31 (0%)	0/33 (0%)	0/25 (0%)	2/35 (6%)
First incidence (days)[f]	—[g]	—	—	729 (T)
Poly-3 test[h]	P=0.046	—[i]	—	P=0.246
Female				
Number Necropsied	50	50	50	50
Glands, Hyperplasia	0	0	0	2 (1.5)
Glands, Ectasia	10 (1.0)	4 (1.0)	10 (1.0)	26** (1.1)

** Significantly different ($P \leq 0.01$) from the control group by the Poly-3 test
(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year drinking water studies: 0/300; for all routes: 0/1,199
[d] Number of animals with neoplasm/number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable; no neoplasms in animal group
[h] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.
[i] Value of statistic cannot be computed

Esophagus: The incidences of epithelial hyperkeratosis were significantly increased in 100 and 200 mg/L males (6/48, 8/50, 34/50, 46/50; Table A4) and females (10/49, 8/50, 28/50, 48/50; Table B4). The severity grade of the hyperkeratosis was similar to that of the controls in the exposed males (1.8, 1.6, 1.8, 1.9) but was slightly increased in exposed females (1.7, 1.9, 2.0, 2.0). Microscopically, the hyperkeratosis was characterized by an increase in the thickness of the keratin layer on the luminal surface of the esophageal epithelium up to approximately two to three times normal thickness (Plate 8).

Skin: The incidences of squamous cell papilloma or keratoacanthoma (combined) (0/50, 0/50, 1/50, 3/50; (Table B2) and squamous cell papilloma, keratoacanthoma, basal cell adenoma, or basal cell carcinoma (combined) (0/50, 0/50, 3/50, 4/50) occurred with significant positive trends in females. The incidence of squamous cell papilloma or keratoacanthoma in 200 mg/L females exceeded the historical control range for drinking water studies [historical incidence for drinking water studies: 2/250 (0.8% ± 1.1%); range, 0% to 2%].

Kidney: The incidences of nephropathy were slightly increased with a significant trend in exposed groups of females (33/50, 36/49, 44/50, 43/49; Table B4) and reached statistical significance in the 100 and 200 mg/L groups. The increased nephropathy in female rats was considered to be of limited biological significance because the severity of nephropathy was of a minimal to mild degree and the severity in exposed animals was not appreciably increased over that of the controls (1.1 in controls versus 1.3 in the 200 mg/L group). The incidence of chronic inflammation of the renal pelvis was significantly increased in 200 mg/L females (1/50, 0/49, 0/50, 7/49). This finding was considered to be of limited biological significance because the inflammation was mild and because lymphocyte infiltrates are occasionally seen in the renal pelvis of the F344/N rat.

Mice

2-Week Study

All mice survived to the end of the study (Table 10). Final mean body weights and body weight gains of all exposed groups were similar to those of the controls. Decreases in water consumption were most apparent in mice exposed to 200 mg/L. Average compound consumption values for males in the 12.5, 25, 50, 100, and 200 mg/L groups were 2, 4, 8, 15, and 21 mg/kg per day, respectively. Average compound consumption values for females in the 12.5, 25, 50, 100, and 200 mg/L groups were 2, 3, 10, 14, and 22 mg/kg per day, respectively. There were no clinical findings related to dibromoacetonitrile exposure.

Liver glutathione-*S*-transferase activity was not affected in males or females exposed to dibromoacetonitrile (Table G2).

The absolute and relative liver weights of 50, 100, and 200 mg/L females were significantly decreased relative to controls (Table H3).

No chemical-related microscopic lesions were seen in the liver, kidney, lungs, or testes of 200 mg/L groups.

Exposure Concentration Selection Rationale: Based on the absence of significant toxicity, the exposure concentrations selected for the 3-month drinking water study of dibromoacetonitrile in mice were the same as those used for the 2-week study (12.5, 25, 50, 100, and 200 mg/L).

Table 10
Survival, Body Weights, and Water Consumption of Mice in the 2-Week Drinking Water Study of Dibromoacetonitrile

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	20.9 ± 0.6	23.4 ± 1.0	2.5 ± 0.6		3.9	3.8
12.5	5/5	21.0 ± 0.5	24.6 ± 0.4	3.6 ± 0.4	105	4.3	3.0
25	5/5	20.8 ± 0.6	24.5 ± 0.5	3.7 ± 0.8	105	3.7	3.9
50	5/5	21.1 ± 0.7	23.7 ± 0.8	2.6 ± 0.9	102	3.7	3.5
100	5/5	20.6 ± 0.5	23.8 ± 0.6	3.2 ± 0.3	102	3.3	2.9
200	5/5	20.3 ± 0.8	22.1 ± 0.9	1.8 ± 0.4	95	2.2	2.2
Female							
0	5/5	17.6 ± 0.3	19.8 ± 0.5	2.3 ± 0.3		2.6	4.8
12.5	5/5	18.1 ± 0.2	19.5 ± 0.3	1.4 ± 0.4	98	2.8	3.3
25	5/5	17.9 ± 0.7	19.1 ± 0.4	1.2 ± 0.6	96	2.3	2.7
50	5/5	17.4 ± 0.2	18.7 ± 0.3	1.3 ± 0.1	95	2.5	4.8
100	5/5	17.1 ± 0.4	19.0 ± 0.3	2.0 ± 0.2	96	2.2	2.8
200	5/5	18.1 ± 0.2	19.3 ± 0.3	1.2 ± 0.2	97	1.4	2.5

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

3-Month Study

All mice survived to the end of the study (Table 11). The final mean body weights and body weight gains of exposed groups were similar to those of the controls. Water consumption by 200 mg/L females was less than that by the controls. Drinking water concentrations of 12.5, 25, 50, 100, and 200 mg/L resulted in average daily doses of 2, 3, 6, 11, and 18 mg/kg per day to male and female mice. There were no clinical findings related to dibromoacetonitrile exposure.

There were no hematological effects in mice administered dibromoacetonitrile (Table F2).

No changes in absolute or relative organ weights in males or females were attributed to dibromoacetonitrile exposure (Table H4).

There were no significant changes in reproductive organ weights in male or female mice or in estrous cyclicity in female mice at any dose (Tables I3 and I4). Decreases in epididymal sperm motility in all exposed groups of male mice were 5% to 6% less than that of the controls. No gross or microscopic lesions were attributed to dibromoacetonitrile exposure.

Exposure Concentration Selection Rationale: Based on the absence of significant toxicity in the 3-month study, the exposure concentrations selected for the 2-year drinking water study of dibromoacetonitrile in mice were 50, 100, and 200 mg/L. Higher concentrations could not be used because of decreased water consumption and a moderate effect on body weight gain at 200 mg/L.

Table 11
Survival, Body Weights, and Water Consumption of Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile

Concentration (mg/L)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Water Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	22.4 ± 0.2	39.1 ± 0.8	16.7 ± 0.7		3.8	3.6
12.5	10/10	21.6 ± 0.4*	38.5 ± 0.7	16.9 ± 0.8	98	4.2	3.3
25	10/10	22.1 ± 0.1	36.7 ± 1.0	14.6 ± 1.1	94	4.2	3.2
50	10/10	22.3 ± 0.2	38.4 ± 0.6	16.2 ± 0.5	98	3.2	2.9
100	10/10	22.5 ± 0.2	37.3 ± 1.0	14.8 ± 1.0	95	2.8	3.2
200	10/10	22.2 ± 0.2	36.8 ± 1.0	14.5 ± 1.0	94	2.8	3.0
Female							
0	10/10	18.7 ± 0.2	30.5 ± 0.7	11.8 ± 0.7		3.0	3.0
12.5	10/10	18.7 ± 0.1	32.6 ± 0.9	14.0 ± 0.9	107	2.8	2.8
25	10/10	18.8 ± 0.2	31.0 ± 0.5	12.2 ± 0.4	102	3.0	2.6
50	10/10	18.8 ± 0.2	30.5 ± 0.4	11.6 ± 0.4	100	2.5	2.5
100	10/10	18.7 ± 0.1	29.4 ± 1.0	10.7 ± 0.9	96	2.2	2.3
200	10/10	18.0 ± 0.4*	29.2 ± 0.8	11.3 ± 0.7	96	1.8	1.9

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

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 12 and in the Kaplan-Meier survival curves (Figure 3). Survival of female mice exposed to 100 or 200 mg/L was significantly greater than that of the controls.

Body Weights, Water and Compound Consumption, and Clinical Findings

Mean body weights of 200 mg/L males and females were less than those of the controls throughout most of the study (Figure 4; Tables 13 and 14). Mean body weights of 200 mg/L females recovered to 97% that of the controls at the end of the study, while mean body weights of 200 mg/L males were less than 90% that of the controls. Water consumption by exposed groups was less than that of controls throughout most of the study. Drinking water concentrations of 50, 100, and 200 mg/L resulted in average daily doses of approximately 4, 7, and 13 mg/kg per day to males and 3, 6, and 11 mg/kg per day to females (Tables K3 and K4). There were no clinical findings related to dibromoacetonitrile exposure.

Table 12
Survival of Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Male				
Animals initially in study	50	50	50	50
Accidental death[a]	0	0	0	1
Moribund	4	5	2	3
Natural deaths	6	5	13	4
Animals surviving to study termination	40	40	35	42
Percent probability of survival at end of study[b]	80	80	70	86
Mean survival (days)[c]	697	714	696	695
Survival analysis[d]	P=0.671N	P=1.000N	P=0.349	P=0.630N
Female				
Animals initially in study	50	50	50	50
Accidental deaths	0	3	3	0
Moribund	3	5	0	1
Natural deaths	11	6	4	2
Animals surviving to study termination	36[e]	36	43[e]	47[e]
Percent probability of survival at end of study	72	77	92	94
Mean survival (days)	703	671	701	726
Survival analysis	P=0.002N	P=0.825N	P=0.028N	P=0.007N

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

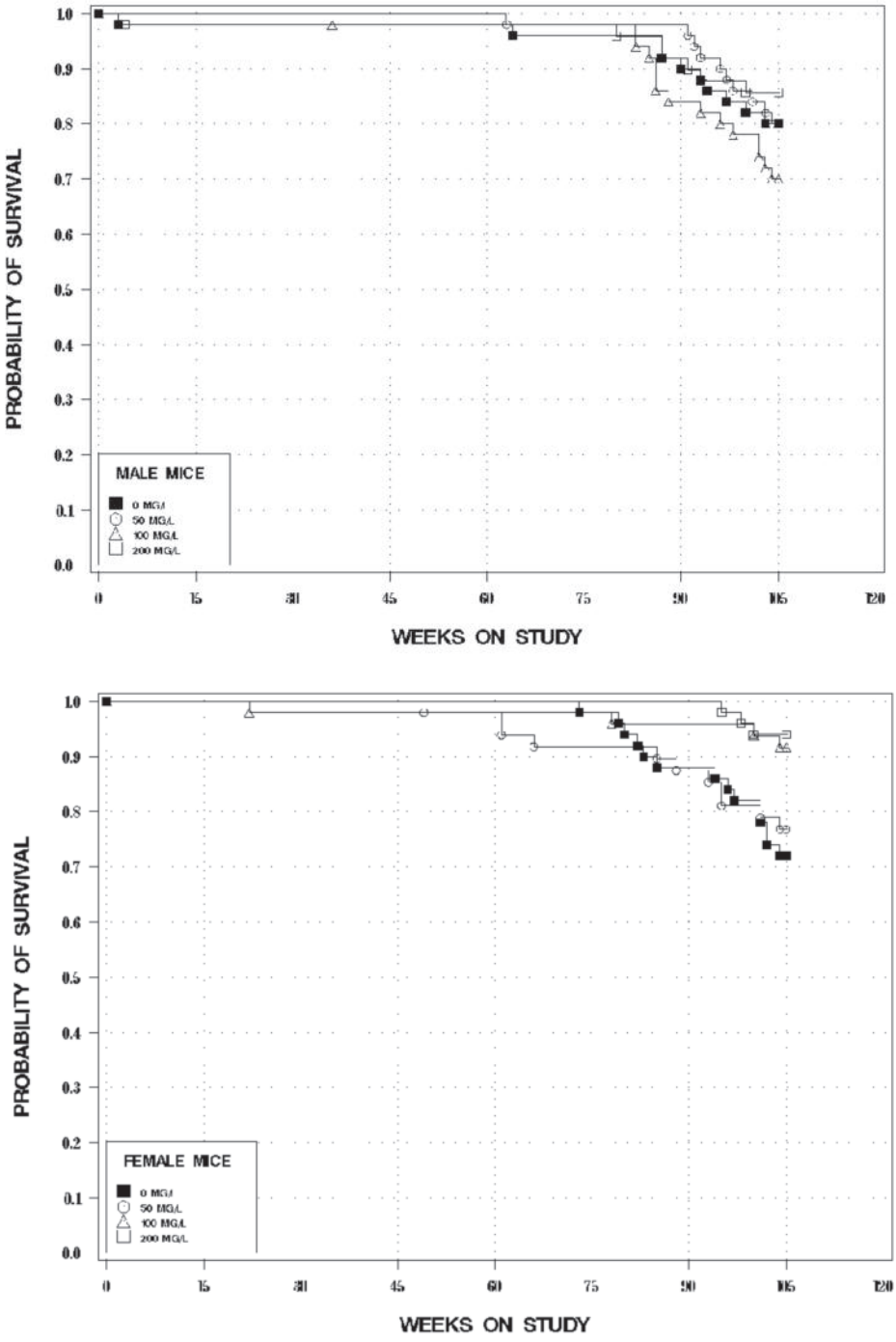


Figure 3
Kaplan-Meier Survival Curves for Male and Female Mice Exposed to Dibromoacetonitrile in Drinking Water for 2 Years

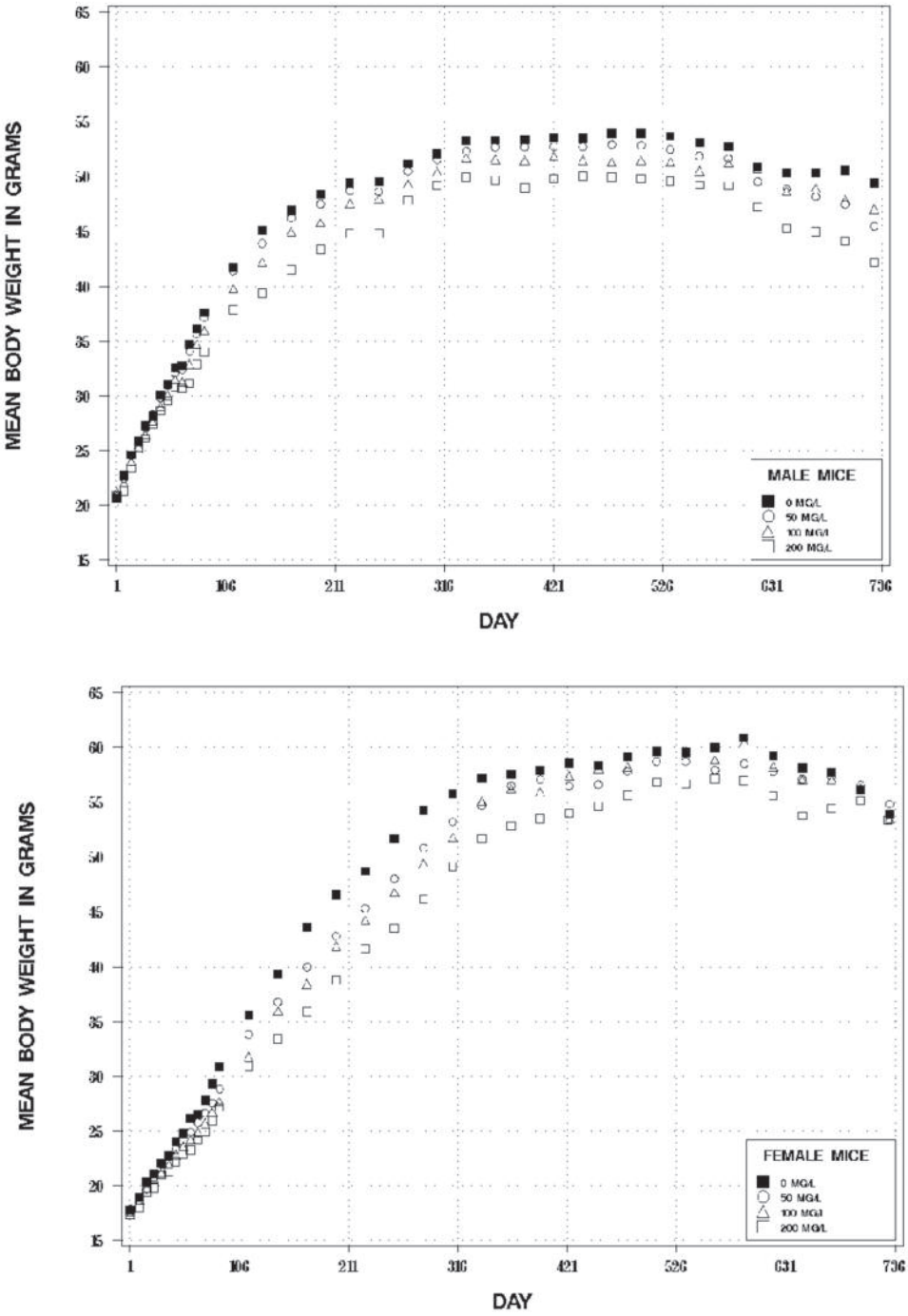


FIGURE 4
Growth Curves for Male and Female Mice Exposed to Dibromoacetonitrile in Drinking Water for 2 Years

Table 13
Mean Body Weights and Survival of Male Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

Days on Study	0 mg/L		50 mg/L			100 mg/L			200 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	20.6	50	20.8	101	50	21.3	103	50	20.8	101	50
8	22.7	50	22.4	99	50	22.2	98	50	21.3	94	50
15	24.6	50	24.5	100	50	24.1	98	50	23.4	95	50
22	25.9	49	25.9	100	50	25.3	98	50	25.1	97	49
29	27.3	49	27.1	99	50	26.5	97	50	26.1	96	49
36	28.2	49	28.3	101	50	27.7	98	50	27.4	97	49
43	30.1	49	29.8	99	50	29.0	97	50	28.6	95	49
50	31.0	49	30.9	100	50	30.0	97	50	29.6	95	49
57	32.5	49	32.1	99	50	31.5	97	50	30.8	95	49
64	32.7	49	32.4	99	50	31.3	96	50	30.7	94	49
71	34.7	49	34.0	98	50	32.9	95	50	31.1	90	49
78	36.1	49	35.6	99	50	34.7	96	50	32.9	91	49
85	37.6	49	37.1	99	50	36.0	96	50	34.0	91	49
113	41.7	49	41.4	99	50	39.8	95	50	37.9	91	49
141	45.1	49	43.9	97	50	42.2	93	50	39.3	87	49
169	46.9	49	46.2	99	50	45.0	96	50	41.5	88	49
197	48.4	49	47.5	98	50	45.8	95	50	43.4	90	49
225	49.4	49	48.7	99	50	47.5	96	50	44.8	91	49
253	49.5	49	48.6	98	50	48.0	97	49	44.8	91	49
281	51.2	49	50.5	99	50	49.4	96	49	47.8	93	49
309	52.1	49	51.5	99	50	50.5	97	49	49.2	94	49
337	53.3	49	52.3	98	50	51.7	97	49	49.9	94	49
365	53.3	49	52.7	99	50	51.6	97	49	49.7	93	48
393	53.3	49	52.7	99	50	51.5	97	49	49.0	92	48
421	53.5	49	52.7	99	50	51.9	97	49	49.8	93	48
449	53.5	48	52.7	99	49	51.5	96	49	50.0	94	48
477	54.0	48	52.9	98	49	51.3	95	49	50.0	93	48
505	54.0	48	52.9	98	49	51.4	95	49	49.8	92	48
533	53.7	48	52.5	98	49	51.3	96	49	49.6	92	48
561	53.1	48	51.9	98	49	50.5	95	49	49.2	93	47
589	52.8	48	51.6	98	49	51.2	97	47	49.2	93	47
617	50.9	46	49.5	97	49	50.8	100	42	47.2	93	45
645	50.3	45	48.9	97	47	48.7	97	42	45.3	90	44
673	50.3	43	48.2	96	45	48.9	97	40	45.0	89	43
701	50.6	41	47.5	94	43	47.9	95	39	44.1	87	42
Mean for weeks											
1-13	29.5		29.3	99		28.7	97		27.8	95	
14-52	48.6		47.8	98		46.7	96		44.3	91	
53-101	52.6		51.3	98		50.7	96		48.3	92	

Table 14
Mean Body Weights and Survival of Female Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

Days on Study	0 mg/L		50 mg/L			100 mg/L			200 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	17.8	50	17.8	100	50	17.4	98	50	17.6	99	50
10	18.9	50	18.7	99	50	18.7	99	50	18.0	95	50
17	20.3	50	19.7	97	50	19.8	97	50	19.4	96	50
24	21.1	50	20.8	99	50	20.7	98	50	19.8	94	50
31	22.1	50	21.9	99	50	21.3	96	50	21.0	95	50
38	22.8	50	22.1	97	50	22.0	97	50	21.3	93	50
45	24.0	50	23.3	97	50	22.9	95	50	22.2	93	50
52	24.8	50	23.5	95	50	23.6	95	50	22.9	93	50
59	26.1	50	24.9	95	50	24.3	93	50	23.3	89	50
66	26.5	50	25.8	97	50	24.9	94	50	24.2	91	50
73	27.8	50	26.7	96	50	25.7	93	50	24.9	90	50
80	29.3	50	27.5	94	50	26.8	91	50	25.9	88	50
87	30.9	50	28.8	94	50	27.6	90	50	27.2	88	50
115	35.6	50	33.8	95	50	31.8	89	50	30.9	87	50
143	39.3	50	36.8	94	49	36.0	92	50	33.4	85	50
171	43.6	50	40.0	92	49	38.4	88	49	35.9	82	50
199	46.5	50	42.8	92	49	41.9	90	49	38.8	83	50
227	48.7	50	45.3	93	49	44.2	91	49	41.7	86	50
255	51.7	50	48.0	93	49	46.8	91	49	43.5	84	50
283	54.3	50	50.8	94	49	49.4	91	49	46.2	85	50
311	55.8	50	53.2	95	49	51.8	93	49	49.1	88	50
339	57.2	50	54.7	96	48	55.1	96	49	51.7	90	50
367	57.5	50	56.5	98	48	56.3	98	49	52.8	92	50
395	57.9	50	57.1	99	48	55.9	97	49	53.5	93	50
423	58.6	50	56.5	96	48	57.4	98	49	54.0	92	50
451	58.4	50	56.6	97	44	58.1	100	49	54.6	94	50
479	59.2	50	57.8	98	43	58.2	98	49	55.6	94	50
507	59.7	50	58.7	98	43	59.6	100	49	56.9	95	50
535	59.5	49	58.7	99	43	59.6	100	46	56.7	95	50
563	60.0	47	57.9	97	43	58.9	98	45	57.1	95	50
591	60.9	45	58.5	96	42	60.5	99	45	57.0	94	50
619	59.2	44	57.8	98	41	58.2	98	45	55.6	94	50
647	58.1	44	57.1	98	40	57.1	98	45	53.8	93	50
675	57.7	42	57.4	99	38	57.1	99	45	54.5	94	49
703	56.6	40	56.5	100	37	56.3	99	44	55.1	97	47
Mean for weeks											
1-13	24.0		23.2	97		22.7	95		22.1	93	
14-52	48.1		45.0	94		43.9	91		41.2	86	
53-101	58.7		57.5	98		57.9	97		55.2	94	

Pathology and Statistical Analyses

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

Forestomach: The incidences of squamous cell papilloma or squamous cell carcinoma (combined) in males occurred with a positive trend, and the incidence in the 200 mg/L group was significantly increased and exceeded the historical control ranges for drinking water studies and for all study routes (Tables 15, C1, C2, and C3a). The incidences of squamous cell papilloma and squamous cell carcinoma in males occurred with a positive trend. Squamous cell papilloma in females occurred with a positive trend, was significantly increased in the 200 mg/L group, and the incidences in the 100 and 200 mg/L groups exceeded the historical control ranges for drinking water studies and all study routes (Tables 15, D1, D2, and D3).

Microscopically, the squamous cell papillomas consisted of multiple papillary fronds radiating from a central stalk. The papillary structures were composed of a central connective tissue core covered by a layer of thickened, well differentiated, stratified squamous epithelium, sometimes with secondary branching (Plate 9). The squamous cell carcinomas were characterized by prominent thickening and folding of the stratified squamous epithelium, with clusters of basal type or keratinizing epithelial cells invading downward from the lower surface of the thickened area of epithelium into the forestomach wall. One well differentiated carcinoma also invaded a submucosal vessel (Plates 10 and 11) and one moderately differentiated carcinoma invaded the adjacent glandular stomach.

The incidences of squamous epithelial hyperplasia of the forestomach were slightly increased in 50 and 200 mg/L males. Squamous epithelial hyperplasia was a focal lesion characterized by an increase in the thickness of the forestomach stratified squamous epithelium, accompanied by an increase in surface keratin. The thickened epithelium was often thrown into folds which sometimes formed a papillary surface projecting into the forestomach lumen (Plate 12).

Liver: The incidences of hepatoblastoma were increased in males, and were significantly increased in the 50 mg/L group (0 mg/L, 1/50; 50 mg/L, 7/50; 100 mg/L, 3/50; 200 mg/L, 2/50) (Table C2). Incidences of hepatocellular adenoma or carcinoma (combined) were significantly increased in 50 mg/L males (37/50, 45/50, 42/50, 42/50) and exceeded the historical control ranges for drinking water studies (57% to 85%) and for all study routes (20% to 85%) (Tables C2 and C3b). Incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) were increased in all exposed groups of males, and were significantly increased in the 50 and 100 mg/L groups (37/50, 46/50, 43/50, 42/50). The decreased incidences of hepatocellular carcinoma (10/50, 10/50, 7/50, 4/50) and hepatocellular adenoma or carcinoma (combined) (27/50, 25/50, 26/50, 20/50) in 200 mg/L females (Table D2) may have been related to the reduction in body weight (Haseman *et al*., 1997).

Kidney: In the standard evaluation of the kidneys, a slight increase in the incidence of renal tubule adenoma in exposed males (0 mg/L, 0/50; 50 mg/L, 1/50; 100 mg/L, 3/50; 200 mg/L, 2/50) suggested a potential treatment effect (Tables C1 and C2). Therefore, additional step sections of the male kidneys were prepared from the residual formalin-fixed tissues. The kidney step section review revealed additional animals with renal tubule adenoma, but the combined single section and step section incidences (1/50, 3/50, 3/50, 3/50) were not significantly increased in exposed animals, nor was there an exposure concentration-related trend.

Table 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Male				
Number Necropsied	50	50	50	50
Epithelium, Hyperplasia[a]	1 (3.0)[b]	4 (2.5)	1 (2.0)	6 (1.7)
Squamous Cell Papilloma, Multiple	0	1	0	0
Squamous Cell Papilloma, (includes multiple)	0	1	0	3
Squamous Cell Carcinoma	0	0	0	2
Squamous Cell Papilloma or Squamous Cell Carcinoma[c]				
Overall rate[d]	0/50 (0%)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted rate[e]	0.0%	2.1%	0.0%	10.8%
Terminal rate[f]	0/40 (0%)	0/40 (0%)	0/35 (0%)	3/42 (7%)
First incidence (days)	—[g]	437	—	647
Poly-3 test[h]	P=0.003	P=0.509	—[i]	P=0.031
Female				
Number Necropsied	50	50	50	50
Squamous Cell Papilloma, Multiple	0	0	0	1
Squamous Cell Papilloma (includes multiple)[j]				
Overall rate	1/50 (2%)	0/50 (0%)	5/50 (10%)	14/50 (28%)
Adjusted rate	2.2%	0.0%	10.8%	28.3%
Terminal rate	1/36 (3%)	0/36 (0%)	5/43 (12%)	14/47 (30%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P<0.001	P=0.515N	P=0.105	P<0.001

(T) Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[c] Historical incidence for 2-year drinking water studies (mean ± standard deviation): 3/249 (1.2% ± 1.8%), range 0%-4%; all routes: 13/1,149 (1.1% ± 1.5%), range 0%-4%
[d] Number of animals with neoplasm per number of animals necropsied
[e] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Not applicable, no neoplasms in animal group
[h] Beneath the control incidence is the P value associated with the trend test. Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in an exposure group is indicated by **N**.
[i] Value of statistic cannot be computed
[j] Historical incidence for drinking water studies: 3/300 (1.0% ± 1.1%), range 0%-2%; all routes: 20/1,249 (1.6% ± 1.4%), range 0%-4%

Genetic Toxicology

Dibromoacetonitrile was tested in five independent bacterial mutagenicity assays, in multiple strains of bacteria, and in the presence and absence of induced rat or hamster liver activation enzymes (S9). In four of the five assays, concentrations of dibromoacetonitrile dissolved in dimethylsulfoxide ranged up to 10,000 µg/plate (Table E1; Mortelmans *et al.*, 1986); in three of the assays, weak mutagenicity was observed in *Salmonella typhimurium* strains TA100, TA1535, and/or TA97 in the presence of hamster S9 liver enzymes and occasionally in the presence of rat liver S9. In the fourth study, small increases in revertant colonies were observed in TA97 in the presence of hamster and rat liver S9, and results of this study were judged to be equivocal. In the fifth study, mutagenic activity was observed in *Escherichia coli* strain WP2 *uvrA*/pKM101 in the presence of induced hamster S9. Across all studies, induced hamster liver S9 enzymes were generally more effective than rat liver S9 in generating the mutagenic metabolite.

Dibromoacetonitrile did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* exposed by feeding (50 or 75 ppm) or by injection (200 ppm) (Table E2; Valencia *et al.,* 1985). No increases in the frequencies of micronucleated normochromatic erythrocytes or significant alterations in the percentages of polychromatic erythrocytes were seen in peripheral blood of male or female mice in the 3-month study (Table E3), indicating no chemical-induced toxicity to the bone marrow. One female mouse in the 12.5 mg/L group had an extraordinarily high frequency of immature erythrocytes (38%), and this one value caused the group mean percentage of polychromatic erythrocytes to be significantly increased over the background frequency. However, recalculating the mean without this one outlier gave a value of 4.7 for the group, which is not significantly higher than the control value.

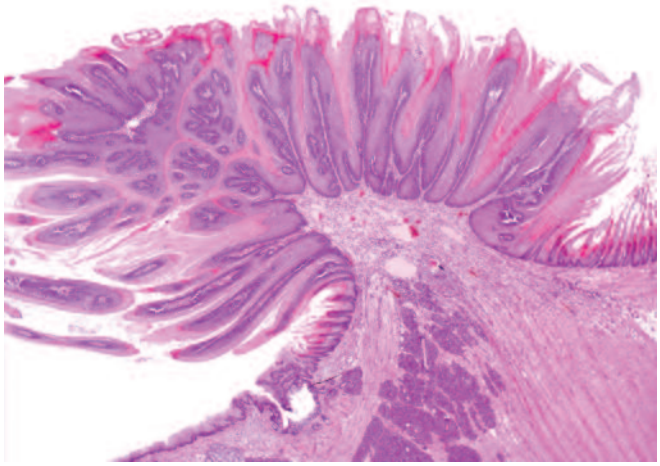


Plate 1
Squamous cell papilloma projecting from the dorsal surface of the tongue in a male F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm forms a large protruding mass with multiple papillary fronds and a central connective tissue core. H&E

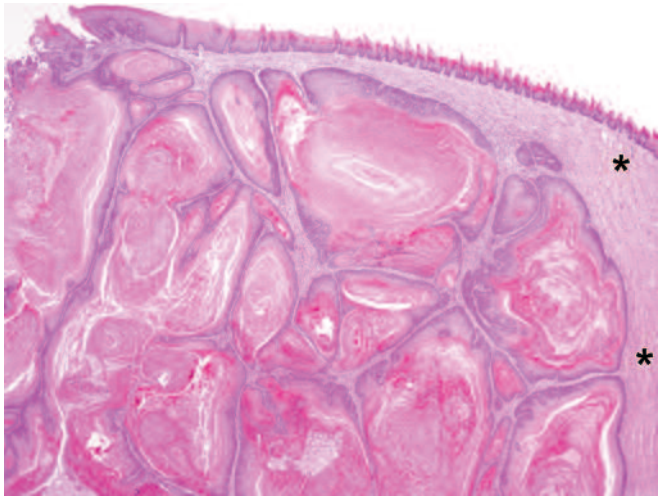


Plate 2
Squamous cell carcinoma, well differentiated, arising from the dorsal surface of the tongue at the lateral margin in a male F344/N rat exposed to 100 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm is forming large, cohesive, keratin-filled nests that have invaded deeply into the muscle (asterisks) of the tongue. H&E

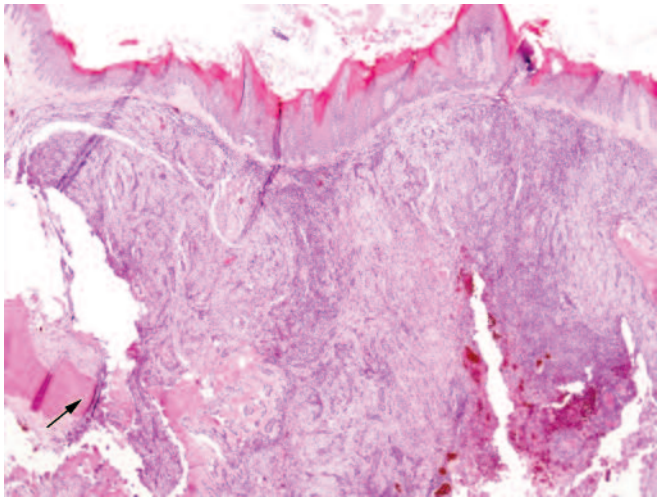


Plate 3
Squamous cell carcinoma, poorly differentiated, arising from the oral mucosa of the hard palate in a male F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm exhibits a diffuse, aggressive pattern and has infiltrated deeply into the underlying bone (arrow). H&E

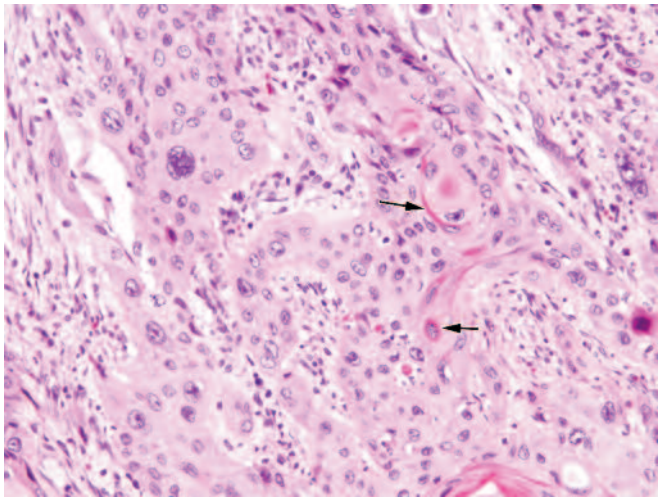


Plate 4
Higher magnification of Plate 3. The invasive squamous cell carcinoma forms coalescing cords and nests of pleomorphic cells within an inflamed, reactive stroma. The architectural pattern and the lack of squamous differentiation other than foci of individual cell keratinization (arrows) are histologic features of high grade neoplasia. H&E

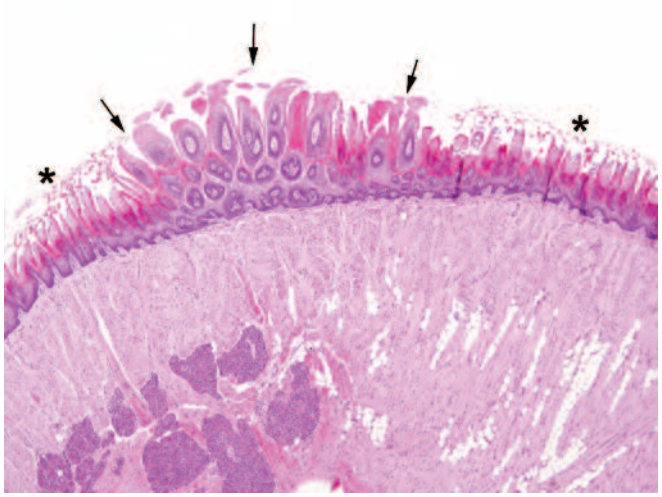


Plate 5
Squamous epithelial hyperplasia (arrows), projecting focally above the adjacent normal filiform papillae (asterisks) of the dorsal surface of the tongue in a female F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. H&E

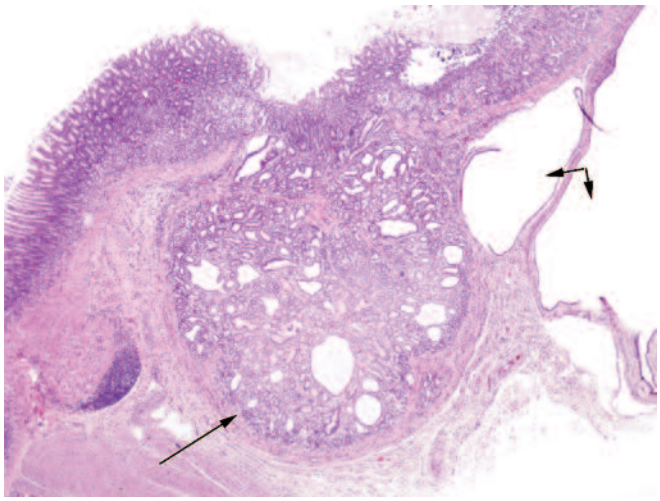


Plate 6
Adenoma (long arrow) in the glandular stomach of a male F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm forms a circumscribed nodule that has arisen in the mucosa and protruded into the submucosa. It is composed of well-differentiated glands that are variably sized and sometimes dilated. Two submucosal cysts (short arrows) are also present. H&E

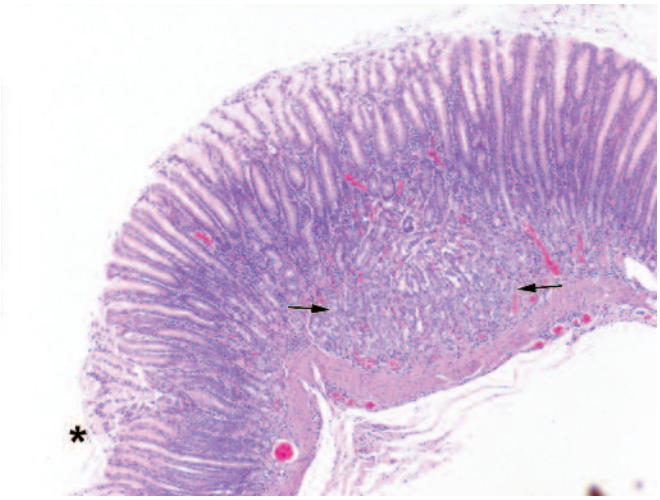


Plate 7
Glandular hyperplasia (arrows) developing within the basal portion of the mucosa of the glandular stomach in a male F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. Normal mucosa is indicated by an asterisk to the left of the hyperplastic lesion. H&E

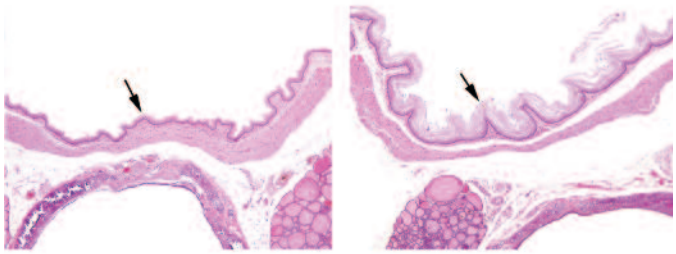


Plate 8
Normal esophagus (left arrow) in a control female F344/N rat at 2 years. Hyperkeratosis in the esophageal squamous mucosa (right arrow) of a female F344/N rat exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. Trachea and thyroid gland lie beneath the esophagus in both photos. H&E

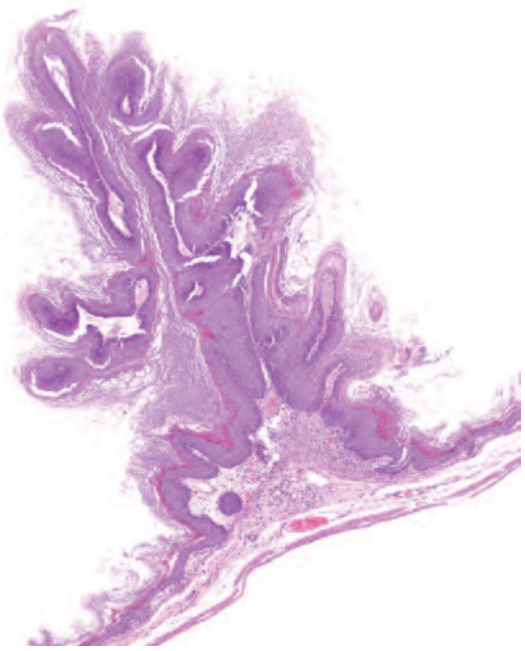


Plate 9
Squamous cell papilloma in the forestomach of a female B6C3F1 mouse exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm forms a delicate arborescent structure with a central connective tissue core. H&E

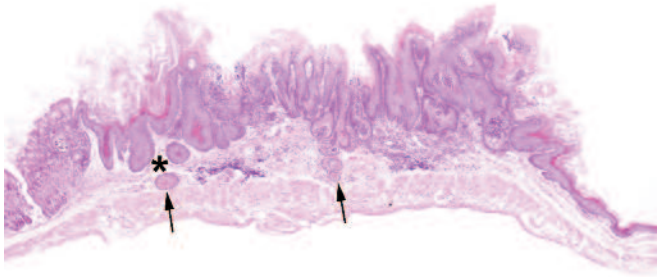


Plate 10
Squamous cell carcinoma, well differentiated, in the forestomach of a male B6C3F1 mouse exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The neoplasm has invaded beneath the muscularis mucosa (asterisk) into the submucosa (arrows). H&E

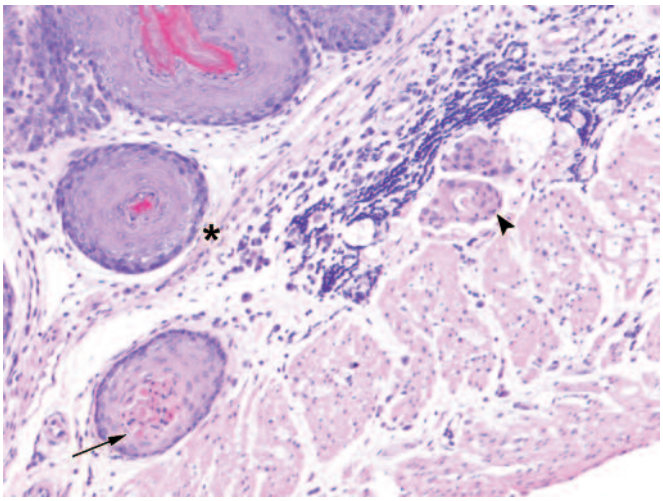


Plate 11
Higher magnification of Plate 10. Nests of invasive squamous cell carcinoma are noted in the stroma of the submucosa (arrow) as well as within the lumen of a submucosal vessel (arrowhead). The asterisk marks the muscularis mucosa, which separates the mucosa (above and to the left) from the submucosa (below and to the right). H&E

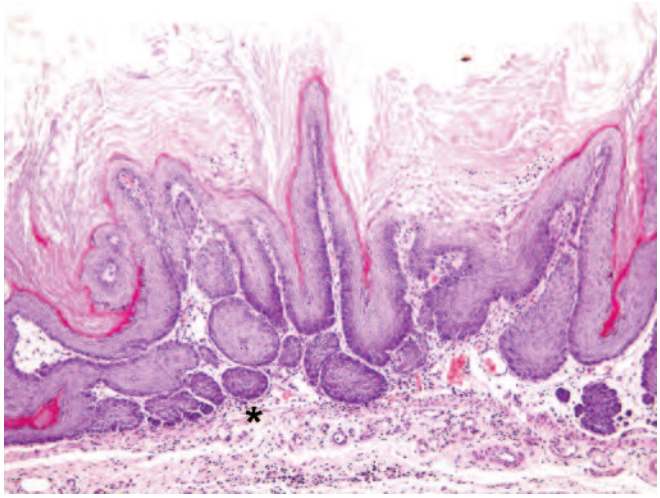


Plate 12
Squamous epithelial hyperplasia in the forestomach of a female B6C3F1 mouse exposed to 200 mg/L dibromoacetonitrile in drinking water for 2 years. The epithelium is hyperkeratotic, thickened, and thrown into folds that are sometimes papillary. The hyperplastic epithelium also protrudes into the underlying lamina propria with the appearance of separate nests due to tangential cuts of the epithelial downgrowths; note, however, that these squamous nests do not extend below the muscularis mucosa (asterisk). H&E

DISCUSSION AND CONCLUSIONS

Dibromoacetonitrile is a drinking water disinfection by-product formed by the reaction of chlorine disinfection compounds with natural organic matter, particularly nitrogen-containing organic compounds, in source water containing bromine. Dibromoacetonitrile is also a by-product of ozone disinfection of drinking water. While levels of trihalomethanes and several haloacetic acids in drinking water are regulated by the United States Environmental Protection Agency (USEPA), no drinking water standard has been established in the United States for exposure to dibromoacetonitrile or other halogenated acetonitriles. Toxicity and carcinogenicity studies of dibromoacetonitrile were nominated to the NTP by the USEPA as part of an interagency initiative on potential health effects of various disinfection by-products in drinking water and as a representative member of the family of haloacetonitriles. Brominated acetonitriles were considered to be potentially more active than chlorinated acetonitriles. Dibromoacetonitrile was administered in drinking water to mimic the major route of human exposure to this chemical.

In 2-week and 3-month studies, rats and mice were exposed to dibromoacetonitrile in drinking water at concentrations ranging from 12.5 to 200 mg/L. The highest exposure concentration selected for the 2-week study was based on previous observations of increased mortality and decreased body weight gain in Sprague-Dawley rats exposed to 45 mg/kg of dibromoacetonitrile for 90 days (Hayes *et al.*, 1986) and reduced water consumption and decreased mean body weight in Sprague-Dawley rats exposed to dibromoacetonitrile in drinking water at a concentration of 200 mg/L (NTP, 1997). The decrease in final mean body weight and body weight gain in male rats exposed to 200 mg/L in the 2-week study as well as the decreases in water consumption by 100 and 200 mg/L rats were consistent with previous studies.

Hepatic glutathione-*S*-transferase (GST) activity was not reduced in rats exposed to dibromoacetonitrile for 2 weeks. This result contrasts with studies by Ahmed *et al*. (1991b), who observed significant decreases in hepatic GST activity in Sprague-Dawley rats administered single gavage doses of dibromoacetonitrile ranging from 50 to 100 mg/kg. The differing results may be due to differences in administered dose of dibromoacetonitrile, the mode of chemical administration, strains of rats used, or the time at which livers were collected and analyzed for GST activity. The highest daily dose of dibromoacetonitrile in the 2-week drinking water study was slightly less than 20 mg/kg, and Ahmed *et al*. (1991b) did not detect an effect of dibromoacetonitrile on GST activity with a gavage dose of 25 mg/kg.

In the 2-week rat study, atrophy of the testicular germinal epithelium was observed in two of five male rats exposed to 200 mg/L; however, this finding is of questionable significance since testicular atrophy was not observed in male rats exposed to the same concentration of dibromoacetonitrile in the 3-month study. In addition, a previous study in Sprague-Dawley rats found no effects in reproductive organs in male or female rats exposed to dibromoacetonitrile in drinking water at concentrations up to 150 mg/L (NTP, 1997). In the latter study, male rats were exposed to dibromoacetonitrile during cohabitation with female rats and through 30 days after mating.

In the 3-month study, rats were exposed to the same concentrations of dibromoacetonitrile as in the 2-week study. Once again, marginal decreases in body weight gain in male and female rats and decreases in water consumption were observed in the 200 mg/L groups. Hence, reduced water consumption and consequent effects on body weight set practical limits for drinking water exposures of rats to dibromoacetonitrile. The small decreases in epididymal sperm motility in male rats were unlikely to have a significant biological consequence. No exposure-related gross or microscopic lesions were observed in rats.

In the 2-year study, water consumption was less by the 100 and 200 mg/L groups of male and female rats than by controls throughout the study, and this decrease was associated with slightly lower mean body weight of 200 mg/L males compared to controls. Neoplasms and nonneoplastic lesions induced by dibromoacetonitrile

were observed predominantly in the oral cavity, esophagus, and the glandular stomach. Neoplasms of the oral cavity are uncommon in untreated male or female F344/N rats. In the present study, squamous cell neoplasms of the oral cavity (oral mucosa or tongue) were observed at a significantly increased incidence in male rats exposed to 200 mg/L. The neoplasms included squamous cell papillomas and squamous cell carcinomas. In female rats, a nonsignificant increase in the incidence of squamous cell papilloma of the oral mucosa or tongue was observed in the 100 mg/L group (4/50, 8%), while an increased incidence of squamous epithelial hyperplasia of the tongue was observed in 200 mg/L females (six versus one in controls). Squamous epithelial hyperplasia is considered to be part of the continuum of proliferative changes in oral cavity neoplasia (Brown and Hardisty, 1990). Thus, the findings of increased incidences of oral cavity neoplasms in males, squamous cell papillomas in females, and squamous epithelial hyperplasia of the tongue in females were considered clear evidence of carcinogenicity of dibromoacetonitrile in male rats and some evidence of carcinogenicity in female rats. Among the nearly 550 chemicals in the NTP database, 15 chemicals that were studied in both male and female rats produced some or clear evidence of carcinogenicity in the oral cavity (NTP, 2007). Eleven of these chemicals were active in both male and female rats, indicating a low likelihood of sex specificity in the induction of oral cavity neoplasms. The lack of oral cavity neoplasms in mice exposed to dibromoacetonitrile may be due to a species difference in susceptibility to tumor inductions at this site. Only one NTP study (1,2,3-trichloropropane) demonstrated some or clear evidence of chemically induced carcinogenicity in the oral cavity of mice. In that study, the incidence of squamous cell neoplasms in the oral cavity was much higher in rats administered 30 mg/kg than in mice administered 60 mg/kg (NTP, 1993; Irwin *et al.*, 1995).

In the current study, dibromoacetonitrile also induced hyperkeratosis of the esophagus in both sexes of rats; the cause of this lesion is uncertain. Maintenance of a constant thickness of the keratin layer in the esophagus depends on mechanical abrasion; rats that are not eating may accumulate excessive keratin in the esophagus without evidence of a proliferative change in the epithelium (Brown and Hardisty, 1990). However, mean body weights of 100 and 200 mg/L rats differed from controls by less than 10%. Larger reductions in body weight would have been expected if the hyperkeratosis was due to substantial reduction in feed consumption. The likely cause of this lesion was a direct interaction of the esophagus with dibromoacetonitrile in the drinking water.

Two adenomas of the glandular stomach were observed in 200 mg/L male rats. Neoplasms at this site occur rarely and none have been observed in 1,199 male rats in the NTP historical control database for all routes of exposure. Glandular hyperplasias were also observed in a small number of male and female rats exposed to dibromoacetonitrile. Based on the rarity of these neoplasms, the occurrence of two glandular stomach adenomas in 200 mg/L male rats was considered to be related to exposure to dibromoacetonitrile.

Neoplasms of the skin occurred with a positive trend in female rats, but not in male rats. The skin neoplasms were not present at any consistent location. Because the incidence in the 200 mg/L group (4/50) was not significantly different from that in the controls and was only slightly above the range for these lesions in historical control female rats in drinking water studies (0%-6%) and equivalent to the upper range in control female rats by all routes of exposure (0%-8%), the relationship between these lesions and dibromoacetonitrile exposure is uncertain.

In mice exposed to dibromoacetonitrile for 2 weeks, water consumption was reduced compared to controls and mean body weight gains were moderately but not significantly less than those of the controls in the 200 mg/L groups. There were no effects on hepatic GST activity. The highest average daily dose of dibromoacetonitrile in this study was 22 mg/kg. Decreases in absolute and relative liver weights of female mice exposed to 50 mg/L or greater were not accompanied by any histopathological changes. The reason for the decreases in liver weights of female mice is not known.

Water consumption was also less by female mice exposed to 200 mg/L compared to controls in the 3-month study, and mean body weight gains were moderately but not significantly less in both sexes of mice in the 100 and 200 mg/L groups. The small decreases in epididymal sperm motility in male mice were unlikely to have a significant biological consequence. As with rats, reduced water consumption and moderate effects on body weight gain set practical limits for drinking water exposures of mice to dibromoacetonitrile.

In the 2-year mouse study, water consumption by exposed groups was less than that by controls and mean

body weights of 200 mg/L males and females were less than those of controls throughout most of the study. The forestomach was a target of dibromoacetonitrile-induced carcinogenicity in male and female mice. Squamous cell neoplasms, and particularly squamous cell carcinomas of the forestomach, are uncommon in male and female B6C3F1 mice fed the NTP-2000 diet. Thus the significant increases in the incidences of squamous cell papillomas or carcinomas (combined) in male mice and in squamous cell papillomas of the forestomach in female mice were clearly associated with exposure to dibromoacetonitrile. The increase in the incidence of squamous epithelial hyperplasia of the forestomach in 200 mg/L male mice (six versus one in controls) contributed to the evidence of carcinogenicity since the latter lesion is considered to be part of the continuum of proliferative changes in forestomach neoplasia (Leininger *et al.*, 1999).

The forestomach neoplasm response (incidence and exposure-related trend) was stronger in female mice than in male mice; however, unlike male mice, no squamous cell carcinomas were observed in female mice. The determination of clear evidence of carcinogenic activity of dibromoacetonitrile in female mice is supported by results from previous NTP studies that demonstrate the potential of forestomach neoplasms to become malignant; squamous cell carcinomas were observed with 19 or 22 chemicals (86%) in the NTP database associated with forestomach neoplasms in mice and evaluated as having some evidence or clear evidence of carcinogenic activity. Most relevant to the issue of possible progression to malignancy is the finding of squamous cell carcinomas of the forestomach in male mice exposed to dibromoacetonitrile. In addition, multiple reviews in toxicologic pathology concluded that forestomach papillomas in mice can progress to carcinomas (Leininger and Jokinen, 1994; Leininger *et al*., 1999).

The incidences of liver neoplasms, including hepatocellular adenoma or carcinoma (combined) and hepatoblastoma, were increased in 50 mg/L males. In the higher exposure groups, the incidences of liver neoplasms were marginally greater than those in the controls. The relationship between liver neoplasms in male mice and exposure to dibromoacetonitrile was considered to be uncertain because of the relatively flat dose-response, the high rate of these neoplasms in historical control male mice and in the controls in this study, and the lack of an increased incidence of liver neoplasms in female mice. The decreased incidence of hepatocellular neoplasms in 200 mg/L female mice compared to controls was likely due to the reduction in body weight (Haseman *et al.*, 1997) in this group.

Step sections of the kidneys from male mice were examined because the standard single-section evaluation indicated a possible treatment-related effect on the incidence of renal tubule adenomas. The step-section review did not support a chemical-related effect in the male mouse kidney; the incidence of renal tubule adenomas was not increased significantly in any of the exposed male mouse groups compared to controls, and there was no exposure-related trend.

The carcinogenic effects of dibromoacetonitrile observed in this study are predominantly in the upper alimentary system; this pattern of response may be a consequence of the reactivity of this compound. In rats, increased incidences of neoplasms were observed in the oral cavity and glandular stomach, while nonneoplastic lesions were also observed in the esophagus, tongue, and glandular stomach. In mice, increased incidences of neoplasms were observed in the forestomach, and possibly also in the liver of male mice. Because none of these sites were identified as targets of dibromoacetonitrile toxicity in the 3-month studies, it is evident that early toxic effects at these sites are not required for tumor induction.

The finding of radioactivity in feces from orally administered [2-^{14}C]-dibromoacetonitrile in rats and mice provides direct evidence that the parent compound or metabolite(s) reached the intestines (NTP, 2002). The finding that the percentage of dose recovered in feces from rats was higher after oral administration than after intravenous administration indicates that a portion (approximately 10%) of the oral dose was not absorbed. Because the primary route of elimination of radioactivity is in the urine and low levels of parent compound were measured in the blood of exposed animals, systemic effects, such as increased nephropathy in female rats, were probably caused by a dibromoacetonitrile metabolite.

The mechanisms of toxicity or carcinogenicity of dibromoacetonitrile are not known. The finding of thiocyanate in the urine of rats exposed to dibromoacetonitrile suggests that cyanide is released from a hydroxyacetonitrile intermediate (Pereira *et al.*, 1984). This may account for the ataxia and depressed respiration observed in acute toxicity studies (Hayes *et al.*, 1986).

In an initiation/promotion study in Sencar mice using 12-*O*-tetradecanoylphorbol-13-acetate as the tumor promoter, dibromoacetonitrile induced squamous cell papillomas and carcinomas of the skin when applied topically but not after oral administration (Bull *et al.*, 1985). Thus, the parent compound or unstable metabolites are likely to be involved in the induction of squamous cell neoplasms by dibromoacetonitrile. Gavage administration of dibromoacetonitrile to Sprague-Dawley rats caused reductions in glutathione levels and glutathione-*S*-transferase activities in the liver and glandular stomach (Ahmed *et al.*, 1991b). The lack of an effect on liver glutathione-*S*-transferase activity in the present drinking water study probably reflects differences in dose and method of administration. It is likely that a greater amount of parent compound reached the liver in the gavage study. Oral administration of [2-^{14}C]-dibromoacetonitrile by intragastric gavage resulted in a portion of the radiolabel in the stomach and liver not being extractable with organic solvents (NTP, 2002). This suggests covalent binding occurs in these tissues, possibly by glutathione-mediated activation of dibromoacetonitrile. The finding of rare glandular stomach neoplasms in male rats in the present study may be a consequence of oxidative stress associated with reduced glutathione levels and deficiency in glutathione-*S*-transferase activity and/or binding to protein or other macromolecules. The carcinogenicity of dibromoacetonitrile may also involve DNA damaging effects because it has been shown that this compound induces DNA strand breaks in Chinese hamster ovary cells (Bull *et al*., 1985), human lymphoblastic cells (Daniel *et al*., 1986), and HeLa cells (Mueller-Pillet *et al*., 2000), sister chromatid exchange in Chinese hamster ovary cells (Bull *et al*., 1985), and DNA repair response in *E. coli* (Le Curieux *et al*., 1995). No measurements were made of retention of radioactivity in the oral cavity of rats. The effects of dibromoacetonitrile in the stomach after gavage administrations may be pertinent to the induction of neoplasms and nonneoplastic lesions in the oral cavity after drinking water exposures. The finding of forestomach neoplasms in mice rather than oral cavity neoplasms may be due to species differences in tissue reactivity with dibromoacetonitrile or in organ susceptibility to changes induced by dibromoacetonitrile. Certainly, much more research will be needed to understand the mechanisms of tumor induction by dibromoacetonitrile.

Dibromoacetonitrile is the only halogenated acetonitrile ever evaluated for chronic toxicity and carcinogenicity by the NTP. In a 2-year inhalation study of acetonitrile in F344/N rats and B6C3F1 mice (NTP, 1996), the evidence of carcinogenic activity was equivocal in male rats based on a marginally increased incidence of hepatocellular neoplasms. There was no evidence of carcinogenic activity in female rats or in mice of either sex, although there were increased incidences of squamous cell hyperplasia of the forestomach in mice. The differences in response between the NTP (1996) study of acetonitrile and the present study of dibromoacetonitrile are likely due to differences in route of exposure as well as the greater reactivity of the brominated compound with glutathione or cellular macromolecules.

Conclusions

Under the conditions of these 2-year drinking water studies there was *clear evidence of carcinogenic activity** of dibromoacetonitrile in male rats based on increased incidences of squamous cell papillomas or carcinomas of the oral cavity; adenomas in the glandular stomach of male rats were also considered to be exposure-related. There was *some evidence of carcinogenic activity* of dibromoacetonitrile in female rats based on an increased incidence of squamous cell papillomas of the oral cavity; increased incidences of basal cell or squamous cell neoplasms of the skin in female rats may have been related to dibromoacetonitrile exposure. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in male mice based on increased incidences of squamous cell papillomas or carcinomas of the forestomach. Increased incidences of neoplasms in the liver of male mice may have been related to dibromoacetonitrile exposure. There was *clear evidence of carcinogenic activity* of dibromoacetonitrile in female mice based on increased incidences of squamous cell papilloma of the forestomach.

Exposure to dibromoacetonitrile for 2 years caused increased incidences of epithelial hyperkeratosis in the esophagus of male and female rats, glandular ectasia of the glandular stomach and squamous epithelial hyperplasia of the tongue in female rats, and squamous epithelial hyperplasia of the forestomach in male mice.

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

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	11	22	10
Natural deaths	7	6	3	5
Survivors				
Terminal sacrifice	31	33	25	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(48)	(50)	(50)	(50)
Intestine large, cecum	(48)	(49)	(50)	(47)
Intestine large, colon	(48)	(50)	(49)	(47)
Adenoma			1 (2%)	2 (4%)
Carcinoma	1 (2%)			
Intestine large, rectum	(49)	(50)	(50)	(49)
Adenoma		1 (2%)	1 (2%)	
Intestine small, duodenum	(49)	(49)	(50)	(48)
Intestine small, ileum	(46)	(48)	(50)	(47)
Intestine small, jejunum	(47)	(46)	(49)	(47)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			1 (2%)	
Hepatocellular carcinoma			1 (2%)	
Mesentery	(14)	(18)	(18)	(15)
Oral mucosa	(50)	(50)	(50)	(50)
Squamous cell carcinoma				2 (4%)
Squamous cell papilloma			1 (2%)	1 (2%)
Pancreas	(49)	(50)	(50)	(50)
Acinus, adenoma	1 (2%)		2 (4%)	
Duct, carcinoma			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(49)	(50)	(50)
Squamous cell papilloma		1 (2%)		1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Adenoma, multiple				1 (2%)
Tongue	(50)	(50)	(50)	(50)
Squamous cell carcinoma			1 (2%)	1 (2%)
Squamous cell papilloma			1 (2%)	1 (2%)
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(1)	(0)	(1)	(0)
Heart	(50)	(50)	(50)	(50)
Schwannoma benign				2 (4%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				1 (2%)
Adrenal medulla	(50)	(50)	(49)	(49)
Ganglioneuroma			1 (2%)	
Pheochromocytoma benign	9 (18%)	5 (10%)	7 (14%)	7 (14%)
Pheochromocytoma complex				1 (2%)
Pheochromocytoma malignant			3 (6%)	
Bilateral, pheochromocytoma benign	1 (2%)	1 (2%)		1 (2%)
Islets, pancreatic	(49)	(50)	(50)	(50)
Adenoma	5 (10%)	4 (8%)	3 (6%)	3 (6%)
Carcinoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Parathyroid gland	(46)	(44)	(43)	(47)
Adenoma	1 (2%)			1 (2%)
Pituitary gland	(50)	(50)	(48)	(49)
Pars distalis, adenoma	24 (48%)	14 (28%)	20 (42%)	24 (49%)
Pars distalis, carcinoma		1 (2%)		
Pars intermedia, carcinoma		1 (2%)	1 (2%)	
Thyroid gland	(49)	(47)	(49)	(46)
C-cell, adenoma	10 (20%)	5 (11%)	4 (8%)	9 (20%)
C-cell, carcinoma	1 (2%)	2 (4%)		
Follicular cell, carcinoma			1 (2%)	1 (2%)
General Body System				
Peritoneum	(2)	(6)	(0)	(2)
Tissue NOS	(1)	(0)	(2)	(1)
Genital System				
Coagulating gland	(1)	(0)	(1)	(0)
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(48)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Carcinoma		1 (2%)	2 (4%)	
Prostate	(49)	(50)	(50)	(50)
Seminal vesicle	(50)	(49)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	34 (68%)	38 (76%)	38 (76%)	40 (80%)
Interstitial cell, adenoma	15 (30%)	6 (12%)	4 (8%)	8 (16%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(26)	(24)	(19)	(21)
Mediastinal, fibrous histiocytoma, metastatic, skin	1 (4%)			
Lymph node, mandibular	(1)	(0)	(3)	(0)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Spleen	(49)	(50)	(50)	(50)
Thymus	(43)	(50)	(44)	(45)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Integumentary System				
Mammary gland	(46)	(49)	(47)	(46)
Carcinoma	1 (2%)		1 (2%)	
Fibroadenoma	6 (13%)	3 (6%)	3 (6%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma	2 (4%)		2 (4%)	2 (4%)
Basal cell carcinoma	2 (4%)	1 (2%)		1 (2%)
Keratoacanthoma	6 (12%)	6 (12%)	5 (10%)	5 (10%)
Keratoacanthoma, multiple		1 (2%)		
Squamous cell carcinoma		1 (2%)		1 (2%)
Squamous cell papilloma				1 (2%)
Trichoepithelioma			1 (2%)	
Pinna, neural crest tumor		1 (2%)	1 (2%)	
Pinna, squamous cell papilloma	1 (2%)			
Sebaceous gland, adenoma			1 (2%)	
Subcutaneous tissue, fibroma	7 (14%)	6 (12%)	1 (2%)	4 (8%)
Subcutaneous tissue, fibroma, multiple	1 (2%)		3 (6%)	2 (4%)
Subcutaneous tissue, fibrous histiocytoma	1 (2%)			
Subcutaneous tissue, lipoma	1 (2%)			
Subcutaneous tissue, osteosarcoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, chordoma			1 (2%)	
Skeletal muscle	(1)	(3)	(1)	(1)
Squamous cell carcinoma, metastatic, skin		1 (33%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland		2 (4%)		
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Peripheral nerve	(2)	(0)	(4)	(0)
Schwannoma malignant			1 (25%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	3 (6%)		
Alveolar/bronchiolar adenoma, multiple	1 (2%)			
Alveolar/bronchiolar carcinoma	1 (2%)	1 (2%)	1 (2%)	
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Chordoma, metastatic, uncertain primary site			1 (2%)	
Fibrous histiocytoma, metastatic, skin	1 (2%)			
Neural crest tumor, metastatic, skin		1 (2%)	1 (2%)	
Nose	(50)	(50)	(50)	(50)
Osteochrondroma		1 (2%)		
Squamous cell carcinoma, metastatic, oral mucosa				1 (2%)
Trachea	(50)	(50)	(50)	(50)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Special Senses System				
Eye	(48)	(47)	(48)	(47)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(2)	(0)
Carcinoma			2 (100%)	
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Urethra	(0)	(1)	(1)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	2 (4%)
Leukemia mononuclear	21 (42%)	19 (38%)	20 (40%)	20 (40%)
Lymphoma malignant		1 (2%)		
Mesothelioma malignant		6 (12%)		1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	50	49	50	49
Total primary neoplasms	157	135	142	152
Total animals with benign neoplasms	50	47	47	48
Total benign neoplasms	128	97	103	120
Total animals with malignant neoplasms	27	29	31	25
Total malignant neoplasms	29	37	38	32
Total animals with metastatic neoplasms	1	4	3	1
Total metastatic neoplasms	3	4	5	1
Total animals with malignant neoplasms of uncertain primary site			1	
Total animals with uncertain neoplasms - benign or malignant		1	1	
Total uncertain neoplasms		1	1	

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	10/50 (20%)	6/50 (12%)	7/49 (14%)	8/49 (16%)
Adjusted rate[b]	22.8%	13.5%	16.4%	17.9%
Terminal rate[c]	9/31 (29%)	5/33 (15%)	2/24 (8%)	8/35 (23%)
First incidence (days)	721	714	616	729 (T)
Poly-3 test[d]	P=0.430N	P=0.194N	P=0.316N	P=0.380N
Adrenal Medulla: Malignant Pheochromocytoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/49 (6%)	0/49 (0%)
Adjusted rate	0.0%	0.0%	7.2%	0.0%
Terminal rate	0/31 (0%)	0/33 (0%)	3/24 (13%)	0/35 (0%)
First incidence (days)	—[e]	—	729 (T)	—
Poly-3 test	P=0.561	—[f]	P=0.108	—
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	10/50 (20%)	6/50 (12%)	10/49 (20%)	9/49 (18%)
Adjusted rate	22.8%	13.5%	23.4%	20.1%
Terminal rate	9/31 (29%)	5/33 (15%)	5/24 (21%)	8/35 (23%)
First incidence (days)	721	714	616	714
Poly-3 test	P=0.524	P=0.194N	P=0.574	P=0.481N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	6.8%	6.7%	0.0%	0.0%
Terminal rate	2/31 (7%)	2/33 (6%)	0/25 (0%)	0/35 (0%)
First incidence (days)	610	710	—	—
Poly-3 test	P=0.033N	P=0.660N	P=0.126N	P=0.113N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	1/50 (2%)	0/50 (0%)
Adjusted rate	9.0%	9.0%	2.4%	0.0%
Terminal rate	3/31 (10%)	3/33 (9%)	0/25 (0%)	0/35 (0%)
First incidence (days)	610	710	674	—
Poly-3 test	P=0.020N	P=0.640N	P=0.190N	P=0.056N
Mammary Gland: Fibroadenoma				
Overall rate	6/50 (12%)	3/50 (6%)	3/50 (6%)	2/50 (4%)
Adjusted rate	13.6%	6.7%	7.1%	4.4%
Terminal rate	2/31 (7%)	2/33 (6%)	1/25 (4%)	2/35 (6%)
First incidence (days)	697	710	709	729 (T)
Poly-3 test	P=0.106N	P=0.237N	P=0.261N	P=0.122N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	6/50 (12%)	3/50 (6%)	4/50 (8%)	2/50 (4%)
Adjusted rate	13.6%	6.7%	9.3%	4.4%
Terminal rate	2/31 (7%)	2/33 (6%)	1/25 (4%)	2/35 (6%)
First incidence (days)	697	710	619	729 (T)
Poly-3 test	P=0.120N	P=0.237N	P=0.388N	P=0.122N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Oral Cavity (Oral Mucosa or Tongue): Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.4%	6.6%
Terminal rate	0/31 (0%)	0/33 (0%)	0/25 (0%)	2/35 (6%)
First incidence (days)	—	—	709	704
Poly-3 test	P=0.021	—	P=0.493	P=0.126
Oral Cavity (Oral Mucosa or Tongue): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	5/50 (10%)
Adjusted rate	0.0%	0.0%	4.7%	10.9%
Terminal rate	0/31 (0%)	0/33 (0%)	1/25 (4%)	3/35 (9%)
First incidence (days)	—	—	709	636
Poly-3 test	P=0.003	—	P=0.230	P=0.035
Pancreatic Islets: Adenoma				
Overall rate	5/49 (10%)	4/50 (8%)	3/50 (6%)	3/50 (6%)
Adjusted rate	11.5%	9.0%	7.0%	6.6%
Terminal rate	3/31 (10%)	4/33 (12%)	1/25 (4%)	1/35 (3%)
First incidence (days)	606	729 (T)	616	699
Poly-3 test	P=0.258N	P=0.485N	P=0.361N	P=0.328N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	6/49 (12%)	5/50 (10%)	4/50 (8%)	5/50 (10%)
Adjusted rate	13.8%	11.2%	9.3%	10.9%
Terminal rate	4/31 (13%)	5/33 (15%)	2/25 (8%)	3/35 (9%)
First incidence (days)	606	729 (T)	616	699
Poly-3 test	P=0.409N	P=0.483N	P=0.374N	P=0.463N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	24/50 (48%)	14/50 (28%)	20/48 (42%)	24/49 (49%)
Adjusted rate	51.6%	30.7%	45.1%	52.5%
Terminal rate	12/31 (39%)	10/33 (30%)	9/24 (38%)	18/35 (51%)
First incidence (days)	606	563	568	592
Poly-3 test	P=0.263	P=0.030N	P=0.339N	P=0.549
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	24/50 (48%)	15/50 (30%)	20/48 (42%)	24/49 (49%)
Adjusted rate	51.6%	32.4%	45.1%	52.5%
Terminal rate	12/31 (39%)	10/33 (30%)	9/24 (38%)	18/35 (51%)
First incidence (days)	606	496	568	592
Poly-3 test	P=0.279	P=0.044N	P=0.339N	P=0.549
Preputial Gland: Adenoma				
Overall rate	1/48 (2%)	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rate	2.4%	4.5%	7.0%	2.2%
Terminal rate	1/30 (3%)	2/33 (6%)	1/25 (4%)	1/35 (3%)
First incidence (days)	729 (T)	729 (T)	652	729 (T)
Poly-3 test	P=0.547N	P=0.519	P=0.311	P=0.743N
Preputial Gland: Adenoma or Carcinoma				
Overall rate	1/48 (2%)	3/50 (6%)	5/50 (10%)	1/50 (2%)
Adjusted rate	2.4%	6.7%	11.5%	2.2%
Terminal rate	1/30 (3%)	2/33 (6%)	2/25 (8%)	1/35 (3%)
First incidence (days)	729 (T)	714	568	729 (T)
Poly-3 test	P=0.503N	P=0.325	P=0.107	P=0.743N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Skin: Keratoacanthoma				
Overall rate	6/50 (12%)	7/50 (14%)	5/50 (10%)	5/50 (10%)
Adjusted rate	13.6%	15.4%	11.8%	10.9%
Terminal rate	5/31 (16%)	4/33 (12%)	3/25 (12%)	4/35 (11%)
First incidence (days)	710	563	709	636
Poly-3 test	P=0.346N	P=0.525	P=0.524N	P=0.469N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	7/50 (14%)	7/50 (14%)	5/50 (10%)	6/50 (12%)
Adjusted rate	15.9%	15.4%	11.8%	13.0%
Terminal rate	6/31 (19%)	4/33 (12%)	3/25 (12%)	5/35 (14%)
First incidence (days)	710	563	709	636
Poly-3 test	P=0.379N	P=0.589N	P=0.402N	P=0.466N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	7/50 (14%)	8/50 (16%)	5/50 (10%)	7/50 (14%)
Adjusted rate	15.9%	17.6%	11.8%	15.2%
Terminal rate	6/31 (19%)	5/33 (15%)	3/25 (12%)	6/35 (17%)
First incidence (days)	710	563	709	636
Poly-3 test	P=0.463N	P=0.527	P=0.402N	P=0.579N
Skin: Trichoepithelioma or Basal Cell Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted rate	4.6%	0.0%	7.0%	4.4%
Terminal rate	2/31 (7%)	0/33 (0%)	1/25 (4%)	2/35 (6%)
First incidence (days)	729 (T)	—	647	729 (T)
Poly-3 test	P=0.439	P=0.234N	P=0.488	P=0.680N
Skin: Trichoepithelioma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	3/50 (6%)	3/50 (6%)
Adjusted rate	9.1%	2.3%	7.0%	6.6%
Terminal rate	4/31 (13%)	1/33 (3%)	1/25 (4%)	2/35 (6%)
First incidence (days)	729 (T)	729 (T)	647	689
Poly-3 test	P=0.550N	P=0.174N	P=0.514N	P=0.477N
Skin: Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma				
Overall rate	11/50 (22%)	9/50 (18%)	8/50 (16%)	10/50 (20%)
Adjusted rate	25.0%	19.8%	18.6%	21.7%
Terminal rate	10/31 (32%)	6/33 (18%)	4/25 (16%)	8/35 (23%)
First incidence (days)	710	563	647	636
Poly-3 test	P=0.445N	P=0.369N	P=0.323N	P=0.450N
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	8/50 (16%)	6/50 (12%)	4/50 (8%)	6/50 (12%)
Adjusted rate	18.0%	13.3%	9.4%	13.0%
Terminal rate	6/31 (19%)	5/33 (15%)	3/25 (12%)	3/35 (9%)
First incidence (days)	619	580	619	681
Poly-3 test	P=0.314N	P=0.374N	P=0.192N	P=0.357N
Skin (Subcutaneous Tissue): Fibroma or Fibrous Histiocytoma				
Overall rate	9/50 (18%)	6/50 (12%)	4/50 (8%)	6/50 (12%)
Adjusted rate	20.2%	13.3%	9.4%	13.0%
Terminal rate	6/31 (19%)	5/33 (15%)	3/25 (12%)	3/35 (9%)
First incidence (days)	619	580	619	681
Poly-3 test	P=0.234N	P=0.279N	P=0.130N	P=0.264N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Testes: Adenoma				
Overall rate	49/50 (98%)	44/50 (88%)	42/50 (84%)	48/50 (96%)
Adjusted rate	98.3%	92%	91.8%	99.7%
Terminal rate	31/31 (100%)	31/33 (94%)	25/25 (100%)	35/35 (100%)
First incidence (days)	491	535	583	538
Poly-3 test	P=0.298	P=0.140N	P=0.109N	P=0.630
Thyroid Gland (C-Cell): Adenoma				
Overall rate	10/49 (20%)	5/47 (11%)	4/49 (8%)	9/46 (20%)
Adjusted rate	23.1%	11.6%	9.5%	21.3%
Terminal rate	9/31 (29%)	3/33 (9%)	2/25 (8%)	9/35 (26%)
First incidence (days)	652	563	568	729 (T)
Poly-3 test	P=0.521	P=0.127N	P=0.076N	P=0.522N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	11/49 (22%)	7/47 (15%)	4/49 (8%)	9/46 (20%)
Adjusted rate	25.4%	16.2%	9.5%	21.3%
Terminal rate	10/31 (32%)	5/33 (15%)	2/25 (8%)	9/35 (26%)
First incidence (days)	652	563	568	729 (T)
Poly-3 test	P=0.416N	P=0.214N	P=0.046N	P=0.422N
All Organs: Mononuclear Leukemia				
Overall rate	21/50 (42%)	19/50 (38%)	20/50 (40%)	20/50 (40%)
Adjusted rate	44.1%	40.5%	43.9%	41.4%
Terminal rate	9/31 (29%)	12/33 (36%)	5/25 (20%)	11/35 (31%)
First incidence (days)	491	461	494	383
Poly-3 test	P=0.474N	P=0.443N	P=0.575N	P=0.477N
All Organs: Malignant Mesothelioma				
Overall rate	0/50 (0%)	6/50 (12%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	13.3%	0.0%	2.2%
Terminal rate	0/31 (0%)	3/33 (9%)	0/25 (0%)	1/35 (3%)
First incidence (days)	—	594	—	729 (T)
Poly-3 test	P=0.351N	P=0.017	—	P=0.508
All Organs: Benign Neoplasms				
Overall rate	50/50 (100%)	47/50 (94%)	47/50 (94%)	48/50 (96%)
Adjusted rate	100.0%	96.8%	98.6%	99.7%
Terminal rate	31/31 (100%)	32/33 (97%)	25/25 (100%)	35/35 (100%)
First incidence (days)	491	535	568	538
Poly-3 test	P=0.590	P=0.291N	P=0.761N	P=1.000N
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	29/50 (58%)	31/50 (62%)	25/50 (50%)
Adjusted rate	56.3%	59.7%	64.2%	51.4%
Terminal rate	14/31 (45%)	16/33 (49%)	11/25 (44%)	14/35 (40%)
First incidence (days)	491	461	251	383
Poly-3 test	P=0.319N	P=0.448	P=0.280	P=0.389N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	49/50 (98%)	50/50 (100%)	49/50 (98%)
Adjusted rate	100%	98%	100%	100%
Terminal rate	31/31 (100%)	32/33 (97%)	25/25 (100%)	35/35 (100%)
First incidence (days)	491	461	251	383
Poly-3 test	P=0.568	P=0.500N	—	P=1.000N

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

Table A3
Historical Incidence of Oral Cavity Neoplasms in Control Male F344/N Rats[a]

	Incidence in Controls				
	Squamous Cell Papilloma		Squamous Cell Carcinoma		Papilloma, Squamous Cell Papilloma, or Squamous Cell Carcinoma
	Oral Mucosa	Tongue	Oral Mucosa	Tongue	All Sites[b]
Historical Incidence: Drinking Water Studies					
Bromochloroacetic acid	0/50	0/50	0/50	0/50	0/50
Bromodichloromethane	0/50	1/50	0/50	0/50	1/50
Dibromoacetic acid	0/50	0/50	0/50	0/50	0/50
Dibromoacetonitrile	0/50	0/50	0/50	0/50	0/50
Sodium chlorate	0/50	0/50	0/50	0/50	0/50
Sodium dichromate dihydrate	0/50	0/50	0/50	0/50	0/50
Total	0/300	1/300 (0.3%)	0/300	0/300	1/300 (0.3%)
Mean ± standard deviation		0.3% ± 0.8%			0.3% ± 0.8%
Range		0%-2%			0%-2%
Overall Historical Incidence: All Routes					
Total (%)	3/1,199 (0.1%)	4/1,199 (0.3%)	3/1,199 (0.3%)	0/1,199	8/1,199 (0.7%)
Mean ± standard deviation	0.1% ± 0.4%	0.3% ± 0.8%	0.3% ± 0.7%		0.7% ± 1.0%
Range	0%-2%	0%-2%	0%-2%		0%-2%

[a] Data as of October 4, 2007
[b] All sites includes the oral mucosa, tongue, pharynx, tooth, and gingiva.

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	12	11	22	10
Natural deaths	7	6	3	5
Survivors				
Terminal sacrifice	31	33	25	35
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(48)	(50)	(50)	(50)
Epithelium, hyperkeratosis	6 (13%)	8 (16%)	34 (68%)	46 (92%)
Intestine large, cecum	(48)	(49)	(50)	(47)
Inflammation, chronic	1 (2%)			
Ulcer			1 (2%)	
Intestine large, colon	(48)	(50)	(49)	(47)
Intestine large, rectum	(49)	(50)	(50)	(49)
Intestine small, duodenum	(49)	(49)	(50)	(48)
Congestion			1 (2%)	
Intestine small, ileum	(46)	(48)	(50)	(47)
Intestine small, jejunum	(47)	(46)	(49)	(47)
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal	1 (2%)	5 (10%)	3 (6%)	3 (6%)
Congestion		4 (8%)	2 (4%)	6 (12%)
Degeneration, cystic, focal	5 (10%)	5 (10%)	8 (16%)	4 (8%)
Fibrosis, focal	1 (2%)			
Hematopoietic cell proliferation	1 (2%)	3 (6%)		
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	5 (10%)	6 (12%)	3 (6%)	2 (4%)
Inflammation, chronic	28 (56%)	31 (62%)	29 (58%)	34 (68%)
Necrosis	3 (6%)			
Bile duct, hyperplasia	45 (90%)	47 (94%)	47 (94%)	46 (92%)
Hepatocyte, basophilic focus	26 (52%)	32 (64%)	23 (46%)	25 (50%)
Hepatocyte, clear cell focus	24 (48%)	22 (44%)	19 (38%)	22 (44%)
Hepatocyte, cytologic alterations, focal	1 (2%)	5 (10%)	2 (4%)	6 (12%)
Hepatocyte, cytoplasmic alteration, focal	2 (4%)			
Hepatocyte, eosinophilic focus	7 (14%)	8 (16%)	4 (8%)	7 (14%)
Hepatocyte, mixed cell focus	26 (52%)	24 (48%)	19 (38%)	29 (58%)
Hepatocyte, necrosis		8 (16%)	7 (14%)	4 (8%)
Hepatocyte, necrosis, focal	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	37 (74%)	37 (74%)	36 (72%)	29 (58%)
Serosa, fibrosis		1 (2%)		
Mesentery	(14)	(18)	(18)	(15)
Accessory spleen	1 (7%)	1 (6%)		
Fibrosis			1 (6%)	
Pigmentation			1 (6%)	
Fat, necrosis, focal	9 (64%)	10 (56%)	12 (67%)	7 (47%)
Oral mucosa	(50)	(50)	(50)	(50)

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Alimentary System (continued)				
Pancreas	(49)	(50)	(50)	(50)
Hyperplasia, lymphoid	1 (2%)			1 (2%)
Inflammation		2 (4%)		
Lipomatosis				1 (2%)
Vacuolization cytoplasmic			1 (2%)	
Acinus, atrophy, focal	24 (49%)	37 (74%)	33 (66%)	30 (60%)
Acinus, atrophy, diffuse				1 (2%)
Acinus, cytoplasmic alteration	1 (2%)			
Acinus, hyperplasia, focal		1 (2%)		1 (2%)
Duct, cyst	24 (49%)	21 (42%)	24 (48%)	16 (32%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal	1 (2%)			1 (2%)
Vacuolization cytoplasmic				1 (2%)
Stomach, forestomach	(50)	(49)	(50)	(50)
Inflammation			2 (4%)	
Ulcer	1 (2%)	1 (2%)	4 (8%)	3 (6%)
Epithelium, cyst				1 (2%)
Epithelium, hyperkeratosis				1 (2%)
Epithelium, hyperplasia	4 (8%)	2 (4%)	8 (16%)	7 (14%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst				1 (2%)
Erosion	5 (10%)	4 (8%)	5 (10%)	6 (12%)
Fibrosis			1 (2%)	1 (2%)
Inflammation				1 (2%)
Inflammation, focal	1 (2%)			
Ulcer	2 (4%)	2 (4%)	3 (6%)	3 (6%)
Epithelium, hyperplasia				1 (2%)
Glands, ectasia	4 (8%)	3 (6%)	3 (6%)	10 (20%)
Glands, hyperplasia			2 (4%)	2 (4%)
Tongue	(50)	(50)	(50)	(50)
Epithelium, hyperplasia			1 (2%)	2 (4%)
Tooth	(1)	(0)	(0)	(0)
Malformation	1 (100%)			
Cardiovascular System				
Blood vessel	(1)	(0)	(1)	(0)
Aneurysm	1 (100%)			
Hemorrhage	1 (100%)			
Inflammation, chronic			1 (100%)	
Thrombosis			1 (100%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	39 (78%)	44 (88%)	38 (76%)	45 (90%)
Thrombosis	2 (4%)		3 (6%)	1 (2%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Angiectasis		1 (2%)		3 (6%)
Cytoplasmic alteration, focal	3 (6%)		2 (4%)	3 (6%)
Hematopoietic cell proliferation	1 (2%)			
Hyperplasia, focal	5 (10%)	7 (14%)	1 (2%)	8 (16%)
Infiltration cellular, mixed cell		1 (2%)		1 (2%)
Necrosis		1 (2%)		
Vacuolization cytoplasmic	6 (12%)	3 (6%)	5 (10%)	11 (22%)
Capsule, inflammation, focal	1 (2%)			
Adrenal medulla	(50)	(50)	(49)	(49)
Hyperplasia	1 (2%)		1 (2%)	
Hyperplasia, focal	4 (8%)	9 (18%)	5 (10%)	4 (8%)
Necrosis		1 (2%)		
Islets, pancreatic	(49)	(50)	(50)	(50)
Hyperplasia	3 (6%)		2 (4%)	5 (10%)
Parathyroid gland	(46)	(44)	(43)	(47)
Hyperplasia			2 (5%)	2 (4%)
Pituitary gland	(50)	(50)	(48)	(49)
Angiectasis	7 (14%)	8 (16%)	3 (6%)	6 (12%)
Cyst	2 (4%)	3 (6%)	4 (8%)	2 (4%)
Hemorrhage	5 (10%)		2 (4%)	2 (4%)
Pars distalis, cyst		2 (4%)	3 (6%)	
Pars distalis, cytoplasmic alteration, focal	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Pars distalis, degeneration, cystic, focal	1 (2%)			
Pars distalis, hyperplasia			1 (2%)	1 (2%)
Pars distalis, hyperplasia, focal	6 (12%)	8 (16%)	2 (4%)	5 (10%)
Rathke's cleft, cyst		1 (2%)		
Thyroid gland	(49)	(47)	(49)	(46)
Cyst		1 (2%)		
C-cell, hyperplasia	38 (78%)	38 (81%)	45 (92%)	44 (96%)
Follicle, degeneration, cystic, focal	1 (2%)	1 (2%)	1 (2%)	
Follicle, pigmentation, focal				1 (2%)
Follicular cell, hyperplasia, focal	5 (10%)	6 (13%)		4 (9%)
General Body System				
Peritoneum	(2)	(6)	(0)	(2)
Inflammation				1 (50%)
Mesothelium, tunica vaginalis, hyperplasia	2 (100%)			
Tissue NOS	(1)	(0)	(2)	(1)
Abdominal, fibrosis			1 (50%)	1 (100%)
Thoracic, hemorrhage	1 (100%)			

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Genital System				
Coagulating gland	(1)	(0)	(1)	(0)
Inflammation	1 (100%)			
Epididymis	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Fibrosis		1 (2%)		
Granuloma sperm				1 (2%)
Inflammation	2 (4%)		1 (2%)	
Epithelium, vacuolization cytoplasmic	1 (2%)			1 (2%)
Preputial gland	(48)	(50)	(50)	(50)
Cyst			1 (2%)	
Degeneration, cystic	1 (2%)		2 (4%)	1 (2%)
Hyperplasia, cystic	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Inflammation	27 (56%)	27 (54%)	24 (48%)	22 (44%)
Prostate	(49)	(50)	(50)	(50)
Inflammation	36 (73%)	29 (58%)	34 (68%)	33 (66%)
Epithelium, hyperplasia	7 (14%)	15 (30%)	5 (10%)	10 (20%)
Seminal vesicle	(50)	(49)	(50)	(50)
Atrophy, focal		1 (2%)		
Epithelium, hyperplasia			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Atrophy	14 (28%)	10 (20%)	4 (8%)	6 (12%)
Cyst		1 (2%)		
Hemorrhage			1 (2%)	1 (2%)
Inflammation, granulomatous				1 (2%)
Interstitial cell, hyperplasia, focal	3 (6%)	4 (8%)	7 (14%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	2 (4%)	5 (10%)	4 (8%)	6 (12%)
Hyperplasia, histiocytic, focal		1 (2%)		1 (2%)
Lymph node	(26)	(24)	(19)	(21)
Ectasia				1 (5%)
Fibrosis				1 (5%)
Bronchial, hyperplasia, lymphoid				1 (5%)
Deep cervical, hemorrhage	1 (4%)	1 (4%)		
Mediastinal, ectasia	1 (4%)	1 (4%)	3 (16%)	1 (5%)
Mediastinal, hemorrhage	4 (15%)	2 (8%)	1 (5%)	4 (19%)
Mediastinal, hyperplasia, lymphoid		2 (8%)	4 (21%)	1 (5%)
Mediastinal, pigmentation		1 (4%)		
Pancreatic, ectasia		2 (8%)	1 (5%)	2 (10%)
Pancreatic, hemorrhage	1 (4%)	3 (13%)	1 (5%)	4 (19%)
Pancreatic, hyperplasia, lymphoid	1 (4%)	1 (4%)		
Pancreatic, pigmentation			1 (5%)	
Lymph node, mandibular	(1)	(0)	(3)	(0)
Ectasia	1 (100%)			

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Hematopoietic System (continued)				
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Ectasia	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Fibrosis	1 (2%)			
Hemorrhage		1 (2%)	1 (2%)	2 (4%)
Hyperplasia, histiocytic			1 (2%)	1 (2%)
Hyperplasia, lymphoid	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Spleen	(49)	(50)	(50)	(50)
Accessory spleen		1 (2%)	2 (4%)	
Congestion	1 (2%)		2 (4%)	
Fibrosis, focal	3 (6%)		3 (6%)	1 (2%)
Hematopoietic cell proliferation	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Hemorrhage			1 (2%)	1 (2%)
Hyperplasia, histiocytic, focal		1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, mixed cell			1 (2%)	
Necrosis, focal	1 (2%)		2 (4%)	
Pigmentation			1 (2%)	
Lymphoid follicle, atrophy			1 (2%)	1 (2%)
Red pulp, depletion cellular	1 (2%)			
Thymus	(43)	(50)	(44)	(45)
Atrophy	1 (2%)			
Ectopic parathyroid gland		1 (2%)		
Hemorrhage	1 (2%)			
Hyperplasia, lymphoid				1 (2%)
Integumentary System				
Mammary gland	(46)	(49)	(47)	(46)
Dilatation	13 (28%)	9 (18%)	14 (30%)	9 (20%)
Fibrosis	1 (2%)			
Inflammation, granulomatous, focal	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	2 (4%)	2 (4%)		1 (2%)
Hyperkeratosis, focal		2 (4%)		2 (4%)
Inflammation, focal		1 (2%)	1 (2%)	
Ulcer				1 (2%)
Epidermis, hyperplasia, focal		1 (2%)	2 (4%)	1 (2%)
Subcutaneous tissue, edema				1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrous osteodystrophy				1 (2%)
Hyperostosis		1 (2%)		
Femur, hyperostosis			1 (2%)	
Skeletal muscle	(1)	(3)	(1)	(1)
Fibrosis			1 (100%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	5 (10%)	8 (16%)	12 (24%)	9 (18%)
Hemorrhage			1 (2%)	
Hemorrhage, focal	4 (8%)	2 (4%)	3 (6%)	2 (4%)
Peripheral nerve	(2)	(0)	(4)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Hemorrhage			1 (2%)	1 (2%)
Infiltration cellular, mixed cell		2 (4%)		
Inflammation	2 (4%)	2 (4%)		2 (4%)
Metaplasia, osseous, focal	1 (2%)			
Metaplasia, squamous, focal			1 (2%)	
Alveolar epithelium, hyperplasia	12 (24%)	10 (20%)	8 (16%)	6 (12%)
Serosa, fibrosis				1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Inflammation			1 (2%)	2 (4%)
Nasolacrimal duct, inflammation	2 (4%)			
Trachea	(50)	(50)	(50)	(50)
Inflammation				1 (2%)
Special Senses System				
Eye	(48)	(47)	(48)	(47)
Atrophy	1 (2%)		1 (2%)	
Cataract			1 (2%)	
Edema			1 (2%)	
Retina, degeneration			1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, lymphoid, focal	1 (2%)			
Inflammation		1 (2%)		
Epithelium, hyperplasia, focal	3 (6%)	3 (6%)	1 (2%)	6 (12%)
Zymbal's gland	(0)	(0)	(2)	(0)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Infarct	2 (4%)	1 (2%)	2 (4%)	
Mineralization			1 (2%)	
Nephropathy	48 (98%)	47 (94%)	45 (90%)	45 (90%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	7 (14%)	1 (2%)	1 (2%)
Renal tubule, cyst	2 (4%)			1 (2%)
Renal tubule, pigmentation	2 (4%)	2 (4%)		2 (4%)
Urethra	(0)	(1)	(1)	(0)
Urinary bladder	(50)	(50)	(49)	(50)
Hemorrhage	1 (2%)		1 (2%)	

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

Table B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile 84

Table B2 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile 88

Table B3 Historical Incidence of Papillomas of the Oral Cavity in Control Female F344/N Rats . . . 91

Table B4 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile 92

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	11	16	12
Natural deaths	12	4	5	7
Survivors				
Terminal sacrifice	29	35	29	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Intestine large, cecum	(46)	(50)	(47)	(50)
Intestine large, colon	(48)	(50)	(49)	(50)
Adenoma				1 (2%)
Intestine large, rectum	(49)	(49)	(48)	(49)
Adenoma		1 (2%)		
Carcinoma	1 (2%)			
Schwannoma malignant, metastatic, uterus	1 (2%)			
Intestine small, duodenum	(49)	(50)	(50)	(49)
Intestine small, ileum	(45)	(48)	(49)	(49)
Intestine small, jejunum	(42)	(48)	(48)	(46)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			1 (2%)	
Mesentery	(20)	(11)	(17)	(15)
Schwannoma malignant, metastatic, uterus	1 (5%)			
Oral mucosa	(50)	(50)	(50)	(50)
Squamous cell papilloma			1 (2%)	
Pancreas	(50)	(50)	(50)	(50)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(49)	(50)	(50)	(50)
Tongue	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		3 (6%)	
Tooth	(1)	(0)	(0)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma benign	1 (2%)			1 (2%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma				2 (4%)
Adrenal medulla	(49)	(50)	(50)	(50)
Ganglioneuroma			1 (2%)	
Pheochromocytoma benign	1 (2%)	2 (4%)	1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	1 (2%)	1 (2%)		2 (4%)
Parathyroid gland	(42)	(42)	(43)	(43)
Pituitary gland	(49)	(48)	(50)	(50)
Pars distalis, adenoma	27 (55%)	31 (65%)	29 (58%)	22 (44%)
Pars distalis, carcinoma			1 (2%)	
Thyroid gland	(46)	(48)	(48)	(46)
Bilateral, C-cell, adenoma	2 (4%)			
C-cell, adenoma	4 (9%)	6 (13%)	6 (13%)	5 (11%)
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma	1 (2%)		2 (4%)	
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(47)	(50)
Adenoma	4 (8%)	1 (2%)	1 (2%)	3 (6%)
Carcinoma	1 (2%)	5 (10%)	4 (9%)	3 (6%)
Bilateral, adenoma	1 (2%)	1 (2%)		
Ovary	(50)	(50)	(50)	(50)
Granulosa-theca tumor benign	1 (2%)			
Oviduct	(1)	(0)	(1)	(0)
Uterus	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			
Schwannoma malignant			1 (2%)	
Endometrium, adenoma		1 (2%)		1 (2%)
Endometrium, polyp stromal	12 (24%)	10 (20%)	4 (8%)	11 (22%)
Endometrium, polyp stromal, multiple	1 (2%)			
Endometrium, schwannoma malignant	1 (2%)			
Vagina	(6)	(2)	(7)	(7)
Polyp		1 (50%)		
Schwannoma malignant, metastatic, uterus	1 (17%)		1 (14%)	
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Lymph node	(21)	(15)	(23)	(15)
Deep cervical, carcinoma, metastatic, thyroid gland		1 (7%)		
Lymph node, mandibular	(3)	(2)	(2)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Thymus	(48)	(49)	(48)	(49)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Adenoma			1 (2%)	
Carcinoma	2 (4%)	5 (10%)	3 (6%)	3 (6%)
Carcinoma, multiple			2 (4%)	
Fibroadenoma	14 (29%)	19 (38%)	21 (42%)	19 (38%)
Fibroadenoma, multiple	17 (35%)	17 (34%)	10 (20%)	6 (12%)
Skin	(50)	(50)	(50)	(49)
Basal cell adenoma			1 (2%)	1 (2%)
Basal cell carcinoma			1 (2%)	
Keratoacanthoma				1 (2%)
Squamous cell papilloma			1 (2%)	2 (4%)
Subcutaneous tissue, fibroma			3 (6%)	
Subcutaneous tissue, fibroma, multiple			1 (2%)	
Subcutaneous tissue, fibrosarcoma	1 (2%)			
Subcutaneous tissue, lipoma			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Vertebra, chondrosarcoma	1 (2%)			
Skeletal muscle	(3)	(0)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland			1 (2%)	
Glioma malignant		1 (2%)		
Spinal cord	(5)	(3)	(2)	(4)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	1 (2%)		1 (2%)	
Alveolar/bronchiolar adenoma, multiple				1 (2%)
Alveolar/bronchiolar carcinoma				1 (2%)
Nose	(50)	(50)	(50)	(50)
Respiratory epithelium, adenoma				1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(47)	(50)	(50)	(48)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(50)	(49)	(50)	(49)
Urinary bladder	(50)	(50)	(49)	(50)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		2 (4%)		
Leukemia mononuclear	14 (28%)	9 (18%)	10 (20%)	12 (24%)
Lymphoma malignant	1 (2%)	1 (2%)		
Neoplasm Summary				
Total animals with primary neoplasms[c]	48	49	49	47
Total primary neoplasms	112	115	112	99
Total animals with benign neoplasms	44	48	49	43
Total benign neoplasms	89	91	89	79
Total animals with malignant neoplasms	20	20	21	18
Total malignant neoplasms	23	24	23	20
Total animals with metastatic neoplasms	1	1	2	
Total metastatic neoplasms	3	1	2	

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Clitoral Gland: Adenoma				
Overall rate[a]	5/50 (10%)	2/50 (4%)	1/47 (2%)	3/50 (6%)
Adjusted rate[b]	11.6%	4.4%	2.3%	6.7%
Terminal rate[c]	4/29 (14%)	2/35 (6%)	0/29 (0%)	2/31 (7%)
First incidence (days)	722	729 (T)	566	714
Poly-3 test[d]	P=0.321N	P=0.191N	P=0.101N	P=0.334N
Clitoral Gland: Carcinoma				
Overall rate	1/50 (2%)	5/50 (10%)	4/47 (9%)	3/50 (6%)
Adjusted rate	2.3%	10.9%	9.4%	6.7%
Terminal rate	0/29 (0%)	5/35 (14%)	2/29 (7%)	2/31 (7%)
First incidence (days)	656	729 (T)	596	702
Poly-3 test	P=0.423	P=0.114	P=0.175	P=0.318
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	6/50 (12%)	7/50 (14%)	5/47 (11%)	6/50 (12%)
Adjusted rate	13.9%	15.3%	11.6%	13.4%
Terminal rate	4/29 (14%)	7/35 (20%)	2/29 (7%)	4/31 (13%)
First incidence (days)	656	729 (T)	566	702
Poly-3 test	P=0.486N	P=0.543	P=0.498N	P=0.596N
Mammary Gland: Fibroadenoma				
Overall rate	31/50 (62%)	36/50 (72%)	31/50 (62%)[e]	25/50 (50%)
Adjusted rate	68.9%	76.2%	65.2%	53.3%
Terminal rate	22/29 (76%)	28/35 (80%)	18/29 (62%)	12/31 (39%)
First incidence (days)	535	600	594	596
Poly-3 test	P=0.021N	P=0.279	P=0.434N	P=0.086N
Mammary Gland: Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	5/50 (10%)	3/50 (6%)
Adjusted rate	4.6%	10.8%	11.0%	6.7%
Terminal rate	1/29 (3%)	3/35 (9%)	3/29 (10%)	1/31 (3%)
First incidence (days)	535	600	596	657
Poly-3 test	P=0.537	P=0.241	P=0.234	P=0.515
Mammary Gland: Adenoma or Carcinoma				
Overall rate	2/50 (4%)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted rate	4.6%	10.8%	13.2%	6.7%
Terminal rate	1/29 (3%)	3/35 (9%)	4/29 (14%)	1/31 (3%)
First incidence (days)	535	600	596	657
Poly-3 test	P=0.520	P=0.241	P=0.147	P=0.515
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	31/50 (62%)	36/50 (72%)	33/50 (66%)	26/50 (52%)
Adjusted rate	68.9%	76.2%	68.7%	55.4%
Terminal rate	22/29 (76%)	28/35 (80%)	19/29 (66%)	13/31 (42%)
First incidence (days)	535	600	594	596
Poly-3 test	P=0.040N	P=0.279	P=0.584N	P=0.124N
Oral Cavity (Oral Mucosa or Tongue): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	8.8%	0.0%
Terminal rate	1/29 (3%)	0/35 (0%)	2/29 (7%)	0/31 (0%)
First incidence (days)	729 (T)	—[f]	596	—
Poly-3 test	P=0.526N	P=0.487N	P=0.197	P=0.492N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	27/49 (55%)	31/48 (65%)	29/50 (58%)	22/50 (44%)
Adjusted rate	59.4%	65.7%	61.1%	47.0%
Terminal rate	16/29 (55%)	21/35 (60%)	16/29 (55%)	14/31 (45%)
First incidence (days)	535	561	594	590
Poly-3 test	P=0.074N	P=0.338	P=0.520	P=0.159N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	27/49 (55%)	31/48 (65%)	30/50 (60%)	22/50 (44%)
Adjusted rate	59.4%	65.7%	63.2%	47.0%
Terminal rate	16/29 (55%)	21/35 (60%)	17/29 (59%)	14/31 (45%)
First incidence (days)	535	561	594	590
Poly-3 test	P=0.077N	P=0.338	P=0.436	P=0.159N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.2%	6.7%
Terminal rate	0/29 (0%)	0/35 (0%)	1/29 (3%)	2/31 (7%)
First incidence (days)	—	—	729 (T)	672
Poly-3 test	P=0.020	—[g]	P=0.510	P=0.126
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Adjusted rate	0.0%	0.0%	6.5%	8.9%
Terminal rate	0/29 (0%)	0/35 (0%)	1/29 (3%)	2/31 (7%)
First incidence (days)	—	—	520	672
Poly-3 test	P=0.011	—	P=0.131	P=0.066
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	0.0%	8.8%	0.0%
Terminal rate	0/29 (0%)	0/35 (0%)	3/29 (10%)	0/31 (0%)
First incidence (days)	—	—	672	—
Poly-3 test	P=0.512	—	P=0.067	—
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma				
Overall rate	1/50 (2%)	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.3%	0.0%	8.8%	0.0%
Terminal rate	0/29 (0%)	0/35 (0%)	3/29 (10%)	0/31 (0%)
First incidence (days)	656	—	672	—
Poly-3 test	P=0.527N	P=0.489N	P=0.193	P=0.494N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	6/46 (13%)	6/48 (13%)	6/48 (13%)	5/46 (11%)
Adjusted rate	14.9%	13.3%	13.8%	11.6%
Terminal rate	6/29 (21%)	4/35 (11%)	3/29 (10%)	3/31 (10%)
First incidence (days)	729 (T)	596	664	590
Poly-3 test	P=0.397N	P=0.538N	P=0.564N	P=0.450N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	6/46 (13%)	7/48 (15%)	6/48 (13%)	5/46 (11%)
Adjusted rate	14.9%	15.5%	13.8%	11.6%
Terminal rate	6/29 (21%)	5/35 (14%)	3/29 (10%)	3/31 (10%)
First incidence (days)	729 (T)	596	664	590
Poly-3 test	P=0.352N	P=0.589	P=0.564N	P=0.450N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Uterus: Stromal Polyp				
Overall rate	13/50 (26%)	10/50 (20%)	4/50 (8%)	11/50 (22%)
Adjusted rate	29.0%	21.7%	8.9%	24.2%
Terminal rate	7/29 (24%)	8/35 (23%)	3/29 (10%)	9/31 (29%)
First incidence (days)	550	600	701	477
Poly-3 test	P=0.332N	P=0.286N	P=0.013N	P=0.393N
All Organs: Mononuclear Leukemia				
Overall rate	14/50 (28%)	9/50 (18%)	10/50 (20%)	12/50 (24%)
Adjusted rate	30.3%	19.4%	21.1%	25.6%
Terminal rate	7/29 (24%)	6/35 (17%)	3/29 (10%)	6/31 (19%)
First incidence (days)	400	596	520	380
Poly-3 test	P=0.449N	P=0.163N	P=0.217N	P=0.391N
All Organs: Benign Neoplasms				
Overall rate	44/50 (88%)	48/50 (96%)	49/50 (98%)	43/50 (86%)
Adjusted rate	91.9%	96.4%	98.0%	88.5%
Terminal rate	27/29 (93%)	34/35 (97%)	28/29 (97%)	26/31 (84%)
First incidence (days)	535	561	520	477
Poly-3 test	P=0.221N	P=0.282	P=0.155	P=0.413N
All Organs: Malignant Neoplasms				
Overall rate	20/50 (40%)	20/50 (40%)	21/50 (42%)	18/50 (36%)
Adjusted rate	42.4%	42.4%	42.9%	38.1%
Terminal rate	10/29 (35%)	14/35 (40%)	8/29 (28%)	10/31 (32%)
First incidence (days)	400	596	520	380
Poly-3 test	P=0.362N	P=0.582N	P=0.562	P=0.412N
All Organs: Benign or Malignant Neoplasms				
Overall rate	48/50 (96%)	49/50 (98%)	49/50 (98%)	47/50 (94%)
Adjusted rate	96.0%	98.0%	98.0%	94.0%
Terminal rate	27/29 (93%)	34/35 (97%)	28/29 (97%)	28/31 (90%)
First incidence (days)	400	561	520	380
Poly-3 test	P=0.325N	P=0.500	P=0.500	P=0.500N

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

Table B3
Historical Incidence of Papillomas of the Oral Cavity in Control Female F344/N Rats[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma		Papilloma or Squamous Cell Papilloma
	Oral Mucosa	Tongue	All Sites[b]
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	1/50	0/50	1/50
Dibromoacetic acid	0/50	0/50	0/50
Dibromoacetonitrile	0/50	1/50	1/50
Sodium chlorate	0/50	0/50	0/50
Sodium dichromate dihydrate	0/50	1/50	0/50
Total	1/250 (0.4%)	2/250 (0.8%)	3/250 (1.2%)
Mean ± standard deviation	0.4% ± 0.9%	0.8% ± 1.1%	1.2% ± 1.1%
Range	0%-2%	0%-2%	0%-2%
Overall Historical Incidence: All Routes			
Total (%)	1/1,100 (0.1%)	5/1,100 (0.5%)	6/1,100 (0.6%)
Mean ± standard deviation	0.1% ± 0.4%	0.5% ± 1.1%	0.6% ± 1.1%
Range	0%-2%	0%-4%	0%-4%

[a] Data as of October 4, 2007
[b] All sites includes the oral mucosa, tongue, pharynx, tooth, and gingiva.

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	9	11	16	12
Natural deaths	12	4	5	7
Survivors				
Terminal sacrifice	29	35	29	31
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(49)	(50)	(50)	(50)
Ulcer				1 (2%)
Epithelium, hyperkeratosis	10 (20%)	8 (16%)	28 (56%)	48 (96%)
Intestine large, cecum	(46)	(50)	(47)	(50)
Intestine large, colon	(48)	(50)	(49)	(50)
Intestine large, rectum	(49)	(49)	(48)	(49)
Intestine small, duodenum	(49)	(50)	(50)	(49)
Intestine small, ileum	(45)	(48)	(49)	(49)
Intestine small, jejunum	(42)	(48)	(48)	(46)
Liver	(50)	(50)	(50)	(50)
Angiectasis, focal	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Congestion	3 (6%)	5 (10%)	4 (8%)	6 (12%)
Degeneration, cystic, focal	1 (2%)		1 (2%)	
Hematopoietic cell proliferation			3 (6%)	1 (2%)
Hepatodiaphragmatic nodule	7 (14%)	5 (10%)	6 (12%)	3 (6%)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation, chronic	35 (70%)	41 (82%)	30 (60%)	34 (68%)
Thrombosis	1 (2%)			
Bile duct, hyperplasia	29 (58%)	29 (58%)	32 (64%)	29 (58%)
Hepatocyte, basophilic focus	39 (78%)	47 (94%)	41 (82%)	41 (82%)
Hepatocyte, clear cell focus	11 (22%)	15 (30%)	14 (28%)	14 (28%)
Hepatocyte, cytologic alterations, focal	6 (12%)	10 (20%)	16 (32%)	16 (32%)
Hepatocyte, eosinophilic focus	2 (4%)	3 (6%)	6 (12%)	1 (2%)
Hepatocyte, mixed cell focus	14 (28%)	25 (50%)	15 (30%)	25 (50%)
Hepatocyte, necrosis	2 (4%)	1 (2%)	6 (12%)	3 (6%)
Hepatocyte, syncytial alteration, focal	1 (2%)			
Hepatocyte, vacuolization cytoplasmic	22 (44%)	27 (54%)	29 (58%)	23 (46%)
Mesentery	(20)	(11)	(17)	(15)
Accessory spleen		1 (9%)		1 (7%)
Fat, necrosis, focal	14 (70%)	8 (73%)	10 (59%)	11 (73%)
Oral mucosa	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		2 (4%)
Pancreas	(50)	(50)	(50)	(50)
Acinus, atrophy, focal	12 (24%)	20 (40%)	19 (38%)	17 (34%)
Acinus, atrophy, diffuse		1 (2%)		
Acinus, cytoplasmic alteration				1 (2%)
Acinus, hyperplasia	1 (2%)			
Acinus, hyperplasia, focal	1 (2%)			
Duct, cyst	8 (16%)	13 (26%)	7 (14%)	4 (8%)

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Atrophy, focal	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Erosion			1 (2%)	1 (2%)
Inflammation		1 (2%)	1 (2%)	
Ulcer	5 (10%)		1 (2%)	2 (4%)
Epithelium, hyperplasia	4 (8%)	4 (8%)	8 (16%)	4 (8%)
Stomach, glandular	(49)	(50)	(50)	(50)
Erosion	4 (8%)		4 (8%)	1 (2%)
Inflammation, focal				1 (2%)
Mineralization		1 (2%)		
Ulcer			2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)			
Glands, ectasia	10 (20%)	4 (8%)	10 (20%)	26 (52%)
Glands, hyperplasia				2 (4%)
Neuroendocrine cell, hyperplasia				1 (2%)
Tongue	(50)	(50)	(50)	(50)
Epithelium, hyperplasia	1 (2%)	1 (2%)	2 (4%)	6 (12%)
Tooth	(1)	(0)	(0)	(0)
Peridontal tissue, inflammation	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	34 (68%)	36 (72%)	36 (72%)	35 (70%)
Thrombosis	3 (6%)		2 (4%)	1 (2%)
Endocardium, hyperplasia		1 (2%)		
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	6 (12%)	1 (2%)	1 (2%)	2 (4%)
Angiectasis	5 (10%)	1 (2%)	3 (6%)	1 (2%)
Cytoplasmic alteration, focal	2 (4%)		4 (8%)	1 (2%)
Hyperplasia	1 (2%)			
Hyperplasia, focal	2 (4%)	9 (18%)	10 (20%)	8 (16%)
Infiltration cellular, mixed cell			1 (2%)	2 (4%)
Necrosis				1 (2%)
Pigmentation	1 (2%)			
Vacuolization cytoplasmic	9 (18%)	8 (16%)	9 (18%)	6 (12%)
Adrenal medulla	(49)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Hyperplasia, focal	2 (4%)	2 (4%)	1 (2%)	5 (10%)
Infiltration cellular, mixed cell				1 (2%)
Vacuolization cytoplasmic, focal			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia		1 (2%)	1 (2%)	
Parathyroid gland	(42)	(42)	(43)	(43)
Atrophy	1 (2%)			
Hyperplasia	1 (2%)			

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System (continued)				
Pituitary gland	(49)	(48)	(50)	(50)
Angiectasis	6 (12%)	3 (6%)	5 (10%)	7 (14%)
Cyst	5 (10%)	3 (6%)	2 (4%)	4 (8%)
Fibrosis, focal				1 (2%)
Hemorrhage	6 (12%)	4 (8%)		2 (4%)
Hyperplasia, histiocytic, focal	1 (2%)			
Pars distalis, cyst	4 (8%)	6 (13%)	5 (10%)	12 (24%)
Pars distalis, cytoplasmic alteration, focal	1 (2%)	2 (4%)		2 (4%)
Pars distalis, hyperplasia				2 (4%)
Pars distalis, hyperplasia, focal	8 (16%)	7 (15%)	9 (18%)	10 (20%)
Pars intermedia, hyperplasia, focal	1 (2%)			
Pars intermedia, vacuolization cytoplasmic, focal			1 (2%)	
Rathke's cleft, cyst	1 (2%)		1 (2%)	1 (2%)
Thyroid gland	(46)	(48)	(48)	(46)
C-cell, hyperplasia	38 (83%)	44 (92%)	45 (94%)	42 (91%)
General Body System				
None				
Genital System				
Clitoral gland	(50)	(50)	(47)	(50)
Cyst				1 (2%)
Degeneration, cystic	2 (4%)	2 (4%)	4 (9%)	6 (12%)
Hyperplasia		1 (2%)		
Hyperplasia, cystic	4 (8%)	3 (6%)	8 (17%)	4 (8%)
Inflammation	7 (14%)	2 (4%)	1 (2%)	3 (6%)
Metaplasia, squamous			1 (2%)	
Ovary	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Cyst	3 (6%)	4 (8%)	3 (6%)	6 (12%)
Oviduct	(1)	(0)	(1)	(0)
Uterus	(50)	(50)	(50)	(50)
Decidual reaction		1 (2%)		
Hemorrhage	1 (2%)	2 (4%)		1 (2%)
Cervix, hypertrophy	1 (2%)			
Endometrium, hyperplasia, cystic	32 (64%)	38 (76%)	39 (78%)	32 (64%)
Vagina	(6)	(2)	(7)	(7)
Inflammation		1 (50%)	1 (14%)	2 (29%)
Inflammation, suppurative	1 (17%)			
Epithelium, cyst			1 (14%)	1 (14%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Hematopoietic System				
Bone marrow	(49)	(50)	(50)	(50)
Hyperplasia	3 (6%)	1 (2%)	5 (10%)	2 (4%)
Hyperplasia, histiocytic, focal	1 (2%)	1 (2%)		1 (2%)
Hyperplasia, reticulum cell, diffuse		1 (2%)		
Inflammation, granulomatous		1 (2%)		
Lymph node	(21)	(15)	(23)	(15)
Congestion			1 (4%)	
Deep cervical, hemorrhage	1 (5%)		1 (4%)	
Mediastinal, ectasia	1 (5%)		4 (17%)	
Mediastinal, hemorrhage	7 (33%)	6 (40%)	6 (26%)	3 (20%)
Mediastinal, hyperplasia, histiocytic			1 (4%)	
Mediastinal, hyperplasia, lymphoid	1 (5%)	2 (13%)	4 (17%)	2 (13%)
Mediastinal, inflammation, granulomatous	1 (5%)			
Mediastinal, pigmentation		1 (7%)		1 (7%)
Pancreatic, amyloid deposition			1 (4%)	
Pancreatic, ectasia	1 (5%)			
Pancreatic, hemorrhage	3 (14%)	2 (13%)	2 (9%)	2 (13%)
Pancreatic, inflammation, granulomatous	1 (5%)			
Lymph node, mandibular	(3)	(2)	(2)	(2)
Ectasia	1 (33%)			
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	1 (2%)		2 (4%)	1 (2%)
Hemorrhage			1 (2%)	
Hyperplasia, lymphoid	2 (4%)	1 (2%)		2 (4%)
Inflammation, granulomatous	1 (2%)	1 (2%)		
Pigmentation	1 (2%)			
Spleen	(50)	(50)	(50)	(50)
Hematopoietic cell proliferation	4 (8%)	2 (4%)	4 (8%)	5 (10%)
Hyperplasia, histiocytic, focal	2 (4%)			
Inflammation, granulomatous	1 (2%)	1 (2%)		
Pigmentation	6 (12%)			1 (2%)
Lymphoid follicle, atrophy				1 (2%)
Thymus	(48)	(49)	(48)	(49)
Hemorrhage				1 (2%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)		
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Dilatation	38 (78%)	41 (82%)	33 (66%)	32 (64%)
Fibrosis		1 (2%)	2 (4%)	1 (2%)
Galactocele	2 (4%)			1 (2%)
Skin	(50)	(50)	(50)	(49)
Cyst epithelial inclusion			1 (2%)	
Ulcer	1 (2%)	1 (2%)		1 (2%)
Subcutaneous tissue, fibrosis, focal	1 (2%)			
Subcutaneous tissue, necrosis, fatty			1 (2%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis				1 (2%)
Cranium, hyperostosis			1 (2%)	
Skeletal muscle	(3)	(0)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression, focal	12 (24%)	12 (24%)	19 (38%)	6 (12%)
Hemorrhage, focal	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Spinal cord	(5)	(3)	(2)	(4)
Atrophy, focal	1 (20%)			
Hemorrhage, focal				1 (25%)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Hemorrhage	1 (2%)		1 (2%)	2 (4%)
Hyperplasia, histiocytic, focal		1 (2%)		
Inflammation	3 (6%)	1 (2%)	3 (6%)	7 (14%)
Metaplasia, squamous, focal	1 (2%)			
Alveolar epithelium, hyperplasia	10 (20%)	5 (10%)	2 (4%)	5 (10%)
Interstitium, edema	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Nasolacrimal duct, inflammation		1 (2%)		1 (2%)
Trachea	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(47)	(50)	(50)	(48)
Atrophy		2 (4%)	1 (2%)	3 (6%)
Cataract	3 (6%)	2 (4%)		
Exudate	1 (2%)			
Retina, degeneration	2 (4%)	2 (4%)		
Harderian gland	(50)	(50)	(50)	(50)
Degeneration, cystic, focal				1 (2%)
Hyperplasia, lymphoid, focal	1 (2%)			
Inflammation			1 (2%)	
Epithelium, hyperplasia, focal	4 (8%)		1 (2%)	1 (2%)
Zymbal's gland	(0)	(0)	(1)	(0)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Urinary System				
Kidney	(50)	(49)	(50)	(49)
Congestion				1 (2%)
Infarct	1 (2%)	1 (2%)		4 (8%)
Infiltration cellular, lymphocyte			1 (2%)	
Mineralization		1 (2%)		
Nephropathy	33 (66%)	36 (73%)	44 (88%)	43 (88%)
Pelvis, inflammation, chronic	1 (2%)			7 (14%)
Renal tubule, accumulation, hyaline droplet	11 (22%)	18 (37%)	17 (34%)	14 (29%)
Renal tubule, cyst	1 (2%)	1 (2%)		1 (2%)
Renal tubule, hyperplasia, focal			1 (2%)	
Renal tubule, pigmentation	1 (2%)			
Urinary bladder	(50)	(50)	(49)	(50)

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	4	5	2	3
Natural deaths	6	5	13	4
Survivors				
Terminal sacrifice	40	40	35	42
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(48)	(48)	(50)
Leiomyoma			1 (2%)	
Intestine large, colon	(50)	(50)	(50)	(50)
Intestine small, duodenum	(47)	(48)	(47)	(49)
Adenoma	1 (2%)			
Carcinoma		1 (2%)	2 (4%)	
Intestine small, ileum	(47)	(50)	(48)	(49)
Carcinoma			1 (2%)	
Intestine small, jejunum	(47)	(49)	(48)	(49)
Adenoma			1 (2%)	
Carcinoma	1 (2%)	1 (2%)	1 (2%)	
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, salivary glands	1 (2%)			
Hemangioma	1 (2%)			1 (2%)
Hemangiosarcoma		1 (2%)		3 (6%)
Hepatoblastoma	1 (2%)	7 (14%)	3 (6%)	2 (4%)
Hepatocellular adenoma	15 (30%)	20 (40%)	18 (36%)	12 (24%)
Hepatocellular adenoma, multiple	14 (28%)	18 (36%)	17 (34%)	15 (30%)
Hepatocellular carcinoma	18 (36%)	17 (34%)	14 (28%)	19 (38%)
Hepatocellular carcinoma, multiple	6 (12%)	7 (14%)	7 (14%)	10 (20%)
Ito cell tumor malignant	2 (4%)			
Mesentery	(12)	(12)	(5)	(2)
Pancreas	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell carcinoma				2 (4%)
Squamous cell papilloma				3 (6%)
Squamous cell papilloma, multiple		1 (2%)		
Stomach, glandular	(50)	(49)	(50)	(50)
Mast cell tumor malignant				1 (2%)
Tooth	(3)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(0)
Heart	(50)	(50)	(50)	(50)
Carcinoma, metastatic, salivary glands	1 (2%)			
Hepatocellular carcinoma, metastatic, liver			1 (2%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Subcapsular, adenoma	6 (12%)	2 (4%)	1 (2%)	3 (6%)
Subcapsular, carcinoma		1 (2%)		
Adrenal medulla	(49)	(49)	(50)	(50)
Neuroblastoma			1 (2%)	
Pheochromocytoma benign	1 (2%)		1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)		1 (2%)	
Parathyroid gland	(35)	(28)	(37)	(26)
Pituitary gland	(49)	(50)	(50)	(50)
Pars intermedia, adenoma				1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma	2 (4%)	4 (8%)	1 (2%)	2 (4%)
Follicular cell, adenoma, multiple	1 (2%)		1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(49)	(50)	(50)	(50)
Adenoma	1 (2%)			
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Hemangiosarcoma				1 (2%)
Interstitial cell, adenoma	1 (2%)		1 (2%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Mast cell tumor malignant				1 (2%)
Lymph node	(0)	(2)	(1)	(0)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung			1 (100%)	
Lymph node, mandibular	(46)	(49)	(45)	(45)
Lymph node, mesenteric	(48)	(48)	(47)	(50)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)			1 (2%)
Mast cell tumor malignant				1 (2%)
Thymus	(44)	(47)	(43)	(47)
Alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)	
Hepatocellular carcinoma, metastatic, liver			1 (2%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Carcinoma, metastatic, salivary glands	1 (2%)			
Sebaceous gland, adenoma				1 (2%)
Subcutaneous tissue, hemangiosarcoma		1 (2%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(0)	(0)	(2)	(0)
Hepatocellular carcinoma, metastatic, liver			2 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(1)	(1)	(1)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	8 (16%)	5 (10%)	4 (8%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)	1 (2%)		
Alveolar/bronchiolar carcinoma	3 (6%)	8 (16%)	5 (10%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple	1 (2%)		1 (2%)	
Carcinoma, metastatic, harderian gland			1 (2%)	
Carcinoma, metastatic, salivary glands	1 (2%)			
Hepatocellular carcinoma, metastatic, liver	5 (10%)	6 (12%)	6 (12%)	5 (10%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Ear	(0)	(1)	(0)	(0)
Pinna, neural crest tumor		1 (100%)		
Eye	(50)	(50)	(50)	(50)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	5 (10%)	9 (18%)	4 (8%)	5 (10%)
Carcinoma		2 (4%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, salivary glands	1 (2%)			
Renal tubule, adenoma		1 (2%)	3 (6%)	2 (4%)
Urethra	(0)	(0)	(0)	(1)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma		1 (2%)	1 (2%)	
Lymphoma malignant		2 (4%)	4 (8%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	41	49	47	44
Total primary neoplasms	89	115	97	95
Total animals with benign neoplasms	32	45	39	33
Total benign neoplasms	55	64	56	51
Total animals with malignant neoplasms	29	38	29	33
Total malignant neoplasms	34	50	41	44
Total animals with metastatic neoplasms	6	6	8	5
Total metastatic neoplasms	10	6	13	5
Total animals with uncertain neoplasms - benign or malignant		1		
Total uncertain neoplasms		1		

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Adrenal Cortex: Adenoma				
Overall rate[a]	6/50 (12%)	2/50 (4%)	1/50 (2%)	3/50 (6%)
Adjusted rate[b]	13%	4.2%	2.2%	6.5%
Terminal rate[c]	6/40 (15%)	2/40 (5%)	1/35 (3%)	3/42 (7%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.217N	P=0.123N	P=0.060N	P=0.243N
Harderian Gland: Adenoma				
Overall rate	5/50 (10%)	9/50 (18%)	4/50 (8%)	5/50 (10%)
Adjusted rate	10.9%	18.7%	8.7%	10.9%
Terminal rate	5/40 (13%)	6/40 (15%)	2/35 (6%)	4/42 (10%)
First incidence (days)	729 (T)	633	575	699
Poly-3 test	P=0.373N	P=0.217	P=0.503N	P=0.630N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	11/50 (22%)	5/50 (10%)	5/50 (10%)
Adjusted rate	10.9%	22.9%	10.9%	10.9%
Terminal rate	5/40 (13%)	8/40 (20%)	3/35 (9%)	4/42 (10%)
First incidence (days)	729 (T)	633	575	699
Poly-3 test	P=0.312N	P=0.099	P=0.628	P=0.630N
Small Intestine (Duodenum, Ileum, or Jejunum): Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	2.2%	4.2%	8.8%	0.0%
Terminal rate	1/40 (3%)	2/40 (5%)	2/35 (6%)	0/42 (0%)
First incidence (days)	729 (T)	729 (T)	602	—[e]
Poly-3 test	P=0.377N	P=0.511	P=0.175	P=0.500N
Small Intestine (Duodenum, Ileum, or Jejunum): Adenoma or Carcinoma				
Overall rate	1/50 (2%)	2/50 (4%)	5/50 (10%)	0/50 (0%)
Adjusted rate	2.2%	4.2%	11.0%	0.0%
Terminal rate	1/40 (3%)	2/40 (5%)	3/35 (9%)	0/42 (0%)
First incidence (days)	729 (T)	729 (T)	602	—
Poly-3 test	P=0.409N	P=0.511	P=0.098	P=0.500N
Kidney (Renal Tubule): Adenoma (Single Sections)				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rate	0.0%	2.1%	6.7%	4.4%
Terminal rate	0/40 (0%)	1/40 (3%)	3/35 (9%)	2/42 (5%)
First incidence (days)	—	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.160	P=0.506	P=0.114	P=0.237
Kidney (Renal Tubule): Adenoma (Step Sections)				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	2.2%	6.3%	0.0%	2.2%
Terminal rate	1/40 (3%)	3/40 (8%)	0/35 (0%)	0/42 (0%)
First incidence (days)	729 (T)	729 (T)	—	554
Poly-3 test	P=0.408N	P=0.317	P=0.505N	P=0.758N
Kidney (Renal Tubule): Adenoma (Single and Step Sections)				
Overall rate	1/50 (2%)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate	2.2%	6.3%	6.7%	6.5%
Terminal rate	1/40 (3%)	3/40 (8%)	3/35 (9%)	2/42 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	554
Poly-3 test	P=0.294	P=0.317	P=0.297	P=0.309

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Liver: Hemangiosarcoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	0.0%	6.4%
Terminal rate	0/40 (0%)	1/40 (3%)	0/35 (0%)	1/42 (2%)
First incidence (days)	—	729 (T)	—	632
Poly-3 test	P=0.043	P=0.506	—[f]	P=0.121
Liver: Hepatocellular Adenoma				
Overall rate	29/50 (58%)	38/50 (76%)	35/50 (70%)	27/50 (54%)
Adjusted rate	61.4%	78.8%	75.6%	58.6%
Terminal rate	26/40 (65%)	33/40 (83%)	30/35 (86%)	26/42 (62%)
First incidence (days)	605	633	575	699
Poly-3 test	P=0.248N	P=0.045	P=0.097	P=0.476N
Liver: Hepatocellular Carcinoma				
Overall rate	24/50 (48%)	24/50 (48%)	21/50 (42%)	29/50 (58%)
Adjusted rate	49.1%	48.8%	44.5%	61.3%
Terminal rate	16/40 (40%)	16/40 (40%)	13/35 (37%)	26/42 (62%)
First incidence (days)	445	633	575	554
Poly-3 test	P=0.129	P=0.567N	P=0.401N	P=0.158
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	37/50 (74%)	45/50 (90%)	42/50 (84%)	42/50 (84%)
Adjusted rate	75.7%	91.4%	88.5%	88.6%
Terminal rate	29/40 (73%)	36/40 (90%)	32/35 (91%)	38/42 (91%)
First incidence (days)	445	633	575	554
Poly-3 test	P=0.096	P=0.031	P=0.080	P=0.079
Liver: Hepatoblastoma				
Overall rate	1/50 (2%)	7/50 (14%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.2%	14.6%	6.6%	4.4%
Terminal rate	1/40 (3%)	5/40 (13%)	1/35 (3%)	2/42 (5%)
First incidence (days)	729 (T)	633	651	729 (T)
Poly-3 test	P=0.443N	P=0.035	P=0.301	P=0.499
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	24/50 (48%)	29/50 (58%)	23/50 (46%)	29/50 (58%)
Adjusted rate	49.1%	58.9%	48.2%	61.3%
Terminal rate	16/40 (40%)	20/40 (50%)	13/35 (37%)	26/42 (62%)
First incidence (days)	445	633	575	554
Poly-3 test	P=0.204	P=0.220	P=0.545N	P=0.158
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	37/50 (74%)	46/50 (92%)	43/50 (86%)	42/50 (84%)
Adjusted rate	75.7%	93.5%	90.0%	88.6%
Terminal rate	29/40 (73%)	37/40 (93%)	32/35 (91%)	38/42 (91%)
First incidence (days)	445	633	575	554
Poly-3 test	P=0.102	P=0.013	P=0.050	P=0.079
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	6/50 (12%)	9/50 (18%)	5/50 (10%)	4/50 (8%)
Adjusted rate	13.0%	18.8%	11.0%	8.7%
Terminal rate	6/40 (15%)	7/40 (18%)	4/35 (11%)	4/42 (10%)
First incidence (days)	729 (T)	633	590	729 (T)
Poly-3 test	P=0.194N	P=0.316	P=0.510N	P=0.371N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	8/50 (16%)	6/50 (12%)	2/50 (4%)
Adjusted rate	8.7%	16.8%	13.2%	4.3%
Terminal rate	2/40 (5%)	7/40 (18%)	3/35 (9%)	1/42 (2%)
First incidence (days)	699	672	614	647
Poly-3 test	P=0.171N	P=0.193	P=0.359	P=0.337N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	10/50 (20%)	15/50 (30%)	11/50 (22%)	6/50 (12%)
Adjusted rate	21.6%	31.2%	24.0%	13.0%
Terminal rate	8/40 (20%)	12/40 (30%)	7/35 (20%)	5/42 (12%)
First incidence (days)	699	633	590	647
Poly-3 test	P=0.089N	P=0.207	P=0.493	P=0.205N
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	2.1%	0.0%	6.5%
Terminal rate	0/40 (0%)	0/40 (0%)	0/35 (0%)	2/42 (5%)
First incidence (days)	—	437	—	699
Poly-3 test	P=0.042	P=0.509	—	P=0.119
Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	5/50 (10%)
Adjusted rate	0.0%	2.1%	0.0%	10.8%
Terminal rate	0/40 (0%)	0/40 (0%)	0/35 (0%)	3/42 (7%)
First incidence (days)	—	437	—	647
Poly-3 test	P=0.003	P=0.509	—	P=0.031
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	4/50 (8%)	2/50 (4%)	2/50 (4%)
Adjusted rate	6.5%	8.4%	4.5%	4.4%
Terminal rate	3/40 (8%)	4/40 (10%)	2/35 (6%)	2/42 (5%)
First incidence (days)	729 (T)	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.325N	P=0.517	P=0.511N	P=0.501N
All Organs: Hemangiosarcoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	4/50 (8%)
Adjusted rate	2.2%	6.3%	0.0%	8.6%
Terminal rate	1/40 (3%)	2/40 (5%)	0/35 (0%)	2/42 (5%)
First incidence (days)	729 (T)	704	—	632
Poly-3 test	P=0.163	P=0.317	P=0.505N	P=0.182
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	1/50 (2%)	5/50 (10%)
Adjusted rate	4.3%	6.3%	2.2%	10.7%
Terminal rate	2/40 (5%)	2/40 (5%)	1/35 (3%)	3/42 (7%)
First incidence (days)	729 (T)	704	729 (T)	632
Poly-3 test	P=0.158	P=0.515	P=0.508N	P=0.221
All Organs: Malignant Lymphoma				
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted rate	0.0%	4.1%	8.9%	0.0%
Terminal rate	0/40 (0%)	1/40 (3%)	4/35 (11%)	0/42 (0%)
First incidence (days)	—	437	729 (T)	—
Poly-3 test	P=0.561N	P=0.248	P=0.057	—

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
All Organs: Benign Neoplasms				
Overall rate	32/50 (64%)	45/50 (90%)	39/50 (78%)	33/50 (66%)
Adjusted rate	67.7%	91.6%	82.6%	70.8%
Terminal rate	29/40 (73%)	38/40 (95%)	32/35 (91%)	31/42 (74%)
First incidence (days)	605	437	575	554
Poly-3 test	P=0.372N	P=0.002	P=0.067	P=0.460
All Organs: Malignant Neoplasms				
Overall rate	29/50 (58%)	38/50 (76%)	29/50 (58%)	33/50 (66%)
Adjusted rate	59.2%	76.0%	59.2%	69.4%
Terminal rate	20/40 (50%)	28/40 (70%)	16/35 (46%)	29/42 (69%)
First incidence (days)	445	437	575	554
Poly-3 test	P=0.350	P=0.057	P=0.580N	P=0.203
All Organs: Benign or Malignant Neoplasms				
Overall rate	41/50 (82%)	49/50 (98%)	47/50 (94%)	44/50 (88%)
Adjusted rate	83.7%	98.0%	95.8%	92.3%
Terminal rate	32/40 (80%)	39/40 (98%)	33/35 (94%)	39/42 (93%)
First incidence (days)	445	437	575	554
Poly-3 test	P=0.188	P=0.015	P=0.047	P=0.161

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

Table C3a
Historical Incidence of Forestomach Neoplasms in Control Male B6C3F1 Mice[a]

Study	Incidence in Controls		
	Squamous Cell Papilloma	Squamous Cell Carcinoma	Squamous Cell Papilloma or Carcinoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	2/50	1/50	2/50
Dibromoacetic acid	0/49	1/49	1/49
Dibromoacetonitrile	0/50	0/50	0/50
Sodium chlorate	0/50	0/50	0/50
Sodium dichromate dihydrate	0/50	0/50	0/50
Total (%)	2/249 (0.8%)	2/249 (0.8%)	3/249 (1.2%)
Mean ± standard deviation	0.8% ± 1.8%	0.8% ± 1.1%	1.2% ± 1.8%
Range	0%-4%	0%-2%	0%-4%
Overall Historical Incidence: All Routes			
Total (%)	11/1,149 (1.0%)	3/1,149 (0.3%)	13/1,149 (1.1%)
Mean ± standard deviation	1.0% ± 1.5%	0.3% ± 0.7%	1.1% ± 1.5%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of October 4, 2007

Table C3b
Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice[a]

Study	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatoblastoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	27/50	19/50	4/50
Dibromoacetic acid	18/49	14/49	0/49
Dibromoacetonitrile	29/50	24/50	1/50
Dipropylene glycol	30/48	20/48	6/48
Sodium chlorate	36/50	14/50	17/50
Total (%)	140/247 (56.8%)	91/247 (36.8%)	28/247 (11.3%)
Mean ± standard deviation	56.7% ± 13.0%	36.9% ± 8.6%	11.3% ± 13.6%
Range	37%-72%	28%-48%	0%-34%
Total (%)	544/1,146 (47.5%)	317/1,146 (27.7%)	43/1,146 (3.8%)
Mean ± standard deviation	47.5% ± 14.9%	27.7% ± 9.2%	3.8% ± 7.4%
Range	14%-72%	8%-48%	0%-34%

	Hepatocellular Adenoma or Hepatocellular Carcinoma	Hepatocellular Carcinoma or Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Drinking Water Studies			
Bromochloroacetic acid	34/50	21/50	35/50
Dibromoacetic acid	28/49	14/49	28/49
Dibromoacetonitrile	37/50	24/50	37/50
Sodium chlorate	41/48	23/48	41/48
Sodium dichromate dihydrate	42/50	25/50	46/50
Total (%)	182/247 (73.7%)	107/247 (43.3%)	187/247 (75.7%)
Mean ± standard deviation	73.7% ± 11.7%	43.3% ± 8.8%	75.7% ± 13.6%
Range	57%-85%	29%-50%	57%-92%
Overall Historical Incidence: All Routes			
Total (%)	729/1,146 (63.6%)	346/1,146 (30.2%)	740/1,146 (64.6%)
Mean ± standard deviation	63.6% ± 15.6%	30.2% ± 10.7%	64.6% ± 16.3%
Range	20%-85%	8%-50%	20%-92%

[a] Data as of October 4, 2007

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental death				1
Moribund	4	5	2	3
Natural deaths	6	5	13	4
Survivors				
Terminal sacrifice	40	40	35	42
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, cecum	(48)	(48)	(48)	(50)
Edema		5 (10%)		4 (8%)
Inflammation, chronic		1 (2%)		
Intestine large, colon	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Intestine small, duodenum	(47)	(48)	(47)	(49)
Hemorrhage	1 (2%)			
Intestine small, ileum	(47)	(50)	(48)	(49)
Intestine small, jejunum	(47)	(49)	(48)	(49)
Hyperplasia, lymphoid	1 (2%)	2 (4%)		
Liver	(50)	(50)	(50)	(50)
Basophilic focus	2 (4%)	3 (6%)	4 (8%)	1 (2%)
Clear cell focus	27 (54%)	23 (46%)	20 (40%)	16 (32%)
Cyst		2 (4%)		
Eosinophilic focus	7 (14%)	8 (16%)	8 (16%)	10 (20%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage		2 (4%)		
Infiltration cellular, mixed cell	1 (2%)	4 (8%)	1 (2%)	
Inflammation, chronic		1 (2%)		
Mixed cell focus	2 (4%)	4 (8%)	4 (8%)	4 (8%)
Necrosis, focal	3 (6%)	1 (2%)	6 (12%)	3 (6%)
Centrilobular, necrosis	2 (4%)	2 (4%)	2 (4%)	
Hepatocyte, vacuolization cytoplasmic	12 (24%)	6 (12%)	7 (14%)	2 (4%)
Mesentery	(12)	(12)	(5)	(2)
Hemorrhage			1 (20%)	1 (50%)
Fat, necrosis	9 (75%)	10 (83%)	4 (80%)	
Pancreas	(50)	(50)	(50)	(50)
Cyst	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			1 (2%)
Hyperplasia, lymphoid	7 (14%)	1 (2%)	3 (6%)	2 (4%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active	1 (2%)			
Necrosis				2 (4%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	1 (2%)			
Erosion		1 (2%)		
Ulcer	1 (2%)		1 (2%)	1 (2%)
Epithelium, hyperplasia	1 (2%)	4 (8%)	1 (2%)	6 (12%)

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Alimentary System (continued)				
Stomach, glandular	(50)	(49)	(50)	(50)
Cyst			1 (2%)	1 (2%)
Erosion	1 (2%)	1 (2%)	4 (8%)	
Necrosis				1 (2%)
Glands, hyperplasia	1 (2%)			
Tooth	(3)	(0)	(1)	(0)
Malformation	2 (67%)		1 (100%)	
Cardiovascular System				
Blood vessel	(0)	(1)	(0)	(0)
Aorta, inflammation, chronic		1 (100%)		
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy		1 (2%)	1 (2%)	1 (2%)
Inflammation, acute	1 (2%)	1 (2%)		
Inflammation, chronic	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Mineralization	3 (6%)	4 (8%)	2 (4%)	
Thrombosis	1 (2%)	1 (2%)	1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	5 (10%)	4 (8%)	5 (10%)	1 (2%)
Hyperplasia, focal	1 (2%)	3 (6%)	2 (4%)	4 (8%)
Hypertrophy, focal	12 (24%)	11 (22%)	3 (6%)	5 (10%)
Subcapsular, hyperplasia	7 (14%)	7 (14%)	7 (14%)	2 (4%)
Adrenal medulla	(49)	(49)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	3 (6%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	27 (54%)	35 (70%)	24 (48%)	13 (26%)
Parathyroid gland	(35)	(28)	(37)	(26)
Cyst		1 (4%)	1 (3%)	1 (4%)
Pituitary gland	(49)	(50)	(50)	(50)
Pars distalis, cyst		3 (6%)	2 (4%)	
Pars distalis, hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst	1 (2%)	3 (6%)	5 (10%)	
Follicle, degeneration, focal	7 (14%)	14 (28%)	8 (16%)	10 (20%)
Follicular cell, hyperplasia	14 (28%)	20 (40%)	15 (30%)	11 (22%)
General Body System				
None				

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Fibrosis			1 (2%)	
Granuloma sperm		1 (2%)	1 (2%)	
Inflammation, chronic			1 (2%)	1 (2%)
Preputial gland	(49)	(50)	(50)	(50)
Cyst	13 (27%)	14 (28%)	4 (8%)	7 (14%)
Inflammation, chronic	17 (35%)	15 (30%)	10 (20%)	11 (22%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	2 (4%)	3 (6%)	
Epithelium, hyperplasia			2 (4%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Degeneration	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Inflammation, chronic		3 (6%)	2 (4%)	1 (2%)
Testes	(50)	(50)	(50)	(50)
Mineralization	1 (2%)			
Germinal epithelium, atrophy	9 (18%)	6 (12%)	4 (8%)	6 (12%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	21 (42%)	15 (30%)	18 (36%)	14 (28%)
Lymph node	(0)	(2)	(1)	(0)
Iliac, hyperplasia, lymphoid		1 (50%)		
Lymph node, mandibular	(46)	(49)	(45)	(45)
Atrophy			2 (4%)	1 (2%)
Ectasia		1 (2%)		
Hyperplasia, lymphoid	5 (11%)	7 (14%)	7 (16%)	3 (7%)
Pigmentation		3 (6%)	3 (7%)	1 (2%)
Lymph node, mesenteric	(48)	(48)	(47)	(50)
Atrophy	2 (4%)	3 (6%)	2 (4%)	6 (12%)
Ectasia		2 (4%)		1 (2%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	2 (4%)	3 (6%)
Hemorrhage	3 (6%)	4 (8%)	3 (6%)	8 (16%)
Hyperplasia, lymphoid	4 (8%)	5 (10%)	1 (2%)	3 (6%)
Pigmentation				1 (2%)
Spleen	(50)	(50)	(50)	(50)
Fibrosis				1 (2%)
Hematopoietic cell proliferation	18 (36%)	20 (40%)	22 (44%)	20 (40%)
Hyperplasia, lymphoid				1 (2%)
Lymphoid follicle, atrophy	2 (4%)		2 (4%)	2 (4%)
Lymphoid follicle, hyperplasia	5 (10%)	4 (8%)	1 (2%)	1 (2%)
Red pulp, atrophy, focal	1 (2%)			
Thymus	(44)	(47)	(43)	(47)
Atrophy	9 (20%)	7 (15%)	9 (21%)	7 (15%)
Cyst	5 (11%)	8 (17%)	6 (14%)	1 (2%)
Hyperplasia, lymphoid		1 (2%)		1 (2%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	1 (2%)	1 (2%)	1 (2%)	
Inflammation, chronic			2 (4%)	
Metaplasia, osseous		1 (2%)		
Ulcer			1 (2%)	1 (2%)
Epidermis, hyperplasia		1 (2%)	3 (6%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis	1 (2%)	1 (2%)	1 (2%)	
Skeletal muscle	(0)	(0)	(2)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	1 (2%)		1 (2%)	
Hemorrhage		2 (4%)		
Necrosis	1 (2%)	2 (4%)	1 (2%)	
Peripheral nerve	(1)	(1)	(1)	(0)
Atrophy	1 (100%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Foreign body			1 (2%)	
Hemorrhage	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid		1 (2%)		
Infiltration cellular, histiocyte	4 (8%)	11 (22%)	6 (12%)	6 (12%)
Infiltration cellular, polymorphonuclear				1 (2%)
Metaplasia, osseous		1 (2%)		
Thrombosis				1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)	6 (12%)	3 (6%)	3 (6%)
Nose	(50)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	1 (2%)		1 (2%)
Special Senses System				
Ear	(0)	(1)	(0)	(0)
Eye	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Cataract		3 (6%)	1 (2%)	
Inflammation, chronic	2 (4%)	1 (2%)		
Cornea, hyperplasia	2 (4%)			
Retina, degeneration		2 (4%)	1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hyperplasia, focal		2 (4%)		

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Casts granular			1 (2%)	
Infarct	7 (14%)	7 (14%)	10 (20%)	7 (14%)
Inflammation			1 (2%)	
Inflammation, suppurative		1 (2%)	1 (2%)	
Metaplasia, osseous	5 (10%)	1 (2%)	4 (8%)	4 (8%)
Nephropathy	47 (94%)	46 (92%)	47 (94%)	48 (96%)
Glomerulus, dilatation			1 (2%)	
Glomerulus, hyalinization			1 (2%)	
Pelvis, dilatation		1 (2%)	3 (6%)	3 (6%)
Pelvis, inflammation		1 (2%)		1 (2%)
Renal tubule, accumulation, hyaline droplet			1 (2%)	
Renal tubule, cyst	11 (22%)	13 (26%)	8 (16%)	2 (4%)
Renal tubule, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Renal tubule, hypertrophy		1 (2%)	2 (4%)	
Renal tubule, pigmentation		2 (4%)		1 (2%)
Urethra	(0)	(0)	(0)	(1)
Inflammation, chronic				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Calculus microscopic observation only			1 (2%)	
Hemorrhage				2 (4%)
Inflammation, chronic		1 (2%)	1 (2%)	2 (4%)
Transitional epithelium, hyperplasia		2 (4%)	1 (2%)	1 (2%)

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		3	3	
Moribund	3	5		1
Natural deaths	11	6	4	2
Survivors				
Died last week of study	1		1	1
Terminal sacrifice	35	36	42	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(49)	(49)	(45)
Intestine large, cecum	(46)	(47)	(48)	(48)
Leiomyoma				1 (2%)
Leiomyosarcoma			1 (2%)	
Intestine small, duodenum	(47)	(47)	(47)	(50)
Adenoma			1 (2%)	
Fibrosarcoma, metastatic, skin				1 (2%)
Intestine small, ileum	(47)	(47)	(49)	(50)
Intestine small, jejunum	(45)	(47)	(49)	(50)
Carcinoma			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin	1 (2%)			
Hemangiosarcoma	2 (4%)		2 (4%)	2 (4%)
Hepatocellular adenoma	16 (32%)	15 (30%)	11 (22%)	9 (18%)
Hepatocellular adenoma, multiple	3 (6%)	6 (12%)	13 (26%)	11 (22%)
Hepatocellular carcinoma	9 (18%)	10 (20%)	5 (10%)	3 (6%)
Hepatocellular carcinoma, multiple	1 (2%)		2 (4%)	1 (2%)
Hepatocholangiocarcinoma				1 (2%)
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Mesentery	(12)	(13)	(15)	(7)
Sarcoma			1 (7%)	
Sarcoma, metastatic, skeletal muscle			1 (7%)	
Sarcoma, metastatic, skin				1 (14%)
Pancreas	(49)	(50)	(48)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		5 (10%)	13 (26%)
Squamous cell papilloma, multiple				1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin				1 (2%)
Tooth	(0)	(1)	(0)	(0)
Odontoma		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Subcapsular, adenoma		1 (2%)		1 (2%)
Adrenal medulla	(50)	(49)	(50)	(50)
Pheochromocytoma benign		2 (4%)	1 (2%)	1 (2%)
Islets, pancreatic	(49)	(50)	(48)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	
Parathyroid gland	(25)	(19)	(29)	(25)
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, adenoma	2 (4%)	4 (8%)	2 (4%)	3 (6%)
Thyroid gland	(49)	(50)	(50)	(50)
Follicular cell, adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
General Body System				
None				
Genital System				
Clitoral gland	(47)	(49)	(50)	(49)
Ovary	(50)	(46)	(47)	(48)
Choriocarcinoma	1 (2%)			
Cystadenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Granulosa cell tumor benign	1 (2%)	1 (2%)		1 (2%)
Hemangiosarcoma		1 (2%)		
Thecoma malignant				1 (2%)
Tubulostromal adenoma	1 (2%)			2 (4%)
Uterus	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Hemangiosarcoma			1 (2%)	
Polyp stromal	1 (2%)	1 (2%)	1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(11)	(9)	(6)	(3)
Mediastinal, choriocarcinoma, metastatic, ovary	1 (9%)			
Mediastinal, fibrosarcoma, metastatic, skin			1 (17%)	
Renal, fibrosarcoma, metastatic, skin		1 (11%)		
Lymph node, mandibular	(48)	(50)	(49)	(49)
Lymph node, mesenteric	(48)	(49)	(49)	(50)
Spleen	(48)	(50)	(48)	(50)
Hemangiosarcoma			1 (2%)	3 (6%)
Thymus	(46)	(47)	(50)	(49)
Sarcoma, metastatic, skeletal muscle			1 (2%)	

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Carcinoma	1 (2%)		1 (2%)	
Skin	(50)	(50)	(50)	(50)
Sebaceous gland, adenoma	1 (2%)			
Subcutaneous tissue, fibrosarcoma	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Subcutaneous tissue, hemangiosarcoma	1 (2%)			1 (2%)
Subcutaneous tissue, sarcoma		1 (2%)	1 (2%)	1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteoma		1 (2%)		
Skeletal muscle	(2)	(4)	(1)	(0)
Sarcoma			1 (100%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Peripheral nerve	(2)	(2)	(0)	(0)
Spinal cord	(2)	(2)	(0)	(0)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	3 (6%)	3 (6%)	7 (14%)	2 (4%)
Alveolar/bronchiolar carcinoma	6 (12%)	1 (2%)	1 (2%)	2 (4%)
Alveolar/bronchiolar carcinoma, multiple		1 (2%)		
Fibrosarcoma, metastatic, skin	2 (4%)		1 (2%)	
Hepatocellular carcinoma, metastatic, liver	2 (4%)			1 (2%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)	
Sarcoma, metastatic, skin		1 (2%)		1 (2%)
Nose	(50)	(50)	(50)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	6 (12%)	4 (8%)	3 (6%)	6 (12%)
Carcinoma	2 (4%)	1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	4 (8%)	1 (2%)	2 (4%)	
Lymphoma malignant	9 (18%)	7 (14%)	6 (12%)	7 (14%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Neoplasm Summary				
Total animals with primary neoplasms[c]	44	40	41	38
Total primary neoplasms	78	67	76	78
Total animals with benign neoplasms	29	33	32	34
Total benign neoplasms	38	42	47	53
Total animals with malignant neoplasms	31	22	20	19
Total malignant neoplasms	40	25	29	25
Total animals with metastatic neoplasms	5	2	3	3
Total metastatic neoplasms	6	2	8	6
Total animals with malignant neoplasms of uncertain primary site			1	

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Harderian Gland: Adenoma				
Overall rate[a]	6/50 (12%)	4/50 (8%)	3/50 (6%)	6/50 (12%)
Adjusted rate[b]	12.9%	9.4%	6.5%	12.1%
Terminal rate[c]	3/36 (8%)	4/36 (11%)	3/43 (7%)	6/47 (13%)
First incidence (days)	548	729 (T)	729 (T)	729 (T)
Poly-3 test[d]	P=0.557N	P=0.427N	P=0.242N	P=0.575N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	8/50 (16%)	5/50 (10%)	4/50 (8%)	6/50 (12%)
Adjusted rate	17.1%	11.8%	8.6%	12.1%
Terminal rate	4/36 (11%)	5/36 (14%)	4/43 (9%)	6/47 (13%)
First incidence (days)	548	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.312N	P=0.343N	P=0.182N	P=0.346N
Liver: Hepatocellular Adenoma				
Overall rate	19/50 (38%)	21/50 (42%)	24/50 (48%)	20/50 (40%)
Adjusted rate	41.5%	49.2%	51.8%	40.5%
Terminal rate	18/36 (50%)	20/36 (56%)	24/43 (56%)	20/47 (43%)
First incidence (days)	710	660	729 (T)	729 (T)
Poly-3 test	P=0.417N	P=0.302	P=0.216	P=0.541N
Liver: Hepatocellular Carcinoma				
Overall rate	10/50 (20%)	10/50 (20%)	7/50 (14%)	4/50 (8%)
Adjusted rate	21.3%	23.3%	14.9%	8.1%
Terminal rate	7/36 (19%)	9/36 (25%)	6/43 (14%)	4/47 (9%)
First incidence (days)	570	590	542	729 (T)
Poly-3 test	P=0.026N	P=0.511	P=0.295N	P=0.058N
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	27/50 (54%)	25/50 (50%)	26/50 (52%)	20/50 (40%)
Adjusted rate	57.5%	58.0%	55.4%	40.5%
Terminal rate	23/36 (64%)	23/36 (64%)	25/43 (58%)	20/47 (43%)
First incidence (days)	570	590	542	729 (T)
Poly-3 test	P=0.035N	P=0.570	P=0.499N	P=0.068N
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	7/50 (14%)	2/50 (4%)
Adjusted rate	6.6%	7.1%	14.9%	4.1%
Terminal rate	3/36 (8%)	1/36 (3%)	6/43 (14%)	2/47 (4%)
First incidence (days)	729 (T)	703	520	729 (T)
Poly-3 test	P=0.400N	P=0.629	P=0.169	P=0.464N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	6/50 (12%)	2/50 (4%)	1/50 (2%)	2/50 (4%)
Adjusted rate	13.0%	4.6%	2.1%	4.0%
Terminal rate	4/36 (11%)	0/36 (0%)	0/43 (0%)	1/47 (2%)
First incidence (days)	581	611	519	696
Poly-3 test	P=0.078N	P=0.157N	P=0.053N	P=0.112N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	9/50 (18%)	5/50 (10%)	8/50 (16%)	4/50 (8%)
Adjusted rate	19.5%	11.6%	16.8%	8.1%
Terminal rate	7/36 (19%)	1/36 (3%)	6/43 (14%)	3/47 (6%)
First incidence (days)	581	611	519	696
Poly-3 test	P=0.099N	P=0.232N	P=0.473N	P=0.091N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	2/48 (4%)	4/49 (8%)	2/48 (4%)	3/49 (6%)
Adjusted rate	4.6%	9.4%	4.4%	6.2%
Terminal rate	1/34 (3%)	4/36 (11%)	2/43 (5%)	3/46 (7%)
First incidence (days)	668	729 (T)	729 (T)	729 (T)
Poly-3 test	P=0.577	P=0.321	P=0.685N	P=0.544
Skin (Subcutaneous Tissue): Fibrosarcoma				
Overall rate	4/50 (8%)	2/50 (4%)	2/50 (4%)	2/50 (4%)
Adjusted rate	8.5%	4.6%	4.3%	4.0%
Terminal rate	1/36 (3%)	1/36 (3%)	2/43 (5%)	1/47 (2%)
First incidence (days)	511	427	729 (T)	659
Poly-3 test	P=0.261N	P=0.376N	P=0.343N	P=0.312N
Skin (Subcutaneous Tissue): Fibrosarcoma or Sarcoma				
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	3/50 (6%)
Adjusted rate	8.5%	6.9%	6.5%	6.0%
Terminal rate	1/36 (3%)	1/36 (3%)	3/43 (7%)	2/47 (4%)
First incidence (days)	511	427	729 (T)	659
Poly-3 test	P=0.399N	P=0.545N	P=0.507N	P=0.469N
Spleen: Hemangiosarcoma				
Overall rate	0/48 (0%)	0/50 (0%)	1/48 (2%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	2.2%	6.1%
Terminal rate	0/36 (0%)	0/36 (0%)	0/43 (0%)	3/47 (6%)
First incidence (days)	—[e]	—	700	729 (T)
Poly-3 test	P=0.026	—[f]	P=0.503	P=0.137
Stomach (Forestomach): Squamous Cell Papilloma				
Overall rate	1/50 (2%)	0/50 (0%)	5/50 (10%)	14/50 (28%)
Adjusted rate	2.2%	0.0%	10.8%	28.3%
Terminal rate	1/36 (3%)	0/36 (0%)	5/43 (12%)	14/47 (30%)
First incidence (days)	729 (T)	—	729 (T)	729 (T)
Poly-3 test	P<0.001	P=0.515N	P=0.105	P<0.001
All Organs: Hemangiosarcoma				
Overall rate	3/50 (6%)	1/50 (2%)	3/50 (6%)	4/50 (8%)
Adjusted rate	6.6%	2.3%	6.5%	8.1%
Terminal rate	3/36 (8%)	0/36 (0%)	2/43 (5%)	4/47 (9%)
First incidence (days)	729 (T)	660	700	729 (T)
Poly-3 test	P=0.336	P=0.330N	P=0.654N	P=0.543
All Organs: Histiocytic Sarcoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	8.5%	2.4%	4.3%	0.0%
Terminal rate	0/36 (0%)	1/36 (3%)	1/43 (2%)	0/47 (0%)
First incidence (days)	548	729 (T)	542	—
Poly-3 test	P=0.041N	P=0.212N	P=0.339N	P=0.055N
All Organs: Malignant Lymphoma				
Overall rate	9/50 (18%)	7/50 (14%)	6/50 (12%)	7/50 (14%)
Adjusted rate	19.7%	15.7%	12.9%	14.2%
Terminal rate	8/36 (22%)	3/36 (8%)	5/43 (12%)	7/47 (15%)
First incidence (days)	706	426	700	729 (T)
Poly-3 test	P=0.290N	P=0.416N	P=0.276N	P=0.330N

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
All Organs: Benign Neoplasms				
Overall rate	29/50 (58%)	33/50 (66%)	32/50 (64%)	34/50 (68%)
Adjusted rate	62.1%	74.6%	68.1%	68.8%
Terminal rate	24/36 (67%)	27/36 (75%)	31/43 (72%)	34/47 (72%)
First incidence (days)	548	339	520	729 (T)
Poly-3 test	P=0.395	P=0.138	P=0.347	P=0.317
All Organs: Malignant Neoplasms				
Overall rate	31/50 (62%)	22/50 (44%)	20/50 (40%)	19/50 (38%)
Adjusted rate	62.6%	47.7%	41.9%	38.1%
Terminal rate	20/36 (56%)	14/36 (39%)	16/43 (37%)	17/47 (36%)
First incidence (days)	511	426	519	659
Poly-3 test	P=0.011N	P=0.101N	P=0.030N	P=0.011N
All Organs: Benign or Malignant Neoplasms				
Overall rate	44/50 (88%)	40/50 (80%)	41/50 (82%)	38/50 (76%)
Adjusted rate	88.5%	84.3%	84.8%	76.3%
Terminal rate	31/36 (86%)	29/36 (81%)	36/43 (84%)	36/47 (77%)
First incidence (days)	511	339	519	659
Poly-3 test	P=0.064N	P=0.377N	P=0.403N	P=0.088N

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

Table D3
Historical Incidence of Squamous Cell Papilloma of the Forestomach in Control Female B6C3F1 Mice[a]

Study	Incidence in Controls
Historical Incidence: Drinking Water Studies	
Bromochloroacetic acid	0/50
Bromodichloromethane	0/50
Dibromoacetic acid	1/50
Dibromoacetonitrile	1/50
Sodium chlorate	0/50
Sodium dichromate dihydrate	1/50
Total (%)	3/300 (1.0%)
Mean ± standard deviation	1.0% ± 1.1%
Range	0%-2%
Overall Historical Incidence: All Routes	
Total (%)	20/1,249 (1.6%)
Mean ± standard deviation	1.6% ± 1.4%
Range	0%-4%

[a] Data as of October 4, 2007

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths		3	3	
Moribund	3	5		1
Natural deaths	11	6	4	2
Survivors				
Died last week of study	1		1	1
Terminal sacrifice	35	36	42	46
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(46)	(49)	(49)	(45)
Cyst				1 (2%)
Intestine large, cecum	(46)	(47)	(48)	(48)
Edema	1 (2%)	1 (2%)		
Ulcer	1 (2%)			
Intestine small, duodenum	(47)	(47)	(47)	(50)
Erosion	1 (2%)			
Ulcer		1 (2%)		
Intestine small, ileum	(47)	(47)	(49)	(50)
Intestine small, jejunum	(45)	(47)	(49)	(50)
Inflammation, suppurative	1 (2%)			
Peyer's patch, necrosis		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	2 (4%)	5 (10%)	3 (6%)	1 (2%)
Clear cell focus	4 (8%)	1 (2%)	2 (4%)	5 (10%)
Cyst	1 (2%)	1 (2%)		1 (2%)
Eosinophilic focus	6 (12%)	12 (24%)	1 (2%)	4 (8%)
Hematopoietic cell proliferation	3 (6%)	2 (4%)	3 (6%)	1 (2%)
Hemorrhage	1 (2%)	2 (4%)		1 (2%)
Infiltration cellular, lymphoid				1 (2%)
Infiltration cellular, mixed cell	7 (14%)	6 (12%)	4 (8%)	7 (14%)
Mixed cell focus			1 (2%)	2 (4%)
Necrosis	2 (4%)	5 (10%)		1 (2%)
Necrosis, focal	2 (4%)			
Tension lipidosis			1 (2%)	
Centrilobular, atrophy, hepatocyte			1 (2%)	
Centrilobular, necrosis	2 (4%)	1 (2%)		
Hepatocyte, vacuolization cytoplasmic	6 (12%)	7 (14%)	2 (4%)	2 (4%)
Mesentery	(12)	(13)	(15)	(7)
Hemorrhage	1 (8%)			1 (14%)
Infiltration cellular, lymphoid			1 (7%)	
Fat, necrosis	8 (67%)	10 (77%)	10 (67%)	4 (57%)
Pancreas	(49)	(50)	(48)	(50)
Atrophy	1 (2%)	1 (2%)		2 (4%)
Cyst		2 (4%)		1 (2%)
Inflammation, chronic			1 (2%)	

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

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Alimentary System (continued)				
Salivary glands	(50)	(50)	(50)	(50)
Atrophy			2 (4%)	1 (2%)
Hyperplasia, lymphoid	8 (16%)	10 (20%)	9 (18%)	10 (20%)
Infiltration cellular, lymphoid				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum	1 (2%)			3 (6%)
Inflammation, chronic	1 (2%)			1 (2%)
Ulcer	4 (8%)	1 (2%)		
Epithelium, hyperplasia	8 (16%)	5 (10%)	4 (8%)	4 (8%)
Epithelium, hyperplasia, lymphoid	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Erosion	2 (4%)	1 (2%)	1 (2%)	
Hyperplasia	1 (2%)			
Metaplasia, squamous			1 (2%)	
Ulcer			1 (2%)	
Epithelium, degeneration	1 (2%)			
Epithelium, dysplasia	1 (2%)			
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	2 (4%)	1 (2%)		1 (2%)
Inflammation, chronic				1 (2%)
Mineralization	2 (4%)	1 (2%)	1 (2%)	
Thrombosis		1 (2%)	1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	8 (16%)	9 (18%)	10 (20%)	11 (22%)
Infiltration cellular, lymphoid				1 (2%)
Vacuolization cytoplasmic				1 (2%)
Subcapsular, hyperplasia	1 (2%)			3 (6%)
Adrenal medulla	(50)	(49)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		5 (10%)
Islets, pancreatic	(49)	(50)	(48)	(50)
Hyperplasia	2 (4%)	1 (2%)	2 (4%)	2 (4%)
Parathyroid gland	(25)	(19)	(29)	(25)
Cyst		1 (5%)	1 (3%)	1 (4%)
Pituitary gland	(48)	(49)	(48)	(49)
Pars distalis, angiectasis		1 (2%)	1 (2%)	
Pars distalis, cyst	1 (2%)		2 (4%)	2 (4%)
Pars distalis, hyperplasia				1 (2%)
Pars distalis, hyperplasia, focal	5 (10%)	9 (18%)	6 (13%)	10 (20%)
Thyroid gland	(49)	(50)	(50)	(50)
Follicle, cyst	3 (6%)	2 (4%)	1 (2%)	3 (6%)
Follicle, degeneration, focal	15 (31%)	17 (34%)	22 (44%)	19 (38%)
Follicular cell, hyperplasia	11 (22%)	13 (26%)	12 (24%)	6 (12%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
General Body System				
None				
Genital System				
Clitoral gland	(47)	(49)	(50)	(49)
Inflammation, chronic				1 (2%)
Ovary	(50)	(46)	(47)	(48)
Angiectasis		1 (2%)	2 (4%)	2 (4%)
Cyst	14 (28%)	13 (28%)	13 (28%)	16 (33%)
Hemorrhage	5 (10%)	4 (9%)	5 (11%)	5 (10%)
Hyperplasia, tubular, focal			1 (2%)	
Corpus luteum, hyperplasia				1 (2%)
Granulosa cell, hyperplasia			1 (2%)	
Thecal cell, hyperplasia				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)		
Pigmentation		1 (2%)		1 (2%)
Endometrium, fibrosis		1 (2%)		
Endometrium, hyperplasia, cystic	45 (90%)	43 (86%)	45 (90%)	49 (98%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)			
Hemorrhage			1 (2%)	
Hyperplasia	15 (30%)	10 (20%)	12 (24%)	8 (16%)
Hyperplasia, histiocytic				1 (2%)
Lymph node	(11)	(9)	(6)	(3)
Iliac, ectasia		1 (11%)		
Iliac, hematopoietic cell proliferation			1 (17%)	
Iliac, hemorrhage	1 (9%)			
Iliac, hyperplasia, lymphoid	1 (9%)	1 (11%)	1 (17%)	
Inguinal, hyperplasia, lymphoid			1 (17%)	
Mediastinal, hyperplasia, lymphoid	2 (18%)		1 (17%)	
Renal, hemorrhage	1 (9%)			
Renal, hyperplasia, lymphoid		1 (11%)	1 (17%)	
Lymph node, mandibular	(48)	(50)	(49)	(49)
Ectasia		1 (2%)		
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage			1 (2%)	2 (4%)
Hyperplasia, lymphoid	6 (13%)	3 (6%)	8 (16%)	5 (10%)
Pigmentation	9 (19%)	14 (28%)	8 (16%)	5 (10%)
Lymph node, mesenteric	(48)	(49)	(49)	(50)
Atrophy		2 (4%)		1 (2%)
Ectasia		1 (2%)		
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hemorrhage	3 (6%)	3 (6%)		1 (2%)
Hyperplasia, lymphoid	3 (6%)	6 (12%)	4 (8%)	5 (10%)

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Hematopoietic System (continued)				
Spleen	(48)	(50)	(48)	(50)
Hematopoietic cell proliferation	33 (69%)	32 (64%)	26 (54%)	25 (50%)
Pigmentation	3 (6%)	1 (2%)		
Lymphoid follicle, atrophy		3 (6%)		
Lymphoid follicle, hyperplasia	14 (29%)	18 (36%)	21 (44%)	25 (50%)
Thymus	(46)	(47)	(50)	(49)
Atrophy	4 (9%)	5 (11%)	5 (10%)	2 (4%)
Cyst		1 (2%)	2 (4%)	
Hyperplasia, lymphoid	6 (13%)	1 (2%)	7 (14%)	6 (12%)
Integumentary System				
Mammary gland	(50)	(50)	(49)	(50)
Hyperplasia	6 (12%)	2 (4%)	2 (4%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Edema		1 (2%)		
Inflammation, chronic		2 (4%)	1 (2%)	
Ulcer	1 (2%)	1 (2%)		
Epidermis, hyperplasia		3 (6%)	1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Myelofibrosis	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Cranium, osteopetrosis	1 (2%)			
Skeletal muscle	(2)	(4)	(1)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	1 (2%)	1 (2%)		1 (2%)
Cyst epithelial inclusion			1 (2%)	1 (2%)
Hemorrhage		1 (2%)	1 (2%)	
Necrosis	1 (2%)			
Peripheral nerve	(2)	(2)	(0)	(0)
Atrophy	1 (50%)	1 (50%)		
Inflammation, chronic	1 (50%)			
Spinal cord	(2)	(2)	(0)	(0)
Necrosis		1 (50%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	3 (6%)	3 (6%)	1 (2%)	3 (6%)
Hyperplasia, lymphoid	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Infiltration cellular, histiocyte	5 (10%)	7 (14%)		5 (10%)
Alveolar epithelium, hyperplasia	2 (4%)	1 (2%)		
Nose	(50)	(50)	(50)	(50)
Foreign body	2 (4%)	1 (2%)		
Hemorrhage	1 (2%)			
Inflammation, chronic	1 (2%)	1 (2%)		

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

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Special Senses System				
Eye	(50)	(50)	(50)	(49)
Atrophy		1 (2%)		
Cataract				2 (4%)
Inflammation, chronic			2 (4%)	2 (4%)
Cornea, hyperplasia			2 (4%)	2 (4%)
Retina, degeneration	1 (2%)		2 (4%)	2 (4%)
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	2 (4%)	1 (2%)	1 (2%)	
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Casts granular			1 (2%)	1 (2%)
Infarct	6 (12%)	8 (16%)	7 (14%)	4 (8%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic		4 (8%)		
Metaplasia, osseous	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Nephropathy	9 (18%)	7 (14%)	3 (6%)	7 (14%)
Pelvis, dilatation	1 (2%)		1 (2%)	
Renal tubule, accumulation, hyaline droplet	1 (2%)	1 (2%)	1 (2%)	
Renal tubule, cyst	1 (2%)		1 (2%)	1 (2%)
Renal tubule, pigmentation	1 (2%)			
Urinary bladder	(50)	(50)	(50)	(50)
Inflammation, chronic	4 (8%)	5 (10%)	7 (14%)	4 (8%)

APPENDIX E
GENETIC TOXICOLOGY

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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Five independent mutagenicity assays were conducted with dibromoacetonitrile. Testing was performed for the first four assays as reported by Mortelmans *et al.* (1986) or Zeiger *et al.* (1992). Dibromoacetonitrile was sent to the laboratories as a coded aliquot from Radian Corporation (Austin, TX). The fifth assay, conducted with the same lot of dibromoacetonitrile tested in the 2-year study (sent to the laboratory as a coded aliquot from Battelle Columbus Laboratories, Columbus, OH) also employed *Escherichia coli* strain WP2 *uvrA*/pKM101 as a bacterial tester strain in addition to *Salmonella typhimurium* strains TA98 and TA100. It was incubated with the *S. typhimurium* tester strains TA97, TA98, TA100, TA1535, and TA1537 and with the *E. coli* tester strain either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least four doses of dibromoacetonitrile. The high dose was limited by toxicity. Trials that were not negative were repeated at the same or a higher S9 fraction; some negative trials were also repeated.

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

Drosophila melanogaster Test Protocol

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Valencia *et al.* (1985). Dibromoacetonitrile was supplied as a coded aliquot by Radian Corporation. Dibromoacetonitrile was assayed in the SLRL test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, dibromoacetonitrile was retested by injection into adult males.

To administer dibromoacetonitrile by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament, and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 µL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector that automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of dibromoacetonitrile at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Canton-S males were allowed to feed for 72 hours on a solution of dibromoacetonitrile in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of dibromoacetonitrile dissolved in distilled water and allowed to recover for 24 hours. A concurrent vehicle control group was also included. In the adult exposures, treated males were mated to three Basc females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from

successive matings was treated at successively earlier postmeiotic stages). F_1 heterozygous females were mated with their siblings and then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls (Mason *et al.,* 1992) using a normal approximation to the binomial test (Margolin *et al.,* 1983). A test result was considered positive if the P value was less than or equal to 0.01 and the mutation frequency in the tested group was greater than 0.10% or if the P value was less than or equal to 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if the P value was between 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or if the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if the P value was greater than or equal to 0.10 or if the frequency in the treatment group was less than 0.10%.

Mouse Peripheral Blood Micronucleus Test Protocol

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

The results were tabulated as the mean of the pooled results from all animals within an exposure group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed by a statistical software package that tested for increasing trend over exposure groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each exposure group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single exposed group is less than or equal to 0.025 divided by the number of exposed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Results of the 3-month study were accepted without repeat tests. Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

Evaluation Protocol

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation.

Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

Results

Dibromoacetonitrile was tested in five independent bacterial mutagenicity assays, in multiple strains of bacteria, and in the presence and absence of induced rat or hamster liver activation enzymes (S9). In four of the five assays, concentrations of dibromoacetonitrile dissolved in dimethylsulfoxide ranged up to 10,000 µg/plate (Table E1; Mortelmans *et al.*, 1986); in three of the assays, weak mutagenicity was observed in *S. typhimurium* strains TA100, TA1535, and/or TA97 in the presence of hamster S9 liver enzymes and occasionally in the presence of rat liver S9. In the fourth study, small increases in revertant colonies were observed in TA97 in the presence of hamster and rat liver S9, and results of this study were judged to be equivocal. In the fifth study, mutagenic activity was observed in *E. coli* strain WP2 *uvrA*/pKM101 in the presence of induced hamster liver S9. Across all studies induced hamster liver S9 enzymes were generally more effective than rat liver S9 in generating the mutagenic metabolite.

Dibromoacetonitrile did not induce SLRL mutations in germ cells of male *Drosophila melanogaster* exposed by feeding (50 or 75 ppm) or by injection (200 ppm) (Table E2; Valencia *et al.,* 1985). No increases in the frequencies of micronucleated NCEs or significant alterations in the percentages of PCEs were seen in peripheral blood of male or female mice in the 3-month study (Table E3), indicating no chemical-induced toxicity to the bone marrow. It should be noted that one female mouse in the 12.5 mg/L group had an extraordinarily high frequency of immature erythrocytes (38%), and this one value caused the group mean percentage of PCEs to be significantly increased over the background frequency. However, recalculating the mean without this one outlier gave a value of 4.7 for the group, which is not significantly higher than the control value.

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*[a]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	Trial 2	Trial 3	Trial 4
Study performed at BioReliance Corporation					
TA100	0	126 ± 9.0	151 ± 5.0	118 ± 3.0	105 ± 7.0
	1				98 ± 4.0
	3.3	135 ± 6.0		124 ± 2.0	111 ± 4.0
	10	150 ± 12.0	183 ± 13.0	177 ± 2.0	141 ± 6.0
	20		194 ± 3.0		
	33	226 ± 10.0	237 ± 10.0	228 ± 8.0	194 ± 9.0
	67		226 ± 7.0		209 ± 7.0
	100	161 ± 11.0	190 ± 11.0	119 ± 11.0	
	200	0 ± 0.0[c]		0 ± 0.0[c]	
Trial summary		Equivocal	Equivocal	Weakly Positive	Weakly Positive
Positive control[d]		501 ± 25.0	442 ± 26.0	516 ± 17.0	840 ± 20.0

		+hamster S9 10%	10%	30%	+rat S9 10%	10%	30%
TA100 (continued)	0	107 ± 2.0	131 ± 13.0	105 ± 7.0	132 ± 6.0	128 ± 6.0	120 ± 3.0
	3.3	116 ± 2.0	133 ± 6.0		125 ± 7.0		
	10	120 ± 8.0	136 ± 9.0	117 ± 4.0	132 ± 2.0	134 ± 3.0	106 ± 2.0
	25		160 ± 6.0				
	33	269 ± 5.0	157 ± 1.0	123 ± 6.0	162 ± 4.0	147 ± 4.0	130 ± 6.0
	67		195 ± 6.0			161 ± 5.0	
	100	160 ± 1.0		131 ± 7.0	274 ± 7.0	216 ± 5.0	143 ± 1.0
	200					198 ± 3.0[c]	
	333	0 ± 0.0[c]		0 ± 0.0[c]	0 ± 0.0[c]		0 ± 0.0[c]
	666			Toxic			Toxic
Trial summary		Positive	Equivocal	Negative	Weakly Positive	Equivocal	Negative
Positive control		728 ± 14.0	3,670 ± 62.0	627 ± 33.0	1,638 ± 13.0	2,089 ± 50.0	1,348 ± 51.0

		–S9 Trial 1	Trial 2
TA1535	0	25 ± 2.0	24 ± 3.0
	3.3		25 ± 4.0
	10	18 ± 1.0	20 ± 0.0
	20	17 ± 3.0	
	33	20 ± 4.0	27 ± 3.0
	67	23 ± 2.0	
	100	7 ± 4.0	9 ± 3.0
	200		0 ± 0.0[c]
Trial summary		Negative	Negative
Positive control		318 ± 19.0	276 ± 18.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		+hamster S9			+rat S9		
		10%	10%	30%	10%	10%	30%
Study performed at BioReliance Corporation (continued)							
TA1535 (continued)	0	11 ± 2.0	15 ± 5.0	13 ± 2.0	13 ± 3.0	11 ± 2.0	13 ± 2.0
	3.3	21 ± 3.0			14 ± 3.0		
	10	17 ± 3.0	16 ± 3.0	17 ± 1.0	14 ± 1.0	7 ± 1.0	18 ± 1.0
	33	18 ± 1.0	20 ± 1.0	12 ± 1.0	16 ± 2.0	14 ± 3.0	16 ± 1.0
	67		28 ± 1.0			16 ± 2.0	
	100	32 ± 3.0	29 ± 1.0	18 ± 2.0	33 ± 3.0	20 ± 2.0	21 ± 2.0
	200		2 ± 1.0			23 ± 2.0[c]	
	333	0 ± 0.0[c]			0 ± 0.0[c]		
	334			19 ± 2.0			19 ± 1.0[c]
	667			0 ± 0.0[c]			Toxic
Trial summary		Equivocal	Equivocal	Negative	Equivocal	Equivocal	Negative
Positive control		105 ± 4.0	238 ± 0.0	133 ± 2.0	406 ± 17.0	210 ± 11.0	204 ± 17.0
		–S9					
		Trial 1					
TA1538	0	8 ± 1.0					
	10	11 ± 2.0					
	20	7 ± 2.0					
	33	8 ± 0.0					
	67	3 ± 1.0					
	100	0 ± 0.0[c]					
Trial summary		Negative					
Positive control		1,512 ± 76.0					
		– S9					
		Trial 1	Trial 2				
TA97	0	128 ± 8.0	103 ± 3.0				
	1		107 ± 4.0				
	3.3	121 ± 14.0	145 ± 3.0				
	10	182 ± 12.0	166 ± 4.0				
	33	244 ± 9.0	219 ± 14.0				
	67		62 ± 30.0				
	100	36 ± 17.0					
	200	0 ± 0.0[c]					
Trial summary		Weakly Positive	Weakly Positive				
Positive control		340 ± 12.0	407 ± 5.0				

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		+hamster S9			+rat S9		
		10%	10%	30%	10%	10%	30%
Study performed at BioReliance Corporation (continued)							
TA97 (continued)	0	126 ± 4.0	132 ± 3.0	203 ± 3.0	121 ± 7.0	110 ± 8.0	108 ± 3.0
	3.3	119 ± 4.0			124 ± 5.0		
	10	116 ± 10.0	137 ± 2.0	166 ± 17.0	130 ± 9.0	112 ± 6.0	113 ± 5.0
	33	149 ± 8.0	156 ± 10.0	214 ± 6.0	162 ± 11.0	122 ± 11.0	136 ± 8.0
	67		222 ± 9.0			179 ± 7.0	
	100	234 ± 5.0	283 ± 10.0	191 ± 14.0	270 ± 7.0	228 ± 12.0	151 ± 6.0
	200		0 ± 0.0[c]			113 ± 27.0[c]	
	333	0 ± 0.0[c]			0 ± 0.0[c]		
	334			74 ± 27.0[c]			108 ± 9.0[c]
	667			Toxic			Toxic
Trial summary		Weakly Positive	Weakly Positive	Negative	Weakly Positive	Weakly Positive	Equivocal
Positive control		1,239 ± 27.0	832 ± 27.0	1,032 ± 25.0	3,413 ± 78.0	3,138 ± 239.0	551 ± 37.0

		–S9			
		Trial 1	Trial 2	Trial 3	Trial 4
TA98	0	21 ± 3.0	16 ± 2.0	23 ± 3.0	19 ± 3.0
	1				13 ± 3.0
	3.3	22 ± 2.0		25 ± 5.0	17 ± 3.0
	10	32 ± 4.0	21 ± 4.0	24 ± 4.0	20 ± 4.0
	20		25 ± 3.0		
	33	43 ± 5.0	28 ± 1.0	37 ± 5.0	30 ± 4.0
	67		20 ± 4.0		24 ± 2.0
	100	30 ± 4.0	24 ± 5.0	22 ± 4.0	
	200	0 ± 0.0[c]		0 ± 0.0[c]	
Trial summary		Equivocal	Negative	Equivocal	Negative
Positive control		294 ± 10.0	341 ± 19.0	416 ± 26.0	262 ± 10.0

		+hamster S9		+rat S9	
		10%	30%	10%	30%
TA98 (continued)	0	31 ± 3.0	39 ± 4.0	31 ± 2.0	35 ± 7.0
	3.3	28 ± 3.0		35 ± 1.0	
	10	31 ± 4.0	44 ± 5.0	36 ± 3.0	39 ± 3.0
	33	35 ± 1.0	43 ± 4.0	33 ± 3.0	35 ± 5.0
	100	33 ± 3.0	44 ± 12.0	45 ± 2.0	37 ± 3.0
	333	0 ± 0.0[c]	0 ± 0.0[c]	0 ± 0.0[c]	1 ± 1.0[c]
	666		0 ± 0.0[c]		0 ± 0.0[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control		521 ± 44.0	108 ± 9.0	784 ± 65.0	432 ± 33.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	Revertants/Plate –S9 Trial 2
Study performed at Case Western Reserve University			
TA100	0	75 ± 8.0	74 ± 5.0
	0.33	78 ± 10.0	78 ± 5.0
	1	92 ± 3.0	70 ± 9.0
	3.3	95 ± 2.0	71 ± 7.0
	10	83 ± 13.0	73 ± 7.0
	33	Toxic	92 ± 2.0
Trial summary		Negative	Negative
Positive control		705 ± 52.0	455 ± 40.0

Strain	Dose (µg/plate)	+10% hamster S9 Trial 1	Trial 2	Trial 3	Trial 4
TA100 (continued)	0	96 ± 8.0	75 ± 2.0	105 ± 4.0	152 ± 14.0
	1		90 ± 1.0		
	3.3	131 ± 1.0	80 ± 5.0		
	10	159 ± 12.0	82 ± 9.0	88 ± 0.0	
	16			99 ± 6.0	231 ± 9.0
	33	138 ± 10.0	110 ± 13.0	103 ± 7.0	231 ± 4.0
	67			149 ± 6.0	271 ± 8.0
	100	119 ± 37.0	142 ± 14.0	155 ± 10.0	206 ± 29.0
	167				2 ± 2.0
	333	0 ± 0.0			
Trial summary		Weakly Positive	Equivocal	Weakly Positive	Weakly Positive
Positive control		1,759 ± 40.0	1,232 ± 44.0	2,063 ± 40.0	1,906 ± 123.0

Strain	Dose (µg/plate)	+10% rat S9 Trial 1	Trial 2	Trial 3	Trial 4
TA100 (continued)	0	112 ± 14.0	86 ± 7.0	103 ± 5.0	213 ± 13.0
	1		84 ± 1.0		
	3.3	134 ± 4.0	87 ± 3.0		
	10	129 ± 1.0	97 ± 15.0	113 ± 8.0	
	16			96 ± 12.0	251 ± 19.0
	33	154 ± 7.0	117 ± 2.0	113 ± 3.0	264 ± 4.0
	67			123 ± 8.0	264 ± 20.0
	100		137 ± 12.0	130 ± 10.0	161 ± 56.0
	167				Toxic
	333	0 ± 0.0			
Trial summary		Equivocal	Equivocal	Equivocal	Equivocal
Positive control		1,040 ± 102.0	661 ± 83.0	2,058 ± 172.0	2,690 ± 53.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate			
		–S9			
		Trial 1	Trial 2	Trial 3	Trial 4
Study performed at Case Western Reserve University (continued)					
TA1535	0	4 ± 1.0	6 ± 1.0	6 ± 1.0	7 ± 2.0
	0.33	5 ± 0.0	6 ± 1.0		
	1	4 ± 1.0	8 ± 0.0		
	3.3	6 ± 1.0	7 ± 2.0		
	10	9 ± 3.0	9 ± 2.0	5 ± 1.0	13 ± 2.0
	16			8 ± 1.0	
	16.7				8 ± 1.0
	33	8 ± 2.0	7 ± 2.0	5 ± 1.0	
	33.3				9 ± 2.0
	66.7				5 ± 1.0
	67			5 ± 1.0	
Trial summary		Negative	Negative	Negative	Negative
Positive control		407 ± 41.0	650 ± 21.0	534 ± 39.0	399 ± 19.0
		+10% hamster S9			
		Trial 1	Trial 2	Trial 3	Trial 4
TA1535 (continued)	0	8 ± 1.0	5 ± 0.0	9 ± 2.0	12 ± 2.0
	3.3	7 ± 2.0			
	10	9 ± 2.0	8 ± 2.0	13 ± 1.0	
	16		10 ± 5.0		52 ± 1.0
	16.7			15 ± 3.0	
	33	13 ± 4.0	15 ± 3.0		48 ± 4.0
	33.3			17 ± 2.0	
	66.7			22 ± 1.0	
	67		25 ± 3.0		50 ± 12.0
	100	Toxic	32 ± 5.0	12 ± 1.0	20 ± 8.0
	167				0 ± 0.0
	333	0 ± 0.0			
Trial summary		Equivocal	Positive	Weakly Positive	Positive
Positive control		63 ± 18.0	96 ± 10.0	108 ± 3.0	174 ± 19.0
		+10% rat S9			
		Trial 1	Trial 2	Trial 3	Trial 4
TA1535 (continued)	0	8 ± 2.0	6 ± 1.0	6 ± 1.0	9 ± 2.0
	3.3	6 ± 1.0			
	10	10 ± 0.0	11 ± 1.0	9 ± 1.0	
	16		13 ± 3.0		12 ± 3.0
	16.7			12 ± 2.0	
	33	14 ± 2.0	17 ± 2.0		14 ± 2.0
	33.3			14 ± 2.0	
	66.7			16 ± 1.0	
	67		36 ± 2.0		21 ± 4.0
	100	Toxic	27 ± 4.0	Toxic	11 ± 4.0
	167				0 ± 0.0
	333	0 ± 0.0			
Trial summary		Negative	Positive	Equivocal	Equivocal
Positive control		51 ± 6.0	51 ± 2.0	32 ± 5.0	146 ± 9.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Study performed at Case Western Reserve University (continued)							
TA1537	0	3 ± 1.0	2 ± 1.0	5 ± 2.0	5 ± 1.0	4 ± 0.0	7 ± 3.0
	0.33	1 ± 1.0	3 ± 1.0				
	1	3 ± 1.0	4 ± 1.0		7 ± 2.0		
	3.3	2 ± 1.0	3 ± 1.0	6 ± 1.0	3 ± 1.0	4 ± 1.0	6 ± 0.0
	10	1 ± 1.0	3 ± 1.0	5 ± 2.0	5 ± 1.0	3 ± 1.0	6 ± 2.0
	33	1 ± 0.0	Toxic	4 ± 0.0	5 ± 1.0	3 ± 1.0	10 ± 3.0
	100			4 ± 1.0	3 ± 1.0	4 ± 1.0	6 ± 0.0
	333			0 ± 0.0		1 ± 0.0	Toxic
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		158 ± 29.0	424 ± 64.0	85 ± 17.0	125 ± 1.0	59 ± 5.0	56 ± 5.0
TA98	0	13 ± 1.0	13 ± 1.0	22 ± 1.0	18 ± 2.0	19 ± 4.0	13 ± 1.0
	0.33	10 ± 3.0	9 ± 1.0				
	1	12 ± 1.0	16 ± 1.0				
	3.3	13 ± 2.0	9 ± 1.0	22 ± 1.0	17 ± 2.0	27 ± 2.0	14 ± 0.0
	10	7 ± 1.0	9 ± 3.0	19 ± 0.0	16 ± 3.0	18 ± 2.0	15 ± 2.0
	33	17 ± 4.0	10 ± 3.0	20 ± 1.0	15 ± 3.0	20 ± 2.0	20 ± 4.0
	100			22 ± 2.0	20 ± 2.0	21 ± 3.0	20 ± 1.0
	333			4 ± 2.0	0 ± 0.0	3 ± 1.0	14 ± 4.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		177 ± 10.0	441 ± 38.0	784 ± 251.0	1,039 ± 24.0	378 ± 78.0	434 ± 45.0

Study performed at EG&G Mason Research Institute

		–S9
		Trial 1
TA100	0	139 ± 7.0
	0.3	106 ± 7.0
	1	130 ± 10.0
	3.3	118 ± 7.0
	10	133 ± 2.0
	22	136 ± 6.0[c]
Trial summary		Negative
Positive control		1,348 ± 33.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		+10% hamster S9				+10% rat S9	
		Trial 1	Trial 2	Trial 3	Trial 4	Trial 1	Trial 2
Study performed at EG&G Mason Research Institute (continued)							
TA100 (continued)	0	130 ± 3.0	140 ± 2.0	159 ± 12.0	170 ± 5.0	113 ± 9.0	134 ± 11.0
	3.3	116 ± 5.0			164 ± 6.0	124 ± 13.0	
	10	125 ± 9.0	149 ± 10.0	166 ± 3.0	160 ± 8.0	128 ± 3.0	147 ± 7.0
	33	162 ± 19.0	161 ± 12.0	208 ± 8.0	162 ± 16.0	141 ± 10.0	131 ± 8.0
	66		195 ± 8.0	257 ± 1.0			146 ± 7.0
	100	197 ± 7.0	221 ± 10.0	241 ± 10.0	19 ± 5.0	169 ± 5.0	158 ± 10.0
	125		201 ± 8.0	120 ± 13.0[c]			174 ± 4.0
	150		196 ± 5.0	54 ± 4.0[c]			176 ± 1.0
	200		112 ± 6.0[c]	0 ± 0.0[c]			135 ± 9.0[c]
	220	26 ± 13.0[c]				55 ± 9.0[c]	
	333				0 ± 0.0[c]		
	667				Toxic		
	1,000				Toxic		
	3,333				Toxic		
	10,000				Toxic		
Trial summary		Equivocal	Weakly Positive	Weakly Positive	Negative	Equivocal	Equivocal
Positive control		1,181 ± 43.0	675 ± 29.0	1,350 ± 27.0	2,524 ± 137.0	1,256 ± 37.0	794 ± 38.0

		–S9	+10%hamster S9	+10% rat S9
		Trial 1	Trial 1	Trial 1
TA1535	0	29 ± 3.0	16 ± 1.0	11 ± 1.0
	0.3	28 ± 2.0		
	1	24 ± 3.0		
	3.3	25 ± 2.0	11 ± 2.0	12 ± 2.0
	10	18 ± 3.0	14 ± 2.0	11 ± 1.0
	22	15 ± 2.0[c]		
	33		15 ± 1.0	11 ± 1.0
	100		18 ± 1.0	21 ± 3.0
	220		3 ± 1.0[c]	6 ± 1.0[c]
Trial summary		Negative	Negative	Negative
Positive control		1,096 ± 29.0	94 ± 1.0	72 ± 5.0
TA1537	0	7 ± 1.0	6 ± 1.0	7 ± 2.0
	0.3	7 ± 2.0		
	1	4 ± 1.0		
	3.3	5 ± 2.0	6 ± 1.0	6 ± 2.0
	10	5 ± 1.0	9 ± 2.0	7 ± 3.0
	22	4 ± 0.0[c]		
	33		8 ± 1.0	5 ± 0.0
	100		3 ± 1.0	3 ± 1.0
	220		0 ± 0.0[c]	3 ± 1.0[c]
Trial summary		Negative	Negative	Negative
Positive control		307 ± 8.0	79 ± 5.0	78 ± 6.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate		
		–S9	+10% hamster S9	+10% rat
		Trial 1	Trial 1	Trial 1
Study performed at EG&G Mason Research Institute (continued)				
TA98	0	17 ± 2.0	22 ± 2.0	28 ± 2.0
	0.3	16 ± 2.0		
	1	17 ± 2.0		
	3.3	15 ± 4.0	28 ± 3.0	25 ± 5.0
	10	21 ± 3.0	33 ± 1.0	23 ± 5.0
	22	19 ± 3.0[c]		
	33		32 ± 2.0	27 ± 4.0
	100		36 ± 2.0	29 ± 4.0
	220		5 ± 1.0[c]	16 ± 5.0[c]
Trial summary		Negative	Negative	Negative
Positive control		1,228 ± 40.0	635 ± 41.0	888 ± 26.0

Study performed at SRI International

		–S9		
		Trial 1	Trial 2	Trial 3
TA100	0	134 ± 8.0	151 ± 2.0	119 ± 3.0
	1		156 ± 2.0	116 ± 4.0
	3	118 ± 10.0	137 ± 6.0	118 ± 21.0
	10	136 ± 11.0	144 ± 3.0	121 ± 9.0
	33	140 ± 13.0	150 ± 10.0	118 ± 6.0
	66		133 ± 13.0	91 ± 33.0
	100	Toxic		
	166	Toxic		
Trial summary		Negative	Negative	Negative
Positive control		319 ± 11.0	414 ± 10.0	429 ± 4.0

		+hamster S9		+rat S9		
		10%	30%	10%	30%	30%
TA100 (continued)	0	123 ± 15.0	139 ± 2.0	122 ± 10.0	127 ± 5.0	163 ± 8.0
	3	120 ± 15.0		123 ± 9.0		144 ± 2.0
	10	124 ± 8.0	132 ± 14.0	129 ± 1.0	137 ± 10.0	153 ± 5.0
	33	128 ± 10.0	159 ± 5.0	136 ± 6.0	133 ± 4.0	150 ± 8.0
	100	119 ± 6.0	164 ± 12.0	132 ± 12.0	152 ± 2.0	157 ± 6.0
	166	96 ± 27.0		129 ± 6.0		133 ± 11.0
	333		114 ± 10.0		65 ± 17.0	
	666		Toxic		Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative
Positive control		670 ± 6.0	339 ± 5.0	603 ± 40.0	447 ± 17.0	240 ± 10.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		–S9		+hamster S9		+rat S9	
		Trial 1	Trial 2	10%	30%	10%	30%
Study performed at SRI International (continued)							
TA1535	0	17 ± 2.0	12 ± 2.0	11 ± 2.0	11 ± 2.0	12 ± 4.0	18 ± 2.0
	0.3		12 ± 4.0				
	1	16 ± 1.0	10 ± 1.0				
	3	11 ± 2.0	8 ± 1.0	11 ± 2.0	8 ± 1.0	9 ± 2.0	12 ± 1.0
	10	12 ± 1.0	9 ± 0.0	10 ± 1.0	8 ± 0.0	8 ± 4.0	11 ± 1.0
	33	13 ± 1.0	8 ± 0.0	10 ± 3.0	12 ± 2.0	11 ± 2.0	12 ± 2.0
	66	0 ± 0.0[c]					
	100			7 ± 1.0	9 ± 2.0	9 ± 3.0	12 ± 2.0
	166			0 ± 0.0[c]	9 ± 1.0	8 ± 2.0[c]	10 ± 5.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		221 ± 3.0	204 ± 6.0	195 ± 4.0	320 ± 41.0	121 ± 10.0	88 ± 6.0
		+hamster S9			+rat S9		
		10%	30%	50%	10%	30%	5%
TA1537	0	10 ± 1.0	10 ± 3.0	17 ± 1.0	14 ± 2.0	6 ± 0.0	16 ± 2.0
	10	9 ± 2.0	8 ± 2.0	20 ± 1.0	17 ± 2.0	14 ± 2.0	15 ± 1.0
	33	13 ± 3.0	8 ± 3.0	13 ± 1.0	16 ± 0.0	12 ± 3.0	11 ± 1.0
	66	10 ± 1.0	10 ± 1.0	9 ± 1.0	13 ± 0.0	10 ± 1.0	12 ± 2.0
	100	12 ± 1.0	7 ± 2.0	8 ± 2.0	12 ± 1.0	9 ± 1.0	13 ± 1.0
	166	7 ± 1.0	8 ± 3.0	0 ± 0.0[c]	12 ± 1.0	9 ± 1.0	3 ± 1.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		48 ± 2.0	57 ± 4.0	86 ± 7.0	53 ± 2.0	79 ± 4.0	73 ± 3.0
		–S9					
		Trial 1	Trial 2				
TA97	0	121 ± 6.0	154 ± 8.0				
	0.3		151 ± 2.0				
	1	119 ± 8.0	167 ± 1.0				
	3	106 ± 4.0	159 ± 6.0				
	10	125 ± 13.0	183 ± 4.0				
	33	136 ± 4.0	172 ± 5.0				
	66	1 ± 1.0[c]					
Trial summary		Negative	Negative				
Positive control		439 ± 31.0	437 ± 7.0				
		+hamster S9					
		10%	10%	30%	5%	30%	
TA97 (continued)	0	171 ± 4.0	173 ± 8.0	147 ± 4.0	218 ± 2.0	200 ± 9.0	
	3	185 ± 7.0		168 ± 1.0			
	10	179 ± 8.0	173 ± 12.0	165 ± 7.0	206 ± 8.0	229 ± 17.0	
	33	188 ± 9.0	137 ± 5.0	191 ± 7.0	254 ± 23.0	211 ± 21.0	
	66		178 ± 6.0		339 ± 33.0	200 ± 5.0	
	100	223 ± 15.0	196 ± 11.0	157 ± 28.0	128 ± 28.0[c]	205 ± 14.0	
	166	0 ± 0.0[c]	32 ± 10.0[c]	218 ± 10.0	0 ± 0.0[c]	346 ± 58.0	
Trial summary		Negative	Negative	Equivocal	Equivocal	Equivocal	
Positive control		505 ± 30.0	395 ± 30.0	332 ± 8.0	459 ± 13.0	359 ± 5.0	

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate				
		+rat S9				
		10%	10%	10%		
Study performed at SRI International (continued)						
TA97 (continued)	0	178 ± 23.0	186 ± 2.0	197 ± 11.0		
	3	181 ± 7.0	175 ± 11.0			
	10	215 ± 3.0	177 ± 13.0	201 ± 8.0		
	33	211 ± 9.0	176 ± 3.0	218 ± 33.0		
	66			215 ± 16.0		
	100	198 ± 19.0	335 ± 11.0	317 ± 17.0		
	166	290 ± 3.0	350 ± 25.0	330 ± 18.0		
Trial summary		Equivocal	Equivocal	Weakly Positive		
Positive control		400 ± 26.0	419 ± 3.0	342 ± 38.0		
		+rat S9				
		5%	5%	10%	30%	30%
TA97 (continued)	0	186 ± 7.0	171 ± 2.0	193 ± 3.0	136 ± 23.0	197 ± 18.0
	3		172 ± 1.0	185 ± 9.0	169 ± 12.0	
	10	227 ± 5.0	165 ± 12.0	178 ± 5.0	165 ± 2.0	172 ± 18.0
	33	282 ± 37.0	194 ± 11.0	191 ± 11.0	160 ± 3.0	168 ± 3.0
	66	348 ± 16.0	219 ± 11.0	166 ± 28.0		179 ± 10.0
	100	291 ± 35.0	204 ± 16.0	209 ± 43.0	85 ± 6.0[e]	171 ± 5.0
	166	386 ± 19.0	0 ± 0.0	4 ± 1.0	232 ± 15.0	194 ± 9.0
Trial summary		Weakly Positive	Negative	Negative	Equivocal	Negative
Positive control		423 ± 23.0	505 ± 14.0	411 ± 10.0	431 ± 13.0	482 ± 10.0
		–S9				
		Trial 1	Trial 2	Trial 3		
TA98	0	22 ± 3.0	24 ± 5.0	17 ± 1.0		
	1		22 ± 3.0	18 ± 2.0		
	3	25 ± 3.0	19 ± 3.0	14 ± 4.0		
	10	23 ± 4.0	23 ± 1.0	13 ± 1.0		
	33	20 ± 2.0	22 ± 2.0	19 ± 2.0		
	66		18 ± 1.0	8 ± 2.0		
	100	0 ± 0.0[c]				
	166	Toxic				
Trial summary		Negative	Negative	Negative		
Positive control		380 ± 6.0	470 ± 8.0	540 ± 37.0		

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		+hamster S9			+rat S9		
		10%	30%	30%	10%	30%	30%
Study performed at SRI International (continued)							
TA98	0	26 ± 1.0	34 ± 5.0	38 ± 5.0	25 ± 6.0	30 ± 1.0	36 ± 2.0
(continued)	3	30 ± 5.0		34 ± 2.0	26 ± 3.0		33 ± 4.0
	10	26 ± 3.0	27 ± 2.0	30 ± 4.0	23 ± 3.0	36 ± 5.0	32 ± 4.0
	33	23 ± 4.0	33 ± 3.0	30 ± 3.0	25 ± 6.0	37 ± 2.0	29 ± 5.0
	100	25 ± 2.0	33 ± 2.0	29 ± 2.0	16 ± 1.0	31 ± 2.0	25 ± 0.0
	166	16 ± 1.0		27 ± 1.0	10 ± 2.0		18 ± 0.0
	333		2 ± 0.0			9 ± 5.0[c]	
	666		Toxic			Toxic	
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		469 ± 41.0	147 ± 9.0	304 ± 5.0	369 ± 38.0	83 ± 7.0	129 ± 11.0

Study performed at SITEK Research Laboratories

		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA100	0	69 ± 3.0	106 ± 8.0	94 ± 2.0	103 ± 2.0	102 ± 5.0	92 ± 5.0
	2.5	69 ± 3.0	104 ± 5.0				
	5	93 ± 5.0	104 ± 12.0				
	10	97 ± 7.0	109 ± 6.0			111 ± 7.0	83 ± 4.0
	20	118 ± 7.0	131 ± 5.0				
	30	33 ± 6.0	64 ± 5.0				
	50		0 ± 0.0	125 ± 3.0	124 ± 2.0	104 ± 8.0	97 ± 2.0
	100			120 ± 7.0	104 ± 1.0	136 ± 8.0	94 ± 2.0
	150			134 ± 2.0	109 ± 5.0		
	200			82 ± 9.0	45 ± 13.0		
	250			25 ± 3.0	10 ± 2.0	132 ± 8.0	108 ± 10.0
	500			Toxic	Toxic	104 ± 4.0	121 ± 9.0
	750						131 ± 7.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		427 ± 4.0	432 ± 7.0	1,839 ± 47.0	1,655 ± 54.0	1,142 ± 57.0	477 ± 43.0

		–S9		+10% hamster S9		+10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
TA98	0	14 ± 1.0	20 ± 2.0	38 ± 1.0	34 ± 0.0	27 ± 1.0	25 ± 1.0
	2.5	11 ± 2.0	21 ± 1.0				
	5	16 ± 3.0	24 ± 3.0				
	10	23 ± 4.0	28 ± 3.0			25 ± 1.0	26 ± 2.0
	20	15 ± 2.0	23 ± 1.0				
	30	16 ± 3.0	21 ± 3.0				
	50		Toxic	46 ± 3.0	38 ± 1.0	25 ± 4.0	25 ± 2.0
	100			43 ± 3.0	35 ± 1.0	23 ± 3.0	24 ± 2.0
	150			36 ± 2.0	34 ± 2.0		
	200			19 ± 4.0	17 ± 5.0		
	250			12 ± 4.0	3 ± 1.0	23 ± 1.0	23 ± 2.0
	500			Toxic	Toxic	32 ± 5.0	22 ± 2.0
	750					15 ± 2.0	10 ± 1.0
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		450 ± 78.0	538 ± 15.0	1,175 ± 38.0	1,657 ± 182.0	1,770 ± 19.0	849 ± 39.0

Table E1
Mutagenicity of Dibromoacetonitrile in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate					
		–S9		10% hamster S9		10% rat S9	
		Trial 1	Trial 2	Trial 1	Trial 2	Trial 1	Trial 2
Study performed at SITEK Research Laboratories (continued)							
***Escherichia coli* WPM *uvrA*/pKM101 (Analogous to *S. typhimurium* TA102)**							
	0	75 ± 7.0	135 ± 4.0	191 ± 10.0	159 ± 7.0	113 ± 7.0	151 ± 20.0
	2.5	88 ± 5.0	141 ± 3.0				
	5	88 ± 7.0	148 ± 7.0				
	10	95 ± 12.0	143 ± 5.0			129 ± 1.0	165 ± 1.0
	20	80 ± 2.0	145 ± 1.0				
	30	101 ± 7.0	149 ± 5.0				
	50	50 ± 8.0	49 ± 2.0	300 ± 14.0	307 ± 5.0	111 ± 8.0	152 ± 2.0
	100			365 ± 9.0	473 ± 10.0	158 ± 15.0	165 ± 9.0
	150			429 ± 11.0	435 ± 45.0		
	200			441 ± 31.0	433 ± 17.0	141 ± 23.0	157 ± 9.0
	250			432 ± 21.0	410 ± 27.0		
	500			16 ± 3.0	2 ± 0.0	143 ± 5.0	164 ± 8.0
	750					195 ± 15.0	73 ± 5.0
Trial summary		Negative	Negative	Positive	Positive	Negative	Negative
Positive control		1,571 ± 153.0	1,653 ± 42.0	933 ± 76.0	954 ± 15.0	705 ± 42.0	705 ± 11.0

[a] The detailed protocol is presented by Mortelmans *et al.* (1986) and Zeiger *et al.* (1992); the Case Western Reserve University and EG&G Mason Research Institute data are published in Mortelmans *et al*. (1986). 0 µg/plate was the solvent control.
[b] Revertants are presented as mean ± standard error from three plates.
[c] Slight toxicity
[d] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA97 and TA1537), 4-nitro-*o*-phenylenediamine (TA98), and methyl methanesulfonate (WP2 *uvrA* pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.
[e] Slight toxicity and precipitate on plate

Table E2
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Dibromoacetonitrile[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested			Total[b]
				Mating 1	Mating 2	Mating 3	
Feeding	75	18	43	0/653	0/609	2/587	2/1,849 (0.11%)
	0			0/1,142	3/1,130	1/1,109	4/3,381 (0.12%)
	50	2	23	1/1,474	2/1,351	5/1,277	4/4,044 (0.10%)[c]
	0			1/1,051	0/989	1/983	2/3,023 (0.07%)
							P=0.438[d]
Injection	200	7	13	3/2,043	10/1,872	2/1,807	8/5,644 (0.14%)[e]
	0			0/2,071	0/2,019	4/1,913	4/6,003 (0.07%)
							P=0.103

[a] Study was performed at the University of Wisconsin-Madison. The detailed protocol and these data are presented by Valencia *et al.* (1985). The mean mutant frequency from 518 negative control experiments is 0.074% (Mason *et al.*, 1992).
[b] Total number of lethal mutations/number of X chromosomes tested for three mating trials
[c] One cluster of four lethals removed
[d] Significance of total number of lethal mutations/total number of X chromosomes tested by a normal approximation to the binomial test (Margolin *et al*., 1983).
[e] One cluster of seven lethals removed

Table E3
Frequency of Micronuclei in Normochromatic Erythrocytes and Percent Polychromatic Erythrocytes in Peripheral Blood of Mice Administered Dibromoacetonitrile in Drinking Water for 3 Months[a]

Compound	Exposure Concentration (mg/L)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Water[d]	0	10	3.40 ± 0.43		4.6 ± 0.3
Dibromoacetonitrile	12.5	10	3.00 ± 0.68	0.6917	4.8 ± 0.2
	25	10	3.30 ± 0.37	0.5487	4.3 ± 0.2
	50	10	3.30 ± 0.40	0.5487	4.1 ± 0.2
	100	10	3.80 ± 0.61	0.3184	4.3 ± 0.2
	200	10	2.70 ± 0.62	0.8153	4.2 ± 0.2
			P=0.715[e]		
Female					
Water	0	10	2.70 ± 0.40		4.0 ± 0.3
Dibromoacetonitrile	12.5	10	2.40 ± 0.34	0.6630	8.1 ± 3.3
	25	10	3.10 ± 0.41	0.2994	4.3 ± 0.3
	50	10	3.50 ± 0.60	0.1544	3.8 ± 0.3
	100	10	2.80 ± 0.39	0.4463	4.2 ± 0.1
	200	10	3.60 ± 0.56	0.1280	4.6 ± 0.4
			P=0.109		

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

APPENDIX F
CLINICAL PATHOLOGY RESULTS

Table F1 Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile 148
Table F2 Hematology Data for Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile 153

Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Male						
Hematology						
n						
Day 3	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 14	8	9	8	10	10	10
Hematocrit (auto) (%)						
Day 3	45.4 ± 0.6	45.3 ± 0.5	45.1 ± 0.5	46.0 ± 0.6	48.1 ± 0.6**	49.4 ± 0.8**
Day 21	48.9 ± 0.7	48.7 ± 0.7	48.9 ± 0.9	49.1 ± 0.4	49.2 ± 0.8	46.8 ± 0.6
Week 14	44.8 ± 0.6	45.5 ± 0.5	45.3 ± 0.4	45.3 ± 0.4	45.1 ± 0.4	45.8 ± 0.4
Hematocrit (spun) (%)						
Day 3	45.4 ± 0.6	45.5 ± 0.5	46.0 ± 0.6	46.1 ± 0.8	48.2 ± 0.6**	48.3 ± 0.8**
Day 21	48.8 ± 0.7	48.6 ± 0.7	49.1 ± 0.9	49.2 ± 0.4	49.5 ± 0.7	47.4 ± 0.7
Week 14	44.3 ± 0.5	45.2 ± 0.5	45.0 ± 0.5	44.9 ± 0.4	44.5 ± 0.4	45.5 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.5 ± 0.3	14.7 ± 0.2	14.5 ± 0.2	14.8 ± 0.2	15.4 ± 0.2**	16.0 ± 0.3**
Day 21	16.0 ± 0.3	15.7 ± 0.2	15.8 ± 0.2	15.9 ± 0.2	15.9 ± 0.2	15.1 ± 0.2*
Week 14	14.8 ± 0.2	15.0 ± 0.2	15.0 ± 0.2	15.0 ± 0.1	14.9 ± 0.1	15.2 ± 0.1
Erythrocytes (10^6/µL)						
Day 3	7.48 ± 0.13	7.54 ± 0.07	7.49 ± 0.07	7.57 ± 0.09	7.95 ± 0.08**	8.25 ± 0.13**
Day 21	8.11 ± 0.11	8.16 ± 0.13	8.21 ± 0.13	8.23 ± 0.10	8.31 ± 0.14	7.87 ± 0.12
Week 14	8.61 ± 0.14	8.81 ± 0.09	8.73 ± 0.09	8.75 ± 0.08	8.76 ± 0.06	8.92 ± 0.07
Reticulocytes (10^5/µL)						
Day 3	6.85 ± 0.20	6.15 ± 0.30	6.97 ± 0.20	7.19 ± 0.34	6.96 ± 0.35	7.29 ± 0.25
Day 21	4.53 ± 0.15	4.31 ± 0.12	4.45 ± 0.16	4.25 ± 0.10	4.40 ± 0.18	4.39 ± 0.13
Week 14	3.06 ± 0.07	2.83 ± 0.08	2.96 ± 0.07	2.89 ± 0.05	2.84 ± 0.08*	2.77 ± 0.06**
Nucleated erythrocytes/100 leukocytes						
Day 3	1.10 ± 0.23	0.70 ± 0.21	0.50 ± 0.22	0.89 ± 0.46	0.50 ± 0.22	0.30 ± 0.21
Day 21	0.40 ± 0.16	0.20 ± 0.13	0.30 ± 0.21	0.30 ± 0.21	0.10 ± 0.10	0.20 ± 0.13
Week 14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)						
Day 3	60.8 ± 0.4	60.1 ± 0.3	60.2 ± 0.3	60.9 ± 0.4	60.5 ± 0.4	59.9 ± 0.2
Day 21	60.2 ± 0.4	59.7 ± 0.3	59.5 ± 0.5	59.7 ± 0.4	59.2 ± 0.4	59.5 ± 0.3
Week 14	52.1 ± 0.2	51.6 ± 0.2	51.9 ± 0.3	51.8 ± 0.2	51.5 ± 0.3	51.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 3	19.5 ± 0.1	19.4 ± 0.2	19.4 ± 0.2	19.5 ± 0.1	19.4 ± 0.2	19.5 ± 0.1
Day 21	19.7 ± 0.2	19.3 ± 0.1	19.2 ± 0.1	19.3 ± 0.1	19.1 ± 0.1	19.2 ± 0.2
Week 14	17.2 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.1 ± 0.1	17.0 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.0 ± 0.3	32.3 ± 0.2	32.2 ± 0.3	32.1 ± 0.3	32.1 ± 0.3	32.5 ± 0.2
Day 21	32.7 ± 0.3	32.3 ± 0.1	32.3 ± 0.2	32.4 ± 0.1	32.3 ± 0.1	32.3 ± 0.2
Week 14	33.1 ± 0.1	33.0 ± 0.1	33.0 ± 0.1	33.0 ± 0.1	33.2 ± 0.1	33.2 ± 0.1
Platelets (10^3/µL)						
Day 3	792.3 ± 19.9	792.6 ± 17.7	756.4 ± 27.1	781.0 ± 27.0	743.3 ± 37.8	819.3 ± 25.5
Day 21	800.5 ± 26.5	797.3 ± 15.7	798.1 ± 19.0	801.5 ± 17.8	812.8 ± 24.8	860.3 ± 25.4
Week 14	578.8 ± 10.5	541.8 ± 17.9	520.1 ± 30.9	545.6 ± 22.4	553.5 ± 13.9	576.7 ± 13.3
Leukocytes (10^3/µL)						
Day 3	8.05 ± 0.31	8.73 ± 0.40	7.88 ± 0.46	8.08 ± 0.32	8.67 ± 0.26	9.14 ± 0.41
Day 21	9.72 ± 0.54	9.23 ± 0.38	9.99 ± 0.37	10.39 ± 0.60	10.95 ± 0.46	9.85 ± 0.39
Week 14	5.99 ± 0.75	4.76 ± 0.31	5.78 ± 0.71	5.37 ± 0.51	5.00 ± 0.49	5.84 ± 0.45
Segmented neutrophils (10^3/µL)						
Day 3	0.72 ± 0.05	0.86 ± 0.03*	0.73 ± 0.05	0.75 ± 0.03	0.73 ± 0.04	0.77 ± 0.03
Day 21	1.01 ± 0.15	0.88 ± 0.05	0.87 ± 0.03	0.91 ± 0.04	1.04 ± 0.05	0.90 ± 0.04
Week 14	0.84 ± 0.09	0.71 ± 0.05	0.86 ± 0.13	0.83 ± 0.09	0.75 ± 0.07	0.85 ± 0.06

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

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Male (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	9	10	10
Day 21	10	10	10	10	10	10
Week 14	8	9	8	10	10	10
Lymphocytes (10^3/μL)						
Day 3	6.70 ± 0.28	7.10 ± 0.34	6.54 ± 0.35	6.65 ± 0.29	7.29 ± 0.22	7.67 ± 0.36
Day 21	8.06 ± 0.40	7.65 ± 0.31	8.45 ± 0.32	8.78 ± 0.54	9.12 ± 0.40	8.27 ± 0.34
Week 14	4.84 ± 0.62	3.81 ± 0.25	4.59 ± 0.55	4.26 ± 0.40	4.00 ± 0.42	4.69 ± 0.37
Activated lymphocytes (10^3/μL)						
Day 3	0.43 ± 0.03	0.54 ± 0.06	0.40 ± 0.04	0.45 ± 0.05	0.44 ± 0.03	0.46 ± 0.03
Day 21	0.47 ± 0.04	0.51 ± 0.05	0.49 ± 0.03	0.52 ± 0.04	0.59 ± 0.04	0.51 ± 0.02
Week 14	0.22 ± 0.05	0.16 ± 0.02	0.21 ± 0.04	0.18 ± 0.02	0.18 ± 0.03	0.21 ± 0.02
Monocytes (10^3/μL)						
Day 3	0.14 ± 0.01	0.16 ± 0.01	0.15 ± 0.02	0.16 ± 0.01	0.14 ± 0.01	0.17 ± 0.01
Day 21	0.13 ± 0.02	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.02	0.11 ± 0.01
Week 14	0.05 ± 0.01	0.03 ± 0.00	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.01	0.04 ± 0.01
Basophils (10^3/μL)						
Day 3	0.039 ± 0.004	0.051 ± 0.005	0.040 ± 0.008	0.046 ± 0.008	0.049 ± 0.004	0.042 ± 0.005
Day 21	0.037 ± 0.003	0.037 ± 0.003	0.042 ± 0.003	0.043 ± 0.004	0.042 ± 0.002	0.036 ± 0.003
Week 14	0.024 ± 0.007	0.022 ± 0.003	0.025 ± 0.004	0.017 ± 0.003	0.017 ± 0.002	0.023 ± 0.005
Eosinophils (10^3/μL)						
Day 3	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Day 21	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.01	0.03 ± 0.00	0.02 ± 0.00	0.03 ± 0.00
Week 14	0.03 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	13.0 ± 0.5	13.6 ± 0.5	14.0 ± 0.4	15.1 ± 0.4**	15.2 ± 0.4**	15.8 ± 0.4**
Day 21	13.6 ± 0.6	12.2 ± 0.3	12.7 ± 0.3	15.9 ± 1.2	15.6 ± 0.8	14.9 ± 0.6
Week 14	11.1 ± 0.6	12.1 ± 0.4	13.2 ± 0.6	12.5 ± 0.3	12.6 ± 0.4	12.9 ± 0.6
Creatinine (mg/dL)						
Day 3	0.46 ± 0.02	0.46 ± 0.02	0.46 ± 0.02	0.44 ± 0.02	0.44 ± 0.02	0.45 ± 0.02
Day 21	0.53 ± 0.02	0.55 ± 0.02	0.57 ± 0.02	0.54 ± 0.02	0.52 ± 0.02	0.56 ± 0.02
Week 14	0.65 ± 0.02	0.67 ± 0.03	0.65 ± 0.02	0.71 ± 0.02	0.68 ± 0.04	0.66 ± 0.04
Total protein (g/dL)						
Day 3	5.7 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	6.0 ± 0.1*	6.3 ± 0.1**
Day 21	6.3 ± 0.1	6.4 ± 0.1	6.4 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.2 ± 0.1
Week 14	6.4 ± 0.1	6.6 ± 0.1	6.5 ± 0.0	6.5 ± 0.1	6.5 ± 0.1	6.5 ± 0.1
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.0 ± 0.0	4.2 ± 0.0**	4.4 ± 0.0**
Day 21	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.3 ± 0.0	4.2 ± 0.0	4.1 ± 0.0
Week 14	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0
Alanine aminotransferase (IU/L)						
Day 3	72 ± 2	75 ± 2	72 ± 1	73 ± 2	61 ± 2**	55 ± 1**
Day 21	58 ± 2	52 ± 2	53 ± 1	58 ± 3	54 ± 2	49 ± 2*
Week 14	56 ± 3	58 ± 2	56 ± 3	56 ± 2	52 ± 2	52 ± 2
Alkaline phosphatase (IU/L)						
Day 3	652 ± 10	653 ± 15	658 ± 24	642 ± 17	621 ± 10	618 ± 18
Day 21	455 ± 7	470 ± 15	489 ± 16	443 ± 14	439 ± 6	435 ± 9
Week 14	186 ± 6	194 ± 7	189 ± 5	182 ± 3	184 ± 6	191 ± 4

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

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Male (continued)						
Clinical Chemistry (continued)						
n	10	10	10	10	10	10
Creatine kinase (IU/L)						
Day 3	391 ± 22	353 ± 28	412 ± 24	400 ± 31	420 ± 19	529 ± 31**
Day 21	232 ± 11	204 ± 16	206 ± 12	235 ± 21	201 ± 10	254 ± 32
Week 14	154 ± 23	167 ± 17	153 ± 23	122 ± 30	194 ± 46	141 ± 18
Sorbitol dehydrogenase (IU/L)						
Day 3	27 ± 1	27 ± 1	27 ± 1	26 ± 1	31 ± 2	28 ± 2
Day 21	24 ± 1	24 ± 1	22 ± 1	24 ± 1	24 ± 1	22 ± 1
Week 14	23 ± 1	22 ± 1	23 ± 2	22 ± 1	21 ± 1	21 ± 1
Bile acids (µmol/L)						
Day 3	22.4 ± 1.6	24.6 ± 1.3	22.8 ± 1.5	22.2 ± 1.5	22.2 ± 1.6	23.8 ± 1.0
Day 21	25.9 ± 1.2	30.2 ± 1.8	23.1 ± 1.6	27.1 ± 2.0	25.9 ± 1.8	27.0 ± 1.9
Week 14	17.1 ± 2.0	14.9 ± 1.3	16.1 ± 2.2	14.1 ± 1.1	14.3 ± 1.4	18.7 ± 2.5
Female						
Hematology						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	9
Week 14	9	10	10	9	10	9
Hematocrit (auto) (%)						
Day 3	44.9 ± 0.6	44.9 ± 0.5	44.4 ± 0.5	45.4 ± 0.4	46.8 ± 0.4**	49.2 ± 0.5**
Day 21	49.1 ± 0.7	48.1 ± 0.5	48.4 ± 0.5	48.9 ± 0.4	48.7 ± 0.6	48.0 ± 0.5
Week 14	42.6 ± 0.3	43.1 ± 0.4	43.0 ± 0.4	43.0 ± 0.3	42.8 ± 1.1	42.4 ± 0.5
Hematocrit (spun) (%)						
Day 3	46.3 ± 0.6	46.1 ± 0.6	45.2 ± 0.5	46.5 ± 0.7	47.5 ± 0.5	49.5 ± 0.6**
Day 21	49.3 ± 0.7	48.3 ± 0.6	48.4 ± 0.5	48.8 ± 0.4	49.2 ± 0.7	48.6 ± 0.5
Week 14	42.8 ± 0.4	43.7 ± 0.5	43.7 ± 0.4	43.0 ± 0.3	43.2 ± 1.2	42.7 ± 0.5
Hemoglobin (g/dL)						
Day 3	14.6 ± 0.2	14.5 ± 0.2	14.7 ± 0.1	15.0 ± 0.2	15.2 ± 0.2	15.9 ± 0.2**
Day 21	16.4 ± 0.2	16.0 ± 0.2	16.1 ± 0.2	16.3 ± 0.1	16.4 ± 0.2	16.1 ± 0.2
Week 14	14.0 ± 0.1	14.2 ± 0.1	14.2 ± 0.1	14.1 ± 0.1	14.1 ± 0.4	14.0 ± 0.2
Erythrocytes (10^6/µL)						
Day 3	7.78 ± 0.08	7.81 ± 0.09	7.72 ± 0.07	7.91 ± 0.06	8.09 ± 0.09**	8.60 ± 0.07**
Day 21	8.24 ± 0.10	8.06 ± 0.09	8.11 ± 0.08	8.15 ± 0.07	8.22 ± 0.09	8.21 ± 0.11
Week 14	8.18 ± 0.07	8.23 ± 0.08	8.22 ± 0.07	8.20 ± 0.07	8.16 ± 0.19	8.14 ± 0.10
Reticulocytes (10^5/µL)						
Day 3	5.12 ± 0.29	5.40 ± 0.34	5.23 ± 0.26	5.21 ± 0.17	5.21 ± 0.27	5.38 ± 0.32
Day 21	2.92 ± 0.11	2.68 ± 0.08	2.87 ± 0.15	2.99 ± 0.09	2.89 ± 0.11	2.90 ± 0.13
Week 14	2.17 ± 0.10	2.23 ± 0.08	2.14 ± 0.07	2.26 ± 0.06	2.21 ± 0.12	2.18 ± 0.07
Nucleated erythrocytes/100 leukocytes						
Day 3	0.40 ± 0.16	0.44 ± 0.24[b]	0.30 ± 0.15	0.30 ± 0.21	0.20 ± 0.13	0.20 ± 0.13
Day 21	0.10 ± 0.10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.11 ± 0.11
Week 14	0.11 ± 0.11	0.00 ± 0.00	0.00 ± 0.00	0.22 ± 0.15	0.30 ± 0.15	0.22 ± 0.15
Mean cell volume (fL)						
Day 3	57.7 ± 0.2	57.4 ± 0.2	57.4 ± 0.2	57.4 ± 0.3	57.9 ± 0.3	57.2 ± 0.2
Day 21	59.6 ± 0.4	59.7 ± 0.2	59.7 ± 0.3	60.0 ± 0.4	59.4 ± 0.5	58.5 ± 0.6
Week 14	52.1 ± 0.1	52.4 ± 0.1	52.3 ± 0.2	52.4 ± 0.2	52.4 ± 0.2	52.0 ± 0.2

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

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Female (continued)						
Hematology (continued)						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	9
Week 14	9	10	10	9	10	9
Mean cell hemoglobin (pg)						
Day 3	18.8 ± 0.2	18.6 ± 0.1	19.0 ± 0.1	18.9 ± 0.2	18.7 ± 0.1	18.5 ± 0.2
Day 21	19.9 ± 0.1	19.8 ± 0.1	19.8 ± 0.1	20.0 ± 0.1	19.9 ± 0.1	19.6 ± 0.2
Week 14	17.1 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.2 ± 0.1	17.3 ± 0.1	17.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 3	32.6 ± 0.4	32.3 ± 0.2	33.1 ± 0.1	33.0 ± 0.2	32.4 ± 0.2	32.3 ± 0.3
Day 21	33.4 ± 0.2	33.2 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.5 ± 0.2	33.5 ± 0.1
Week 14	32.9 ± 0.1	32.9 ± 0.1	33.0 ± 0.1	32.8 ± 0.1	32.9 ± 0.2	33.1 ± 0.2
Platelets (10^3/µL)						
Day 3	803.6 ± 25.8	867.5 ± 19.1	856.1 ± 24.9	806.2 ± 19.2	833.0 ± 21.0	763.8 ± 14.5
Day 21	691.8 ± 15.6	707.5 ± 20.5	729.1 ± 22.2	704.1 ± 12.0	706.6 ± 14.7	747.3 ± 24.4
Week 14	585.8 ± 16.5	585.0 ± 14.2	584.0 ± 22.1	577.8 ± 20.5	568.7 ± 24.5	580.8 ± 13.3
Leukocytes (10^3/µL)						
Day 3	9.81 ± 0.25	9.08 ± 0.33	9.72 ± 0.32	9.47 ± 0.27	10.19 ± 0.43	11.08 ± 0.35*
Day 21	10.19 ± 0.40	9.44 ± 0.39	9.52 ± 0.31	10.00 ± 0.35	9.60 ± 0.46	9.92 ± 0.43
Week 14	4.58 ± 0.63	4.85 ± 0.42	4.48 ± 0.52	4.30 ± 0.47	4.27 ± 0.44	3.43 ± 0.24
Segmented neutrophils (10^3/µL)						
Day 3	0.79 ± 0.03	0.68 ± 0.02	0.78 ± 0.04	0.82 ± 0.04	0.75 ± 0.03	0.85 ± 0.04
Day 21	0.85 ± 0.04	0.88 ± 0.06	0.77 ± 0.03	0.89 ± 0.07	0.91 ± 0.08	0.91 ± 0.07
Week 14	0.80 ± 0.13	0.73 ± 0.06	0.73 ± 0.07	0.73 ± 0.06	0.65 ± 0.05	0.65 ± 0.08
Lymphocytes (10^3/µL)						
Day 3	8.16 ± 0.20	7.74 ± 0.28	8.20 ± 0.25	7.87 ± 0.25	8.62 ± 0.39	9.29 ± 0.29
Day 21	8.60 ± 0.35	7.92 ± 0.32	8.12 ± 0.28	8.41 ± 0.29	8.05 ± 0.36	8.32 ± 0.38
Week 14	3.46 ± 0.47	3.82 ± 0.34	3.50 ± 0.43	3.35 ± 0.41	3.34 ± 0.38	2.60 ± 0.15
Activated lymphocytes (10^3/µL)						
Day 3	0.62 ± 0.04	0.46 ± 0.03*	0.54 ± 0.04	0.56 ± 0.04	0.59 ± 0.04	0.67 ± 0.03
Day 21	0.53 ± 0.03	0.46 ± 0.02	0.47 ± 0.03	0.52 ± 0.04	0.44 ± 0.03	0.51 ± 0.04
Week 14	0.22 ± 0.05	0.19 ± 0.03	0.16 ± 0.03	0.14 ± 0.02	0.19 ± 0.03	0.12 ± 0.01
Monocytes (10^3/µL)						
Day 3	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.12 ± 0.01	0.16 ± 0.03
Day 21	0.13 ± 0.01	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.12 ± 0.01
Week 14	0.04 ± 0.01	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00
Basophils (10^3/µL)						
Day 3	0.056 ± 0.004	0.044 ± 0.004	0.058 ± 0.010	0.050 ± 0.003	0.070 ± 0.008	0.063 ± 0.004
Day 21	0.040 ± 0.004	0.035 ± 0.003	0.035 ± 0.003	0.041 ± 0.004	0.039 ± 0.003	0.038 ± 0.005
Week 14	0.020 ± 0.006	0.028 ± 0.006	0.012 ± 0.001	0.017 ± 0.003	0.015 ± 0.003	0.013 ± 0.002
Eosinophils (10^3/µL)						
Day 3	0.06 ± 0.03	0.03 ± 0.01	0.04 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.00
Day 21	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.00
Week 14	0.03 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.03 ± 0.01	0.04 ± 0.01	0.03 ± 0.01

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

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Female (continued)						
Clinical Chemistry						
n						
Day 3	10	10	10	10	10	10
Day 21	10	10	10	10	10	9
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 3	14.1 ± 0.8	13.4 ± 0.8	14.3 ± 0.5	15.5 ± 0.3	14.9 ± 0.6	15.5 ± 0.5
Day 21	12.9 ± 0.5[b]	14.2 ± 0.3	12.4 ± 0.4	14.1 ± 0.5	15.1 ± 0.5**	14.7 ± 0.6*
Week 14	13.3 ± 0.6	14.0 ± 0.5	15.2 ± 0.5	14.1 ± 0.5	15.3 ± 0.5	13.5 ± 0.6
Creatinine (mg/dL)						
Day 3	0.44 ± 0.02	0.47 ± 0.02	0.43 ± 0.02	0.45 ± 0.02	0.44 ± 0.02	0.41 ± 0.02
Day 21	0.49 ± 0.02	0.51 ± 0.01	0.50 ± 0.00	0.52 ± 0.01	0.52 ± 0.01	0.50 ± 0.00
Week 14	0.56 ± 0.02	0.58 ± 0.02	0.59 ± 0.02	0.58 ± 0.01	0.60 ± 0.02	0.53 ± 0.02
Total protein (g/dL)						
Day 3	5.9 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.0	6.0 ± 0.1	6.3 ± 0.0**
Day 21	6.0 ± 0.1[b]	6.1 ± 0.1	6.1 ± 0.1	6.1 ± 0.0	6.1 ± 0.1	6.0 ± 0.1
Week 14	6.7 ± 0.1	6.9 ± 0.0	7.0 ± 0.2	6.8 ± 0.1	6.6 ± 0.2	6.4 ± 0.1
Albumin (g/dL)						
Day 3	4.0 ± 0.0	4.1 ± 0.0	4.1 ± 0.0	4.2 ± 0.0	4.2 ± 0.0	4.3 ± 0.0**
Day 21	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.1	4.2 ± 0.0	4.2 ± 0.0	4.2 ± 0.0
Week 14	4.6 ± 0.1	4.8 ± 0.0	4.9 ± 0.1	4.8 ± 0.1	4.6 ± 0.1	4.5 ± 0.1
Alanine aminotransferase (IU/L)						
Day 3	58 ± 1	60 ± 2	57 ± 2	60 ± 2	55 ± 1	47 ± 3**
Day 21	42 ± 2	39 ± 1	40 ± 1	41 ± 1	38 ± 1	34 ± 3*
Week 14	54 ± 2	62 ± 4	54 ± 4	49 ± 3	61 ± 4	45 ± 2
Alkaline phosphatase (IU/L)						
Day 3	491 ± 8	542 ± 17	486 ± 9	508 ± 12	499 ± 14	489 ± 17
Day 21	340 ± 6	344 ± 10	328 ± 7	346 ± 10	343 ± 11	327 ± 11
Week 14	150 ± 3	162 ± 6	150 ± 5	158 ± 5	149 ± 5	151 ± 5
Creatine kinase (IU/L)						
Day 3	219 ± 16	241 ± 24	241 ± 15	266 ± 24	242 ± 10	300 ± 23**
Day 21	208 ± 13	196 ± 14	208 ± 18	205 ± 18	280 ± 53	208 ± 15
Week 14	236 ± 49	148 ± 23	217 ± 49	134 ± 18[b]	295 ± 87	109 ± 19*
Sorbitol dehydrogenase (IU/L)						
Day 3	19 ± 1	18 ± 1	18 ± 1	18 ± 1	18 ± 1	20 ± 1
Day 21	25 ± 1	25 ± 1	26 ± 1	23 ± 1	23 ± 1	25 ± 1
Week 14	20 ± 1	20 ± 2	22 ± 1	18 ± 1	28 ± 4	19 ± 1
Bile acids (μmol/L)						
Day 3	17.2 ± 0.8	19.9 ± 1.9	20.5 ± 2.3	18.4 ± 1.3	18.8 ± 1.3	20.9 ± 1.5
Day 21	23.0 ± 2.4[b]	19.4 ± 1.5	23.1 ± 2.1	22.4 ± 1.9	22.4 ± 1.7	17.1 ± 1.0
Week 14	22.3 ± 3.1	24.9 ± 2.5	22.0 ± 3.3	18.5 ± 1.9	23.5 ± 3.7	17.9 ± 2.1

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

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

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
Male						
n	10	10	9	10	10	10
Hematocrit (%) (auto)	51.0 ± 0.7	52.1 ± 0.6	51.0 ± 0.7	50.8 ± 0.5	51.8 ± 0.6	51.1 ± 0.2
Hematocrit (%) (spun)	50.4 ± 0.7	51.5 ± 0.6	49.8 ± 1.1[b]	50.6 ± 0.6	51.0 ± 0.6	50.6 ± 0.3
Hemoglobin (g/dL)	16.7 ± 0.2	17.1 ± 0.3	16.7 ± 0.2	16.7 ± 0.2	17.1 ± 0.2	16.9 ± 0.1
Erythrocytes (10^6/µL)	11.31 ± 0.13	11.51 ± 0.12	11.21 ± 0.15	11.26 ± 0.13	11.43 ± 0.13	11.34 ± 0.06
Reticulocytes (10^6/µL)	4.28 ± 0.09	4.35 ± 0.10	4.11 ± 0.08	4.06 ± 0.05	4.00 ± 0.10	4.06 ± 0.11
Nucleated erythrocytes/ 100 leukocytes	0.00 ± 0.00	0.00 ± 0.00	0.10 ± 0.10*b*	0.00 ± 0.00	0.10 ± 0.10	0.10 ± 0.10
Mean cell volume (fL)	45.1 ± 0.2	45.2 ± 0.2	45.5 ± 0.2	45.1 ± 0.2	45.4 ± 0.2	45.1 ± 0.1
Mean cell hemoglobin (pg)	14.8 ± 0.1	14.8 ± 0.1	14.9 ± 0.1	14.8 ± 0.0	15.0 ± 0.1	14.9 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.8 ± 0.2	32.8 ± 0.2	32.8 ± 0.1	33.0 ± 0.1	33.0 ± 0.2
Platelets (10^3/µL)	804.8 ± 52.5	755.7 ± 27.8	706.4 ± 62.9	791.7 ± 44.8	682.0 ± 23.1	751.9 ± 43.7
Leukocytes (10^3/µL)	4.92 ± 0.32	4.55 ± 0.22	4.05 ± 0.32	4.46 ± 0.44	4.66 ± 0.22	3.90 ± 0.38
Segmented neutrophils (10^3/µL)	0.52 ± 0.04	0.56 ± 0.05	0.47 ± 0.02	0.55 ± 0.03[c]	0.64 ± 0.11	0.52 ± 0.06
Lymphocytes (10^3/µL)	4.21 ± 0.28	3.82 ± 0.21	3.42 ± 0.30	4.06 ± 0.28[c]	3.83 ± 0.17	3.24 ± 0.32
Activated lymphocytes (10^3/µL)	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.00	0.04 ± 0.00[c]	0.04 ± 0.01	0.04 ± 0.01
Monocytes (10^3/µL)	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01[c]	0.05 ± 0.01	0.04 ± 0.01
Basophils (10^3/µL)	0.010 ± 0.003	0.011 ± 0.002	0.009 ± 0.002	0.011 ± 0.002[c]	0.009 ± 0.002	0.008 ± 0.001
Eosinophils (10^3/µL)	0.09 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.10 ± 0.02[c]	0.09 ± 0.01	0.07 ± 0.01
Female						
n	10	9	10	10	10	10
Hematocrit (%) (auto)	50.8 ± 0.4	48.6 ± 0.8	49.6 ± 0.6	49.5 ± 0.5	49.3 ± 0.7	49.7 ± 0.5
Hematocrit (%) (spun)	50.8 ± 0.5	49.0 ± 0.7	49.7 ± 0.6	50.1 ± 0.6	49.3 ± 0.6	50.0 ± 0.5
Hemoglobin (g/dL)	16.9 ± 0.2	16.1 ± 0.3*	16.4 ± 0.2	16.5 ± 0.2	16.3 ± 0.2	16.5 ± 0.1
Erythrocytes (10^6/µL)	11.02 ± 0.10	10.46 ± 0.18*	10.76 ± 0.12	10.75 ± 0.11	10.74 ± 0.15	10.87 ± 0.09
Reticulocytes (10^6/µL)	4.14 ± 0.12	4.43 ± 0.27	4.33 ± 0.27	3.67 ± 0.17	3.82 ± 0.09	4.09 ± 0.16
Nucleated erythrocytes/ 100 leukocytes	0.10 ± 0.10	0.11 ± 0.11	0.20 ± 0.13	0.30 ± 0.15	0.00 ± 0.00	0.10 ± 0.10
Mean cell volume (fL)	46.0 ± 0.2	46.5 ± 0.2	46.1 ± 0.3	46.1 ± 0.2	45.9 ± 0.1	45.7 ± 0.1
Mean cell hemoglobin (pg)	15.3 ± 0.1	15.4 ± 0.1	15.3 ± 0.1	15.3 ± 0.0	15.2 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.3 ± 0.2	33.1 ± 0.1	33.2 ± 0.1	33.1 ± 0.1	33.1 ± 0.1
Platelets (10^3/µL)	700.6 ± 46.6	775.9 ± 79.5	763.5 ± 50.4	738.8 ± 50.3	753.9 ± 61.6	715.5 ± 38.8
Leukocytes (10^3/µL)	5.01 ± 0.29	5.18 ± 0.33	5.20 ± 0.27	4.90 ± 0.29	4.46 ± 0.40	4.73 ± 0.36
Segmented neutrophils (10^3/µL)	0.44 ± 0.04	0.51 ± 0.05	0.44 ± 0.04	0.42 ± 0.03	0.40 ± 0.04	0.50 ± 0.07
Lymphocytes (10^3/µL)	4.39 ± 0.25	4.44 ± 0.31	4.56 ± 0.23	4.29 ± 0.26	3.88 ± 0.36	4.01 ± 0.28
Activated lymphocytes (10^3/µL)	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.01	0.05 ± 0.01	0.05 ± 0.01
Monocytes (10^3/µL)	0.05 ± 0.00	0.07 ± 0.01	0.05 ± 0.01	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01
Basophils (10^3/µL)	0.011 ± 0.002	0.013 ± 0.002	0.013 ± 0.002	0.010 ± 0.002	0.010 ± 0.002	0.018 ± 0.009
Eosinophils (10^3/µL)	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.08 ± 0.01	0.10 ± 0.01

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=10
[c] n=9

APPENDIX G
GLUTATHIONE-*S*-TRANSFERASE ACTIVITY

Table G1 Glutathione-*S*-transferase Activity in the Liver of Rats in the 2-Week Drinking Water Study of Dibromoacetonitrile 156
Table G2 Glutathione-*S*-transferase Activity in the Liver of Mice in the 2-Week Drinking Water Study of Dibromoacetonitrile 156

Table G1
Glutathione-*S*-transferase Activity in the Liver of Rats in the 2-Week Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	5	5	5	5	5	5
Male						
Glutathione-*S*-transferase (nmol/min/mg cytosolic protein)	820.2 ± 32.9	725.6 ± 23.3	755.6 ± 50.2	716.6 ± 34.8	737.6 ± 31.1	1,029.6 ± 31.4
Glutathione-*S*-transferase (nmol/min/g liver)	64,002 ± 2,968	56,740 ± 2,285	58,840 ± 1,510	56,678 ± 2,636	54,412 ± 786	82,441 ± 4,119
Female						
Glutathione-*S*-transferase (nmol/min/mg cytosolic protein)	872.4 ± 66.7	799.4 ± 22.0	857.8 ± 23.5	816.2 ± 35.7	867.4 ± 40.0	1,018.8 ± 59.1
Glutathione-*S*-transferase (nmol/min/g liver)	68,784 ± 3,651	61,870 ± 1,321	73,393 ± 2,045	64,134 ± 2,947	62,881 ± 3,738	72,276 ± 3,594

[a] Mean ± standard error. Statistical tests were performed on unrounded data. Differences between exposed and control groups were not significant by Dunn's test.

Table G2
Glutathione-*S*-transferase Activity in the Liver of Mice in the 2-Week Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	5	5	5	5	5	5
Male						
Glutathione-*S*-transferase (nmol/min/mg cytosolic protein)	3,307 ± 95	3,159 ± 114	3,352 ± 97	3,048 ± 112	3,420 ± 128	3,381 ± 158
Glutathione-*S*-transferase (nmol/min/g liver)	297,119 ± 11,464	266,358 ± 12,661	326,241 ± 10,152	294,139 ± 11,752	315,035 ± 9,788	264,429 ± 12,861
Female						
Glutathione-*S*-transferase (nmol/min/mg cytosolic protein)	1,424 ± 70	1,434 ± 52	1,432 ± 67	1,354 ± 41	1,432 ± 32	1,482 ± 97
Glutathione-*S*-transferase (nmol/min/g liver)	106,467 ± 2,098	103,127 ± 2,047	109,356 ± 3,897	109,445 ± 3,826	105,345 ± 2,441	114,768 ± 6,107

[a] Mean ± standard error. Statistical tests were performed on unrounded data. Differences between exposed and control groups were not significant by Dunn's test.

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

Table H1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Drinking Water Study of Dibromoacetonitrile 158
Table H2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile 159
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Table H4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile 161

Table H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Drinking Water Studies of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	5	5	5	5	5	5
Male						
Necropsy body weight	154 ± 2	161 ± 6	161 ± 6	156 ± 4	152 ± 3	128 ± 3**
Heart						
Absolute	0.534 ± 0.010	0.556 ± 0.017	0.594 ± 0.036	0.524 ± 0.021	0.532 ± 0.020	0.472 ± 0.010
Relative	3.460 ± 0.042	3.451 ± 0.050	3.698 ± 0.205	3.348 ± 0.054	3.489 ± 0.073	3.704 ± 0.085
R. Kidney						
Absolute	0.686 ± 0.017	0.688 ± 0.038	0.696 ± 0.039	0.680 ± 0.026	0.680 ± 0.022	0.586 ± 0.019
Relative	4.445 ± 0.094	4.256 ± 0.110	4.316 ± 0.080	4.356 ± 0.158	4.463 ± 0.091	4.590 ± 0.069
Liver						
Absolute	7.800 ± 0.196	8.282 ± 0.391	8.416 ± 0.682	8.312 ± 0.254	8.388 ± 0.199	6.038 ± 0.244*
Relative	50.529 ± 0.980	51.301 ± 1.232	51.992 ± 2.183	53.179 ± 0.826	55.122 ± 1.365	47.287 ± 1.318
Lung						
Absolute	0.938 ± 0.062	0.976 ± 0.022	1.050 ± 0.082	0.906 ± 0.030	0.904 ± 0.031	0.904 ± 0.017
Relative	6.076 ± 0.389	6.079 ± 0.244	6.533 ± 0.476	5.801 ± 0.153	5.941 ± 0.208	7.094 ± 0.143
R. Testis						
Absolute	0.842 ± 0.019	0.914 ± 0.050	0.947 ± 0.047	0.934 ± 0.062	0.852 ± 0.047	0.638 ± 0.097
Relative	5.456 ± 0.090	5.654 ± 0.138	5.884 ± 0.148	5.949 ± 0.260	5.580 ± 0.206	4.988 ± 0.742
Thymus						
Absolute	0.370 ± 0.026	0.411 ± 0.010	0.407 ± 0.011	0.383 ± 0.019	0.393 ± 0.016	0.381 ± 0.008
Relative	2.403 ± 0.176	2.555 ± 0.087	2.540 ± 0.097	2.458 ± 0.152	2.578 ± 0.092	2.991 ± 0.106*
Female						
Necropsy body weight	121 ± 1	116 ± 1	125 ± 3	121 ± 5	120 ± 3	118 ± 3
Heart						
Absolute	0.434 ± 0.007	0.430 ± 0.007	0.444 ± 0.010	0.430 ± 0.013	0.444 ± 0.012	0.424 ± 0.009
Relative	3.576 ± 0.050	3.722 ± 0.070	3.550 ± 0.060	3.571 ± 0.062	3.693 ± 0.094	3.590 ± 0.096
R. Kidney						
Absolute	0.542 ± 0.014	0.558 ± 0.007	0.554 ± 0.010	0.508 ± 0.033	0.540 ± 0.015	0.534 ± 0.020
Relative	4.468 ± 0.136	4.829 ± 0.071	4.431 ± 0.076	4.199 ± 0.124	4.488 ± 0.101	4.527 ± 0.219
Liver						
Absolute	5.486 ± 0.128	5.364 ± 0.136	5.466 ± 0.107	5.346 ± 0.180	5.304 ± 0.081	5.406 ± 0.193
Relative	45.200 ± 1.011	46.399 ± 0.938	43.707 ± 0.544	44.410 ± 1.110	44.126 ± 0.845	45.651 ± 0.718
Lung						
Absolute	0.760 ± 0.022	0.864 ± 0.037	0.790 ± 0.044	0.774 ± 0.048	0.780 ± 0.030	0.738 ± 0.032
Relative	6.263 ± 0.188	7.480 ± 0.337*	6.314 ± 0.315	6.401 ± 0.229	6.491 ± 0.278	6.239 ± 0.225
Thymus						
Absolute	0.332 ± 0.017	0.328 ± 0.010	0.326 ± 0.018	0.338 ± 0.007	0.351 ± 0.016	0.361 ± 0.011
Relative	2.740 ± 0.158	2.838 ± 0.088	2.604 ± 0.131	2.813 ± 0.068	2.918 ± 0.122	3.065 ± 0.149

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

** $P \leq 0.01$

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

Table H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Drinking Water Studies of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body weight	339 ± 6	335 ± 6	353 ± 5	331 ± 9	331 ± 9	317 ± 5
Heart						
Absolute	0.848 ± 0.015	0.866 ± 0.013	0.916 ± 0.022	0.833 ± 0.025	0.797 ± 0.041	0.841 ± 0.009
Relative	2.502 ± 0.012	2.592 ± 0.041	2.594 ± 0.048	2.518 ± 0.026	2.405 ± 0.108	2.654 ± 0.037
R. Kidney						
Absolute	0.955 ± 0.025	0.929 ± 0.024	0.977 ± 0.021	0.930 ± 0.027	0.927 ± 0.028	0.963 ± 0.027
Relative	2.815 ± 0.033	2.773 ± 0.033	2.767 ± 0.041	2.813 ± 0.030	2.801 ± 0.047	3.031 ± 0.054**
Liver						
Absolute	10.156 ± 0.372	9.999 ± 0.217	11.123 ± 0.335	10.156 ± 0.391	10.318 ± 0.341	10.278 ± 0.325
Relative	29.875 ± 0.585	29.872 ± 0.290	31.471 ± 0.639	30.653 ± 0.581	31.137 ± 0.410	32.322 ± 0.586**
Lung						
Absolute	1.408 ± 0.051	1.339 ± 0.037	1.502 ± 0.065	1.330 ± 0.040	1.362 ± 0.056	1.346 ± 0.066
Relative	4.147 ± 0.110	4.005 ± 0.100	4.256 ± 0.181	4.024 ± 0.053	4.115 ± 0.123	4.231 ± 0.167
R. Testis						
Absolute	1.409 ± 0.027	1.305 ± 0.026*	1.385 ± 0.019	1.346 ± 0.027	1.370 ± 0.029	1.355 ± 0.023
Relative	4.160 ± 0.069	3.915 ± 0.113	3.928 ± 0.056	4.081 ± 0.065	4.145 ± 0.040	4.273 ± 0.050
Thymus						
Absolute	0.267 ± 0.015	0.219 ± 0.009*	0.270 ± 0.011	0.245 ± 0.008	0.242 ± 0.011	0.232 ± 0.009
Relative	0.786 ± 0.035	0.655 ± 0.025**	0.768 ± 0.036	0.740 ± 0.015	0.731 ± 0.022	0.730 ± 0.027
Female						
Necropsy body weight	191 ± 3	195 ± 2	189 ± 4	191 ± 2	191 ± 3	181 ± 3*
Heart						
Absolute	0.544 ± 0.010	0.559 ± 0.006	0.557 ± 0.012	0.573 ± 0.012	0.558 ± 0.015	0.550 ± 0.009
Relative	2.854 ± 0.050	2.866 ± 0.031	2.946 ± 0.056	3.007 ± 0.055	2.927 ± 0.069	3.045 ± 0.062
R. Kidney						
Absolute	0.600 ± 0.009	0.614 ± 0.012	0.601 ± 0.013	0.619 ± 0.014	0.642 ± 0.014	0.630 ± 0.010
Relative	3.149 ± 0.057	3.149 ± 0.066	3.176 ± 0.042	3.248 ± 0.063	3.368 ± 0.056**	3.485 ± 0.053**
Liver						
Absolute	5.367 ± 0.109	5.783 ± 0.086	5.393 ± 0.238	5.597 ± 0.173	5.722 ± 0.160	5.320 ± 0.103
Relative	28.129 ± 0.390	29.634 ± 0.332	28.418 ± 0.818	29.343 ± 0.746	29.989 ± 0.565	29.408 ± 0.384
Lung						
Absolute	0.929 ± 0.028	0.938 ± 0.031	0.976 ± 0.022	0.954 ± 0.034	1.045 ± 0.083	0.933 ± 0.030
Relative	4.871 ± 0.139	4.806 ± 0.146	5.180 ± 0.170	5.003 ± 0.152	5.486 ± 0.432	5.150 ± 0.116
Thymus						
Absolute	0.204 ± 0.007	0.205 ± 0.009	0.205 ± 0.006	0.210 ± 0.004	0.208 ± 0.009	0.200 ± 0.008
Relative	1.070 ± 0.029	1.051 ± 0.049	1.083 ± 0.022	1.101 ± 0.017	1.087 ± 0.037	1.109 ± 0.043

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

** $P \leq 0.01$

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

Table H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Drinking Water Studies of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	5	5	5	5	5	5
Male						
Necropsy body weight	23.4 ± 1.0	24.6 ± 0.4	24.5 ± 0.5	23.7 ± 0.8	23.8 ± 0.6	22.1 ± 0.9
Heart						
Absolute	0.108 ± 0.004	0.114 ± 0.004	0.122 ± 0.007	0.120 ± 0.005	0.118 ± 0.005	0.106 ± 0.002
Relative	4.634 ± 0.085	4.641 ± 0.133	4.977 ± 0.271	5.068 ± 0.232	4.946 ± 0.137	4.817 ± 0.128
R. Kidney						
Absolute	0.238 ± 0.010	0.236 ± 0.007	0.252 ± 0.010	0.232 ± 0.014	0.230 ± 0.010	0.210 ± 0.005
Relative	10.207 ± 0.270	9.605 ± 0.174	10.289 ± 0.364	9.752 ± 0.273	9.629 ± 0.221	9.537 ± 0.211
Liver						
Absolute	1.226 ± 0.087	1.368 ± 0.029	1.332 ± 0.044	1.372 ± 0.073	1.266 ± 0.054	1.156 ± 0.087
Relative	52.235 ± 1.601	55.753 ± 1.431	54.333 ± 1.166	57.753 ± 1.844	53.018 ± 1.052	52.114 ± 2.047
Lung						
Absolute	0.150 ± 0.003	0.146 ± 0.002	0.152 ± 0.002	0.154 ± 0.008	0.144 ± 0.004	0.146 ± 0.002
Relative	6.455 ± 0.213	5.949 ± 0.120	6.212 ± 0.127	6.481 ± 0.188	6.044 ± 0.132	6.640 ± 0.190
R. Testis						
Absolute	0.093 ± 0.002	0.089 ± 0.004	0.099 ± 0.003	0.096 ± 0.002	0.097 ± 0.004	0.093 ± 0.004
Relative	4.014 ± 0.098	3.626 ± 0.106	4.036 ± 0.142	4.040 ± 0.109	4.078 ± 0.122	4.191 ± 0.094
Thymus						
Absolute	0.049 ± 0.004	0.049 ± 0.003	0.056 ± 0.004	0.051 ± 0.003	0.053 ± 0.002	0.047 ± 0.003
Relative	2.083 ± 0.103	2.008 ± 0.099	2.276 ± 0.166	2.143 ± 0.153	2.222 ± 0.066	2.117 ± 0.162
Female						
Necropsy body weight	19.8 ± 0.5	19.5 ± 0.3	19.1 ± 0.4	18.7 ± 0.3	19.0 ± 0.3	19.3 ± 0.3
Heart						
Absolute	0.110 ± 0.013	0.094 ± 0.002	0.100 ± 0.003	0.092 ± 0.004	0.092 ± 0.004	0.090 ± 0.010
Relative	5.522 ± 0.530	4.830 ± 0.104	5.254 ± 0.221	4.917 ± 0.235	4.831 ± 0.171	4.659 ± 0.493
R. Kidney						
Absolute	0.146 ± 0.007	0.144 ± 0.002	0.143 ± 0.005	0.144 ± 0.005	0.150 ± 0.004	0.142 ± 0.004
Relative	7.357 ± 0.211	7.407 ± 0.172	7.468 ± 0.239	7.678 ± 0.200	7.878 ± 0.188	7.369 ± 0.102
Liver						
Absolute	1.028 ± 0.044	0.996 ± 0.026	0.974 ± 0.033	0.860 ± 0.025**	0.864 ± 0.021**	0.906 ± 0.038**
Relative	51.785 ± 1.095	51.168 ± 0.900	51.039 ± 1.231	45.906 ± 1.359**	45.510 ± 1.901**	47.016 ± 1.623**
Lung						
Absolute	0.138 ± 0.007	0.130 ± 0.004	0.140 ± 0.003	0.132 ± 0.004	0.136 ± 0.005	0.128 ± 0.004
Relative	6.955 ± 0.231	6.677 ± 0.185	7.360 ± 0.281	7.053 ± 0.252	7.142 ± 0.229	6.650 ± 0.195
Thymus						
Absolute	0.056 ± 0.009	0.068 ± 0.003	0.062 ± 0.005	0.075 ± 0.003	0.065 ± 0.001	0.070 ± 0.004
Relative	2.809 ± 0.434	3.495 ± 0.211	3.252 ± 0.214	3.996 ± 0.183*	3.404 ± 0.061	3.664 ± 0.250

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

** $P \leq 0.01$

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

Table H4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Drinking Water Studies of Dibromoacetonitrile[a]

	0 mg/L	12.5 mg/L	25 mg/L	50 mg/L	100 mg/L	200 mg/L
n	10	10	10	10	10	10
Male						
Necropsy body weight	40.3 ± 0.9	39.7 ± 0.8	38.2 ± 1.0	40.0 ± 0.7	38.4 ± 0.9	37.9 ± 1.0
Heart						
Absolute	0.157 ± 0.003	0.164 ± 0.003	0.159 ± 0.005	0.162 ± 0.004	0.154 ± 0.003	0.154 ± 0.003
Relative	3.901 ± 0.076	4.149 ± 0.126	4.186 ± 0.143	4.057 ± 0.071	4.028 ± 0.124	4.092 ± 0.145
R. Kidney						
Absolute	0.308 ± 0.007	0.335 ± 0.009	0.328 ± 0.007	0.327 ± 0.007	0.304 ± 0.018	0.319 ± 0.006
Relative	7.652 ± 0.177	8.467 ± 0.278	8.629 ± 0.211	8.188 ± 0.119	7.948 ± 0.509	8.468 ± 0.270
Liver						
Absolute	1.612 ± 0.036	1.610 ± 0.040	1.590 ± 0.043	1.649 ± 0.036	1.566 ± 0.037	1.547 ± 0.047
Relative	40.014 ± 0.716	40.556 ± 0.496	41.718 ± 0.710	41.283 ± 0.579	40.763 ± 0.478	40.889 ± 0.935
Lung						
Absolute	0.230 ± 0.008	0.253 ± 0.016	0.233 ± 0.008	0.237 ± 0.011	0.266 ± 0.013	0.238 ± 0.014
Relative	5.710 ± 0.187	6.361 ± 0.346	6.122 ± 0.183	5.941 ± 0.287	6.921 ± 0.304*	6.299 ± 0.355
R. Testis						
Absolute	0.123 ± 0.003	0.128 ± 0.002	0.126 ± 0.003	0.124 ± 0.003	0.128 ± 0.001	0.119 ± 0.003
Relative	3.048 ± 0.087	3.222 ± 0.071	3.334 ± 0.142	3.101 ± 0.082	3.348 ± 0.078	3.170 ± 0.113
Thymus						
Absolute	0.040 ± 0.002	0.037 ± 0.002	0.036 ± 0.001	0.038 ± 0.001	0.037 ± 0.002	0.036 ± 0.002
Relative	1.001 ± 0.062	0.931 ± 0.029	0.949 ± 0.042	0.963 ± 0.027	0.958 ± 0.059	0.952 ± 0.050
Female						
Necropsy body weight	30.9 ± 0.9	33.0 ± 1.0	31.3 ± 0.5	30.5 ± 0.3	30.3 ± 0.9	29.5 ± 0.7
Heart						
Absolute	0.126 ± 0.004	0.135 ± 0.004	0.129 ± 0.002	0.132 ± 0.003	0.131 ± 0.004	0.125 ± 0.002
Relative	4.109 ± 0.165	4.120 ± 0.176	4.136 ± 0.106	4.323 ± 0.093	4.351 ± 0.175	4.257 ± 0.129
R. Kidney						
Absolute	0.185 ± 0.003	0.192 ± 0.004	0.191 ± 0.002	0.186 ± 0.007	0.191 ± 0.004	0.184 ± 0.005
Relative	6.018 ± 0.125	5.840 ± 0.132	6.115 ± 0.110	6.092 ± 0.203	6.322 ± 0.125	6.281 ± 0.275
Liver						
Absolute	1.214 ± 0.037	1.266 ± 0.037	1.282 ± 0.041	1.216 ± 0.040	1.225 ± 0.037	1.206 ± 0.025
Relative	39.391 ± 0.832	38.364 ± 0.345	40.904 ± 0.871	39.840 ± 1.278	40.526 ± 1.116	40.915 ± 0.622
Lung						
Absolute	0.241 ± 0.011	0.225 ± 0.019	0.243 ± 0.011	0.234 ± 0.014	0.239 ± 0.020	0.244 ± 0.008
Relative	7.890 ± 0.457	6.806 ± 0.508	7.752 ± 0.285	7.676 ± 0.458	7.898 ± 0.641	8.292 ± 0.295
Thymus						
Absolute	0.047 ± 0.002	0.041 ± 0.004	0.047 ± 0.002	0.046 ± 0.002	0.045 ± 0.002	0.045 ± 0.002
Relative	1.534 ± 0.068	1.232 ± 0.099*	1.503 ± 0.081	1.496 ± 0.077	1.489 ± 0.073	1.541 ± 0.090

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

APPENDIX I
REPRODUCTIVE TISSUE EVALUATIONS AND ESTROUS CYCLE CHARACTERIZATION

Table I1 Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile 164

Table I2 Estrous Cycle Characterization for Female Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile 164

Table I3 Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile 165

Table I4 Estrous Cycle Characterization for Female Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile 165

Table I1
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	339 ± 6	331 ± 9	331 ± 9	317 ± 5
L. Cauda epididymis	0.200 ± 0.005	0.190 ± 0.007	0.173 ± 0.007*	0.192 ± 0.007
L. Epididymis	0.466 ± 0.016	0.450 ± 0.008	0.425 ± 0.021	0.459 ± 0.010
L. Testis	1.513 ± 0.022	1.439 ± 0.023	1.433 ± 0.026*	1.477 ± 0.020
Spermatid measurements				
Spermatid heads (10^3/mg testis)	118.6 ± 3.7	120.5 ± 5.3	117.0 ± 3.1	117.9 ± 3.8
Spermatid heads (10^6/testis)	167.4 ± 6.6	159.6 ± 8.2	154.1 ± 2.3	159.4 ± 5.5
Epididymal spermatozoal measurements				
Sperm motility (%)	68.98 ± 1.12	65.05 ± 0.80**	64.01 ± 1.02**	59.82 ± 1.71**
Sperm (10^3/mg cauda)	584.3 ± 39.0	617.5 ± 32.4	595.7 ± 39.7	557.9 ± 28.1
Sperm (10^6/cauda)	117.2 ± 9.0	115.6 ± 3.5	102.8 ± 7.9	106.9 ± 6.1

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test (tissue weights) or Shirley's test (spermatozoal measurements)
**$P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weights) or Dunn's test (spermatid measurements).

Table I2
Estrous Cycle Characterization for Female Rats in the 3-Month Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	191 ± 3	191 ± 2	191 ± 3	181 ± 3*
Proportion of regular cycling females[b]	8/10	10/10	10/10	9/10
Estrous cycle length (days)	5.05 ± 0.19	4.85 ± 0.17	4.65 ± 0.15	5.05 ± 0.12
Estrous stages (% of cycle)				
Diestrus	37.5	38.3	39.2	45.8
Proestrus	10.0	10.0	10.0	12.5
Estrus	33.3	31.7	30.0	24.2
Metestrus	19.2	20.0	20.8	17.5

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling

Table I3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
n	10	10	10	10
Weights (g)				
Necropsy body wt	40.3 ± 0.9	39.8 ± 0.5	38.4 ± 0.9	37.9 ± 1.0
L. cauda epididymis	0.0240 ± 0.0014	0.0249 ± 0.0015	0.0270 ± 0.0022	0.0213 ± 0.0013
L. epididymis	0.0531 ± 0.0020	0.0565 ± 0.0021	0.0571 ± 0.0021	0.0521 ± 0.0017
L. testis	0.1195 ± 0.0023	0.1246 ± 0.0016	0.1265 ± 0.0027	0.1166 ± 0.0025
Spermatid measurements				
Spermatid heads (10^3/mg testis)	179.7 ± 7.1	185.6 ± 10.5	190.1 ± 8.7	197.9 ± 6.1
Spermatid heads (10^6/testis)	19.50 ± 0.66	20.77 ± 1.30	21.18 ± 0.85	20.38 ± 0.77
Epididymal spermatozoal measurements				
Sperm motility (%)	68.88 ± 0.97	63.10 ± 3.26*	63.11 ± 1.77**	63.38 ± 1.13**
Sperm (10^6/cauda)	18.40 ± 0.75	23.83 ± 0.92**	22.46 ± 0.73**	20.32 ± 0.93
Sperm (10^3/mg cauda)	792.6 ± 63.0	973.7 ± 52.2	877.5 ± 70.2	969.8 ± 47.1

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test (spermatozoal measurements)
**$P \leq 0.01$
[a] Data are presented as mean ± standard error. Differences from the control group are not significant by Williams' or Dunnett's test (body and tissue weights) or Dunn's test (spermatid measurements).

Table I4
Estrous Cycle Characterization for Female Mice in the 3-Month Drinking Water Study of Dibromoacetonitrile[a]

	0 mg/L	50 mg/L	100 mg/L	200 mg/L
Number weighed at necropsy	10	10	10	10
Necropsy body wt	30.9 ± 0.9	30.5 ± 0.3	30.3 ± 0.9	29.5 ± 0.7
Proportion of regular cycling females[b]	7/10	7/10	8/10	4/10
Estrous cycle length (days)	4.91 ± 0.53[c]	5.25 ± 0.63	4.60 ± 0.39	4.13 ± 0.25[d]
Estrous stages (% of cycle)				
Diestrus	25.8	35.8	31.7	35.8
Proestrus	1.7	3.3	1.7	2.5
Estrus	52.5	42.5	45.0	44.2
Metestrus	20.0	18.3	21.7	17.5

[a] Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the control group are not significant by Dunnett's test (body weights) or Dunn's test (estrous cycle length). By multivariate analysis of variance, exposed females did not differ significantly from the control females in the relative length of time spent in the estrous stages.
[b] Number of females with a regular cycle/number of females cycling
[c] Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
[d] Estrous cycle was longer than 12 days or unclear in 2 of 10 animals.

APPENDIX J
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Dibromoacetonitrile

Dibromoacetonitrile was obtained from Oakwood Products, Inc. (West Columbia, SC) in one lot (S11C) which was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory at Battelle Columbus Operations (Columbus, OH) and by the study laboratory at Southern Research Institute (Birmingham, AL). Karl Fischer titration and elemental analysis were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on analyses performed in support of the dibromoacetonitrile studies are on file at the National Institute of Environmental Health Sciences.

Lot S11C, a pale yellow to amber liquid, was identified as dibromoacetonitrile by the study laboratory using infrared and proton nuclear magnetic resonance (NMR) spectroscopy and by the analytical chemistry laboratory using infrared and proton and carbon-13 NMR spectroscopy. All spectra were consistent with the literature spectra (*Aldrich*, 1985, 1992) and spectra from a frozen reference standard of the same lot and were compatible between laboratories. A proton NMR spectrum obtained at the analytical chemistry laboratory after the addition of deuterium oxide indicated that the single proton in the compound was nonexchangeable. Representative infrared and nuclear magnetic resonance spectra are presented in Figures J1, J2, and J3.

The purity of lot S11C was determined by elemental analysis and gas chromatography (GC) by the analytical chemistry laboratory (system A) and the study laboratory (system B) (Table J1); the moisture content was determined by Karl Fischer titration.

Karl Fischer titration indicated less than 0.01% water. Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values for dibromoacetonitrile. Prior to the 2-week studies, GC by system A indicated one major peak and four impurities with peak area percents greater than or equal to 0.1% of the total peak area; the impurities had a combined area of 1.4% (0.3%, 0.6%, 0.4%, 0.1%). GC by system B indicated an area percent purity of 99.8% and a 94.6% purity relative to a frozen reference standard from the same lot. The overall purity was determined to be 98.6%.

Periodic reanalyses of the bulk chemical were performed by the study laboratory using GC by system B prior to the start of the 2-week studies, prior to and at the end of the 3-month studies, and prior to the study start, at least every 6 months thereafter, and at the end of the 2-year studies. No degradation of the bulk chemical occurred.

The bulk chemical was stored at room temperature in glass carboys, protected from light from February 15, 2000, until July 29, 2000, when storage was changed to 5° C to ensure stability.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared twice during the 2-week studies, four times during the 3-month studies, and approximately every 2 weeks throughout the 2-year studies. The dose formulations were prepared by mixing dibromoacetonitrile with tap water (Table J2). Formulations were stored at 5° C in glass bottles sealed with Teflon®-lined lids and protected from light.

The analytical chemistry laboratory performed stability studies of 5 and 30 µg/mL dose formulations in sealed stainless steel drums or in amber glass bottles with minimal headspace sealed with Teflon®-lined lids stored at approximately 25° and 5° C for up to 39 days and under simulated animal room conditions for 7 days. Samples were analyzed by GC using system C (Table J1). Stability was confirmed for 5 and 30 µg/mL dose formulations

stored at 5° C for at least 14 days; animal room samples were stable for 1 day and usable for 3 days with some degradation. These stability studies indicate that, over time, dibromoacetonitrile dose formulations at these concentrations will probably experience temperature dependent degradation.

Periodic analyses of the dose formulations of dibromoacetonitrile were conducted by the study laboratory using GC by system D. During the 2-week studies, the dose formulations were analyzed twice; eight of 10 dose formulations for rats and mice were within 10% of the target concentrations (Table J3). For the 3-month studies, dose formulations were analyzed at the beginning, middle, and end of the studies; all 20 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table J4). For the 2-year study, dose formulations were analyzed at least every 6 months; all 65 dose formulations analyzed for rats and mice were within 10% of the target concentrations (Table J5). Animal room samples were also analyzed; dose formulations were generally 80% or above the target concentrations, except during the 2-week studies where results as low as 70% of the target concentrations were attributed, in part, to poor recovery with the extraction method.

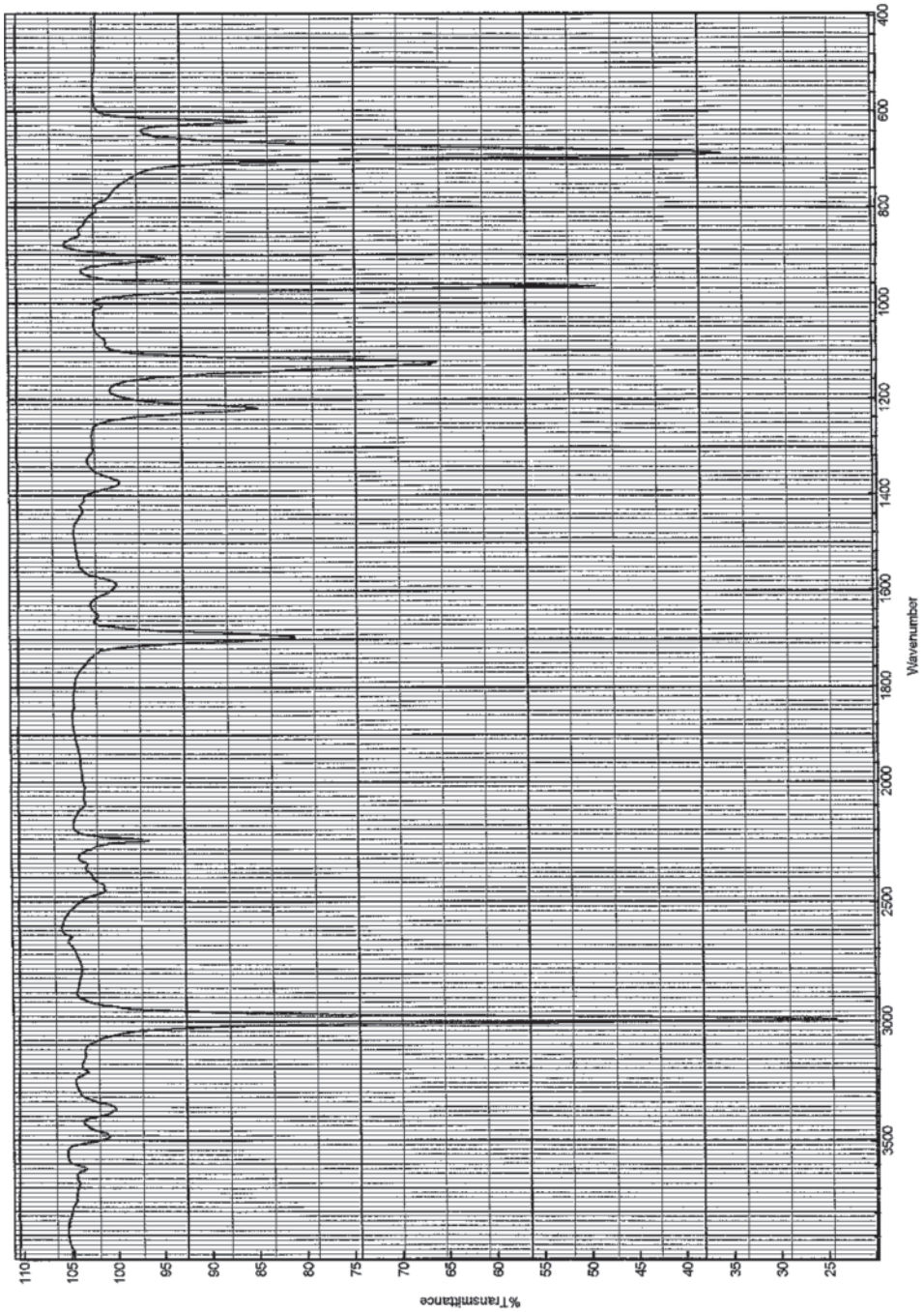

Figure J1
Infrared Absorption Spectrum of Dibromoacetonitrile

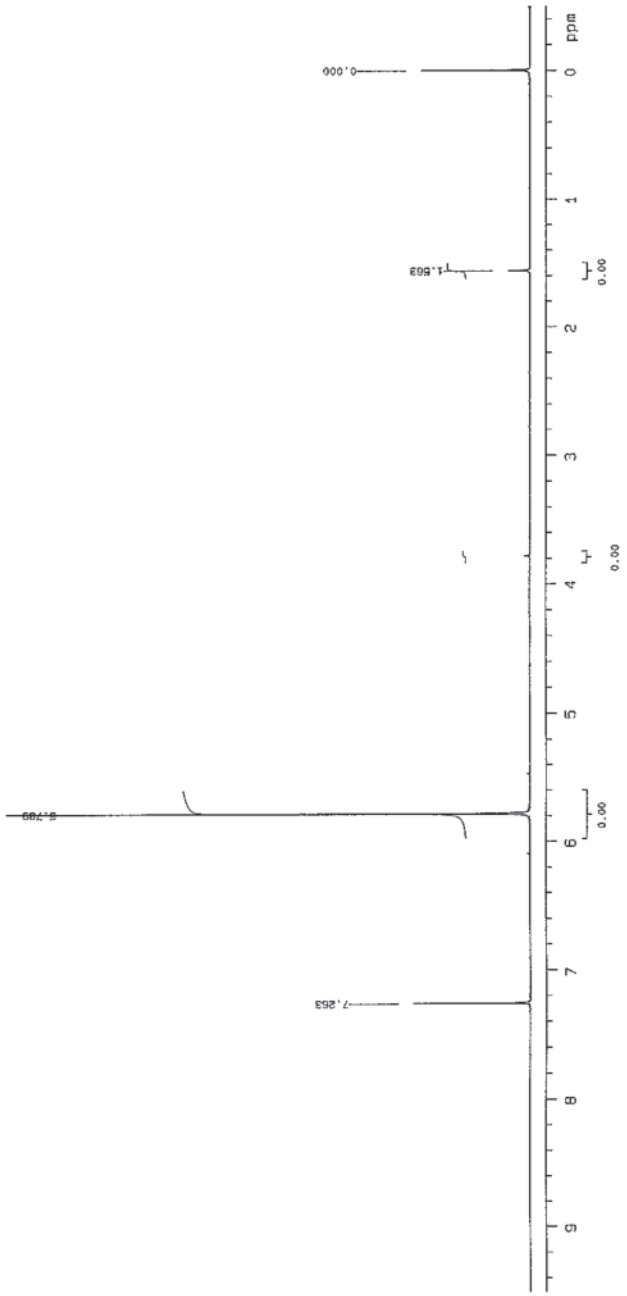

Figure J2
Proton Nuclear Magnetic Resonance Spectrum of Dibromoacetonitrile

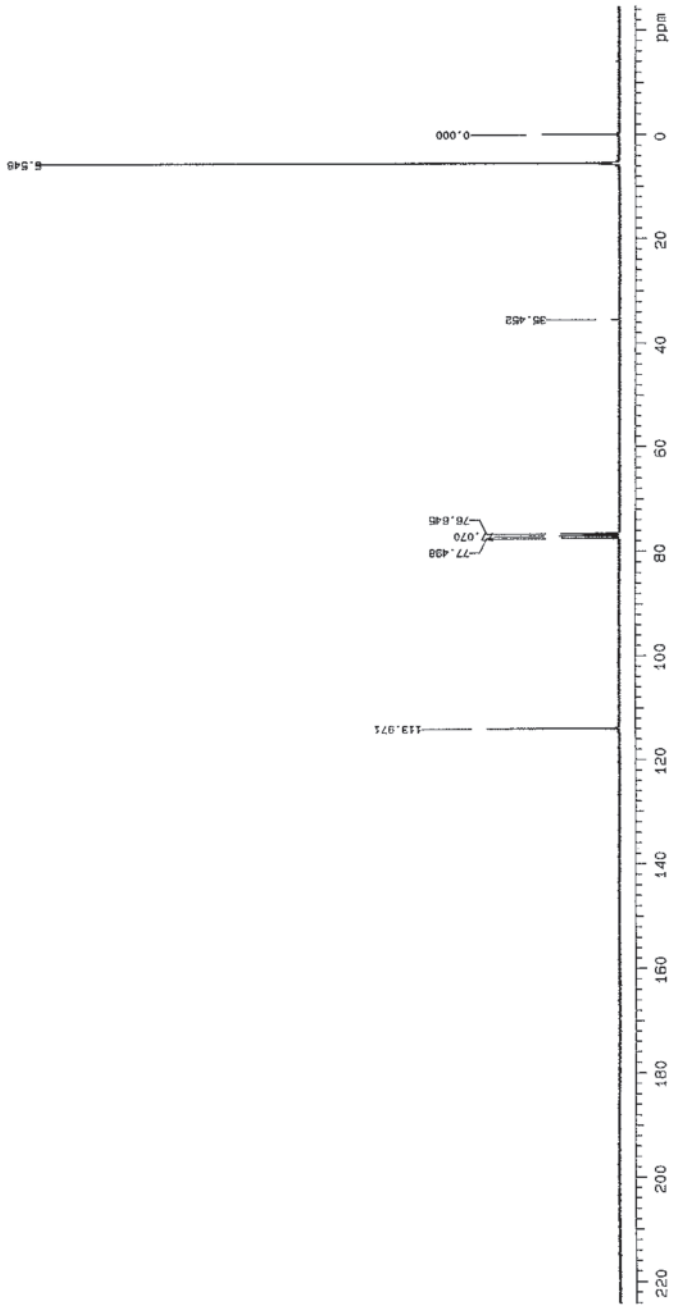

Figure J3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Dibromoacetonitrile

Table J1
Gas Chromatography Systems Used in the Drinking Water Studies of Dibromoacetonitrile[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Flame ionization	SPB-5, 30 m × 0.32 mm (0.25-µm film) (Supelco, Bellefonte, PA)	Helium at 3 mL/minute	35° C for 5 minutes, then 7° C/minute to 225° C
System B			
Flame ionization	Restek Rtx 5, 30 m × 0.53 mm (0.25-µm film) (Restek, Bellefonte, PA)	Helium at 5 mL/minute	35° C for 5 minutes, then 7° C/minute to 225° C
System C			
Electron capture	DB-5, 30 m × 0.53 mm (1.5-µm film) (J&W Scientific, Folsom, CA)	Helium at 4 mL/minute	70° C for 1 minute, then 3° C/minute to 100° C, held 8 minutes, then 70° C/minute to 150° C, held 3 minutes
System D			
Electron capture	Restek Rtx 5, 30 m × 0.53 mm (1.0-µm film) (Restek)	Nitrogen at 9 mL/minute	60° C for 2 minutes, then 5° C/minute to 100° C, held 5 minutes

[a] Gas chromatographs were manufactured by Hewlett-Packard, Palo Alto, CA.

Table J2
Preparation and Storage of Dose Formulations in the Drinking Water Studies of Dibromoacetonitrile

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
A premix was prepared by adding the appropriate amount of dibromoacetonitrile to tap water in a glass container; the container was sealed and stirred with a magnetic stir bar for at least 2 hours or until in solution. The premix was transferred to a mixing tank partially filled with tap water, filled to volume, and mixed for at least 30 minutes. Dose formulations were prepared twice during the study.	Same as 2-week studies. The dose formulations were prepared four times during the studies.	Same as 2-week studies. The dose formulations were prepared approximately every 2 weeks during the studies.
Chemical Lot Number		
S11C	S11C	S11C
Maximum Storage Time		
14 days	40 days	14 days
Storage Conditions		
Stored in glass bottles sealed with Teflon®-lined lids, protected from light, and refrigerated at 5° C	Stored in glass bottles sealed with Teflon®-lined lids, protected from light, and refrigerated at 5° C	Stored in glass bottles sealed with Teflon®-lined lids, protected from light, and refrigerated at 5° C
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

Table J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
September 19, 2000	September 20, 2000	12.5	11.7	–6
		25	26.2	+5
		50	48.8	–2
		100	93.1	–7
		200	189	–6
September 27, 2000	September 28-29, 2000	12.5	11.0	–12
		25	23.9	–4
		50	46.3	–7
		100	88.8	–11
		200	183	–9
Animal Room Samples				
Rats				
September 19, 2000	September 28, 2000	12.5	8.9	–29
		25	21.3	–15
		50	40.5	–19
		100	78.3	–22
		200	161	–20
September 27, 2000	October 10, 2000	12.5	10.2	–18
		25	22.4	–10
		50	43.9	–12
		100	87.5	–13
		200	177	–12
Mice				
September 19, 2000	September 28, 2000	12.5	8.38	–33
		25	20.3	–19
		50	38.4	–23
		100	69.6	–30
		200	154	–23

[a] Results of duplicate analyses

Table J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
December 7, 2000	December 8, 2000	12.5	12.5	0
		25	24.9	0
		50	48.5	–3
		100	95.1	–5
		200	193	–4
January 3, 2001	January 4-5, 2001	25	24.2	–3
		50	47.1	–6
		100	95.2	–5
		200	193	–4
January 9, 2001	January 16-17, 2001	12.5	11.9	–5
March 1, 2001	March 2, 2001	12.5	11.8	–6
		25	24.3	–3
		50	48.7	–3
		100	96.8	–3
		200	199	–1
Animal Room Samples				
Rats				
December 7, 2000	January 16-17, 2001	12.5	10.4	–17
		25	21	–16
		50	42.6	–15
		100	85.6	–14
		200	177	–12
January 9, 2001	February 13-14, 2001	12.5	9.66	–23
January 3, 2001	February 13-14, 2001	25	20.5	–18
		50	41.5	–17
		100	84.2	–16
		200	168	–16
March 1, 2001	March 22-23, 2001	12.5	10.0	–20
		25	21.7	–13
		50	45.5	–9
		100	90.5	–10
		200	186	–7

Table J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Animal Room Samples (continued)				
Mice				
December 7, 2000	January 16-17, 2001	12.5	10.3	–18
		25	21.4	–14
		50	41.9	–16
		100	83.6	–16
		200	172	–14
January 3, 2001	February 13-14, 2001	25	19.6	–22
		50	40.6	–19
		100	81.7	–18
		200	165	–18
January 9, 2001	February 13-14, 2001	12.5	9.53	–24
March 1, 2001	March 22-23, 2001	12.5	9.34	–25
		25	20.2	–19
		50	42.2	–16
		100	87.2	–13
		200	181	–10

[a] Results of duplicate analyses

Table J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration[a] (mg/L)	Difference from Target (%)
Rats and Mice				
August 23, 2001	August 24, 2001	50	49.8	0
		50	47.6	–5
		100	94	–6
		100	94.5	–6
		200	189	–6
		200	189	–6
October 18, 2001	October 19, 2001	50	52.0	+4
		50	50.1	0
		100	100	0
		200	191	–5
		200	195	–3
January 10, 2002	January 11, 2002	50	53.0	+6
		50	52.1	+4
		100	101	+1
		100	101	+1
		200	196	–2
		200	199	–1
March 7, 2002	March 11, 2002	50	49.2	–2
		50	48.9	–2
		100	99	–1
		100	98.5	–2
		200	190	–5
		200	191	–5
May 30, 2002	May 31, 2002	50	52.3	+5
		50	51.6	+3
		100	97.4	–3
		100	97.2	–3
		200	194	–3
		200	191	–5
July 24, 2002	July 26, 2002	50	48.6	–3
		50	50.9	+2
		100	97.3	–3
		100	100	0
		200	190	–5
		200	189	–6
October 15, 2002	October 16, 2002	50	52.6	+5
		50	50	0
		100	100	0
		100	101	+1
		200	200	0
		200	197	–2

Table J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Rats and Mice (continued)				
December 10, 2002	December 11, 2002	50	50.3	+1
		50	50.7	+1
		100	101	+1
		100	102	+2
		200	206	+3
		200	203	+2
March 4, 2003	March 5, 2003	50	49.4	–1
		50	50.6	+1
		100	95.8	–4
		100	94.1	–6
		200	187	–7
		200	189	–6
April 29, 2003	April 30-May 1, 2003	50	51.1	+2
		50	48.1	–4
		100	98.8	–1
		100	100	0
		200	197	–2
		200	202	+1
July 22, 2003	July 23-24, 2003	50	51.0	+2
		50	51.9	+4
		100	101	+1
		100	101	+1
		200	205	+3
		200	197	–2
Animal Room Samples				
Rats				
August 23, 2001	September 17, 2001	50	46.3	–7
		100	89.1	–11
		200	176	–12
March 7, 2002	April 1, 2002	50	45.6	–9
		100	94.7	–5
		200	182	–9
October 15, 2002	November 11, 2002	50	42.4	–15
		100	83	–17
		200	163	–19
April 29, 2003	May 26, 2003	50	40.3	–19
		100	77.6	–22
		200	155	–23

Table J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Drinking Water Studies of Dibromoacetonitrile

Date Prepared	Date Analyzed	Target Concentration (mg/L)	Determined Concentration (mg/L)	Difference from Target (%)
Animal Room Samples (continued)				
Mice				
August 23, 2001	September 17, 2001	50	45.9	–8
		100	89.8	–10
		200	170	–15
March 7, 2002	April 1, 2002	50	45.6	–9
		100	91.2	–9
		200	178	–11
October 15, 2002	November 11, 2002	50	41.7	–17
		100	85.2	–15
		200	167	–17
April 29, 2003	May 26, 2003	50	39.7	–21
		100	77.7	–22
		200	160	–20

[a] Results of duplicate analyses

APPENDIX K
WATER AND COMPOUND CONSUMPTION IN THE 2-YEAR DRINKING WATER STUDIES OF DIBROMOACETONITRILE

Table K1 Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile 182

Table K2 Water and Compound Consumption by Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile 183

Table K3 Water and Compound Consumption by Male Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile 184

Table K4 Water and Compound Consumption by Female Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile 185

Table K1
Water and Compound Consumption by Male Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L		50 mg/L			100 mg/L			200 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	15.1	94	14.0	94	7	11.1	94	12	8.5	94	18
2	15.3	132	14.2	133	5	13.0	128	10	11.5	122	19
3	17.5	164	16.0	166	5	14.7	162	9	13.4	156	17
4	17.9	193	16.6	196	4	15.2	191	8	13.3	185	14
5	17.9	217	16.2	220	4	15.1	214	7	13.4	208	13
6	18.1	239	16.6	241	3	15.4	235	7	14.1	231	12
7	17.8	258	17.0	259	3	15.2	250	6	14.6	246	12
8	18.7	270	16.6	272	3	16.0	264	6	15.0	262	11
9	18.9	285	16.0	286	3	15.3	277	6	14.6	276	11
10	18.9	300	16.3	300	3	15.9	290	6	14.2	289	10
11	19.0	311	17.3	311	3	15.1	299	5	14.1	299	9
12	19.8	319	17.1	322	3	15.6	309	5	15.0	308	10
13	19.9	327	17.1	330	3	15.4	315	5	15.2	312	10
17	17.3	352	16.2	357	2	15.3	339	5	14.6	337	9
22	15.9	384	15.2	386	2	14.2	371	4a	13.1	364	7
25	16.9	400	15.6	399	2	14.7	385	4	14.1	380	7
29	16.3	413	14.9	413	2	14.3	397	4	13.4	391	7
34	16.4	437	15.0	434	2	14.2	420	3	13.1	412	6
37	15.6	441	15.9	439	2	14.1	425	3	13.1	415	6
41	15.6	453	15.2	451	2	14.1	435	3	14.6	428	7
45	15.6	465	14.3	461	2	13.6	445	3	12.7	436	6
49	15.7	472	14.7	468	2	13.7	451	3	13.1	441	6
53	15.6	480	14.5	473	2	13.6	457	3	13.6	448	6
57	16.4	489	14.8	483	2	13.9	466	3	13.8	459	6
61	15.8	487	15.0	481	2	14.0	467	3	13.5	454	6
65	16.1	494	14.6	489	2	13.8	474	3	12.8	462	6
69	15.7	500	14.2	492	1	13.6	476	3	12.1	464	5
73	15.7	502	14.7	495	2	13.5	484	3	12.5	466	5
77	16.2	500	15.0	489	2	13.9	478	3	13.1	464	6
81	16.6	504	14.7	494	2	13.6	476	3	13.2	464	6
85	17.0	504	15.3	495	2	14.5	481	3	13.1	466	6
89	17.1	497	16.0	495	2	15.0	472	3	14.1	457	6
93	17.7	497	15.7	496	2	14.6	472	3	14.1	462	6
97	16.5	502	14.3	492	2	14.0	473	3	12.1	457	5
101	17.1	488	15.3	491	2	14.3	466	3	12.6	457	6
Mean for weeks											
1-13	18.1	239	16.2	241	4	14.9	233	7	13.6	230	13
14-52	16.1	424	15.2	423	2	14.2	408	4	13.5	400	7
53-101	16.4	496	14.9	490	2	14.0	473	3	13.1	460	6

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

Table K2
Water and Compound Consumption by Female Rats in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L		50 mg/L			100 mg/L			200 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	12.5	86	11.1	87	6	9.3	86	11	7.4	87	17
2	12.3	119	11.5	121	5	10.2	117	9	9.2	114	16
3	13.5	133	12.7	135	5	11.3	133	9	9.6	128	15
4	13.0	142	12.3	144	4	11.3	141	8	9.4	138	14
5	12.5	152	11.7	153	4	10.3	150	7	9.0	146	12
6	12.9	160	11.9	161	4	10.6	157	7	9.6	155	12
7	13.2	167	12.3	168	4	10.6	164	7	9.4	161	12
8	13.2	171	12.1	173	4	11.3	170	7	9.8	165	12
9	13.2	175	11.5	176	3	10.9	174	6	10.2	168	12
10	13.0	181	11.4	182	3	11.5	178	7	9.5	173	11
11	12.8	184	11.4	186	3	11.0	183	6	9.5	176	11
12	12.7	184	11.6	187	3	10.6	182	6	9.3	177	11
13	12.6	192	11.0	193	3	9.4	187	5	9.4	184	10
17	11.9	199	11.2	202	3	10.3	200	5	10.2	191	11
22	10.9	210	10.7	210	3	9.3	208	5	8.6	203	9
25	11.8	216	11.1	216	3	9.4	213	4	9.0	206	9
29	10.8	223	10.3	224	2	9.5	222	4	8.9	215	8
34	11.2	233	10.4	235	2	9.5	231	4	8.6	223	8
37	10.5	239	11.1	242	2	9.7	237	4	8.6	228	8
41	11.2	247	10.6	249	2	11.0	243	5	10.1	238	9
45	10.8	256	10.2	257	2	9.6	252	4	8.5	244	7
49	11.1	263	10.2	266	2	9.2	258	4	8.4	248	7
53	10.7	271	9.6	272	2	9.6	266	4	9.5	259	7
57	11.9	276	11.1	282	2	10.3	275	4	9.6	267	7
61	11.5	288	10.6	290	2	9.6	281	3	9.2	273	7
65	11.5	298	10.9	301	2	9.9	293	3	9.8	282	7
69	11.5	306	10.8	311	2	9.4	301	3	9.0	290	6
73	11.0	310	10.8	319	2	9.7	308	3	8.9	298	6
77	11.7	311	11.3	320	2	10.3	310	3	10.3	296	7
81	11.5	319	11.5	327	2	10.6	319	3	10.0	302	7
85	12.5	325	11.0	329	2	11.0	324	3	10.3	307	7
89	12.8	326	11.7	335	2	11.6	325	4	10.9	307	7
93	14.6	328	12.7	337	2	11.8	327	4	11.5	311	7
97	13.4	335	11.9	343	2	11.0	337	3	10.8	318	7
101	14.0	338	12.3	347	2	11.0	337	3	11.3	317	7
Mean for weeks											
1-13	12.9	157	11.7	159	4	10.6	156	7	9.3	152	13
14-52	11.1	232	10.6	233	2	9.7	229	4	9.0	222	8
53-101	12.2	310	11.3	316	2	10.5	308	3	10.1	294	7

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

Table K3
Water and Compound Consumption by Male Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L		50 mg/L			100 mg/L			200 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	3.8	20.6	3.2	20.8	8	2.2	21.3	10	2.0	20.8	19
2	3.3	22.7	3.0	22.4	7	2.8	22.2	13	2.2	21.3	21
3	3.5	24.6	3.1	24.5	6	2.9	24.1	12	2.4	23.4	21
4	3.7	25.9	3.6	25.9	7	2.8	25.3	11	2.6	25.1	21
5	3.6	27.3	3.3	27.1	6	3.0	26.5	11	2.6	26.1	20
6	4.2	28.2	3.6	28.3	6	3.2	27.7	12	3.0	27.4	22
7	3.9	30.1	3.6	29.8	6	3.2	29.0	11	2.7	28.6	19
8	3.6	31.0	3.3	30.9	5	3.0	30.0	10	2.5	29.6	17
9	3.8	32.5	3.3	32.1	5	3.0	31.5	10	2.6	30.8	17
10	3.9	32.7	3.5	32.4	5	3.0	31.3	10	2.7	30.7	18
11	4.6	34.7	3.6	34.0	5	3.1	32.9	9	2.7	31.1	17
12	2.5	36.1	2.9	35.6	4	3.0	34.7	9	2.7	32.9	16
13	3.9	37.6	3.4	37.1	5	3.3	36.0	9	2.7	34.0	16
17	3.7	41.7	3.3	41.4	4	3.1	39.8	8	2.7	37.9	14
21	3.6	45.1	3.1	43.9	4	3.1	42.2	7	2.6	39.3	13
25	3.5	46.9	3.0	46.2	3	2.7	45.0	6	2.2	41.5	11
29	3.4	48.4	2.9	47.5	3	2.5	45.8	6	2.3	43.4	11
33	3.6	49.4	3.1	48.7	3	2.7	47.5	6	2.3	44.8	10
37	3.8	49.5	3.3	48.6	3	3.0	48.0	6	2.3	44.8	10
41	4.0	51.2	3.4	50.5	3	2.8	49.4	6	2.4	47.8	10
45	4.3	52.1	3.4	51.5	3	3.0	50.5	6	2.5	49.2	10
49	4.5	53.3	3.4	52.3	3	2.9	51.7	6	2.4	49.9	10
53	4.2	53.3	3.4	52.7	3	2.8	51.6	5	2.4	49.7	10
57	4.8	53.3	3.7	52.7	4	3.1	51.5	6	2.7	49.0	11
61	4.9	53.5	3.8	52.7	4	3.1	51.9	6	2.7	49.8	11
65	4.8	53.5	3.7	52.7	4	3.0	51.5	6	2.6	50.0	10
69	5.2	54.0	3.8	52.9	4	3.2	51.3	6	2.6	50.0	10
73	5.2	54.0	3.8	52.9	4	3.2	51.4	6	2.4	49.8	10
77	5.1	53.7	3.8	52.5	4	3.3	51.3	6	2.9	49.6	12
81	5.6	53.1	4.0	51.9	4	3.5	50.5	7	3.0	49.2	12
85	5.5	52.8	3.8	51.6	4	4.0	51.2	8	2.7	49.2	11
89	5.6	50.9	3.9	49.5	4	3.5	50.8	7	3.0	47.2	13
93	5.0	50.3	4.0	48.9	4	3.4	48.7	7	3.1	45.3	14
97	4.7	50.3	3.9	48.2	4	3.6	48.9	7	3.3	45.0	15
101	4.9	50.6	3.4	47.5	4	3.3	47.9	7	3.1	44.1	14
Mean for weeks											
1-13	3.7	29.5	3.3	29.3	6	3.0	28.7	11	2.6	27.8	19
14-52	3.8	48.6	3.2	47.8	3	2.9	46.7	6	2.4	44.3	11
53-101	5.0	52.6	3.8	51.3	4	3.3	50.7	7	2.8	48.3	12

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

Table K4
Water and Compound Consumption by Female Mice in the 2-Year Drinking Water Study of Dibromoacetonitrile

	0 mg/L		50 mg/L			100 mg/L			200 mg/L		
Week	Water (g/day)[a]	Body Weight (g)	Water (g/day)	Body Weight (g)	Dose (mg/kg)[b]	Water (g/day)	Body Weight (g)	Dose (mg/kg)	Water (g/day)	Body Weight (g)	Dose (mg/kg)
1	2.8	17.8	2.5	17.8	7	2.5	17.4	14	1.6	17.6	18
2	2.7	18.9	2.3	18.7	6	2.1	18.7	11	1.7	18.0	19
3	2.9	20.3	2.6	19.7	7	2.3	19.8	12	2.2	19.4	23
4	3.1	21.1	2.5	20.8	6	2.3	20.7	11	1.8	19.8	18
5	3.0	22.1	3.0	21.9	7	2.5	21.3	12	2.0	21.0	19
6	3.5	22.8	3.0	22.1	7	2.6	22.0	12	2.4	21.3	23
7	3.3	24.0	2.8	23.3	6	2.7	22.8	12	2.5	22.2	23
8	3.2	24.8	3.2	23.5	7	2.9	23.6	12	2.1	22.9	18
9	3.4	26.1	3.2	24.9	6	2.8	24.2	12	2.4	23.3	21
10	3.6	26.5	3.0	25.8	6	2.9	24.9	12	2.2	24.2	18
11	3.7	27.8	3.0	26.7	6	2.8	25.7	11	2.3	24.9	19
12	2.3	29.3	2.0	27.5	4	2.9	26.8	11	2.4	25.9	19
13	3.6	30.9	3.3	28.8	6	2.9	27.6	11	2.6	27.2	19
17	3.1	35.6	2.7	33.8	4	3.1	31.8	10	3.1	30.9	20
21	3.1	39.3	2.7	36.8	4	3.1	36.0	9	2.4	33.4	14
25	2.6	43.6	2.6	40.0	3	2.2	38.4	6	1.8	35.9	10
29	3.0	46.5	2.4	42.8	3	2.1	41.9	5	2.1	38.8	11
33	2.5	48.7	2.6	45.3	3	2.2	44.2	5	2.0	41.7	10
37	2.6	51.7	2.2	48.0	2	2.3	46.8	5	1.9	43.5	9
41	2.7	54.3	2.7	50.8	3	2.2	49.4	5	1.8	46.2	8
45	2.6	55.8	2.3	53.2	2	2.2	51.8	4	1.9	49.1	8
49	2.9	57.2	2.3	54.7	2	2.5	55.1	5	2.0	51.7	8
53	2.9	57.5	2.3	56.5	2	2.6	56.3	5	2.2	52.8	8
57	2.8	57.9	2.6	57.1	2	2.3	55.9	4	2.0	53.5	8
61	2.7	58.6	2.2	56.5	2	2.3	57.4	4	2.1	54.0	8
65	2.6	58.4	2.3	56.6	2	2.2	58.1	4	1.9	54.6	7
69	2.8	59.2	2.6	57.8	2	2.2	58.2	4	2.0	55.6	7
73	2.8	59.7	2.2	58.7	2	2.5	59.6	4	1.9	56.9	7
77	2.9	59.5	2.7	58.7	2	2.4	59.6	4	2.2	56.7	8
81	3.1	60.0	2.7	57.9	2	2.5	58.9	4	2.9	57.1	10
85	2.8	60.9	2.5	58.5	2	2.6	60.5	4	2.0	57.0	7
89	2.8	59.2	2.5	57.8	2	2.3	58.2	4	1.8	55.6	7
93	2.8	58.1	2.5	57.1	2	2.2	57.1	4	2.0	53.8	7
97	3.3	57.7	2.6	57.4	2	2.5	57.1	4	2.1	54.5	8
101	2.8	56.1	2.5	56.5	2	2.1	56.3	4	1.9	55.1	7
Mean for weeks											
1-13	3.2	24.0	2.8	23.2	6	2.6	22.7	12	2.2	22.1	20
14-52	2.8	48.1	2.5	45.0	3	2.4	43.9	6	2.1	41.2	11
53-101	2.9	58.7	2.5	57.5	2	2.4	57.9	4	2.1	55.2	8

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

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

Table L1 Ingredients of NTP-2000 Rat and Mouse Ration 188
Table L2 Vitamins and Minerals in NTP-2000 Rat and Mouse Ration 188
Table L3 Nutrient Composition of NTP-2000 Rat and Mouse Ration 189
Table L4 Contaminant Levels in NTP-2000 Rat and Mouse Ration 190

Table L1
Ingredients of NTP-2000 Rat and Mouse Ration

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

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

Table L2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

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

[a] Per kg of finished product

Table L3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.7 ± 0.58	13.7 – 15.7	25
Crude fat (% by weight)	8.1 ± 0.26	7.6 – 8.6	25
Crude fiber (% by weight)	9.0 ± 0.45	8.0 – 9.9	25
Ash (% by weight)	5.2 ± 0.28	4.7 – 5.8	25
Amino Acids (% of total diet)			
Arginine	0.750 ± 0.048	0.670 – 0.850	15
Cystine	0.225 ± 0.025	0.150 – 0.250	15
Glycine	0.701 ± 0.039	0.620 – 0.750	15
Histidine	0.365 ± 0.090	0.310 – 0.680	15
Isoleucine	0.533 ± 0.038	0.430 – 0.590	15
Leucine	1.077 ± 0.059	0.960 – 1.150	15
Lysine	0.703 ± 0.125	0.310 – 0.830	15
Methionine	0.402 ± 0.049	0.260 – 0.460	15
Phenylalanine	0.615 ± 0.035	0.540 – 0.660	15
Threonine	0.492 ± 0.040	0.430 – 0.590	15
Tryptophan	0.135 ± 0.018	0.110 – 0.160	15
Tyrosine	0.378 ± 0.048	0.280 – 0.460	15
Valine	0.658 ± 0.043	0.550 – 0.710	15
Essential Fatty Acids (% of total diet)			
Linoleic	3.90 ± 0.256	3.49 – 4.54	15
Linolenic	0.30 ± 0.035	0.21 – 0.35	15
Vitamins			
Vitamin A (IU/kg)	4,804 ± 818	3,060 – 6,920	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 – 110.0	15
Thiamine (ppm)[b]	7.6 ± 1.10	5.9 – 9.2	25
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.26 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	1.007 ± 0.050	0.873 – 1.110	25
Phosphorus (%)	0.608 ± 0.036	0.555 – 0.701	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

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

Table L4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.37 ± 0.151	0.18 – 0.50	25
Cadmium (ppm)	0.04 ± 0.015	0.04 – 0.09	25
Lead (ppm)	0.07 ± 0.026	0.05 – 0.17	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.22 ± 0.055	0.14 – 0.36	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	14.7 ± 3.73	7.88 – 23.2	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	28 ± 71	10 – 360	25
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.3 ± 1.51	2.3 – 8.4	25
N-Nitrosodimethylamine (ppb)[e]	2.8 ± 1.38	1.2 – 6.9	25
N-Nitrosopyrrolidine (ppb)[e]	1.6 ± 0.63	0.9 – 3.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.107 ± 0.063	0.020 – 0.259	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.319 ± 0.470	0.020 – 1.850	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

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

APPENDIX M
SENTINEL ANIMAL PROGRAM

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SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from five male and five female control rats and mice at the end of the 3-month studies, from up to five male and five female sentinel rats and mice at 6, 12, and 18 months in the 2-year studies, and from five male and five female rats and mice in the 200 mg/L groups at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and analyzed at BioReliance Corporation (Rockville, MD) for determination of antibody titers. Fecal samples were taken from sentinel mice at 18 months. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
RATS	
3-Month Study	
ELISA	
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	12 and 18 months, study termination
Mycoplasma pulmonis	12 and 18 months, study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
M. arthritidis	12 months
Parvovirus	6, 12, and 18 months, study termination
PVM	6 months

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

RESULTS

All test results were negative.