FINAL

Report on Carcinogens Background Document for

2,2-bis(Bromomethyl)-1,3-propanediol (Technical Grade)

Meeting of the
NTP Board of Scientific Counselors
Report on Carcinogens Subcommittee

Prepared for the:
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Public Health Services
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Criteria for Listing Agents, Substances or Mixtures in the Report on Carcinogens

US Department of Health and Human Services
National Toxicology Program

Known to be Human Carcinogens:

There is sufficient evidence of carcinogenicity from studies in humans which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated to be Human Carcinogens:

There is limited evidence of carcinogenicity from studies in humans which indicates that causal interpretation is credible but that alternative explanations such as chance, bias or confounding factors could not adequately be excluded; or

There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species, or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor or age at onset; or

There is less than sufficient evidence of carcinogenicity in humans or laboratory animals, however; the agent, substance or mixture belongs to a well defined, structurally-related class of substances whose members are listed in a previous Report on Carcinogens as either a *known to be human carcinogen, or reasonably anticipated to be human carcinogen* or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgment, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

Summary Statement

Technical grade 2,2-bis(bromomethyl)-1,3-propanediol (~79% 2,2-bis[bromomethyl]-1,3-propanediol, ~7% 2,2-bis[hydroxymethyl]-1-bromo-3-hydroxypropane, ~7% 2,2-bis[bromomethyl]-1-bromo-3-hydroxypropane, ~0.2% pentaerythritol, and ~8% dimers and structural isomers)(BBMP)

Carcinogenicity

The flame retardant 2,2-bis(bromomethyl)-1,3-propanediol, technical grade (BBMP) is *reasonably anticipated to be a human carcinogen* based on evidence of tumor induction at multiple organ sites in rats and mice. Two-year dietary studies of the flame retardant BBMP showed a significant increase in the incidence of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, and seminal vesicle and in the incidence of mononuclear cell leukemia in male F344 rats; in the incidence of neoplasms of the oral cavity, esophagus, mammary gland, and thyroid gland in female F344 rats; in the incidence of neoplasms of the Harderian gland, lung, and kidney in male $B6C3F_1$ mice; and in the incidence of neoplasms of the Harderian gland, lung, and subcutaneous tissue in female $B6C3F_1$ mice (NTP 1996; Dunnick *et al.* 1997).

In a stop-exposure study, BBMP was administered in the feed to male F344 rats for three months, followed by maintenance on control diet for up to two years. Neoplasms were observed at the same sites as in the two-year continuous-exposure study of male F344 rats. The incidences of neoplasms in the stop-exposure study were greater than in the continuous-exposure study for the oral cavity, forestomach, small intestine, large intestine, lung, Zymbal gland, thyroid gland, and mesothelium and were considered to be related to treatment (NTP 1996; Dunnick *et al.* 1997).

No case reports or epidemiological studies of the occurrence of human cancer and exposure to BBMP were available.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

BBMP was shown to be mutagenic *in vitro* and *in vivo*, but special conditions were required to induce mutagenicity. BBMP is mutagenic in *Salmonella typhimurium* strains TA100 and TA1535 only when tested in the presence of 30% S9 liver homogenate from induced hamsters (Zeiger *et al*. 1992). In cultured CHO cells, BBMP induced chromosomal aberrations (CA) only in the presence of S9; no induction of sister chromatid exchanges (SCE) was observed with or without S9 mix. *In vivo* exposure to BBMP induced significant increases in the frequencies of micronucleated erythrocytes in male and female mice under varying conditions (MacGregor *et al*. 1990, cited in NTP 1996).

No data are available that would suggest that mechanisms thought to account for tumor induction by BBMP in experimental animals would not also operate in humans.

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1 Introduction

The flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (BBMP) was nominated for listing in the Report on Carcinogens by the NIEHS Report on Carcinogens (RoC) Review Group (RG1) based on the results of a dosed-feed study reported in a 1996 National Toxicology Program (NTP) bioassay technical report that indicated clear evidence of carcinogenicity in rats and mice.

1.1 Chemical identification

The flame retardant BBMP (FR-1138) is a technical-grade mixture of 78.6% 2,2-bis(bromomethyl)-1,3-propanediol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers (NTP 1996). 2,2-bis(Bromomethyl)-1,3-propanediol, the major component of BBMP ($C_5H_{10}Br_2O_2$, mol wt 261.94, CASRN 3296-90-0) also is known by the following names:

dibromoneopentyl glycol
bisbromomethylpropanediol
bis(bromomethyl) propanol
dibromopentaerythritol
pentaerythritol dibromide
pentaerythritol dibromohydrin
dibromohydrin pentaerythritol
1,3-dibromo-2,2-dimethylolpropane.

BBMP is a white solid with a slight musty odor. It is used as a flame retardant for epoxy, polyester, and urethane foams. It also is used as a chemical intermediate for pentaerythritol. The structure of BBMP is illustrated in Figure 1-1.

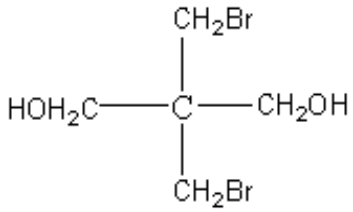

Figure 1-1. Structure of BBMP

1.2 Physical-chemical properties

The RTECS number for BBMP is TY3195500, and its physical and chemical properties are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of BBMP

Property	Information	Reference
Molecular weight	261.94	Budavari *et al*. (1996)
Physical state	off-white powder	Budavari *et al*. (1996)
Odor	mild musty odor	NTP (1996)
Melting point (^{o}C) at 750 mm	109 - 110	Chemfinder (1999)
Boiling point (^{o}C) at 750 mm	235	Budavari *et al*. (1996)
Flash point (^{o}C)	nonflammable	MRI (1978)
Specific gravity	2.2	Dow Chemical (1975)
Solubility in: Water Dimethylsulfoxide 95% Ethanol Acetone Methanol Toluene	 < 1 mg/mL at 19°C ≥ 100 mg/mL at 21°C ≥ 100 mg/mL at 21°C ≥ 100 mg/mL at 21°C ≥ 102.4 g/100g at 20°C 0.6 g/100g at 25°C	 Radian (1991) Radian (1991) Radian (1991) Radian (1991) Miller (1977) Miller (1977)

BBMP is unique in that the aliphatic neopentyl structure contains no hydrogen atoms on the carbon atom adjacent to the carbon bonded to bromine. This results in the compound's being very resistant to dehydrobromination. The remaining hydroxyl groups are reactive sites that allow for polymerization. These –OH groups readily react with organic acids or epoxides to form esters and with isocyanates to form urethanes. BBMP also can react with aldehydes and ketones to form cyclic acetals or ketals, or with phosphorous oxyhalides to form cyclic phosphates or phosphites (Larsen 1969; Larsen and Weaver 1973, both cited in NTP 1996).

The physical and chemical properties of the other components of the flame retardant BBMP (FR-1138), (2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, and pentaerythritol) are summarized in Tables 1-2, 1-3, and 1-4. The chemical structures for these components are illustrated in Figures 1-2, 1-3, and 1-4, respectively.

Table 1-2. Physical and chemical properties of 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane

Property	Information	Reference
Molecular weight	199.04	Chemfinder (1999)
Physical state	np	–
Melting point (^{o}C) at 750 mm	109 - 110	Chemfinder (1999)
Boiling point (^{o}C) at 750 mm	235	Budavari *et al*. (1996)
Flash point (^{o}C)	nonflammable	MRI (1978)
Specific Gravity	2.2	Dow Chemical (1975)

Property	Information	Reference
Solubility in:		
Water	< 1 mg/mL at 19°C	Radian (1991)
Dimethylsulfoxide	≥ 100 mg/mL at 21°C	Radian (1991)
95% Ethanol	≥ 100 mg/mL at 21°C	Radian (1991)
Acetone	≥ 100 mg/mL at 21°C	Radian (1991)
Methanol	≥ 102.4 g/100g at 20°C	Miller (1977)
Toluene	0.6 g/100g at 25°C	Miller (1977)

np: not published

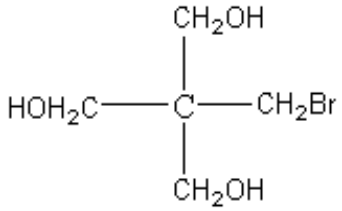

Figure 1-2. Structure of 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane

Table 1-3. Physical and chemical properties of 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane

Property	Information	Reference
Molecular weight	324.84	Chemfinder (1999)
Physical state	white solid	Budavari *et al*. (1996)
Solubility in:		
Water	< 0.1 g/100mL at 21.5°C	Chemfinder (1999)

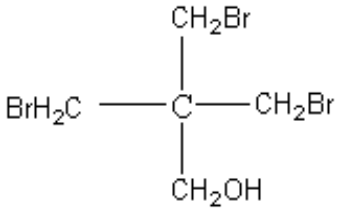

Figure 1-3. Structure of 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane

Table 1-4. Physical and chemical properties of pentaerythritol

Property	Information	Reference
Molecular weight	136.15	Chemfinder (1999)
Physical state	colorless to white crystalline powder	HSDB (1992)
Melting point (^{o}C) at 750 mm	255 - 259	Chemfinder (1999)
Boiling point (^{o}C) at 30 mm	276	Chemfinder (1999)
Flash point (^{o}C)	240	Chemfinder (1999)
Specific gravity	1.396	Chemfinder (1999)

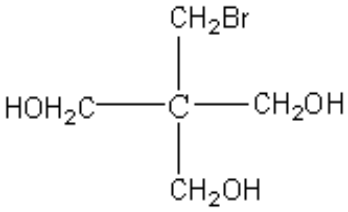

Figure 1-4. Structure of pentaerythritol

2 Human Exposure

2.1 Use

BBMP is used as a flame retardant in unsaturated polyester resins, for molded products, and in the production of rigid polyurethane foam. BBMP also is used to produce the flame retardant, FR-1138. It also is used as a chemical intermediate for pentaerythritol ethers and other derivatives used as flame retardants (Radian 1991; HSDB 1998; NTP 1996).

2.2 Production

Dow Chemical Co., of Midland, MI, was the largest producer of BBMP until the late 1980s. The current producer of BBMP is Albemarle Co., of Baton Rouge, LA. It was estimated that U.S. production in 1977 and 1979 was greater than 2.27×10^6 g (5000 pounds) (SRI 1977, 1979; cited by HSDB 1998). In 1983, U.S. Environmental Protection Agency (EPA) estimated BBMP production to be 3 to 4 million pounds per year (U.S. EPA 1983, cited in NTP 1996). U.S. EPA listed BBMP in the high production volume (HPV) program chemical list, identifying BBMP as being manufactured in or imported into the United States in amounts equal to or greater than 1 million pounds per year. The 1990 HPV list identified BBMP manufacture and importation at 2.21 to 2.95×10^6 lb/yr (U.S. EPA 1990).

2.3 Environmental occurrence

BBMP is found in nature only when it is released into the environment by industry (HSDB 1998). BBMP may enter the environment as fugitive dust and through wastewater (NTP 1996). BBMP was not identified as being released by industry into the environment through the Toxic Release Inventory (TRI 1996).

2.4 Environmental fate

BBMP is expected to remain in water for long periods of time (NTP 1996). No other environmental fate data could be found for BBMP.

2.5 Environmental exposure

The primary modes of potential human exposure to BBMP are inhalation, oral, and dermal contact. Consumer exposure may occur as a result of releases from products containing BBMP.

2.6 Occupational exposure

Occupational exposure to BBMP may occur in industries where it is used as a flame retardant in unsaturated polyester resins, in molded products, and in rigid polyurethane foam (NTP 1996). The National Institute of Occupational Safety and Health (NIOSH) did not survey BBMP to determine occupational exposure (NIOSH 1995, cited in NTP 1996).

2.7 Regulations

The U.S. EPA regulates BBMP under the Toxic Substances Control Act (TSCA). Table 2-1 summarizes the U.S. EPA health and safety data reporting regulations.

Table 2-1. U.S. EPA Regulations

U.S. EPA Regulations	
Regulatory action	Effect of regulation and other comments
40 CFR 716 – PART 716 – HEALTH AND SAFETY DATA REPORTING. Promulgated: 51 FR 32726, 09/15/86. U.S. Codes: 15 U.S.C. 2607(d). 2,2-Bis(bromomethyl)-1,3-propanediol has an effective date of 6/1/87 and a sunset date of 12/19/95.	This subpart sets forth requirements for the submission of lists and copies of health and safety studies on chemical substances and mixtures selected for priority consideration for testing rules under section 4(a) of TSCA and on other chemical substances and mixtures for which U.S. EPA requires health and safety information in fulfilling the purposes of TSCA.

Source: The regulations in this table have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1996; 21 CFR, April 1, 1996; 29 CFR, July 1, 1996.

3 Human Cancer Studies

There were no case reports or epidemiological studies on the occurrence of human cancer and exposure to BBMP or FR-1138.

4 Studies of Cancer in Experimental Animals

4.1 Carcinogenesis studies of BBMP

4.1.1 Carcinogenicity studies in rats

In a study conducted by the Dow Chemical Co., BBMP (FR-1138) was administered in the diet to male and female Sprague-Dawley rats for two years. The test substance (FR-1138) contained 80% 2,2-bis[bromomethyl]-1,3-propanediol, 8% 3-bromo-2,2-bis(bromomethyl)propanol, and 6% 2-(bromomethyl)-2-(hydroxymethyl)-1,3-propanediol. Dietary concentrations of BBMP were sufficient to deliver daily doses of 0, 5, or 100 mg/kg/day. These doses are equivalent to 0, 0.2, and 2.9%, respectively, of the BBMP oral LD_{50} in male rats. The number of rats used for the 0, 5, or 100 mg/kg/day doses were 48, 50, or 50, respectively, for females and 50 for controls and per dose group for males. BBMP administration had no effect on food consumption, weight gain, clinical signs, or survival of the rats, suggesting that the animals may have been able to tolerate higher doses. At the termination of the study, representative samples of all major organs of all surviving rats were necropsied. Upon statistical analysis of tumors found in both control and treated groups of rats, it was concluded that no treatment-related neoplasms were observed in rats of either sex (Keyes *et al.* 1980).

In a range-finding study conducted by the NTP, technical grade BBMP, as the commercial flame retardant FR-1138 (78.6% 2,2-bis[bromomethyl]-1,3-propanediol, 6.6% 2,2-bis[hydroxymethyl]-1-bromo-3-hydroxypropane, 6.9% 2,2-bis[bromomethyl]-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers), was administered in the diet at concentrations of 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm to F344/N rats (10/sex) for 13 weeks.. Based upon food consumption, average daily doses of BBMP in the 13-week study were 100, 200, 400, 800, and 1700 mg/kg-body weight for males, and 100, 200, 400, 800, and 1,630 mg/kg-body weight for females. All rats survived to the end of the 13-week study. Male and female rats fed diets containing BBMP at concentrations ≥ 10,000 ppm exhibited dose-related decrements in body weight gain. BBMP-associated microscopic lesions of the kidney and urinary bladder were observed in rats of both sexes in the 13-week study. Nine of 10 male rats administered BBMP at 20,000 ppm for 13 weeks had hyperplasia of the transitional cells of the urinary bladder, whereas none of the 10 high-dose female rats had a similar change. Papillary degeneration of the kidney was observed in 3/10, 6/10, and 8/10 male rats at BBMP concentrations of 5,000, 10,000, or 20,000 ppm, respectively. One of 10 female rats in the high-dose group had papillary degeneration of the kidney. Based on the body-weight effects and the urinary bladder and/or kidney lesions in rats in the 13-week studies, the high dose selected for the two-year NTP rat study was 10,000 ppm (Elwell *et al.* 1989, cited in NTP 1996).

In the NTP two-year cancer bioassay, groups of F344/N rats (60/sex) received BBMP in feed at concentrations of 2,500, 5,000, or 10,000 ppm for 104 to 105 weeks. The control group for male rats contained 70 animals, and the control group for female rats contained 60 animals. Up to 10 male and female rats from each group were evaluated at 15 months. A 3-month “stop exposure study” was also conducted in male rats. For this study, an

additional group of 70 male rats received 20,000 ppm BBMP in feed for 3 months and then control diet for the remainder of the 2-year dosing period. At 3 months, 10 male rats, each, from the control and 20,000-ppm groups, were evaluated for histopathologic lesions. No neoplastic lesions were observed at this interim sacrifice (data not shown in this report). Based upon food consumption, average daily doses of BBMP in the two-year study were 100, 200, and 430 mg/kg, for males, and 115, 230, and 460 mg/kg, for females, in the continuous exposure study. In the stop-exposure study, male rats received an average daily dose of 800 mg/kg for 13 weeks followed by standard diet only (NTP 1996).

After three weeks of the two-year study, the mean body weights of rats 10,000-ppm groups were approximately 10% and 4% lower than those of the control group for males and females, respectively. The mean body weights of male and female rats administered BBMP at 10,000 ppm remained lower than those of the controls throughout most of the study. After three weeks, mean body weights of males in the 20,000-ppm group were 20% lower than those of controls, and this decrement persisted until the dosed feed was replaced by standard diet (after 13 weeks). Mean body weights for the 20,000-ppm group remained consistently 5% to 15% lower than those of controls for the duration of the study. The survival of male and female rats in the 2,500-ppm groups (20 male and 27 female rats survived) was similar to that of controls (26 male and 36 female rats survived). However, tumor development caused early deaths in the higher-dose groups. Survival of animals in the 5,000-ppm groups (13 male and 23 female rats survived) or 10,000-ppm groups (1 male and 5 female rats survived) was significantly less than that of the controls. None of the animals in the stop-exposure group (20,000-ppm) survived to the scheduled termination of the study. Neoplastic lesions were not observed in control or 20,000-ppm rats evaluated at three months. A few neoplasms were seen in male and female rats at 15 months, but there was no clear treatment-related neoplastic response at that time (data not shown). Due to the marked decrease in survivability of the treated animals, the tumor incidences were partially evaluated using Life Table analysis.

Administration of BBMP for two years caused increased incidences of neoplasms in multiple organs of rats of both sexes, with males exhibiting a wider array of affected organs than females. Treatment-associated neoplasms, appearing exclusively in male rats, are summarized in Table 4-1.

Table 4-1. Treatment-related neoplasms and proliferative, nonneoplastic lesions in male F344/N rats administered BBMP in the diet for up to two years

Tumor type	Dietary concentration of BBMP (ppm)				
	0	2,500	5,000	10,000	20,000[a]
	Tumor response/No. examined				
Skin[b]					
Squamous cell papilloma	1/51	0/53	2/51	5/55	11/59**
Keratoacanthoma	3/51	5/53	11/51**	16/55**	10/59**
Squamous cell carcinoma	0/51	0/53	0/51	0/55	1/59
Trichoepithelioma	0/51	0/53	0/51	1/55	1/59
Sebaceous gland adenoma	0/51	1/53	0/51	2/55	2/59
Basal cell adenoma	0/51	1/53	0/51	3/55**	6/59**
Basal cell carcinoma	0/51	0/53	2/51	2/55	0/59
All skin tumors combined	4/51	6/53	14/51**	24/55**	21/59**
Subcutaneous tissue[c]					
Fibroma	2/51	8/53*	11/51**	15/55**	7/59**
Fibrosarcoma/sarcoma	0/51	1/53	2/51	3/55**	3/59
Fibroma, fibrosarcoma, or sarcoma	2/51	9/53*	13/51**	16/55**	10/59**
Zymbal gland[c]					
Adenoma	0/51	0/53	1/51	3/55*	2/60
Carcinoma	2/51	1/53	3/51	2/55	15/60**
Adenoma/carcinoma	2/51	1/53	4/51	5/55	15/60**
Forestomach[b]					
Squamous cell papilloma	0/51	0/53	0/51	1/55	5/60*
Small intestine[b]					
Adenoma	0/51	0/53	0/51	0/53	1/59
Carcinoma	0/51	0/53	0/51	2/53	4/59
Adenoma/carcinoma	0/51	0/53	0/51	2/53	5/59*
Large intestine[b]					
Adenoma	0/51	0/53	3/51	4/55	10/59**
Carcinoma	0/51	0/53	0/51	0/55	2/59
Adenoma/carcinoma	0/51	0/53	3/51	4/55	11/59**
Peritoneum[b]					
Malignant mesothelioma	0/51	3/53	8/51**	9/55**	26/60**
Urinary bladder					
Transitional cell hyperplasia	0/51	0/53	1/51	3/55	10/59**
Transitional cell papilloma	0/51	0/53	1/51	2/55	1/59
Transitional cell carcinoma	0/51	0/53	0/51	1/55	1/59
Transitional cell papilloma/carcinoma	0/51	0/53	1/51	3/55	2/59

Tumor type	Dietary concentration of BBMP (ppm)				
	0	2,500	5,000	10,000	20,000[a]
	Tumor response/No. examined				
Lung[b]					
Alveolar/bronchiolar adenoma	1/51	0/53	3/51	1/55	4/60
Alveolar/bronchiolar carcinoma	0/51	1/53	0/51	3/55*	3/60
Alveolar/bronchiolar adenoma/carcinoma	1/51	1/53	3/51	4/55*	7/60*
Squamous cell carcinoma	0/51	0/53	0/51	0/55	3/60
Seminal vesicle[b]					
Hyperplasia	1/51	6/53	4/51	16/55**	33/60**
Adenoma/carcinoma	0/51	0/53	0/51	0/55	2/60
Hematopoietic system[c]					
Mononuclear cell leukemia	27/51	29/53	40/51**	34/55**	25/60**
Pancreas[b]					
Acinar cell focal hyperplasia	3/51	9/53*	12/51*	14/53**	27/59**
Acinar cell adenoma	1/51	2/53	4/51*	3/53	3/59

Source: NTP (1996)
[a] Dosing was terminated after 13 weeks for stop-exposure portion of the two-year study.
[b] Statistical significance by logistic regression test: *$P < 0.05$; **$P < 0.01$ vs. controls.
[c] Statistical significance by Life Tables Analysis: *$P < 0.05$; **$P < 0.01$ vs. controls.

In addition to a wide variety of tissues and organs exhibiting proliferative changes in male rats in the continuous-feeding portion of the study, the presence of a high incidence of neoplasms in animals in the stop-exposure portion of the study is noteworthy. Male rats receiving BBMP at 20,000 ppm for only 13 weeks, then fed standard diet for the remainder of the study generally exhibited essentially the same pattern of proliferative changes as male rats in the continuous-feeding study.

The magnitude of the proliferative response was generally similar between the stop- and continuous-exposure groups. In the case of the Zymbal gland, however, the stop-exposure group had approximately three times as many tumors as either the 5,000- or 10,000-ppm continuous-exposure groups. Further, the Zymbal gland lesions in the stop-exposure group were nearly all malignant, whereas in males in the 10,000-ppm continuous-exposure group, the only statistically significant increase in Zymbal gland tumor incidence was in adenomas. The stop-exposure group also had a higher incidence of malignant mesotheliomas (26/60, 43%) than the continuous-exposure groups (controls, 0; 2,500 ppm, 3/53, 6%; 5,000 ppm, 8/51, 16%; 10,000 ppm, 9/55, 16%).

The presence of a statistically significant incidence of alveolar/bronchiolar carcinomas in male rats in the 10,000-ppm continuous-exposure group (3/55, 5%) is noteworthy, as this is a rare tumor in F344/N rats. The historical control incidences of alveolar/bronchiolar carcinomas in untreated-control F344/N male rats in the NTP database is only 12/1,350 (0.9%). Animals in the stop-exposure group exhibited a full continuum of alveolar/bronchiolar changes (i.e., hyperplasia, adenoma, and carcinoma). Furthermore,

three animals in the stop-exposure group had squamous cell carcinoma of the lung, a lesion that has never been observed in untreated F344/N rats in the NTP database.

Proliferative changes in the oral mucosa (squamous-cell papillomas) increased in a dose-related manner, and the incidence in the stop-exposure group was similar to that in the 10,000-ppm continuous-exposure group. Although such changes did not appear in the esophagus of rats in the stop-exposure group, forestomach papillomas, as well as adenomas and carcinomas of the small and large intestines, were significantly increased. Clearly, in the stop-exposure group, cellular changes leading to the development of papillomas of the oral cavity and alimentary canal occurred early and persisted until manifestation of the neoplasms closer to the conclusion of the experiment. There was no evidence of BBMP-associated changes in the oral cavity and alimentary canal of animals sacrificed at the 13-week interim sacrifice.

BBMP administered at 20,000 ppm caused the early deaths of all treated male rats. These early deaths were attributed primarily to the carcinogenic effects of the chemical. Of the 59 animals in the two-year group of the stop-exposure study, 55 (93%) were sacrificed in moribund condition due to development of tumors, and several animals in that group had highly aggressive, life-threatening malignancies.

The administration of BBMP caused increased incidences of several neoplasms in both male and female rats. The incidences of these tumors are summarized in Table 4-2.

Table 4-2. Treatment-related neoplasms and nonneoplastic lesions in male and female F344/N rats administered BBMP in the diet for up to two years

Tumor type	Dietary concentration of BBMP(ppm)				
	0	2,500	5,000	10,000	20,000[a]
	Tumor response/No. examined[b]				
Mammary Gland					
Males					
Fibroadenoma	0/51	4/53*	6/51**	6/55**	5/60**
Adenoma	0/51	0/53	1/51	1/55	0/60
Adenoma/fibrosarcoma	0/51	4/53*	7/51**	7/55**	5/60**
Females					
Fibroadenoma (single or multiple)	25/50	45/51**	46/53**	45/52**	—
Fibroadenoma (multiple)	6/50	37/51**	40/53**	37/52**	—
Adenoma	0/50	2/51	0/53	0/52	—
Carcinoma	4/50	4/51	3/53	4/52	—
All tumors combined	27/50	47/51**	47/53**	47/52**	—
Oral Cavity					
Males					
Squamous cell papilloma	0/51	4/53*	8/51**	10/55**	12/60**
Squamous cell carcinoma	0/51	0/53	1/51	0/55	2/60
Squamous cell papilloma/carcinoma	0/51	4/53*	9/51**	10/55**	13/60**
Females					
Squamous cell papilloma	2/50	2/51	4/53	5/52	—
Squamous cell carcinoma	0/50	1/51	1/53	1/52	—
Squamous cell papilloma/carcinoma	2/50	3/51	5/53	6/52	—
Esophagus					
Males					
Squamous cell papilloma	0/51	0/53	1/51	5/55*	0/60
Squamous cell carcinoma	0/51	0/53	0/51	1/55	0/60
Females					
Squamous cell papilloma	0/50	0/51	1/53	10/52**	—
Kidney					
Males					
Papillary epithelial hyperplasia	10/51	20/53**	25/51**	47/55**	21/59*
Transitional cell hyperplasia	0/51	0/53	0/51	4/55	4/59
Transitional cell carcinoma	0/51	0/53	0/51	0/55	1/59
Renal tubule adenoma	0/51	0/53	1/51	3/55**	1/59
Females					
Papillary epithelial hyperplasia	0/50	1/51	1/53	7/52**	—
Renal tubule adenoma	0/50	1/51	0/53	0/52	—

Tumor type	Dietary concentration of BBMP(ppm)				
	0	2,500	5,000	10,000	20,000[a]
	Tumor response/No. examined[b]				
Thyroid					
Males					
Follicular cell hyperplasia	1/51	0/53	2/51	5/55	6/59*
Follicular cell adenoma	0/51	1/53	2/51	2/55	7/59*
Follicular cell carcinoma	0/51	1/53	4/51*	1/55	2/59
Adenoma/carcinoma	0/51	2/53	6/51*	3/55	9/59**
Females					
Follicular cell adenoma	0/50	0/51	2/53	3/52*	—
Follicular cell carcinoma	0/50	0/51	0/53	1/52	—
Adenoma/carcinoma	0/50	0/51	2/53	4/52**	—

Source: NTP (1996)

[a] Dosing was terminated after 13 weeks for the stop-exposure portion of the two-year study.

[b] Statistical significance by logistic regression test: *$P < 0.05$; **$P < 0.01$ vs. controls.

—, No data.

The variety of tissues and organs showing proliferative effects in response to BBMP was clearly greater in males than in females. In addition, the significantly increased incidences of neoplasms in females tended to be restricted to benign tumors. Explanations for these sex-associated differences are not apparent. The NTP (1996) concluded that under the conditions of this two-year dietary bioassay, BBMP showed *clear evidence of carcinogenic activity* in male and female F344/N rats, based on increased incidences of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, and seminal vesicle and increased incidence of mononuclear cell leukemia in male rats and increased incidences of neoplasms of the oral cavity, esophagus, mammary gland, and thyroid gland in female rats.

4.1.2 Carcinogenicity studies in mice

Technical grade BBMP, as the commercial flame retardant FR-1138 [78.6% 2,2-bis(bromomethyl)-1,3-propanediol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers], was administered in the diet to male and female $B6C3F_1$ mice for 13 weeks in a range-finding study for a cancer bioassay (Elwell *et al.* 1989, cited by NTP 1996). During the 13-week study, groups of 10 mice of each sex were fed diets containing BBMP at concentrations of 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm. Based upon food consumption, male mice received average daily doses of 100, 200, 500, 1300, or 3000 mg/kg-body weight while females received 140, 300, 600, 1200, or 2900 mg/kg-body weight. Five male and four female mice died during the study. All BBMP-dosed female mice and male mice receiving BBMP at concentrations` ≥ 1,250 ppm BBMP exhibited significantly reduced body weight gains. In the 13-week study, BBMP-associated microscopic lesions of the kidney and urinary bladder were observed in mice of both sexes. Urinary bladder transitional cell hyperplasia

was observed in 4/10 and 7/8 male mice in the 5,000-ppm and 10,000-ppm groups, respectively, and in almost all female mice in the 5,000-ppm or 10,000-ppm groups (10/10 and 9/10, respectively). In both male and female mice, the prominent renal lesion was papillary necrosis with evidence of tubular cell regeneration. These effects were more prevalent in males (4 –to 9 of 10 animals in the 2,500, 5,000, or 10,000-ppm groups)and were noted only among high-dose females with 4/10 having tubular cell regeneration and 2/10 having papillary necrosis.

In the two-year cancer bioassay by the NTP, groups of B6C3F_1 mice (60/sex) received diets containing BBMP at concentrations of 0, 312, 625, or 1,250 ppm for 104 to 105 weeks (NTP 1996). Based upon food consumption, average daily doses of BBMP were 35, 70, or 140 mg/kg and 40, 80, or 170 mg/kg for females. Dietary concentrations for the two-year feeding studies were based upon effects on body weight gains and treatment-associated kidney/urinary bladder pathology observed during the 13-week range-finding studies. Survival rates of male and female mice in the 312- or 625-ppm groups were similar to those of the controls. Survival of animals in the 1,250-ppm group was significantly reduced, and females were more severely affected than males. Despite reduced survival at high dietary concentrations of BBMP, mean body weight gains and weight maintenance by exposed animals were similar to those of controls. Reduced survival among high-dose females was related to an increase in the number of rats sacrificed in moribund condition. Of the high-dose females, 29/60 (48%) of the animals were sacrificed in moribund condition, whereas 9/60 (15%), 14/60 (23%), and 14/60 (23%) were sacrificed in the control, low-dose, and mid-dose groups, respectively.

Although a few neoplasms were seen in male and female mice at 15 months, but no clear treatment-related neoplastic response was seen at that time (data not shown). However, administration of BBMP to male and female mice for 2 years increased the incidences of neoplasms in the Harderian gland, lung, and forestomach. While incidences of total Harderian gland tumors (adenomas plus carcinomas) were significantly increased in low-dose females and in mid- and high-dose males and females, the increases in low-dose females and males were generally attributable to changes in the incidence of adenomas. For high-dose female mice, a statistically significant increase in incidence of carcinomas was observed. Additionally, in the case of lung tumors, high-dose males exhibited a significantly increased incidence of alveolar/bronchiolar adenomas and carcinomas and a significant increase in adenoma multiplicity. Mid- and high-dose female mice had significantly increased incidences of alveolar/bronchiolar hyperplasia, adenomas, or carcinomas (combined).

Both male and female mice had increased incidences of squamous-cell tumors of the forestomach; however no tumors of the oral cavity or the intestinal tract were observed. These tumor responses are summarized in Table 4-3.

Table 4-3. Increased incidences of neoplasms and proliferative, nonneoplastic lesions in both male and female B6C3F$_1$ mice administered BBMP in the diet for up to two years

	Dietary concentration of BBMP(ppm)			
	0	313	625	1,250
Tumor type	Tumor response/No. examined[a]			
Harderian gland				
Males				
Adenoma	3/50	6/51	12/50**	18/49**
Carcinoma	1/50	1/51	4/50	4/49
Adenoma/carcinoma	4/50	7/51	16/50**	22/49**
Females				
Adenoma	2/52	6/50	8/51*	15/50**
Carcinoma	1/52	6/50	5/51	7/50*
Adenoma/carcinoma	3/52	12/50**	13/51**	19/50**
Lung				
Males				
Alveolar/bronchiolar adenoma	12/50	4/51	12/50	21/49*
Alveolar/bronchiolar adenoma (multiple)	0/50	0/51	4/50	10/49**
Alveolar/bronchiolar carcinoma	3/50	7/51	8/50	11/49**
Alveolar/bronchiolar adenoma/carcinoma	15/50	11/51	16/50	25/49*
Females				
Alveolar/bronchiolar hyperplasia	1/52	3/50	8/51**	15/50**
Alveolar/bronchiolar adenoma	3/52	3/50	9/51*	17/50**
Alveolar/bronchiolar carcinoma	2/52	2/50	6/51	5/50
Alveolar/bronchiolar adenoma/carcinoma	5/52	5/50	15/51*	19/50**
Forestomach				
Males				
Squamous cell papilloma	0/50	3/51	2/50	2/49
Squamous cell carcinoma	0/50	0/51	1/50	2/49
Squamous cell papilloma/carcinoma	0/50	3/51	3/50	4/49*
Females				
Squamous cell papilloma	0/52	1/50	5/51*	3/50*

Source: NTP (1996)

[a]Statistical significance by logistic regression test: *$P < 0.05$, **$P < 0.01$ vs. controls.

In addition to the neoplasms common to the two sexes, male and female mice exhibited proliferative changes appearing in only one sex. These changes are summarized in Table 4-4.

Table 4-4. Other neoplasms observed in B6C3F$_1$ mice of one sex administered BBMP in the diet for up to two years

	Dietary concentration (ppm)			
	0	312	625	1,250
Tumor type	Tumor response/No. examined[a]			
Males				
Kidney				
Renal tubular adenoma	0/49	0/51	3/50	2/49
Females				
Skin: subcutaneous tissue				
Fibrosarcoma	0/52	0/50	0/51	1/50
Sarcoma	0/52	1/50	4/51	11/50**
Fibrosarcoma/sarcoma	0/52	1/50	4/51	12/50**
Mammary gland				
Carcinoma	0/52	0/50	1/51	3/50
All organs				
Hemangioma/hemangiosarcoma	1/52	2/50	0/51	5/50*

Source: NTP (1996)
[a]Statistical significance by logistic regression test: *$P < 0.05$, **$P < 0.01$ vs. controls.

Although the increase was not statistically significant, the appearance of renal tubule adenomas in the kidneys of mid- and high-dose male mice is noteworthy because of the rarity of occurrence of this neoplasm. In the NTP database, the incidence of renal tubule adenoma was 3/1,466 (0.2%) in untreated control male B6C3F$_1$ mice. In the two-study, 5/99 (5%) males in the mid- and high-dose groups had this tumor.

The statistically significant increase in incidence of skin tumors noted in high-dose female mice was driven by an increased incidence of sarcomas. Like renal tubule adenomas in male mice, subcutaneous sarcomas are extremely rare in untreated female B6C3F$_1$ mice. The NTP database includes only 3/1,470 (0.2%) in untreated females with this tumor.

The NTP (1996) concluded that under the conditions of this two-year dietary bioassay, BBMP showed *clear evidence of carcinogenic activity* in male and female B6C3F$_1$ mice based on increased incidences of neoplasms of the Harderian gland, lung, and kidney in males; and increased incidences of neoplasms of the Harderian gland, lung, and subcutaneous tissue in females.

4.2 Summary

BBMP shows *clear evidence of carcinogenic activity* in F344 rats and B6C3F$_1$ mice of both sexes based on increased incidences of neoplasms in the tissues and organs of both species. In rats, these tissues and organs include the skin, subcutaneous tissue, mammary gland, Zymbal gland, oral cavity, esophagus, forestomach, small and large intestines,

mesothelium, urinary bladder, lung, thyroid gland, seminal vesicle and peripheral blood in males; and the oral cavity, esophagus, mammary gland, and thyroid gland in females. In mice, these tissues and organs include the Harderian gland, lung, and kidney in males; and Harderian gland, lung, and subcutaneous tissue in females. Based on the results of the stop-exposure studies, in which administration of BBMP to the male rats was stopped at 13 weeks and the animals were observed for an additional 100 weeks, BBMP induced early preneoplastic changes that developed to benign and malignant tumors in both species. The variety of tumors attributable to the stop-exposure administration of BBMP was nearly identical to that attributable to the continuous administration of the chemical. The target sites for the tumors differed between the sexes.

5 Genotoxicity

5.1 Prokaryotic systems

5.1.1 Induction of mutation in Salmonella typhimurium

The NTP (1996) conducted two studies to test the mutagenicity of technical grade BBMP in *Salmonella typhimurium* assays. In the first assay (Mortelmans *et al*. 1986), BBMP was tested for mutagenicity at concentrations ranging from 10 to 10,000 µg/plate in various *S. typhimurium* strains without metabolic activation or with metabolic activation by 10% S9 liver homogenate from Aroclor 1254-induced rats or hamsters. BBMP was nonmutagenic in all *S. typhimurium* strains tested with or without, metabolic activation (Mortelmans *et al*. 1986, cited in NTP 1996).

In the second assay (Zeiger *et al*. 1992, cited in NTP 1996), the mutagenicity of BBMP (analyzed purity of 84%) at (concentrations ranging from 10 to 6,666 µg/plate was tested) in *S. typhimurium* strains TA98 and TA100 without metabolic activation or with activation by 30% hamster- or rat- liver S9. BBMP was mutagenic in strain TA100 only in the presence of 30% liver S9 from Aroclor-1254-induced male Syrian hamsters. BBMP was not mutagenic in either *S. typhimurium* strain without metabolic activation, or with 30% rat-liver S9 metabolic activation.

5.2 Mammalian systems *in vitro*

5.2.1 Sister chromatid exchanges

The ability of BBMP to induce sister chromatid exchanges (SCE) was studied *in vitro* in Chinese hamster ovary (CHO) cells (Galloway *et al*. 1987, cited in NTP 1996). The results of this study are summarized in Table 5-1. BBMP was tested in CHO cells at doses ranging from 16.7 to 500 µg/mL in the absence of liver S9 metabolic activation and 800 to 1,200 µg/mL in the presence of liver S9 metabolic activation. No significant increase in the SCE frequency was observed in cultured CHO cells with or without S9 mix; small increases were seen in the presence of S9 but these results were judged equivocal.

5.2.2 Chromosomal aberrations

Galloway *et al*. (1987, cited in NTP 1996) investigated the induction of chromosomal aberrations (CA) in CHO cells exposed to BBMP. A concentration-related increase in CA was observed in CHO cells treated with BBMP at concentrations ranging from 400 to 700 µg/mL in the presence of rat-liver S9 metabolic activation (Table 5-2). The aberrations observed were considered unusual by the researchers because the majority of the breaks were preferentially located in the heterochromatic region of the long arm of the X chromosome. Without metabolic activation, no increase in CA was observed.

Table 5-1. Induction of SCE in CHO cells by BBMP

Conc. (µg/mL)	Total cells	No. of chromo-somes	No. of SCE	SCE/chromo-somes	SCE/ cell	Time in BrdU[a] (h)	Relative change of SCE/chromo-some[b](%)
-S9							
Summary; Negative							
0[f]	50	1,038	496	0.47	9.9	26.3	—
16.7	50	1,041	485	0.46	9.7	26.3	-2.50
16.7	50	1,041	485	0.46	9.7	26.3	-2.50
50	50	1,042	498	0.47	10.0	26.3	0.02
167	50	1,050	545	0.51	10.9	33.5[c]	8.62
500	0	—	—	—	—	33.5[c]	—
				$P = 0.077$[d]			
				$P = 0.077$[d]			
+S9							
Summary; Equivocal							
0[f]	50	1,050	496	0.47	9.9	25.5	—
800	50	1,048	556	0.53	11.1	25.5	12.31
800	50	1,048	556	0.53	11.1	25.5	12.31
1,000	50	1,047	590	0.56	11.8	25.5	19.29
1,200[e]	50	1,046	574	0.54	11.5	25.5	16.17
				$P = 0.004$			

Source: Study performed at Litton Bionetics, Inc. The detailed protocol and these data are presented in Galloway *et al.* (1987, cited in NTP 1996).

[a] BrdU = bromodeoxyuridine.

[b] SCE/chromosome in treated cells versus SCE/chromosome in solvent-control cells (dimethylsulfoxide).

[c] Because of chemical-induced cell cycle delay, incubation time was extended to provide sufficient cells for scoring.

[d] Significance of relative SCE/chromosome tested by the linear regression trend test vs. log of the dose.

[e] Marked toxicity noted at this dose level.

[f] Dimethylsulfoxide – negative control.

—, No data.

Table 5-2. Induction of chromosomal aberrations (CA) in CHO cells by BBMP

-S9					+S9				
Dose (µg/mL)	Total cells	No. of CA	CA/ cell	Cells with CA (%)	Dose (µg/mL)	Total cells	No. of CA	CA/ cell	Cells with CA (%)
Harvest time: 20.5 h[a]					Harvest time: 10.5 h				
Summary: Negative					Summary: Positive				
0[b]	100	1	0.01	1.0	0[b]	100	5	0.05	5.0
400	100	1	0.01	1.0	600	100	8	0.08	4.0
500	100	2	0.02	2.0	800	100	24	0.24	22.0[d]
600	100	0	0.00	0.0	1000	100	17	0.17	16.0[d]
700	0				1200	0			
				$P = 0.833$[c]					$P \leq 0.001$

Source: Study performed at Litton Bionetics, Inc. The detailed protocol and these data are presented in Galloway *et al.* (1987, cited in NTP 1996).

[a] Because of significant chemical-induced cycle delay, incubation time prior to addition of Colcemid was lengthened to provide sufficient metaphase cells at harvest.

[b] Dimethylsulfoxide – negative control.

[c] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose.

[d] Positive: $P < 0.05$.

5.3 Mammalian systems *in vivo*

5.3.1 Mouse bone marrow micronucleus test

The genotoxicity of BBMP was evaluated *in vivo* in male and female mice exposed to BBMP in feed at concentrations ranging from 625 to 10,000 ppm for 13 weeks (MacGregor *et al*. 1990, cited in NTP 1996). BBMP caused significant increases in micronucleated normochromatic erythrocytes (NCEs) in peripheral blood samples obtained from male mice exposed at the two highest BBMP concentrations, 5,000 and 10,000 ppm, and from female mice exposed at the three highest BBMP concentrations, 2,500, 5,000, and 10,000 ppm (Table 5-3).

Table 5-3. Frequency of micronucleated NCEs in mouse peripheral blood following dietary exposure to BBMP for 13 weeks[a]

Dose (ppm)	Micronucleated NCEs/1000 cells (mean ± SE)	Number of mice	Micronucleated NCEs/1000 cells (mean ± SE)	Number of mice
	Male		Female	
0	2.36 ± 0.17	10	1.46 ± 0.26	9
625	2.28 ± 0.29	8	1.86 ± 0.30	9
1,250	2.55 ± 0.18	10	1.86 ± 0.22	9
2,500	2.98 ± 0.21	10	2.72 ± 0.32[b]	9
5,000	3.80 ± 0.19[b]	10	4.26 ± 0.47[b]	9
10,000	9.30 ± 1.26[b]	7	11.81 ± 0.54[b]	9
	$P < 0.001$[c]		$P < 0.001$[c]	

Source: MacGregor *et al.* (1990, cited in NTP 1996).
[a] Ten thousand NCEs scored per animal. 0 ppm is the control.
[b] Significant response by pairwise comparison to control.
[c] Trend test.

Equivocal results were obtained in a mouse bone marrow micronucleus test where male mice received three gavage doses of BBMP (100 to 400 mg/kg) at 24-hour intervals (Table 5-4). The results of the initial trial were negative, but the second trial revealed a clear dose-related increase in micronucleated polychromatic erythrocytes (PCEs) (NTP 1996).

Table 5-4. Frequency of micronuclei in bone marrow cells of mice treated with BBMP by gavage[a]

Dose (mg/kg)	Micronucleated cells/1000 PCEs (mean ± SE)	Micronucleated cells/1000 PCEs (mean ± SE)
	Trial 1—Negative	Trial 2—Positive
0	1.4 ± 0.6	1.5 ± 0.5
100	0.7 ± 0.4	2.3 ± 0.3
200	2.5 ± 0.5	2.6 ± 0.7
300	2.0 ± 0.7	—
400[b]	1.2 ± 1.2	4.8 ± 1.2[d]
	$P = 0.220$[c]	$P = 0.000$[c]

Source: Study performed at Environmental Health Research and Testing Inc (NTP 1996).
[a] Two thousand PCEs scored per animal.
[b] Only 2 mice survived in this dose group.
[c] Trend test.
[d] Significantly different ($P < 0.008$) from control.
—, No data.

The mouse micronucleus test was repeated with male and female mice administered a single intraperitoneal injection (150, 300, or 600 mg/kg) of BBMP. Bone marrow samples were taken 48 hours after dosing. Although male mice showed a two-fold increase in the frequency of micronucleated PCEs, neither the trend test nor pairwise analyses gave statistically significant results. The response in females was stronger (similar to that seen in the 13-week dietary study, Table 5-3) and was considered to be positive evidence of the ability of BBMP to induce micronuclei in bone marrow cells of female mice (NTP 1996). Results of this mouse micronucleus study are summarized in Table 5-5.

Table 5-5. Frequency of micronuclei in bone marrow cells of mice treated with BBMP by intraperitoneal injection[a]

Dose (mg/kg)	Number of mice	Micronucleated cells/1000 PCEs (mean ± SE)	Number of mice	Micronucleated cells/1000 PCEs (mean ± SE)
	Male		Female	
0	4	1.5 ± 0.3	4	2.0 ± 0.4
150	4	3.2 ± 0.8[b]	4	2.7 ± 1.1
300	4	3.0 ± 0.7[b]	3	3.6 ± 0.9[b]
600	3	3.0 ± 1.0[b]	4	5.2 ± 0.5[b]
		$P = 0.150$[c]		$P = 0.003$[c]

Source: NTP (1996)
[a] One thousand PCEs scored per animal.
[b] Significantly different ($P < 0.008$) from control.
[c] Trend test.

5.4 Summary

BBMP was to be mutagenic in several *in vitro* and *in vivo* systems, but specific conditions of metabolic activation were required to observe mutagenicity. BBMP was mutagenic in *S. typhimurium* strains TA100 and TA1535 only in the presence of 30% liver S9 from induced hamsters. In cultured CHO cells, BBMP induced CA only with S9 metabolic activation; no induction of SCE was observed with or without activation. *In vivo* exposure to BBMP induced significant increases in the frequency of micronucleated erythrocytes in male and female mice under various treatment protocols.

6 Other Relevant Data

6.1 Absorption, distribution, metabolism, and excretion of BBMP

BBMP was not detected in tissues of rats orally administered 5 or 100 mg/kg/day BBMP (as the flame retardant FR-1138) in a lifetime oncogenicity study. However, there was a statistically increased level of bromide (< 10-fold over controls) in the liver, kidney, fat, and serum of male rats that received the 100 mg/kg/day dose. The concentration of bromide in the liver was comparable to the concentration in serum although kidney levels exceeded serum levels in a ratio of 2:3 (Keyes *et al.* 1980).

Male F344 rats received single doses of BBMP at 150, 300, or 600 mg/kg by gavage or 15 mg/kg by intravenous (i.v.) injection into the caudal vein. Doses were prepared by diluting [^{14}C]-BBMP (uniformly labeled) with unlabeled BBMP in ethanol, emulphor, and water at a ratio of 1:1:3 by volume, to administer 25 to 50 μCi/kg-body weight. BBMP was rapidly, and nearly completely, absorbed from the gastrointestinal tract of the rats. BBMP was rapidly excreted in the urine of the rats as the glucuronide conjugate, with < 10% of the total dose being excreted in feces and none being detected as exhaled volatiles or CO_2. The ^{14}C in bile consisted of > 99% of the same glucuronide conjugate. The amount of excreted BBMP was determined by analysis for ^{14}C in urine, feces, and tissue. The relative amounts of BBMP and radiolabeled metabolites in rat urine, plasma, and bile were analyzed via high-performance liquid chromatography. The major metabolite derived from BBMP in rat urine was identified as a glucuronide conjugate of BBMP (Sanders *et al.* 1995 [abstract]).

The absorption, tissue distribution, metabolism, and excretion of BBMP in B6C3F$_1$ mice has been studied. Mice received BBMP at either 150 mg/kg by gavage or 15 mg/kg by i.v. injection (N = 4/group). Doses were prepared by diluting [^{14}C]-BBMP (uniformly labeled) with unlabeled BBMP in ethanol, emulphor, and water at a ratio of 1:1:3 by volume, to administer 25 to 50 μCi/kg-body weight. BBMP was rapidly, and nearly completely, absorbed from the gastrointestinal tract of the mice and rapidly excreted in the urine as the glucuronide conjugate, with <10% of the total dose being excreted in feces and none being detected as exhaled volatiles or CO_2. The ^{14}C in bile consisted of >99% of the same glucuronide conjugate. The amount of excreted BBMP was determined by analysis for ^{14}C in urine, feces, and tissue. The relative amounts of BBMP and radiolabeled metabolites in mouse urine were analyzed via high-performance liquid chromatography (Sanders *et al.* 1995 [abstract]).

6.2 Summary

BBMP undergoes rapid conjugation and excretion following absorption from the gut in rats and mice. BBMP did not form reactive metabolites or accumulate in the tissues of either species. However, exposure of rats to BBMP significantly increased bromide concentrations in the liver, kidney, fat, and serum of the exposed rats.

7 References

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL
(FR-1138®)

(CAS NO. 3296-90-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

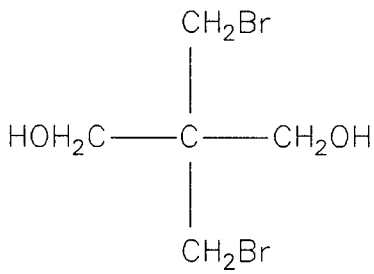
May 1996

NTP TR 452

NIH Publication No. 96-3368

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

ABSTRACT



2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL
(FR-1138®)

(Technical Grade: 78.6% 2,2-bis(bromomethyl)-1,3-propanediol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers)

CAS No. 3296-90-0

Chemical Formula: $C_5H_{10}Br_2O_2$ Molecular Weight: 261.94

Synonyms: 2,2-Bis(2-bromomethyl)-1,3-propanediol; 1,3-dibromo-2,2-dihydroxymethylpropane; 1,3-dibromo-2,2-dimethylolpropane; 2,2-dibromomethyl-1,3-propanediol; dibromopentaerythritol; dibromoneopentyl glycol; pentaerythritol dibromide; pentaerythritol dibromohydrin

2,2-Bis(bromomethyl)-1,3-propanediol is used as a fire retardant in unsaturated polyester resins, in molded products, and in rigid polyurethane foam. 2,2-Bis(bromomethyl)-1,3-propanediol was chosen for study because it is a widely used flame retardant and little toxicity and carcinogenicity data were available.

Groups of male and female F344/N rats and B6C3F$_1$ mice were exposed to technical grade 2,2-bis(bromomethyl)-1,3-propanediol (78.6% pure) in feed for 13 weeks or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, mouse bone marrow, and mouse peripheral blood.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats were fed diets containing 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol for 13 weeks. These levels corresponded to approximately 100, 200, 400, 800, or 1,700 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight (males) and 100, 200, 400, 800, or 1,600 mg/kg (females). No rats died during the studies. The final mean body weights and weight gains of 5,000, 10,000, and 20,000 ppm males and females were significantly lower than those of the controls. Feed consumption by exposed animals was lower than that by controls at week 1, but was generally similar to or slightly higher than that by controls at week 13. No chemical-related clinical findings were observed. Chemical-related differences in clinical pathology parameters included increased urine volumes accompanied by decreased urine specific gravity and minimally increased protein excretion in 10,000 and 20,000 ppm males. In females, urine parameters were less affected than males. Water deprivation tests demonstrated that male and female rats were able to adequately concentrate their urine in response

to decreased water intake. Serum protein and albumin concentrations in female rats exposed to 2,500 ppm and higher were slightly lower than those of the controls. Renal papillary degeneration was present in 5,000 and 10,000 ppm males, and in 20,000 ppm males and females. Hyperplasia of the urinary bladder was present in 20,000 ppm males.

13-Week Study in Mice

Groups of 10 male and 10 female mice were fed diets containing 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol for 13 weeks. These levels corresponded to approximately 100, 200, 500, 1,300, or 3,000 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight (males) and 140, 300, 600, 1,200, or 2,900 mg/kg (females). One control female, two males and one female receiving 625 ppm, one female receiving 1,250 ppm, one female receiving 2,500 ppm, one female receiving 5,000 ppm, and three males receiving 10,000 ppm died during the study. The final mean body weights and body weight gains of males and females receiving 1,250, 2,500, 5,000, or 10,000 ppm and of females receiving 625 ppm were significantly lower than those of the controls. Feed consumption by exposed mice was generally higher than that by controls throughout the study. Clinical findings included abnormal posture and hypoactivity in 10,000 ppm male and female mice. Blood urea nitrogen concentrations of 5,000 ppm females and 10,000 ppm males and females were greater than those of controls. Also, urine specific gravity was lower in 10,000 ppm females. Differences in organ weights generally followed those in body weights. Papillary necrosis, renal tubule regeneration, and fibrosis were observed in the kidneys of 2,500 and 5,000 ppm males and 10,000 ppm males and females. Urinary bladder hyperplasia was observed in 5,000 and 10,000 ppm males and females.

2-Year Study in Rats

Groups of 60 male and 60 female rats received 2,500, 5,000, or 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. Groups of 70 males and 60 females received 0 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. A stop-exposure group of 70 male rats received 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 3 months, after which animals received undosed feed for the remainder of the 2-year study. Average daily doses of 2,2-bis(bromomethyl)-1,3-propanediol were 100, 200, or 430 mg/kg body weight for males and 115, 230, or 460 mg/kg for females. Stop-exposure males received an average daily dose of 800 mg/kg. Ten animals from the 0 ppm male group and the 20,000 ppm stop-exposure group were evaluated at 3 months; nine or 10 control animals and five to nine animals from each of the continuous-exposure groups were evaluated at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Survival of 5,000 and 10,000 ppm continuous-exposure study males and females and 20,000 ppm stop-exposure males was significantly lower than that of the controls. Mean body weights of exposed male and female rats receiving 10,000 ppm and stop-exposure males receiving 20,000 ppm were lower than those of the controls throughout most of the study. In the continuous-exposure study, feed consumption by exposed rats was generally similar to that by controls throughout the study. In 20,000 ppm stop-exposure males, the feed consumption was lower than that by controls. Clinical findings included skin and/or subcutaneous masses on the face, tail, and the ventral and dorsal surfaces of exposed rats.

Pathology Findings

In the 2-year continuous and stop-exposure studies in male rats, exposure to 2,2-bis(bromomethyl)-1,3-propanediol was associated with neoplastic effects in the skin, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, hematopoietic system, and seminal vesicle. Nonneoplastic effects in the kidney, lung, thyroid gland, seminal vesicle, pancreas, urinary bladder, and forestomach were also observed. In females, 2-year exposure to 2,2-bis(bromomethyl)-1,3-propanediol was associated with neoplastic effects in the oral cavity, esophagus, mammary gland, and thyroid gland. Nonneoplastic effects in the kidney were also observed. These findings are outlined in the two summary tables.

2-YEAR STUDY IN MICE

Groups of 60 male and 60 female mice received 0, 312, 625, or 1,250 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. Average daily doses of 2,2-bis(bromomethyl)-1,3-propanediol were 35, 70, or 140 mg/kg (males) and 40, 80, or 170 mg/kg (females). Eight to 10 animals from each group were evaluated at 15 months.

Survival, Body Weights, Feed Consumption, and Clinical Findings

Survival of 1,250 ppm males and females was significantly lower than that of the controls. Mean body weights of exposed male and female mice were similar to controls throughout the study. Final mean body weights were also generally similar to those of controls. Feed consumption by exposed male and female mice was similar to that by controls. Clinical findings included tissue masses involving the eye in exposed mice.

Pathology Findings

Exposure of male mice to 2,2-bis(bromomethyl)-1,3-propanediol for 2 years was associated with neoplastic effects in the harderian gland, lung, and kidney. Exposure of female mice to 2,2-bis(bromomethyl)-1,3-propanediol was associated with increased incidences of neoplasms of the harderian gland, lung, and skin. Nonneoplastic effects in the lung were also observed in exposed females. These findings are outlined in the two summary tables.

GENETIC TOXICOLOGY

2,2-Bis(bromomethyl)-1,3-propanediol was mutagenic in *Salmonella typhimurium* strain TA100 when tested in the presence of induced 30% hamster liver S9; all other strain/activation combinations gave negative results. In cultured Chinese hamster ovary cells, 2,2-bis(bromomethyl)-1,3-propanediol induced chromosomal aberrations only in the presence of S9; no induction of sister chromatid exchanges was observed in cultured Chinese hamster ovary cells after treatment with 2,2-bis(bromomethyl)-1,3-propanediol, with or without S9. *In vivo*, 2,2-bis(bromomethyl)-1,3-propanediol induced significant increases in the frequencies of micronucleated erythrocytes in male and female mice. Significant increases in micronuclei were observed in peripheral blood samples from male and female mice exposed to 2,2-bis(bromomethyl)-1,3-propanediol for 13 weeks via dosed feed. Results of a bone marrow micronucleus test in male mice, where 2,2-bis(bromomethyl)-1,3-propanediol was administered by gavage, were considered to be equivocal due to inconsistent results obtained in two trials. An additional bone marrow micronucleus test was performed with male and female mice and 2,2-bis(bromomethyl)-1,3-propanediol was administered as a single intraperitoneal injection; results of this test were positive in females and negative in males.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of 2,2-bis-(bromomethyl)-1,3-propanediol (FR-1138®) in male F344/N rats based on increased incidences of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, and seminal vesicle, and the increased incidence of mononuclear cell leukemia.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in female F344/N rats based on increased incidences of neoplasms of the oral cavity, esophagus, mammary gland, and thyroid gland.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in male B6C3F$_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and kidney.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in female B6C3F$_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and subcutaneous tissue.

Slight increases in the incidences of neoplasms of the pancreas and kidney in male rats; forestomach in male mice; and forestomach, mammary gland, and circulatory system in female mice may have also been related to treatment.

Exposure of male and female rats to 2,2-bis(bromomethyl)-1,3-propanediol was associated with alveolar/bronchiolar hyperplasia in the lung (males only); focal atrophy, papillary degeneration, transitional epithelial hyperplasia (pelvis), and papillary epithelial hyperplasia in the kidney; follicular cell hyperplasia in the thyroid gland (males only); hyperplasia in the seminal vesicle and pancreas (males only); mucosal hyperplasia in the forestomach (males only); and urinary bladder hyperplasia (males only). Exposure of mice to 2,2-bis(bromomethyl)-1,3-propanediol was associated with hyperplasia of the alveolar epithelium in females.

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

Summary of Site-Specific Carcinogenic Effects in Rats and Mice in the 2-Year Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol

	Male Rats	Female Rats	Male Mice	Female Mice
Site				
Skin	+	−	−	−
Subcutaneous tissue	+	−	−	+
Mammary gland	+	+	−	±
Zymbal's gland	+	−	−	−
Oral cavity	+	+	−	−
Esophagus	+	+	−	−
Forestomach	+	−	±	±
Small intestine	+	−	−	−
Large intestine	+	−	−	−
Mesothelium	+	−	−	−
Kidney	±	−	+	−
Urinary bladder	+	−	−	−
Lung	+	−	+	+
Thyroid gland	+	+	−	−
Seminal vesicle	+	NA	−	NA
Hematopoietic system	+	−	−	−
Pancreas	±	−	−	−
Harderian gland	−	−	+	+
Circulatory system	−	−	−	±

+ = some or clear evidence
± = equivocal evidence
− = no evidence
NA = not applicable

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 2,2-Bis(bromomethyl)-1,3-propanediol

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses	0, 2,500, 5,000, or 10,000 ppm and 20,000 ppm stop-exposure (equivalent to 0, 100, 200, or 430 mg/kg and 800 mg/kg)	0, 2,500, 5,000, or 10,000 ppm (equivalent to 0, 115, 230, or 460 mg/kg)	0, 312, 625, or 1,250 ppm (equivalent to 0, 35, 70, or 140 mg/kg)	0, 312, 625, or 1,250 ppm (equivalent to 0, 40, 80, or 170 mg/kg)
Body weights	10,000 ppm and 20,000 ppm stop-exposure groups lower than controls	10,000 ppm group lower than controls	Exposed groups similar to controls	Exposed groups similar to controls
2-Year survival rates	26/51, 20/53, 13/51, 1/55, 0/60	36/50, 27/51, 23/53, 5/52	42/50, 36/51, 35/50, 30/48	37/52, 30/50, 26/51, 11/50
Nonneoplastic effects	Kidney: focal atrophy (0/51, 0/53, 0/51, 5/55, 0/59); papillary degeneration (0/51, 5/53, 30/51, 29/55, 16/59); papillary epithelial hyperplasia (10/51, 20/53, 25/51, 47/55, 21/59); pelvis, transitional epithelium, hyperplasia (0/51, 0/53, 0/51, 4/55, 4/59) Lung: alveolar/ bronchiolar hyperplasia (3/51, 4/53, 5/51, 7/55, 14/60) Thyroid gland: follicular cell hyperplasia (1/51, 0/53, 2/51, 5/55, 6/59) Seminal vesicle: hyperplasia (1/51, 6/53, 4/51, 16/55, 33/60) Pancreas: focal hyperplasia (3/51, 9/53, 12/51, 14/53, 27/59) Forestomach: mucosal hyperplasia (4/51, 12/53, 6/51, 6/55, 6/59) Urinary bladder: hyperplasia (0/51, 0,53, 1/51, 3/55, 10/59)	Kidney: focal atrophy (0/50, 2/51, 1/53, 7/52); papillary degeneration (0/50, 1/51, 3/53, 17/52); papillary epithelial hyperplasia (0/50, 1/51, 1/53, 7/52)	None	Lung: alveolar epithelium, hyperplasia (1/52, 3/50, 8/51, 15/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects	Skin: squamous cell papilloma, keratoacanthoma, trichoepithelioma, basal cell adenoma, basal cell carcinoma, or squamous cell carcinoma (4/51, 6/53, 14/51, 24/55, 21/60) Skin, subcutaneous tissue: fibroma, fibrosarcoma, or sarcoma (2/51, 9/53, 13/51, 16/55, 10/60) Mammary gland: fibroadenoma or adenoma (0/51, 4/53, 7/51, 7/55, 5/60) Zymbal's gland: adenoma or carcinoma (2/51, 1/53, 4/51, 5/55, 15/60) Oral cavity (pharynx, tongue, or gingiva): squamous cell papilloma or carcinoma (0/51, 4/53, 9/51, 10/55, 13/60) Esophagus: squamous cell papilloma (0/51, 0/53, 1/51, 5/55, 0/60) Forestomach: squamous cell papilloma (0/51, 0/53, 0/51, 1/55, 5/60) Large intestine: adenoma or carcinoma (0/51, 0/53, 3/51, 4/55, 11/59) Small intestine: adenoma or carcinoma (0/51, 0/53, 0/51, 2/53, 5/59) Malignant mesothelioma: (0/51, 3/53, 8/51, 9/55, 26/60) Urinary bladder: transitional cell papilloma or carcinoma (0/51, 0/53, 1/51, 3/55, 2/59)	Oral cavity: squamous cell papilloma or carcinoma (2/50, 3/51, 5/53, 6/52) Esophagus: squamous cell papilloma (0/50, 0/51, 1/53, 10/52) Mammary gland: fibroadenoma (25/50, 45/51, 46/53, 45/52) Thyroid gland: follicular cell adenoma or carcinoma (0/50, 0/51, 2/53, 4/52)	Harderian gland: adenoma or carcinoma (4/50, 7/51, 16/50, 22/49) Lung: alveolar/ bronchiolar adenoma or carcinoma (15/50, 11/51, 16/50, 25/49) Kidney (renal tubule): adenoma (0/50, 0/51, 3/50, 2/49)	Harderian gland: adenoma or carcinoma (3/52, 12/50, 13/51, 19/50) Lung: alveolar/ bronchiolar adenoma or carcinoma (5/52, 5/50, 15/51, 19/50) Skin (subcutaneous tissue): sarcoma (0/52, 1/50, 4/51, 11/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects (continued)	Lung: alveolar/ bronchiolar adenoma or carcinoma (1/51, 1/53, 3/51, 4/55, 7/60); squamous cell carcinoma (0/51, 0/53, 0/51, 0/55, 3/60) Thyroid gland: follicular cell adenoma or carcinoma (0/51, 2/53, 6/51, 3/55, 9/59) Seminal vesicle: adenoma or carcinoma (0/51, 0/53, 0/51, 0/55, 2/60) Hematopoietic system: mononuclear cell leukemia (27/51, 29/53, 40/51, 34/55, 25/60)			
Uncertain effects	Kidney (renal tubule): adenoma (0/51, 0/53, 1/51, 3/55, 1/59) Pancreas: acinar cell adenoma (1/51, 2/53, 4/51, 3/53, 3/59)	None	Forestomach: squamous cell papilloma or carcinoma (0/50, 3/51, 3/50, 4/49)	Mammary gland: carcinoma (0/52, 0/50, 1/51, 3/50) Forestomach: squamous cell papilloma (0/52, 1/50, 5/51, 3/50) Circulatory system: hemangioma and hemangiosarcoma (1/52, 2/50, 0/51, 5/50)
Level of evidence of carcinogenic activity	Clear evidence	Clear evidence	Clear evidence	Clear evidence

Genetic toxicology

Salmonella typhimurium gene mutations:	Positive with S9 in strain TA100; negative in strains TA98, TA1535, and TA1537 with and without S9
Sister chromatid exchanges Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9
Chromosomal aberrations Cultured Chinese hamster ovary cells *in vitro*:	Positive with S9; negative without S9
Micronucleated erythrocytes Mouse bone marrow *in vivo* by gavage:	Equivocal in male mice
Mouse bone marrow *in vivo* by intraperitoneal injection:	Negative in male and positive in female mice
Mouse peripheral blood *in vivo*:	Positive in male and female mice

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.

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 2,2-bis(bromomethyl)-1,3-propanediol on November 29, 1994, 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 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 November 29, 1994, the draft Technical Report on the toxicology and carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol received public review by the National Toxicology Program 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. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of 2,2-bis(bromomethyl)-1,3-propanediol by discussing the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms in rats and mice and possible compound-related nonneoplastic lesions in rats and female mice. The proposed conclusions for the studies were *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in male and female F344/N rats and B6C3F$_1$ mice.

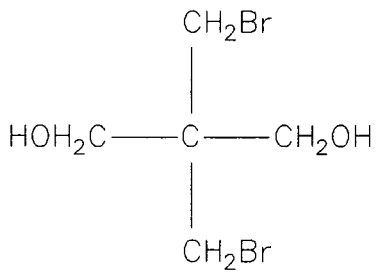
Dr. Russo, a principal reviewer, agreed with the proposed conclusions. She asked if there was more information on possible mutagenic or carcinogenic effects of the impurities detected in the compound or on the metabolism of 2,2-bis(bromomethyl)-1,3-propanediol and its contaminants. (The studies were conducted on commercially available fire retardant from the sole manufacturer, and no attempt was made to study impurities, contaminants, or metabolites.)

Dr. Ryan, the second principal reviewer, agreed with proposed conclusions. She questioned the rationale for dosed feed administration since the text suggested dermal and inhalation exposures were the most likely exposure routes for humans. Dr. Dunnick said the oral route was chosen to provide maximum exposure to the tissues. Dr. Ryan remarked on the large differences between the overall and adjusted incidence rates for several neoplasms and asked for discussion as to why. Dr. J.K. Haseman, NIEHS, said the adjusted rate provides an estimate of overall neoplasm incidence if all animals survive to the end of the study. In many cases this adjusted rate is reasonable, but it is less meaningful when there are only a few survivors as in the high dose groups of rats in the 2,2-bis(bromomethyl)-1,3-propanediol study.

Dr. Miller, the third principal reviewer, agreed with the proposed conclusions. She asked how the rodent doses would compare with likely human exposures and suggested that information be added as to the sources, routes, and degrees of human exposure. Dr. Dunnick responded that the one company that produces 2,2-bis(bromomethyl)-1,3-propanediol had not published information on worker exposure but noted that the Environmental Protection Agency has requested such information. Dr. J. Haartz, NIOSH, added that no information on 2,2-bis(bromomethyl)-1,3-propanediol was found in the National Occupational Exposure Survey, so there was no estimate of potentially exposed workers. Dr. Miller asked whether there should be concerns with vapor or pyrolysis products in the event of a fire. Dr. Dunnick said the chemical volatilizes at temperatures greater than 200° C and at high temperatures would form hydrogen bromide.

Dr. Miller moved that the technical report on 2,2-bis(bromomethyl)-1,3-propanediol be accepted with the revisions discussed and with the conclusions as written for male and female rats and mice, *clear evidence of carcinogenic activity*. Dr. Ryan seconded the motion, which was accepted unanimously with seven votes.

INTRODUCTION



2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL (FR-1138®)

(Technical Grade: 78.6% 2,2-bis(bromomethyl)-1,3-propanediol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers)

CAS No. 3296-90-0

Chemical Formula: $C_5H_{10}Br_2O_2$ Molecular Weight: 261.94

Synonyms: 2,2-Bis(2-bromomethyl)-1,3-propanediol; 1,3-dibromo-2,2-dihydroxymethylpropane; 1,3-dibromo-2,2-dimethylolpropane; 2,2-dibromomethyl-1,3-propanediol; dibromopentaerythritol; dibromoneopentyl glycol; pentaerythritol dibromide; pentaerythritol dibromohydrin

CHEMICAL AND PHYSICAL PROPERTIES

2,2-Bis(bromomethyl)-1,3-propanediol is a white solid material with a slight, mild, musty odor. It has a melting point of 75° to 95° C for technical grade material and 109° to 110° C for pure material. It is soluble in acetone, ethanol, and ether, and slightly soluble in water (2 g/1,000 g water at 25° C). The material is produced by replacement of the hydroxyl groups of pentaerythritol with bromide. In the case of 2,2-bis(bromomethyl)-1,3-propanediol approximately one-half of the hydroxyl groups of pentaerythritol are replaced with bromine-bonded carbon atoms. The compound is unique in that the aliphatic neopentyl structure contains no hydrogen atoms on the carbon atom adjacent to the carbon bonded to the bromine. This provides a compound very resistant to dehydrobromination by elevated temperatures, by chemical reactions, or by photodegradation. The remaining hydroxyl groups provide reactive sites that allow for polymerization. These hydroxyl groups readily react with organic acids to form esters, with isocyanates to form urethanes, or with epoxides to form ethers. In addition, 2,2-bis(bromomethyl)-1,3-propanediol can react with aldehydes and ketones to form cyclic acetals or ketals, or with phosphorous oxyhalides to form cyclic phosphates or phosphites (Larsen, 1969; Larsen and Weaver, 1973).

USE AND HUMAN EXPOSURE

2,2-Bis(bromomethyl)-1,3-propanediol is used as a flame retardant in unsaturated polyester resins, for molded products, and in rigid polyurethane foam. This flame retardant may enter the environment as fugitive dust and through wastewater. 2,2-Bis(bromomethyl)-1,3-propanediol is expected to remain for long periods of time in water (USEPA, 1983).

It is estimated that three to four million pounds of 2,2-bis(bromomethyl)-1,3-propanediol are produced per year (USEPA, 1983), but current production figures are not reported (USITC, 1994). The United States produces 65% of the world's bromine, and the major uses for bromine in the United States are manufacturing of lead scavengers in gasoline (48%), flame retardants (29%), sanitation preparations (16%), and other uses (6%). The demand for bromine-based flame retardant chemicals has increased (Margler, 1982).

The brominated flame retardants (including FR-1138®) are a use-based class of 22 chemicals recommended by the United States Environmental Protection Agency for additional study (*Fed. Regist.*, 1989, 1990) but were withdrawn from testing in 1994 because of the availability of sufficient toxicity data or limited production or use in the United States (*Fed. Regist.*, 1994).

The National Institute for Occupational Safety and Health did not survey any United States facilities for 2,2-bis(bromomethyl)-1,3-propanediol exposure information (NIOSH, 1995).

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

The National Institute of Environmental Health Sciences has ongoing studies on the absorption, distribution, metabolism, and excretion of 2,2-bis(bromomethyl)-1,3-propanediol in rodents. However, there are no published studies on the metabolism of 2,2-bis(bromomethyl)-1,3-propanediol.

Humans

No information on the absorption, distribution, metabolism, and excretion of 2,2-bis(bromomethyl)-1,3-propanediol in humans was found in a search of the available literature.

TOXICITY

Experimental Animals

The oral LD_{50} of 2,2-bis(bromomethyl)-1,3-propanediol in male rats is reported to be 3,458 mg/kg (range 2,810 to 4,257 mg/kg; Keyes *et al.*, 1979). A comparison of the toxicity of 2,2-bis(bromomethyl)-1,3-propanediol in rats and mice by the dosed feed and gavage administration demonstrated similar effects by each route at comparable doses (Elwell *et al.*, 1989). The results of the feed studies are provided in this report.

Humans

No information on 2,2-bis(bromomethyl)-1,3-propanediol toxicity in humans has been reported in the literature.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

The effect of 2,2-bis(bromomethyl)-1,3-propanediol on reproduction in Swiss (CD-1®) mice was evaluated by administering 2,2-bis(bromomethyl)-1,3-propanediol in feed at 1,000, 2,000, or 4,000 ppm in a continuous breeding study in which male and female F_0 mice were exposed 7 days prior to and during a 98-day cohabitation period (Morrissey *et al.*, 1989; Treinen *et al.*, 1989). Although the fertility index was unchanged, 2,2-bis(bromomethyl)-1,3-propanediol exposure caused significantly decreased numbers of litters per pair, pups born alive per litter, and pup weight in mice exposed to 4,000 ppm. Sperm concentration, motility, morphology, and estrual cyclicity were unaffected by treatment. Crossover mating between exposed (4,000 ppm) and control F_0 mice indicated a specific effect on the female reproductive capacity. A decrease in the number of live pups per litter and decrease in pup weight were seen when exposed females were mated to control males but not when exposed males were mated to control females. 2,2-Bis(bromomethyl)-1,3-propanediol at 4,000 ppm caused generalized toxicity in males and females as evidenced by the lower body weight, and this generalized toxicity may have contributed, in part, to the reproductive impairment produced by 2,2-bis(bromomethyl)-1,3-propanediol at the 4,000 ppm concentration.

Humans

No information on the reproductive and developmental toxicity of 2,2-bis(bromomethyl)-1,3-propanediol in humans has been reported in the literature.

CARCINOGENICITY

Experimental Animals

In a 2-year toxicity/carcinogenicity study, Sprague-Dawley rats were administered the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol [FR-1138®: 80% dibromopentyl glycol (2,2-bis(bromomethyl)-1,3-propanediol); 8% tribromoneopentyl alcohol (bis(bromomethyl)-1-bromo-3-hydroxypropane) and 6% monobromoneopentyl triol (2,2-bis (hydroxymethyl)-1-bromo-3-hydroxypropane)] in feed at concentrations that delivered 0, 5, or 100 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight per day (Keyes *et al.*, 1979). No carcinogenic effect was observed. However, degenerative changes in the liver and lens of the eye were attributed to chemical exposure. The article did not provide details on the preparation or stability of the chemical in the feed. No dose-related effects on the feed consumption, weight gain, clinical signs, or mortality were observed, suggesting that the animals may have been able to tolerate higher doses.

Humans

No information on the carcinogenic potential of 2,2-bis(bromomethyl)-1,3-propanediol in humans has been reported in the literature.

GENETIC TOXICITY

There are no mutagenicity data for 2,2-bis(bromomethyl)-1,3-propanediol other than the NTP studies included in Appendix E of this report. These data indicate that 2,2-bis(bromomethyl)-1,3-propanediol is mutagenic, but that specific conditions are required to observe a positive response. 2,2-Bis(bromomethyl)-1,3-propanediol was mutagenic in *Salmonella typhimurium* strain TA100 in the presence of 30% induced hamster liver S9 (Zeiger *et al.*, 1992); in the presence of 30% rat liver S9, no mutagenic response was observed. An earlier *Salmonella* mutation study showed no mutagenicity in strains TA98, TA100, TA1535, or TA1537 with or without 10% induced hamster or rat liver S9 (Mortelmans *et al.*, 1986). In cytogenetic tests with cultured Chinese hamster ovary cells (Galloway *et al.*, 1987), 2,2-bis(bromomethyl)-1,3-propanediol induced a dose-related increase in chromosomal aberrations in the presence of induced rat liver S9; no increase in sister chromatid exchange frequency was noted in cultured Chinese hamster ovary cells treated with 2,2-bis(bromomethyl)-1,3-propanediol, with or without S9.

STUDY RATIONALE

The National Cancer Institute nominated the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol [FR-1138®: 80% dibromopentyl glycol (2,2-bis(bromomethyl)-1,3-propanediol); 8% tribromoneopentyl alcohol (bis(bromomethyl)-1-bromo-3-hydroxypropane) and 6% monobromoneopentyl triol (2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane)] for study because it is a widely used flame retardant, and there was little or no information on the toxicity or carcinogenicity of this flame retardant reported in the literature at the time of nomination for study.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 2,2-BIS(BROMOMETHYL)-1,3-PROPANEDIOL

2,2-Bis(bromomethyl)-1,3-propanediol was obtained from Dow Chemical Company (Rolling Meadows, IL) in one lot (840429-162) which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix I). Reports on analyses performed in support of the 2,2-bis(bromomethyl)-1,3-propanediol studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a fine white powder, was identified as 2,2-bis(bromomethyl)-1,3-propanediol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity was determined by elemental analyses, Karl Fischer water analysis, thin-layer chromatography, and gas chromatography. Elemental analyses for carbon, hydrogen, and bromine were in agreement with the theoretical values for 2,2-bis(bromomethyl)-1,3-propanediol. Karl Fischer water analysis indicated 0.3% ± 0.1% water. Thin-layer chromatography by two systems indicated a major spot and one impurity. Gas chromatography using one system indicated one major peak and three impurities, and a second system indicated a major peak and four impurities. In both cases, the total impurity peak area was less than 3%. High-performance liquid chromatography analyses detected multiple impurities with five impurity peaks having areas of 1% or greater relative to the major peak area. The overall impurity peak area was 21.2%. Four impurities were isolated for identification by mass spectrometry. Two impurities, 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane (6.6%) and 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane (6.9%), were identified. One impurity (1%) was tentatively identified as a dimer of the parent chemical. Another impurity peak (2.8%) consisted of multiple components, including a structural isomer and a dimer of the major component. A quantitative analysis for pentaerythritol, a reactant in the synthesis of 2,2-bis(bromomethyl)-1,3-propanediol, was also conducted. Using a reference standard, 0.2% pentaerythritol was found. The overall purity for lot 840429-162 was determined to be approximately 79%.

Stability studies, performed by the analytical chemistry laboratory using gas chromatography, found that 2,2-bis(bromomethyl)-1,3-propanediol was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at room temperature in sealed containers protected from light. Stability was monitored monthly during the 13-week and 2-year studies using gas chromatography. No degradation of bulk chemical was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared weekly by mixing 2,2-bis(bromomethyl)-1,3-propanediol with feed (Table I1). Homogeneity and stability studies were performed by the analytical chemistry laboratory using gas chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 3 weeks when stored in the dark at −20° C. During the 13-week and 2-year studies the dose formulations were stored in the dark at −20° C for no more than 3 weeks.

Periodic analyses of the dose formulations of 2,2-bis(bromomethyl)-1,3-propanediol were conducted at the study laboratory and analytical chemistry laboratory using gas chromatography. During the 13-week studies, dose formulations were analyzed at the beginning, midpoint, and end of the studies (Table I2). During the 2-year studies, dose formulations were analyzed at least every 10 weeks (Table I3). Of the dose formulations analyzed, 92% (119/130) were within 10% of the target concentration. Results of periodic referee analyses performed

by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table I4).

13-WEEK STUDIES

The 13-week studies were conducted to evaluate the cumulative toxic effects of repeated exposure to 2,2-bis(bromomethyl)-1,3-propanediol and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Taconic Farms (Germantown, NY). On receipt, the rats and mice were 4 weeks old. The animals were quarantined for 11 (mice) or 14 (rats) days and were 6 weeks old on the first day of the studies. Before initiation of the studies, 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 two male and four female control mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

The concentrations for these 13-week feed studies were based on previous 13-week gavage studies where the chemical was administered to F344/N rats at doses of 0, 50, 100, 200, 400, or 800 mg/kg and to B6C3F$_1$ mice at doses of 0, 25, 50, 100, 200, or 400 mg/kg (Elwell *et al.*, 1989). Decreased body weights, urinary bladder transitional cell hyperplasia, and kidney degeneration occurred in male rats receiving 800 mg/kg, and male and female mice receiving 200 or 400 mg/kg. Body weights of female rats receiving 800 mg/kg were only marginally decreased. A high dose of 20,000 ppm was selected for the rat feed study which was estimated to deliver approximately 1,000 mg/kg. The high dose selected for the mouse feed study was 10,000 ppm which was estimated to deliver approximately 4,000 mg/kg. The doses for the 13-week mouse feed study were also selected to allow overlapping doses with the rat study for comparison of species response to 2,2-bis(bromomethyl)-1,3-propanediol.

Groups of 10 male and 10 female rats received 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 13 weeks. Groups of 10 male and 10 female mice received 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 13 weeks. Feed and water were available *ad libitum* except during urine collections. Rats were housed five per cage and mice were housed individually. Clinical findings were recorded weekly for rats and mice. Feed consumption was recorded weekly by cage. The animals were weighed initially, weekly, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Clinical pathology studies were performed on all male and female rats and mice in the 13-week studies. Selected serum chemistry parameters were measured on days 3, 15, 30, 60, and week 13 on rats in a special study group and at week 13 on rats and mice in the core studies. Urinalysis studies were performed on days 3, 15, 30, 60, and week 13 on rats in the special study group and at week 13 on rats and mice in the core studies. Urinalysis water deprivation studies were conducted on days 4, 16, 31, 61, and week 13 on rats in the special study group.

For serum chemistry studies, rats and mice were anesthetized with carbon dioxide and bled from the retroorbital sinus. Blood for serum analyses was collected in containers without anticoagulant, allowed to clot at room temperature, and centrifuged to separate the serum. For all urine studies, rats and mice were placed individually into metabolism cages for 16-hour (rats) or 24-hour (mice) urine collection. The urine containers were kept immersed in an ice water bath during sampling to minimize evaporation and suppress bacterial growth. During urine collection periods feed was removed and, except for during water deprivation studies, water was available *ad libitum*. For water deprivation studies, urine was collected from special study rats for 4 hours following a 16-hour water deprivation period. Water deprivation began approximately 8 hours after the blood collection required for serum chemistry analyses. Serum and urine chemistry end points were determined on a Cobas Fara chemistry analyzer (Roche Diagnostics Systems, Inc., Montclair, NJ) using reagents and methods obtained from the manufacturer. Urine volume was determined volumetrically and urine specific gravity was determined by refractometry. Parameters evaluated are listed in Table 1.

At the end of the studies, samples from 0, 5,000, 10,000, and 20,000 ppm rats and 0, 2,500, 5,000, and 10,000 ppm mice were collected for sperm morphology and vaginal cytology evaluations. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP General Statement of Work (April, 1984). For 7 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and aspirated samples of vaginal fluid and cells were transferred to slides and stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). All males were evaluated for sperm morphology, count, and motility. The right testis and right 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 right cauda epididymis was placed in buffered saline solution and finely minced. 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 hemocytometer. To quantify spermatogenesis, testicular spermatid head count was determined in the left testis by removing the tunica albuginea and homogenizing the testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted.

A necropsy was performed on all animals surviving to the end of the studies. The brain, heart, right kidney, liver, lung, spleen, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on control rats and mice, 20,000 ppm rats, and 10,000 ppm mice. In addition, the kidneys and urinary bladder of all other dose groups of rats and mice were examined. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 60 male and 60 female rats received 2,500, 5,000, or 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. Groups of 70 male and 60 female rats received 0 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. Groups of 60 male and 60 female mice received 0, 312, 625, or 1,250 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 104 to 105 weeks. Up to 10 male and female rats and mice from each group were evaluated at 15 months.

Stop-Exposure Evaluation

A group of 70 male rats received 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 3 months, when ten control and ten 20,000 ppm rats were evaluated. At 3 months, the dosed feed was replaced with a control diet for the remainder of the study.

Source and Specification of Animals

Male and female F344/N rats and B6C3F_1 mice were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year studies. On receipt, the animals were approximately 4 weeks old. The animals were quarantined for 10 to 12 days and were 6 weeks old on the first day of the studies. Before the initiation of the studies, 10 male and 10 female rats and five male and five female mice were selected for parasite evaluation and gross observation of disease. Serology samples were collected for viral screening. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance

Rats were housed five per cage and mice were housed individually. Feed and water were available *ad libitum*. Feed consumption was measured every 4 weeks by cage. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on

feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology

All animals were observed twice daily. Clinical findings and body weights were recorded initially, weekly for 13 weeks, monthly thereafter, and at the end of the studies.

A complete necropsy and microscopic examination were performed on all rats and mice except one 1,250 ppm male mouse that was missing. At the 3-month (male rats) and 15-month interim evaluations, the right kidney and liver of rats and mice were weighed. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (i.e., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The microscopic 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 reviewed the esophagus, kidney, pharynx, thyroid gland, tongue, and Zymbal's gland of male and female rats to confirm the incidences of neoplasms and nonneoplastic lesions. In addition, for male rats, the quality assessment pathologist reviewed ear, eye, forestomach, large and small intestine, liver, pancreas, seminal vesicle/coagulative gland, skin, spleen, teeth, urinary bladder, and multiple organs (mesothelioma) to confirm the incidences of neoplasms and nonneoplastic lesions. For mice, the quality assessment pathologist reviewed the forestomach, harderian gland, and lung of all mice to confirm the incidences of neoplasms and nonneoplastic lesions.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which a disagreement in diagnosis between the laboratory and quality assessment pathologists existed. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologist, or lesions of general interest were presented by the chair 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. Thus, the final diagnoses represent a consensus of contractor pathologists 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 diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

STATISTICAL METHODS

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, B5, C1, C5, D1, and D5 are given as the number of animals bearing

such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3, B3, C3, and D3) and all nonneoplastic lesions are given as the number of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., skin, intestine, harderian gland, and mammary gland) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3, B3, C3, and D3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

In these studies, large numbers of exposed rats died or were killed moribund early in the studies. These deaths were considered to be due primarily to Zymbal's gland neoplasms, subcutaneous tumors, and mononuclear cell leukemia. Consequently, for these particular lesions, primary emphasis in the analysis of neoplasm incidence was given to the life table test (Cox, 1972; Tarone, 1975), a survival-adjusted procedure appropriate for rapidly lethal neoplasms. For incidental neoplasms, the statistical method used was a logistic regression analysis, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidences (McKnight and Crowley, 1984). Other statistical analyses reported in the appendixes include the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures that are based on the overall proportion of neoplasm-bearing animals.

Tests of significance included pairwise comparisons of each exposed group with controls and a test for an overall dose-related trend. Continuity-corrected tests were used in the analysis of neoplasm incidence, and reported P values are one sided. The procedures described in the preceding paragraphs were also used to evaluate selected nonneoplastic lesions. For further discussion of these statistical methods, refer to Haseman (1984).

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Clinical chemistry, urinalysis, spermatid, and epididymal spermatozoa data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) 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). Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Because the 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 normality assumptions. 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 levels.

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, historical control data can be helpful in the overall assessment of neoplasm incidence in certain instances. Consequently, neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. 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, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of 2,2-bis(bromomethyl)-1,3-propanediol was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells, and the frequency of micronucleated erythrocytes in peripheral blood and bone marrow. The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies of 2,2-bis(bromomethyl)-1,3-propanediol are part of a larger effort by the NTP to develop a database that would permit the evaluation of carcinogenicity in experimental animals from the structure and responses of the chemical in short-term *in vitro* and *in vivo* genetic toxicity tests. These genetic toxicity tests were originally developed to study mechanisms of chemically induced DNA damage and to predict carcinogenicity in animals, based on the electrophilic theory of chemical carcinogenesis and the somatic mutation theory (Miller and Miller, 1977; Straus, 1981; Crawford, 1985).

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol

13-Week Studies	2-Year Studies
Study Laboratory	
American Biogenics Corporation (Woburn, MA)	Southern Research Institute (Birmingham, AL)
Strain and Species	
Rats: F344/N	Rats: F344/N
Mice: $B6C3F_1$	Mice: $B6C3F_1$
Animal Source	
Taconic Farms (Germantown, NY)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Studies	
Rats: 14 days	Rats: 10 or 11 days
Special Clinical Chemistry and Urinalysis Study (rats only):	Mice: 12 days
13-15 days (males)	
20-22 days (females)	
Mice: 11 days	
Average Age When Studies Began	
6-7 weeks	6 weeks
Date of First Dose	
Rats: 22 April 1986	Rats: 27 March 1989
Special Clinical Chemistry and Urinalysis Study (rats only):	Mice: 13 March 1989
12-14 May 1986 (males)	
19-21 May 1986 (females)	
Mice: 14 April 1986	
Duration of Dosing	
13 weeks	104 to 105 weeks
Date of Last Dose	
Rats: 22-24 July 1986	Rats: 24 March 1991 (males)
Special Clinical Chemistry and Urinalysis Study (rats only):	26 March 1991 (females)
13-15 August 1986 (males)	Mice: 10 March 1991 (males)
21-22 August 1986 (females)	17 March 1991 (females)
Mice: 14-16 July 1986	
Necropsy Dates	
Rats: 22-24 July 1986	Rats: 3-month interim evaluation - 26 June 1989
Special Clinical Chemistry and Urinalysis Study (rats only):	15-month interim evaluation - 25 June 1990
13-15 August 1986 (males)	terminal sacrifice - 1-5 April 1991
21-22 August 1986 (females)	Mice: 18-26 March 1991
Mice: 14-16 July 1986	
Age at Necropsy	
Rats: 19-20 weeks	Rats: 111 weeks
Mice: 19 weeks	Mice: 111-112 weeks

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

13-Week Studies	2-Year Studies
Size of Study Groups	
10 males and 10 females	Rats: 60 males and 60 females (2,500, 5,000, and 10,000 ppm); 70 males and 60 females (0 ppm); 70 males (20,000 ppm stop-exposure) Mice: 60 males and 60 females
Method of Distribution	
Randomized by weight into cage groups using a computer-generated table of random numbers	Randomized by weight using a random number table
Animals per Cage	
Rats: 5 Mice: 1	Rats: 5 Mice: 1
Method of Animal Identification	
Toe clip	Rats: Tail tattoo Mice: Toe clip
Diet	
NIH-07 open formula mash diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 13-week studies
Water Distribution	
Tap water (Woburn, MA, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI, or Hardco, Cincinnati, OH) available *ad libitum*, except during urine collection	Tap water (Birmingham, AL, municipal supply) via automatic watering system (Edstrom Industries, Waterford, WI) available *ad libitum*
Cages	
Polycarbonate (Lab Products Inc., Garfield, NJ), changed twice weekly	Same as 13-week studies, except mouse cages were changed weekly
Bedding	
Sani-Chip (P.J. Murphy Forestry Products, Corp., Rochelle Park, NJ)	Same as 13-week studies
Cage Filters	
Non-woven filter sheets	Reemay® spun-bonded polyester (Andico, Birmingham, AL) changed every 2 weeks
Racks	
Stainless steel (Lab Products Inc., Garfield, NJ), changed once every 2 weeks	Same as 13-week studies
Animal Room Environment	
Temperature: 18° to 26° C Relative humidity: 48% to 75% Fluorescent light: 12 hours/day Room air: 12 changes per hour	Temperature: 19° to 29° C (rats); 14° to 25° C (mice) Relative humidity: 26.3% to 90% (rats); 25.5% to 85.3% (mice) Fluorescent light: 12 hours/day Room air: 10 changes per hour

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

13-Week Studies	2-Year Studies
Doses	
Rats: 0, 1,250, 2,500, 5,000, 10,000 or 20,000 ppm in feed, available *ad libitum* Mice: 0, 625, 1,250, 2,500, 5,000, or 10,000 ppm in feed, available *ad libitum*	Rats: continuous-exposure study - 0, 2,500, 5,000, or 10,000 ppm; stop-exposure study - 20,000 ppm in feed, available *ad libitum* Mice: 0, 312, 625, or 1,250 ppm in feed, available *ad libitum*
Type and Frequency of Observation	
Observed twice daily; animals were weighed initially, weekly, and at the end of the studies. Clinical observations were recorded weekly. Feed consumption was measured weekly by cage.	Observed twice daily; body weights and clinical observations recorded initially, weekly for weeks 2 to 13, monthly thereafter, and at the end of the studies. Feed consumption was measured every 4 weeks by cage.
Method of Sacrifice	
Anesthetized with CO_2 followed by exsanguination via orbital bleeding	Same as 13-week studies
Necropsy	
Necropsy performed on all animals surviving to the end of the study. Organs weighed included the brain, heart, right kidney, liver, lung, spleen, right testis, and thymus.	All animals (except one 1,250 ppm mouse) were necropsied. Organs weighed at the 3-month (control and stop-exposure male rats) and 15-month interim evaluations were the right kidney and liver.
Clinical Pathology	
At the end of the 13-week studies, blood was collected from the retro-orbital sinus and urine was collected from all rats and mice. In the special study rats, blood was collected on days 3, 15, 30, 60, and at study termination. Urine samples were collected on days 3, 15, 30, 60, and at study termination. Additional urine samples were collected for measurement of urinary concentrating ability following 16-hour water deprivation periods. ***Clinical Chemistry:*** albumin, albumin/globulin ratio, creatinine (rats only), globulin, glucose, total protein, and urea nitrogen ***Urinalysis:*** glucose, protein, specific gravity, and volume	None
Sperm and Vaginal Cytology Evaluation	
Sperm and vaginal fluid samples were evaluated in 0, 5,000, 10,000, and 20,000 ppm rats and 0, 2,500, 5,000, and 10,000 ppm mice at the end of the studies. The parameters evaluated in males were sperm count, morphology, and motility. The right cauda, right epididymis, and right testis were weighed. Vaginal fluid samples were collected for up to 7 consecutive days prior to the end of the studies for vaginal cytology evaluations. The parameters evaluated in females were relative frequency of estrous stages and estrous cycle length.	None

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

13-Week Studies	2-Year Studies
Histopathology	
Complete histopathologic examinations were performed on all control rats and mice, 20,000 ppm rats, and 10,000 ppm mice. In addition to gross lesions, tissue masses and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, and rectum), liver, lung, lymph nodes (mandibular or mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial or clitoral gland (rats), prostate gland, salivary gland, skin, small intestine (duodenum, jejunum, and ileum), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus. The kidney and urinary bladder of all other rats and mice were also examined.	Complete histopathologic examinations were performed on all animals necropsied. In addition to gross lesions, tissue masses and associated lymph nodes, the tissues examined included: adrenal gland, bone and marrow, brain, esophagus, gallbladder (mice), heart, kidney, large intestine (cecum, colon, and rectum), liver, lung, lymph nodes (mandibular or mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial or clitoral gland, prostate gland, salivary gland, skin, small intestine (duodenum, jejunum, and ileum), spleen, stomach (forestomach and glandular), testis with epididymis and seminal vesicle, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

13-WEEK STUDY

All rats survived to the end of the study (Table 2). The final mean body weights and weight gains of 5,000, 10,000, and 20,000 ppm males and females were significantly lower than those of the controls. Feed consumption by exposed animals was lower than that by controls at week 1, but was generally similar to or slightly higher than that by controls at week 13 (Table 2). Dietary levels of 1,250, 2,500, 5,000, 10,000, and 20,000 ppm delivered average daily doses of 100, 200, 400, 800, and 1,700 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight to males, and 100, 200, 400, 800, and 1,630 mg/kg to females. No chemical-related clinical findings were observed.

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 13-Week Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	115 ± 3	353 ± 6	238 ± 4		16.1	17.9
1,250	10/10	116 ± 4	354 ± 6	238 ± 5	100	15.5	19.2
2,500	10/10	114 ± 3	338 ± 5	224 ± 4*	96	15.0	18.1
5,000	10/10	117 ± 4	324 ± 7**	207 ± 7**	92	15.0	19.3
10,000	10/10	116 ± 5	314 ± 6**	198 ± 4**	89	14.3	20.0
20,000	10/10	120 ± 5	269 ± 4**	149 ± 5**	76	13.7	19.3
Female							
0	10/10	96 ± 3	209 ± 4	113 ± 2		11.6	11.9
1,250	10/10	100 ± 2	204 ± 3	105 ± 2*	98	11.5	13.0
2,500	10/10	99 ± 3	200 ± 3	101 ± 1**	96	11.4	11.8
5,000	10/10	93 ± 2	196 ± 3**	102 ± 2**	94	11.4	12.2
10,000	10/10	97 ± 2	191 ± 3**	94 ± 3**	92	11.4	12.1
20,000	10/10	95 ± 2	174 ± 3**	79 ± 2**	83	10.3	11.6

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

Urinalysis and clinical chemistry data for rats in the core study are listed in Table G1. At the end of the study, 16-hour urine volumes in 10,000 and 20,000 ppm male rats were two-fold greater than that in the control group. These higher urine volumes were accompanied by urine specific gravity which was lower than that in the control group. Urine specific gravity in the 5,000 ppm male group was also lower than that in the control group. The female rats were less affected; minimal differences in the urine volume and specific gravity occurred only in the 2,500 ppm group. Renal papillary degeneration occurred in male rats exposed to 5,000 ppm or greater, which would be consistent with the increase in urine volume (polyuria). Minimally increased urine protein excretion (proteinuria) also occurred in the 10,000 and 20,000 ppm groups and may have been related to the renal lesions. In female rats, renal papillary degeneration was present in only one animal in the 20,000 ppm group and could explain the lack of polyuria or proteinuria in females.

Serum total protein and albumin concentration in female rats exposed to 2,500 ppm or greater were slightly lower than those in the controls. Decreased protein values can be caused by several factors including hyperhydration, albumin and/or protein loss associated with renal or intestinal disease (Kaneko, 1989; Nguyen, 1989).

No biologically significant differences in organ weights were observed (Table F1).

There were no treatment-related gross lesions. Treatment-related microscopic lesions were present in the kidney of male and female rats and the urinary bladder of male rats (Table 3). A minimal to mild degeneration of the renal papilla was present in 5,000, 10,000, and 20,000 ppm male rats and in one female rat in the 20,000 ppm group. This degenerative change was characterized by edema of the interstitial tissue at the distal tip of the renal papilla. The interstitial cells of the renal papilla appeared swollen and the nuclei stained less distinctly than in the controls. In the areas of papillary degeneration, there was increased eosinophilic staining of the cytoplasm of the interstitial cells that contained PAS-positive droplets. The cytoplasm of the epithelial cells lining the collecting ducts was vacuolated, and frequently a clear, nonstaining area in the cytoplasm was present around the nuclei of these cells. In the urinary bladder of male rats in the 20,000 ppm group, there was minimal hyperplasia of the transitional epithelium.

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
Kidney[a]	10	10	10	10	10	10
Degeneration, Papillary[b]	0	0	0	3 (1.0)[c]	6** (1.3)	8** (1.3)
Urinary Bladder	10	10	10	10	10	10
Hyperplasia	0	0	0	0	0	9** (1.0)
Female						
Kidney	10	10	10	10	10	10
Degeneration, Papillary	0	0	0	0	0	1 (1.0)

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

Clinical Chemistry and Urinalysis in Special Study Rats

Urinalysis and clinical chemistry data for rats in the special study are listed in Table G1. Similar to the core study animals, changes in urine volume and specific gravity were the major treatment effects, and male rats were more affected than females. On days 3 and 15, urine volume was slightly decreased, and urine specific gravity was increased in 20,000 ppm males compared to controls. On day 3, urine volume was also slightly decreased in the 20,000 ppm females. This would be consistent with a mild transient dehydration related to decreased food and water intake resulting in a smaller, but more concentrated, urine volume. Transient dehydration is supported by the mild increase in serum total protein concentration that occurred in a treatment-related fashion in male rats on days 3 and 15. By day 60, urine volumes were markedly increased (polyuria) in 10,000 and 20,000 ppm males compared to that of the controls. At this time, urine specific gravity decreased to the isosthenuric range (1.008 to 1.012) in these animals and in 5,000 ppm male rats. Additionally, a decreased urine specific gravity occurred in females exposed to 2,500 ppm or greater but was not accompanied by increased urine volume. At the end of the study, 16-hour urine volume was increased in the 20,000 ppm males and was accompanied by decreased urine specific gravity in 10,000 and 20,000 ppm males and 5,000 ppm females. Water deprivation tests demonstrated that male and female rats were able to adequately concentrate their urine in response to dehydration throughout the study. However, by day 61 the urine specific gravity in water-deprived, 20,000 ppm males was lower than that in the control group. Renal papillary degeneration that occurred in males exposed to 5,000 ppm or greater would be consistent with isosthenuric polyuria. On day 61, a minimal increase in the urine protein excretion also occurred in 10,000 and 20,000 ppm males and could be consistent with renal lesions. By day 60, an increase in serum total protein concentration occurred in 5,000 and 20,000 ppm male rats. This could be consistent with excess renal fluid loss resulting in a mild dehydration. Again, the absence of renal papillary degeneration in female rats could explain the lack of polyuria. Changes in other clinical chemistry and urinalysis variables were minor, sporadic, and were not considered relevant.

Dose Selection Rationale: Based on lower final mean body weights in the 20,000 ppm males and females, the incidences of renal papillary degeneration in 20,000 ppm males and females, and hyperplasia of the urinary bladder in 20,000 ppm males, the high dose selected for continuous exposure in the 2-year study was 10,000 ppm; 20,000 ppm was selected as the exposure concentration for a 3-month stop-exposure study in male rats to evaluate the potential for progression or regression of urinary bladder and kidney lesions.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves in Figure 1. Survival of 5,000 and 10,000 ppm continuous-exposure males and females and 20,000 ppm stop-exposure males was significantly lower than that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of males and females receiving 10,000 ppm and stop-exposure males receiving 20,000 ppm were lower than those of the controls throughout most of the study (Tables 5 and 6 and Figure 2). In the continuous-exposure study, feed consumption by exposed rats was generally similar to that by the controls throughout the study (Tables J1 and J2). In 20,000 ppm stop-exposure males the feed consumption was lower than that by controls. Dietary levels of 2,500, 5,000, and 10,000 ppm delivered average daily doses of 100, 200, and 430 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight to males and 115, 230, and 460 mg/kg to females. Dietary levels of 20,000 ppm delivered an average daily dose of 800 mg/kg to stop-exposure males. Clinical findings included skin and subcutaneous tissue masses on the face, tail, and the ventral and dorsal surfaces of exposed rats.

TABLE 4
Survival of Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
Animals initially in study	70	60	60	60	70
3-Month interim evaluation[b]	10	0	0	0	10
15-Month interim evaluation[b]	9	7	9	5	0
Moribund	24	30	36	43	55
Natural deaths	1	3	2	11	5
Animals surviving to study termination	26	20	13	1	0
Percent probability of survival at the end of study[c]	51	38	26	2	0
Mean survival (days)[d]	688	652	669	587	544
Survival analysis[e]	P<0.001	P=0.126	P=0.010	P<0.001	P<0.001
Female					
Animals initially in study	60	60	60	60	
15-Month interim evaluation[b]	10	9	7	8	
Moribund	14	22	27	41	
Natural deaths	0	2	3	6	
Animals surviving to study termination	36	27	23	5	
Percent probability of survival at the end of study	72	53	43	10	
Mean survival (days)	711	701	676	630	
Survival analysis	P<0.001	P=0.095	P=0.005	P<0.001	

a Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats received control feed until the end of the 2-year study.
b Censored from survival analyses
c Kaplan-Meier determinations
d Mean of all deaths (uncensored, censored, and terminal sacrifice).
e The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.

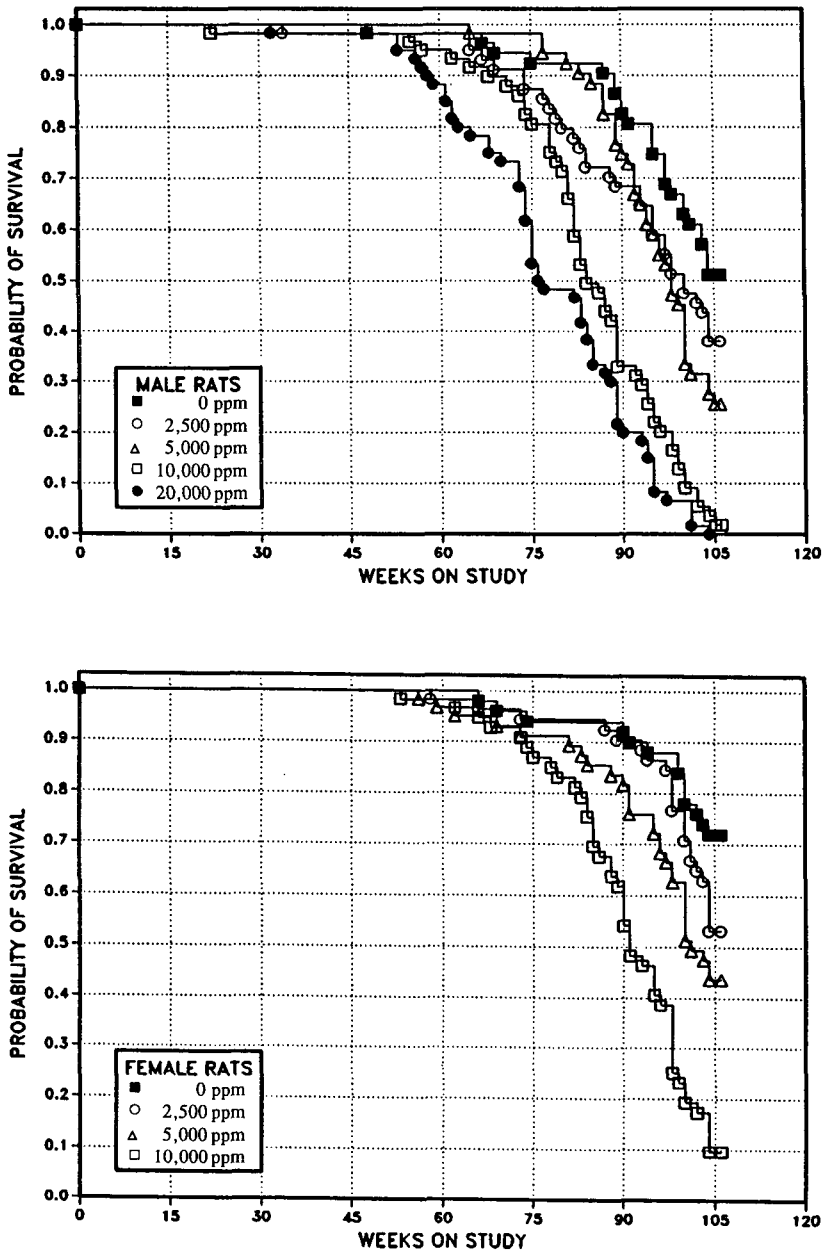


FIGURE 1
Kaplan-Meier Survival Curves for Male and Female Rats Administered 2,2-Bis(bromomethyl)-1,3-propanediol in Feed for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Weeks on Study	0 ppm		2,500 ppm			5,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	114	70	114	101	60	114	100	60
2	163	70	161	99	60	157	97	60
3	198	70	195	99	60	193	98	60
4	231	70	227	98	60	223	97	60
5	249	70	242	98	60	236	95	60
6	267	70	260	98	60	253	95	60
7	284	70	277	98	60	269	95	60
8	295	70	288	98	60	285	97	60
9	306	70	297	97	60	291	95	60
10	314	70	306	97	60	300	96	60
11	321	70	311	97	60	305	95	60
12	330	70	323	98	60	316	96	60
13	341	70	333	98	60	326	96	60
17[a]	365	60	355	97	60	346	95	60
21	386	60	374	97	60	362	94	60
25	399	60	386	97	60	376	94	60
29	412	60	401	97	60	384	93	60
33	424	60	413	97	60	401	95	60
37	432	60	423	98	59	412	95	60
41	442	60	431	97	59	424	96	60
45	440	60	432	98	59	420	96	60
49	452	59	438	97	59	430	95	60
53	454	59	444	98	59	438	96	60
57	458	59	454	99	59	446	97	60
61	461	59	452	98	59	438	95	60
65	463	59	449	97	58	445	96	60
69[a]	457	49	449	98	49	443	97	50
73	455	48	446	98	48	435	96	50
77	450	47	443	98	46	431	96	50
81	444	47	442	100	42	428	96	48
85	443	47	442	100	38	428	97	46
89	440	44	440	100	37	418	95	42
93	435	41	429	99	35	412	95	33
97	432	35	433	100	29	403	93	27
101	432	31	426	99	25	408	94	16
105	425	26	425	100	20	401	95	13
Mean for weeks								
1-13	263		256	97		251	95	
14-52	417		406	97		395	95	
53-105	446		441	99		427	96	

(continued)

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

Weeks on Study	10,000 ppm Av. Wt. (g)	10,000 ppm Wt. (% of controls)	10,000 ppm No. of Survivors	20,000 ppm[b] Av. Wt. (g)	20,000 ppm[b] Wt. (% of controls)	20,000 ppm[b] No. of Survivors
1	109	96	60	105	93	70
2	152	94	60	134	83	70
3	181	91	60	157	79	70
4	208	90	60	176	76	70
5	221	89	60	185	74	70
6	236	89	60	197	74	70
7	249	88	60	206	73	70
8	259	88	60	215	73	70
9	264	86	60	218	71	70
10	273	87	60	222	71	70
11	279	87	60	229	72	70
12	288	87	60	238	72	70
13	298	88	60	245	72	70
17[a]	319	88	60	292	80	60
21	340	88	60	323	84	60
25	355	89	59	347	87	60
29	367	89	59	366	89	60
33	377	89	59	382	90	59
37	388	90	59	399	92	59
41	396	90	59	410	93	59
45	395	90	59	410	93	59
49	401	89	59	419	93	59
53	414	91	59	430	95	57
57	418	91	57	431	94	56
61	415	90	57	422	92	53
65	414	89	56	429	93	48
69[a]	420	92	49	430	94	45
73	406	89	48	423	93	44
77	416	92	44	424	94	30
81	407	92	39	414	93	29
85	402	91	27	410	93	22
89	394	89	20	409	93	15
93	388	89	16	374	86	11
97	384	89	11	402	93	5
101	369	86	5	373	86	3
105						
Mean for weeks						
1-13	232	88		194	74	
14-52	371	89		372	89	
53-105	404	91		413	93	

[a] Interim evaluations occurred during week 14 (0 and 20,000 ppm groups) and week 66 (0, 2,500, 5,000, and 10,000 ppm groups).
[b] Stop-exposure group

TABLE 6
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	2,500 ppm Av. Wt. (g)	2,500 ppm Wt. (% of controls)	2,500 ppm No. of Survivors	5,000 ppm Av. Wt. (g)	5,000 ppm Wt. (% of controls)	5,000 ppm No. of Survivors	10,000 ppm Av. Wt. (g)	10,000 ppm Wt. (% of controls)	10,000 ppm No. of Survivors
1	106	60	105	99	60	106	100	60	106	100	60
2	130	60	127	98	60	126	98	60	127	98	60
3	144	60	142	99	60	139	97	60	138	96	60
4	154	60	151	98	60	150	97	60	147	96	60
5	161	60	159	99	60	156	97	60	152	95	60
6	168	60	165	98	60	165	98	60	159	95	60
7	175	60	172	98	60	171	98	60	166	95	60
8	178	60	174	98	60	172	97	60	168	95	60
9	181	60	178	99	60	175	97	60	170	94	60
10	185	60	180	97	60	178	96	60	172	93	60
11	188	60	183	98	60	181	97	60	175	93	60
12	191	60	187	98	60	184	96	60	179	93	60
13	191	60	187	98	60	186	98	60	180	94	60
17	201	60	198	99	60	193	96	60	186	93	60
21	206	60	203	98	60	198	96	60	192	93	60
25	212	60	209	99	60	203	96	60	199	94	60
29	220	60	214	97	60	211	96	60	205	93	60
33	224	60	220	98	60	214	96	60	209	93	60
37	231	60	229	99	60	221	96	60	215	93	60
41	238	60	234	98	60	237	99	60[a]	224	94	60
45	246	60	240	98	60	234	95	60	228	93	60
49	258	60	254	98	60	247	96	60	239	93	60
53	268	60	265	99	60	257	96	60	247	92	59
57	282	60	277	98	60	270	96	59	259	92	59
61	289	60	284	98	59	275	95	58	262	91	59
65	299	60	293	98	59	283	95	57	269	90	58
69[b]	303	49	300	99	49	288	95	50	274	90	48
73	308	48	300	97	49	291	94	48	277	90	48
77	314	47	305	97	48	295	94	48	284	90	45
81	313	47	308	98	48	299	96	47	291	93	43
85	312	47	307	98	48	296	95	45	286	91	37
89	315	47	314	100	46	305	97	44	293	93	32
93	319	45	318	100	45	311	98	40	297	93	24
97	326	44	325	100	43	323	99	35	301	92	20
101	330	39	327	99	34	322	98	26	307	93	10
105	329	36	328	100	27	320	97	23	313	95	5
Mean for weeks											
1-13	166		162	98		161	97		157	95	
14-52	226		222	98		218	96		211	93	
53-105	308		304	99		295	96		283	92	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.
[b] Interim evaluation occurred during week 66.

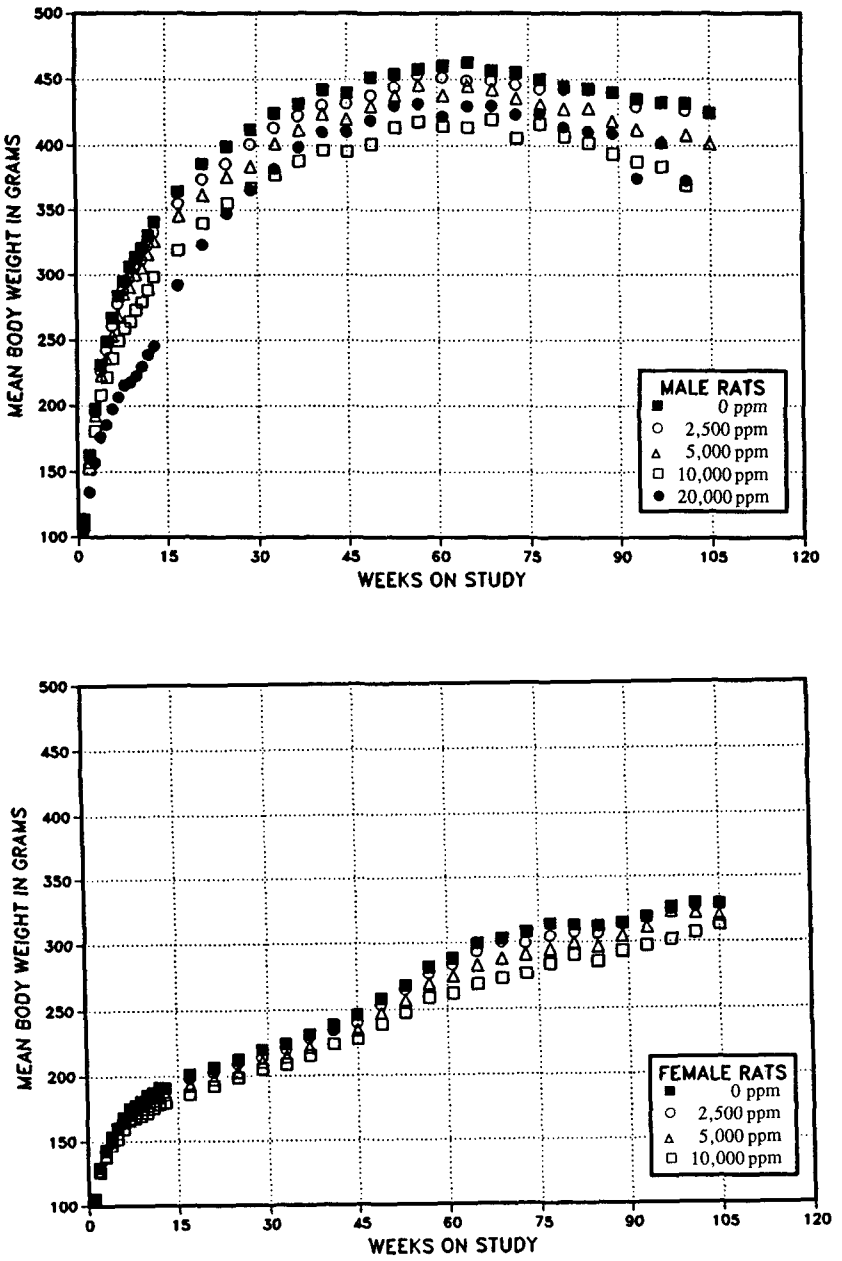


FIGURE 2
Growth Curves for Male and Female Rats Administered 2,2-Bis(bromomethyl)-1,3-propanediol in Feed for 2 Years

Pathology and Statistical Analysis

This section describes statistically significant and biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the skin, mammary gland, Zymbal's gland, oral cavity (pharynx, tongue, and gingiva), esophagus, forestomach, intestine (small and large), kidney, urinary bladder, lung, thyroid gland, seminal vesicle, and pancreas, and in the incidences of malignant mesothelioma and mononuclear cell leukemia. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of 5% in at least one exposure group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Skin: The incidence of squamous cell papilloma in the 20,000 ppm stop-exposure males was significantly greater than that in the control group (Tables 7 and A3). Additionally, the incidences of keratoacanthoma, and of squamous and basal cell neoplasms (combined) in 5,000 and 10,000 ppm continuous-exposure males and in the 20,000 ppm stop-exposure males were significantly greater than that in the control group (Tables 7 and A3). The incidences of keratoacanthoma, and of squamous and basal cell neoplasms (combined) in 5,000 and 10,000 ppm continuous-exposure males and in the 20,000 ppm stop-exposure males exceeded NTP historical control range (Tables 7 and A4a). These masses were all observed at necropsy and occurred at various sites on the body (tail, leg, neck, etc.). Eight of the squamous cell papillomas in exposed male rats occurred on the lips; the one papilloma that occurred in the control group was observed on the tail. Papillomas were exophytic masses of well-differentiated squamous epithelium. Keratoacanthomas extended slightly above the skin surface but generally formed plaque-like masses within the skin and consisted of well-differentiated squamous epithelium with abundant keratin formation. One 20,000 ppm stop-exposure male had squamous cell carcinoma.

The incidences of basal cell and sebaceous gland neoplasms (trichoepithelioma, basal cell adenoma, sebaceous gland adenoma, or basal cell carcinoma [combined]) in 10,000 ppm continuous-exposure males and 20,000 ppm stop-exposure males were greater than that in the control group (Tables 7 and A3). Most of these neoplasms were benign neoplasms and ranged from well-differentiated sebaceous gland adenoma to basal cell adenoma that had morphologic patterns consisting of cords or nests of basal cells as well as areas with sebaceous or squamous differentiation or development of hair follicles (trichoepithelioma).

In addition to epithelial neoplasms of the skin, there were significantly increased incidences of subcutaneous skin neoplasms in all continuous-exposure groups of males (Tables 7 and A3). These subcutaneous masses were located along the lateral and ventral abdominal wall and were also present in the axillary and inguinal areas. Often these large masses were the primary reason for the moribund sacrifice of these rats. In the 5,000 and 10,000 ppm groups of males, there were multiple fibromas in four and six rats, respectively; multiple sarcomas were present in one male in the 10,000 ppm group (Table A1). The incidences of fibroma, fibrosarcoma, or sarcoma (combined) in 2,500, 5,000 and 10,000 ppm continuous-exposure males and in the 20,000 ppm stop-exposure males exceeded the NTP historical control range (Tables 7 and A4b). Fibroma consisted of a uniform mass of spindle-shaped cells within a dense matrix of collagen fibers (Plate 1). Except for the absence of a glandular component, fibromas were morphologically similar to fibroadenomas observed in the same exposure groups of male rats. Sarcomas were composed of anaplastic, spindle-shaped cells that had indistinct and variable growth patterns. Because of the absence of sufficient differentiation of the sarcomas, it was not possible to determine the cell of origin for these malignant mesenchymal tumors.

TABLE 7
Incidences of Skin Neoplasms in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
15-Month Interim Evaluation					
Skin[b]	9	7	9	5	—[d]
Squamous Cell Papilloma[c]	0	1	0	1	
2-Year Study					
Skin	51	53	51	55	59
Squamous Cell Papilloma	1	0	2	5	11**
Keratoacanthoma	3	5	11*	16**	10**
Squamous Cell Carcinoma	0	0	0	0	1
Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma	4	5	13*	20**	19**
Trichoepithelioma	0	0	0	1	1
Sebaceous Gland Adenoma	0	1	0	2	2
Basal Cell Adenoma	0	1	0	3**	6**
Basal Cell Carcinoma	0	0	2	2	0
Trichoepithelioma, Sebaceous Gland Adenoma, Basal Cell Adenoma, or Basal Cell Carcinoma	0	2	2	7**	9**
Squamous Cell Papilloma, Keratoacanthoma, Trichoepithelioma, Basal Cell Adenoma or Carcinoma, or Squamous Cell Carcinoma[e]	4	6	14**	24**	21**
Subcutaneous Tissue	51	53	51	55	59
Fibroma	2	8*	11**	15**	7**
Fibrosarcoma	0	1	0	0	1
Sarcoma	0	0	2	3**	2
Fibroma, Fibrosarcoma, or Sarcoma[f]	2	9*	13**	16**	10**

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test or the life table test (subcutaneous neoplasms).
** $P \leq 0.01$
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with skin examined microscopically
[c] Number of animals with neoplasms
[d] No animals from the stop-exposure group were examined at the 15-month interim evaluation
[e] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 101/1,353 (7.5% ± 3.1%); range 2%-16%
[f] Historical incidence: 89/1,353 (6.6% ± 4.3%); range 0%-16% (includes data for neurofibrosarcoma, fibrosarcoma, sarcoma, neurofibroma, and fibroma)

Mammary Gland: The incidences of benign mammary gland neoplasms (fibroadenoma and fibroadenoma or adenoma [combined]) were significantly greater in the stop-exposure group of male rats and in all continuous-exposure groups of male rats than in the control group (Tables 8 and A3). In female rats, the incidences of fibroadenoma and of fibroadenoma, adenoma, or carcinoma (combined) in all exposed groups were greater than those in the control group (Tables 8 and B3). In male rats, the incidences of fibroadenoma or adenoma (combined) in the 5,000 and 10,000 ppm groups exceeded the NTP historical control range (Tables 8 and A4c). In female rats, the incidence of fibroadenoma, adenoma, or carcinoma (combined) in all exposure groups exceeded the historical control range (Tables 8 and B4a). The incidences of multiple fibroadenoma in all exposed female groups were greater than that in the control group (Table B1). Fibroadenomas were morphologically similar in exposed and control groups and consisted of multiple foci of a well-differentiated epithelial component, forming ductules and alveoli that were surrounded by a dense proliferation of fibrous connective tissue. While the connective tissue component was prominent and sometimes composed the major portion of the fibroadenoma, the adenomas consisted predominantly of glands, ductules, or alveoli with little or no apparent fibrous stroma. Incidences of carcinoma in exposed groups did not differ significantly from those in the control groups (Tables 8, A3, and B3).

TABLE 8
Incidences of Mammary Gland Neoplasms in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
Mammary Gland					
Fibroadenoma					
Overall rate[b]	0/51 (0%)	4/53 (8%)	6/51 (12%)	6/55 (11%)	5/60 (8%)
Adjusted rate[c]	0.0%	18.9%	42.6%	51.6%	57.6%
Terminal rate[d]	0/26 (0%)	3/20 (15%)	5/13 (38%)	0/1 (0%)	0/0
First incidence (days)	—[f]	725	726	576	592
Logistic regression test[e]	P<0.001	P=0.034	P<0.001	P=0.003	P=0.001
Fibroadenoma, Multiple					
Overall rate	0/51 (0%)	1/53 (2%)	2/51 (4%)	0/55 (0%)	1/60 (2%)
Adenoma					
Overall rate	0/51 (0%)	0/53 (0%)	1/51 (2%)	1/55 (2%)	0/60 (0%)
Fibroadenoma or Adenoma[g]					
Overall rate	0/51 (0%)	4/53 (8%)	7/51 (14%)	7/55 (13%)	5/60 (8%)
Adjusted rate	0.0%	18.9%	44.8%	53.2%	57.6%
Terminal rate	0/26 (0%)	3/20 (15%)	5/13 (38%)	0/1 (0%)	0/0
First incidence (days)	—	725	684	576	592
Logistic regression test	P<0.001	P=0.034	P<0.001	P=0.002	P=0.001

(continued)

TABLE 8
Incidences of Mammary Gland Neoplasms in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female				
15-Month Interim				
Mammary Gland	10	9	7	8
Fibroadenoma	1	1	0	3
2-Year Study				
Mammary Gland				
Fibroadenoma				
Overall rate	25/50 (50%)	45/51 (88%)	46/53 (87%)	45/52 (87%)
Adjusted rate	60.7%	95.7%	97.9%	100.0%
Terminal rate	20/36 (56%)	25/27 (93%)	22/23 (96%)	5/5 (100%)
First incidence (days)	516	460	565	460
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001
Fibroadenoma, Multiple				
Overall rate	6/50 (12%)	37/51** (73%)	40/53** (75%)	37/52** (71%)
Adenoma				
Overall rate	0/50 (0%)	2/51 (4%)	0/53 (0%)	0/52 (0%)
Carcinoma				
Overall rate	4/50 (8%)	4/51 (8%)	3/53 (6%)	4/52 (8%)
Adjusted rate	9.7%	12.9%	7.2%	10.6%
Terminal rate	1/36 (3%)	3/27 (11%)	0/23 (0%)	0/5 (0%)
First incidence (days)	624	404	388	432
Logistic regression test	P=0.211N	P=0.603N	P=0.341N	P=0.313N
Fibroadenoma, Adenoma, or Carcinoma[h]				
Overall rate	27/50 (54%)	47/51 (92%)	47/53 (89%)	47/52 (90%)
Adjusted rate	62.5%	97.9%	97.9%	100.0%
Terminal rate	20/36 (56%)	26/27 (96%)	22/23 (96%)	5/5 (100%)
First incidence (days)	516	404	388	432
Logistic regression test	P<0.001	P<0.001	P<0.001	P<0.001

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with neoplasm per number of animals necropsied
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[d] Observed incidence in animals surviving until the end of the study
[e] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[f] Not applicable; no neoplasms in animal group
[g] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 63/1,353 (4.7% ± 3.1%); range 0%-12%
[h] Historical incidence: 568/1,351 (42.0% ± 14.0%); range 8%-64%

Zymbal's Gland: The incidences of Zymbal's gland adenoma in 10,000 ppm males and of adenoma or carcinoma in 20,000 ppm stop-exposure males were significantly greater than those in the controls (Tables 9 and A3). The incidences of adenoma in all other exposed groups, and the incidences of carcinoma, or adenoma or carcinoma (combined) in all continuously exposed male and female rats were not significantly different from those of the control groups. The incidences of adenoma or carcinoma (combined) in 5,000 and 10,000 ppm continuous-exposure males, and in 20,000 ppm stop-exposure males, exceeded the NTP historical control range (Tables 9 and A4d). In the 20,000 ppm stop-exposure group, two rats developed bilateral Zymbal's gland carcinoma (Table A1). The Zymbal's gland neoplasms frequently ulcerated through the skin and in almost all instances were the primary cause of the moribund condition of the rats. These neoplasms are of modified sebaceous gland origin, and those that occurred in exposed groups were morphologically similar to the malignant Zymbal's gland neoplasms that infrequently occur in control rats. Carcinomas were expansile, invasive neoplasms that extended into the adjacent muscle and soft tissues. Two carcinomas in 10,000 ppm males, one in 20,000 ppm males, and one in a control male metastasized to the lung (Table A1).

TABLE 9
Incidences of Zymbal's Gland Neoplasms in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Zymbal's Gland					
Adenoma					
Overall rate[b]	0/51 (0%)	0/53 (0%)	1/51 (2%)	3/55 (5%)	2/60 (3%)
Adjusted rate[c]	0.0%	0.0%	4.3%	52.6%	7.9%
Terminal rate[d]	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—[f]	—	694	556	513
Life table test[e]	P=0.001	—	P=0.422	P=0.020	P=0.129
Carcinoma					
Overall rate	2/51 (4%)	1/53 (2%)	3/51 (6%)	2/55 (4%)	15/60 (25%)
Adjusted rate	4.1%	3.8%	7.2%	5.8%	44.7%
Terminal rate	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	334	696	592	516	222
Life table test	P=0.286	P=0.554N	P=0.467	P=0.582	P<0.001
Adenoma or Carcinoma[g]					
Overall rate	2/51 (4%)	1/53 (2%)	4/51 (8%)	5/55 (9%)	15/60 (25%)
Adjusted rate	4.1%	3.8%	11.2%	55.4%	44.7%
Terminal rate	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	334	696	592	516	222
Life table test	P=0.009	P=0.544N	P=0.286	P=0.067	P<0.001

[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with neoplasm per number of animals necropsied
[c] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[d] Observed incidence in animals surviving until the end of the study
[e] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. A lower incidence in an exposure group is indicated by N.
[f] Not applicable; no neoplasms in animal group
[g] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 16/1,353 (1.2% ± 1.4%); range 0%-4%

Oral Cavity (Pharynx, Tongue, and Gingiva), Esophagus, and Forestomach: The incidences of squamous cell papilloma of the oral cavity in exposed males (continuous- and stop-exposure) were significantly greater than that in the control group (Tables 10 and A3). Additionally, the incidences of squamous cell papilloma of the esophagus in male and female rats exposed to 10,000 ppm were significantly greater than those in the control groups (Tables 10, A3, and B3). These benign neoplasms were observed grossly at necropsy and consisted of well-demarcated exophytic nodular or papillary masses arising from the mucosal surface of the tongue, soft or hard palate of the pharynx, gingiva, or esophagus. In male rats these neoplasms were more commonly observed in the oral cavity (Plate 2), while in female rats the higher incidences occurred in the esophageal mucosa. Some of these esophageal squamous cell neoplasms occurred near the proximal origin of the esophagus at the posterior aspect of the pharynx. In exposed rats, the incidences of squamous cell carcinoma at these sites were not significantly different from those of the control groups; however, these malignant neoplasms occurred only in the oral cavities of exposed rats (Tables 10, A1, and B1). The incidences of squamous cell neoplasms of the oral cavity in 20,000 ppm stop-exposure males were similar to those in 10,000 ppm males (Tables 10 and A1). The incidences of squamous cell papilloma and carcinoma (combined) of the oral cavity in all exposed groups of males and 5,000 and 10,000 ppm females exceeded the NTP historical control ranges (Tables 10, A4g, and B4e).

Squamous cell papilloma of the forestomach (Plate 3) occurred in exposed groups of rats, but the incidence was only significant in 20,000 ppm stop-exposure males (Tables 10, A3, and B3). These benign squamous cell neoplasms in the forestomach were morphologically similar to those that occurred in the oral cavity and esophagus. There were no significantly increased incidences of inflammation, necrosis, or diffuse hyperplasia at these sites, but focal areas of squamous cell hyperplasia in the tongue, palate of the pharynx, or esophagus were present in a few rats from exposed groups (Tables 10, A5, and B5).

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Cavity, Esophagus, and Forestomach in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
Pharynx[b]	—[d]	3	4	5	10
Palate, Epithelium, Hyperplasia, Focal[c]		1 (3.0)[e]	1 (2.0)	3 (2.0)	2 (2.0)
Tongue	—	2	5	13	9
Epithelium, Hyperplasia, Focal		0	0	4 (2.3)	3 (2.3)
Oral Cavity (Pharynx, Tongue, or Gingiva)					
Squamous Cell Papilloma					
Overall rate[f]	0/51 (0%)	4/53 (8%)	8/51 (16%)	10/55 (18%)	12/60 (20%)
Adjusted rate[g]	0.0%	20.0%	35.7%	44.2%	100.0%
Terminal rate[h]	0/26 (0%)	4/20 (20%)	3/13 (23%)	0/1 (0%)	0/0
First incidence (days)	—[j]	736 (T)	536	381	511
Logistic regression test[i]	P<0.001	P=0.033	P=0.004	P=0.005	P<0.001
Squamous Cell Carcinoma					
Overall rate	0/51 (0%)	0/53 (0%)	1/51 (2%)	0/55 (0%)	2/60 (3%)
Squamous Cell Papilloma or Squamous Cell Carcinoma[k]					
Overall rate	0/51 (0%)	4/53 (8%)	9/51 (18%)	10/55 (18%)	13/60 (22%)
Adjusted rate	0.0%	20.0%	39.0%	44.2%	100.0%
Terminal rate	0/26 (0%)	4/20 (20%)	3/13 (23%)	0/1 (0%)	0/0
First incidence (days)	—	736 (T)	536	381	511
Logistic regression test	P<0.001	P=0.033	P=0.002	P=0.005	P<0.001
Esophagus	51	53	51	55	60
Epithelium, Hyperplasia, Focal	0	0	0	1 (2.0)	0
Squamous Cell Papilloma					
Overall rate	0/51 (0%)	0/53 (0%)	1/51 (2%)	5/55 (9%)	0/60 (0%)
Adjusted rate	0.0%	0.0%	3.2%	62.6%	0.0%
Terminal rate	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—	—	662	549	—
Logistic regression test	P=0.001	—	P=0.507	P=0.021	—
Squamous Cell Carcinoma					
Overall rate	0/51 (0%)	0/53 (0%)	0/51 (0%)	1/55 (2%)	0/60 (0%)
Forestomach	51	53	51	55	59
Mucosa, Hyperplasia	4 (1.8)	12 (1.8)	6 (2.3)	6 (2.0)	6 (1.5)
Squamous Cell Papilloma[l]					
Overall rate	0/51 (0%)	0/53 (0%)	0/51 (0%)	1/55 (2%)	5/60 (8%)
Adjusted rate	0.0%	0.0%	0.0%	3.8%	26.7%
Terminal rate	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—	—	—	604	511
Logistic regression test	P<0.001	—	—	P=0.571	P=0.028

(continued)

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Oral Cavity, Esophagus, and Forestomach in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female				
Pharynx	1	1	1	2
Palate, Epithelium, Hyperplasia, Focal	0	0	0	1 (1.0)
Tongue	1	3	6	6
Epithelium, Hyperplasia, Focal	0	1 (2.0)	1 (2.0)	1 (2.0)
Oral Cavity (Pharynx or Tongue)				
Squamous Cell Papilloma				
Overall rate	2/50 (4%)	2/51 (4%)	4/53 (8%)	5/52 (10%)
Adjusted rate	5.6%	7.4%	11.5%	47.0%
Terminal rate	2/36 (6%)	2/27 (7%)	1/23 (4%)	2/5 (40%)
First incidence (days)	738 (T)	738 (T)	627	577
Logistic regression test	P=0.054	P=0.588	P=0.348	P=0.094
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	1/51 (2%)	1/53 (2%)	1/52 (2%)
Adjusted rate	0.0%	3.2%	2.6%	3.6%
Terminal rate	0/36 (0%)	0/27 (0%)	0/23 (0%)	0/5 (0%)
First incidence (days)	—	723	662	631
Logistic regression test	P=0.408	P=0.494	P=0.544	P=0.591
Squamous Cell Papilloma or Squamous Cell Carcinoma[m]				
Overall rate	2/50 (4%)	3/51 (6%)	5/53 (9%)	6/52 (12%)
Adjusted rate	5.6%	10.4%	13.8%	48.9%
Terminal rate	2/36 (6%)	2/27 (7%)	1/23 (4%)	2/5 (40%)
First incidence (days)	738 (T)	723	627	577
Logistic regression test	P=0.042	P=0.424	P=0.236	P=0.064
Esophagus	50	51	53	52
Epithelium, Hyperplasia, Focal	0	0	0	1 (2.0)
Squamous Cell Papilloma				
Overall rate	0/50 (0%)	0/51 (0%)	1/53 (2%)	10/52 (19%)
Adjusted rate	0.0%	0.0%	4.3%	42.4%
Terminal rate	0/36 (0%)	0/27 (0%)	1/23 (4%)	0/5 (0%)
First incidence (days)	—	—	738 (T)	474
Logistic regression test	P<0.001	—	P=0.411	P=0.002

(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with organ examined microscopically
[c] Number of animals with lesion
[d] Organ not examined at this exposure level
[e] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[f] Number of animals with neoplasm per number of animals necropsied
[g] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[h] Observed incidence in animals surviving until the end of the study
[i] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[j] Not applicable; no neoplasms in animal group
[k] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 11/1,353 (0.8% ± 1.4%); range 0%-4% (includes data for oral mucosa, tongue, pharynx, tooth, and lip)
[l] Historical incidence: 3/1351 (0.2% ± 0.6%); range 0%-2%
[m] Historical incidence: 12/1,351 (0.9% ± 1.4%); range 0%-6% (includes data for oral mucosa, tongue, pharynx, tooth, and lip)

Small and Large Intestine: The incidence of adenoma or carcinoma (combined) of the small intestine in 20,000 ppm stop-exposure males was greater than that in the control group (Tables 11 and A1), although the difference was not statistically significant, and the incidence exceeded the NTP historical control range (Tables 11 and A4j). The carcinomas were characterized by extensive invasion of the muscular wall of the intestine and a marked scirrhous response around and within the neoplasm. Several carcinomas contained cystic areas, and one carcinoma contained an area of osseous metaplasia (Table A5). One male from the stop-exposure group exhibited focal hyperplasia with osseous metaplasia in the mucosa of the small intestine (Table A5).

In the large intestine of males, there was a significant positive trend in the incidences of adenoma (adenomatous polyp) (Tables 11 and A3). In 20,000 ppm stop-exposure males, the incidence of adenoma of the large intestine was significantly greater than that in the control group. Additionally, the incidences of adenoma or carcinoma (combined) in the large intestine of 20,000 ppm male rats were significantly greater than that in the control group and exceeded the NTP historical control range (Tables 11, A3, and A4i). Adenomas of the large intestine were all generally similar polypoid masses extending into the intestinal lumen and composed of irregularly shaped, distended glands lined by a tall columnar epithelium (Plate 4).

In females, one rat in the 5,000 ppm group had a carcinoma in the small intestine, and one rat in the 2,500 ppm group had an adenoma of the large intestine (Table B1).

TABLE 11
Incidences of Neoplasms and Nonneoplastic Lesions of the Intestine in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Small Intestine[b]	51	53	51	53	59
Mucosa, Hyperplasia[c]	0	0	0	1 (4.0)[d]	0
Mucosa, Hyperplasia, Cystic	0	0	0	0	1 (3.0)
Adenoma	0	0	0	0	1
Carcinoma	0	0	0	2	4
Adenoma or Carcinoma[e]	0	0	0	2	5*
Large Intestine	51	53	51	55	59
Adenoma	0	0	3	4	10*
Carcinoma	0	0	0	0	2
Adenoma or Carcinoma[f]	0	0	3	4	11**

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with organ examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 7/1,353 (0.5% ± 1.1%); range 0%-4% (includes data for duodenum, ileum, and jejunum)
[f] Historical incidence: 1/1,353 (0.1% ± 0.4%); range 0%-2% (includes data for cecum, colon, and rectum)

Mesothelium: In males in the 5,000 and 10,000 ppm continuous-exposure groups and in the 20,000 ppm stop-exposure group, the incidences of mesothelioma were significantly greater than that in the control group (Tables 12 and A3). In each of these groups the incidence of mesothelioma exceeded the NTP historical control range (0%-8%; Table A4k). In some rats, the more widespread mesotheliomas were considered to be the cause of death. Mesotheliomas typically covered portions or most of the testis and epididymis. Some of these neoplasms extended throughout the abdominal cavity and formed masses on the serosal surfaces of the mesentery, pancreas, intestine, or spleen. Typically, mesothelioma consisted of cuboidal cells that formed papillary and tubular structures as well as multilayered plaques on the testes and abdominal viscera (Plate 5). Invasion into the tissues of abdominal viscera and metastatic lesions in the thoracic lymph nodes was a feature of the more highly malignant mesotheliomas.

TABLE 12
Incidences of Malignant Mesothelioma in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
15-Month Interim Evaluation					
All Organs					
Malignant Mesothelioma					
Overall rate[b]	0/9 (0%)	1/7 (14%)	1/9 (11%)	0/5 (0%)	—[c]
2-Year Study					
All Organs					
Malignant Mesothelioma[d]					
Overall rate	0/51 (0%)	3/53 (6%)	8/51 (16%)	9/55 (16%)	26/60 (43%)
Adjusted rate[e]	0.0%	7.7%	43.3%	100.0%	91.5%
Terminal rate[f]	0/26 (0%)	0/20 (0%)	4/13 (31%)	1/1 (100%)	0/0
First incidence (days)	—[h]	586	681	495	365
Logistic regression test[g]	P<0.001	P=0.157	P<0.001	P=0.003	P<0.001

[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with neoplasm per number of animals necropsied
[c] No animals from the stop-exposure group were examined at the 15-month interim evaluation.
[d] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 40/1,353 (3.0% ± 2.4%); range 0%-8%
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Not applicable; no neoplasms in animal group

Kidney and Urinary Bladder: In male rats, the incidence of renal tubule adenoma in the 10,000 ppm group was marginally but significantly greater than that in the control group; a single adenoma also occurred in one male in the 5,000 ppm group and in one male in the 20,000 ppm stop-exposure group (Tables 13, A1, and A3). However, the incidences of renal tubule adenoma in all exposed groups were within the NTP historical control range (Tables 13 and A4l). The incidences of renal tubule hyperplasia in the exposed groups were similar to the incidence in the control group (Tables 13 and A5). In female rats, renal tubule adenoma occurred in one animal in the 2,500 ppm group. Although renal tubule adenoma is rare in female rats, this single incidence was within the NTP historical control range (Tables 13 and B4i).

In the urinary bladder, a transitional cell papilloma was observed in one 10,000 ppm male at the 15-month interim evaluation (Tables 14 and A1). Transitional cell papillomas were also observed in males from the 5,000 and 10,000 ppm groups and the 20,000 ppm stop-exposure group at 2 years; one 10,000 ppm male and one 20,000 ppm stop-exposure male had transitional cell carcinomas (Table A1). In the current NTP historical database there are no occurrences of transitional cell carcinoma of the urinary bladder; three papillomas have occurred in 1,329 male rats.

Incidences and types of treatment-related non-neoplastic lesions in the kidney and urinary bladder at 15 months and 2 years were similar to those observed at the same sites in the 13-week studies. In the 2-year study, these lesions generally occurred earlier in males than in females, and the incidences and severities in males were greater than those in females. By 15 months and after 2 years, in addition to papillary degeneration, there were increases in the incidences of hyperplasia of the renal papilla epithelium, hyperplasia of the transitional epithelium lining of the renal pelvis, and focal renal tubule atrophy in male rats (Tables 13 and A5). Necrosis, mineralization, and hemorrhage were components of the more severe examples of papillary degeneration that occurred in the 2-year study (Plate 6), but not in the 13-week studies. There were also treatment-related cortical lesions consisting of focal linear or wedge-shaped areas of atrophy or collapse of renal tubules with fibrosis and inflammation. Hyperplasia of the epithelium lining the papilla and pelvis was not always associated with the degree of severity of the papillary degeneration. The incidence and severity of nephropathy were similar in exposed and control groups of male and female rats, although the average severity was slightly decreased in exposed males (Tables 13 and A5). In the urinary bladder of male rats, transitional cell hyperplasia was present, primarily in the 20,000 ppm stop-exposure group (Tables 14 and A5).

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
15-Month Interim Evaluation					
Kidney[b]	9	7	9	5	—[d]
Atrophy, Focal[c]	0	0	0	1 (3.0)[e]	
Papillary Degeneration	0	0	2 (2.5)	4** (1.5)	
Papillary Epithelial Hyperplasia	1 (1.0)	0	1 (2.0)	5** (2.0)	
Transitional Cell Carcinoma	0	0	0	1	
2-Year Study					
Kidney	51	53	51	55	59
Atrophy, Focal	0	0	0	5* (3.0)	0
Papillary Degeneration	0	5 (1.4)	30** (1.5)	29** (2.1)	16** (1.2)
Papillary Epithelial Hyperplasia	10 (1.0)	20** (1.3)	25** (1.3)	47** (1.9)	21* (1.1)
Pelvis, Transitional Epithelium, Hyperplasia	0	0	0	4	4
Nephropathy	51 (2.2)	53 (2.2)	51 (1.8)	53 (1.7)	58 (1.6)
Renal Tubule, Epithelium, Hyperplasia, Focal	0	0	2	0	0
Renal Tubule Adenoma[f]	0	0	1	3**	1
Transitional Cell Carcinoma	0	0	0	0	1
Female					
2-Year Study					
Kidney	50	51	53	52	
Atrophy, Focal	0	2 (1.5)	1 (2.0)	7* (2.9)	
Papillary Degeneration	0	1 (1.0)	3 (1.7)	17** (2.1)	
Papillary Epithelial Hyperplasia	0	1 (1.0)	1 (1.0)	7** (1.4)	
Pelvis, Transitional Epithelium, Hyperplasia	0	1	0	0	
Nephropathy	48 (1.4)	50 (1.2)	50 (1.3)	50 (1.3)	
Renal Tubule Adenoma[g]	0	1	0	0	

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test (interim evaluation) or the logistic regression test (2-year study)
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with kidney examined microscopically
[c] Number of animals with lesion
[d] No animals from the stop-exposure group were examined at the 15-month interim evaluation.
[e] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[f] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 9/1,350 (0.7% ± 1.5%); range 0%-6%
[g] Historical incidence: 1/1,348 (0.1% ± 0.4%); range 0%-2%

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Urinary Bladder in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
15-Month Interim Evaluation					
Urinary Bladder[b]	9	7	9	5	
Transitional Cell Papilloma[c]	0	0	0	1	—[d]
2-Year Study					
Urinary Bladder	51	53	51	55	59
Transitional Cell Hyperplasia	0	0	1 (1.0)[e]	3 (1.3)	10 (1.1)
Transitional Cell Papilloma	0	0	1	2	1
Transitional Cell Carcinoma	0	0	0	1	1
Transitional Cell Papilloma or Carcinoma[f]	0	0	1	3	2
Female					
2-Year Study					
Urinary Bladder	50	51	53	52	
Transitional Cell Hyperplasia	0	0	1 (2.0)	0	
Transitional Cell Papilloma	0	1	0	0	

[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with urinary bladder examined microscopically
[c] Number of animals with lesion
[d] No animals in the stop-exposure group were examined at the 15-month interim evaluation.
[e] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[f] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 3/1,329 (0.23% ± 0.64%); range 0%-2%

Lung: The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 10,000 ppm continuous-exposure males and 20,000 ppm stop-exposure males were significantly greater than that in the control group (Tables 15 and A3) and approached or exceeded the upper limit of the NTP historical control range (Tables 15 and A4m). Multiple carcinomas were present in one 10,000 ppm male and one 20,000 ppm stop-exposure male (Table A1). The adenomas consisted of papillary and solid areas of well-differentiated, cuboidal to columnar epithelium. Carcinomas had increased cellular and nuclear atypia with local invasion and areas of mesenchymal cell proliferation or fibrosis. Metastatic neoplasms were not present. Squamous cell carcinoma was present in the lung of three male rats from the 20,000 ppm stop-exposure group (Table 15). This neoplasm is very rare in control rats, and none appear in the NTP historical database of 1,350 control male rats from dosed feed studies. Squamous cell carcinoma of the lung is morphologically similar to malignant squamous cell neoplasms that occur at other sites. In this study, these were locally invasive neoplasms composed of squamous cells, abundant keratin production, and local scirrhous response. One squamous cell carcinoma was metastatic to the brain, heart, adrenal gland, pancreas, and other abdominal viscera. In male rats there was also a significant increase in the incidence of alveolar/bronchiolar hyperplasia in the 20,000 ppm stop-exposure group. In female rats, a few alveolar/bronchiolar neoplasms occurred only in exposed groups (0/50, 1/51, 0/53, 2/52; Table B1) but the incidences were not significantly different from those in the control group and were within the NTP historical control range (0%-10%; Table B4j).

TABLE 15
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Lung[b]	51	53	51	55	60
Alveolar/bronchiolar Hyperplasia[c]	3 (2.3)[d]	4 (1.8)	5 (1.4)	7 (1.7)	14** (1.6)
Alveolar/bronchiolar Adenoma					
Overall rate[e]	1/51 (2%)	0/53 (0%)	3/51 (6%)	1/55 (2%)	4/60 (7%)
Adjusted rate[f]	2.9%	0.0%	18.6%	10.0%	100.0%
Terminal rate[g]	0/26 (0%)	0/20 (0%)	2/13 (15%)	0/1 (0%)	0/0
First incidence (days)	696	—[i]	684	682	513
Logistic regression test[h]	P=0.182	P=0.515N	P=0.211	P=0.644	P=0.086
Alveolar/bronchiolar Carcinoma					
Overall rate	0/51 (0%)	1/53 (2%)	0/51 (0%)	3/55 (5%)	3/60 (5%)
Adjusted rate	0.0%	5.0%	0.0%	59.3%	21.4%
Terminal rate	0/26 (0%)	1/20 (5%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—	736 (T)	—	620	522
Logistic regression test	P=0.005	P=0.448	—	P=0.024	P=0.118
Alveolar/bronchiolar Carcinoma, Multiple					
Overall rate	0/51 (0%)	0/53 (0%)	0/51 (0%)	1/55 (2%)	1/60 (2%)
Alveolar/bronchiolar Adenoma or Carcinoma[j]					
Overall rate	1/51 (2%)	1/53 (2%)	3/51 (6%)	4/55 (7%)	7/60 (12%)
Adjusted rate	2.9%	5.0%	18.6%	63.4%	100.0%
Terminal rate	0/26 (0%)	1/20 (5%)	2/13 (15%)	0/1 (0%)	0/0
First incidence (days)	696	736 (T)	684	620	513
Logistic regression test	P=0.003	P=0.726	P=0.211	P=0.029	P=0.011
Squamous Cell Carcinoma[k]					
Overall rate	0/51 (0%)	0/53 (0%)	0/51 (0%)	0/55 (0%)	3/60 (5%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	13.2%
Terminal rate	0/26 (0%)	0/20 (0%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—	—	—	—	365
Logistic regression test	P=0.028	—	—	—	P=0.330

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with lung examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Number of animals with neoplasm per number of animals with lung examined microscopically
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] Observed incidence in animals surviving until the end of the study
[h] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 44/1,350 (3.3% ± 1.9%); range 0%-8%
[k] Historical incidence: 0/1,350

Thyroid Gland: In males, the incidence of follicular cell adenoma in the 20,000 ppm stop-exposure group and the incidence of follicular cell carcinoma in the 5,000 ppm group were significantly greater than the incidences in the control group. The combined incidences of follicular cell adenoma or carcinoma in 5,000 ppm continuous-exposure males, 20,000 ppm stop-exposure males, and 10,000 ppm females were significantly greater than those in the control groups and exceeded the NTP historical control range for males and females (Tables 16, A3, A4n, B3, and B4k). In males, the highest incidence occurred in the 20,000 ppm stop-exposure group; 12 of the 20 adenomas or carcinomas that occurred in exposed rats were observed grossly. In exposed females, none of the follicular cell neoplasms were observed at necropsy. Follicular cell neoplasms in exposed rats were morphologically similar to those in control rats. Adenomas were well-demarcated masses that were generally not encapsulated and were composed of cuboidal follicular epithelium forming papillary, solid areas or an atypical follicular pattern. Follicular cell carcinomas were locally invasive neoplasms that often were associated with a scirrhous response; two of the carcinomas in exposed males metastasized to the lung or lymph nodes. Follicular cell hyperplasia was also significantly increased in male rats from the 20,000 ppm stop-exposure group (Tables 16 and A5).

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Male					
2-Year Study					
Thyroid Gland[b]	51	53	51	55	59
Follicular Cell Hyperplasia[c]	1 (3.0)[d]	0	2 (1.5)	5 (2.2)	6* (1.8)
Follicular Cell Adenoma					
Overall rate[e]	0/51 (0%)	1/53 (2%)	2/51 (4%)	2/55 (4%)	7/59 (12%)
Adjusted rate[f]	0.0%	5.0%	8.4%	8.8%	39.9%
Terminal rate[g]	0/26 (0%)	1/20 (5%)	0/13 (0%)	0/1 (0%)	0/0
First incidence (days)	—[i]	736 (T)	666	608	432
Logistic regression test[h]	P=0.113	P=0.448	P=0.233	P=0.274	P=0.021
Follicular Cell Carcinoma					
Overall rate	0/51 (0%)	1/53 (2%)	4/51 (8%)	1/55 (2%)	2/59 (3%)
Adjusted rate	0.0%	2.6%	20.8%	5.9%	26.3%
Terminal rate	0/26 (0%)	0/20 (0%)	2/13 (15%)	0/1 (0%)	0/0
First incidence (days)	—	610	633	647	388
Logistic regression test	P=0.235	P=0.549	P=0.047	P=0.492	P=0.399
Follicular Cell Adenoma or Carcinoma[j]					
Overall rate	0/51 (0%)	2/53 (4%)	6/51 (12%)	3/55 (5%)	9/59 (15%)
Adjusted rate	0.0%	7.5%	27.5%	14.2%	55.7%
Terminal rate	0/26 (0%)	1/20 (5%)	2/13 (15%)	0/1 (0%)	0/0
First incidence (days)	—	610	633	608	388
Logistic regression test	P=0.055	P=0.239	P=0.013	P=0.124	P=0.009

(continued)

TABLE 16
Incidences of Neoplasms and Nonneoplastic Lesions of the Thyroid Gland in Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Female				
15-Month Interim Evaluation				
Thyroid Gland				
Follicular Cell Adenoma				
Overall rate	0/10 (0%)	0/9 (0%)	0/7 (0%)	1/8 (13%)
2-Year Study				
Follicular Cell Adenoma				
Overall rate	0/50 (0%)	0/51 (0%)	2/53 (4%)	3/52 (6%)
Adjusted rate	0.0%	0.0%	6.3%	35.4%
Terminal rate	0/36 (0%)	0/27 (0%)	1/23 (4%)	1/5 (20%)
First incidence (days)	—	—	508	689
Logistic regression test	P=0.021	—	P=0.320	P=0.012
Follicular Cell Carcinoma				
Overall rate	0/50 (0%)	0/51 (0%)	0/53 (0%)	1/52 (2%)
Adjusted rate	0.0%	0.0%	0.0%	20.0%
Terminal rate	0/36 (0%)	0/27 (0%)	0/23 (0%)	1/5 (20%)
First incidence (days)	—	—	—	738 (T)
Logistic regression test	P=0.032	—	—	P=0.124
Follicular Cell Adenoma or Carcinoma[k]				
Overall rate	0/50 (0%)	0/51 (0%)	2/53 (4%)	4/52 (8%)
Adjusted rate	0.0%	0.0%	6.3%	51.5%
Terminal rate	0/36 (0%)	0/27 (0%)	1/23 (4%)	2/5 (40%)
First incidence (days)	—	—	508	689
Logistic regression test	P=0.003	—	P=0.320	P=0.001

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with thyroid gland examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Number of animals with neoplasm per number of animals with thyroid gland examined microscopically
[f] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[g] Observed incidence in animals surviving until the end of the study
[h] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[i] Not applicable; no neoplasms in animal group
[j] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 23/1,343 (1.7% ± 1.6%); range 0%-6%
[k] Historical incidence: 12/1,346 (0.9% ± 1.5%); range 0%-6%

Accessory Sex Glands: There was one adenoma and one carcinoma of the seminal vesicle in male rats from the 20,000 ppm stop-exposure group (Tables 17 and A1). Neoplasms of the seminal vesicle are rare and none have occurred in rats from the current NTP historical database. The adenoma of the seminal vesicle consisted of a focally expansile mass that filled the lumen, with glandular and solid areas formed by a generally well-differentiated, closely packed, tall, columnar epithelium. The carcinoma was a highly invasive neoplasm with marked cellular atypia. Metastatic foci were present in the lung, spleen, and other abdominal viscera. At 15 months, hyperplasia of the seminal vesicle was present in a few rats from exposed groups; at 2 years the incidences of hyperplasia in males from the 10,000 ppm continuous-exposure group and the 20,000 ppm stop-exposure group were greater than the incidence in the control group (Tables 17 and A5). Hyperplasia consisted of one or more focal areas with increased cellularity of the mucosal lining of the seminal vesicle. The hyperplastic epithelium was increased in height and more closely packed compared to the cells forming normal adjacent mucosal lining; the fibrovascular stroma in the foci of hyperplasia was often more prominent than in the normal areas of the mucosa (Plate 7). Although the cellular morphology was similar to adenoma, in the focal hyperplasia, there was a lack of compression or distortion of adjacent tissue in the seminal vesicle. In the coagulating gland, which is attached to the seminal vesicle, there was a slight increase in the incidence of hyperplasia in exposed male rats. The focal areas of hyperplasia of the coagulating gland in exposed male rats were morphologically similar to those seen in the seminal vesicle.

TABLE 17
Incidences of Neoplasms and Nonneoplastic Lesions of the Seminal Vesicle in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
15-Month Interim Evaluation					
Seminal Vesicle[b]	9	7	9	5	—[d]
Hyperplasia[c]	0	2 (1.0)[e]	5* (1.2)	1 (2.0)	
2-Year Study					
Seminal Vesicle	51	53	51	55	60
Hyperplasia	1 (1.0)	6 (1.0)	4 (1.0)	16** (1.4)	33** (1.3)
Adenoma	0	0	0	0	1
Carcinoma	0	0	0	0	1
Adenoma or Carcinoma[f]	0	0	0	0	2
Coagulating gland[b]					
Hyperplasia	0	1 (1.0)	0	2 (1.0)	3 (1.0)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with lesion per number of animals necropsied
[c] Number of animals with lesion
[d] No animals in the stop-exposure group were examined at the 15-month interim.
[e] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[f] Historical incidence for 2-year NTP feed studies with untreated control groups: 0/1,353

Hematopoietic System: The incidences of mononuclear cell leukemia in male rats from the 5,000 and 10,000 ppm continuous-exposure groups and the 20,000 ppm stop-exposure group were significantly greater than that in the control group (Tables 18 and A3). The incidence of mononuclear cell leukemia in the 5,000 ppm group of males exceeded the NTP historical control range (Tables 18 and A4o). Infiltration of leukemic cells generally involved numerous organs and this neoplasm was frequently considered to be the cause of death for exposed and control rats. The incidences of fibrosis of the spleen were slightly increased in 5,000 and 10,000 ppm males, and the 20,000 ppm stop-exposure males (Tables 18 and A5). This lesion is often present in the spleen of rats with mononuclear cell leukemia which was also increased in these three groups. Lymphoid hyperplasia of the mandibular lymph node in 20,000 ppm stop-exposure males and hematopoiesis of the spleen in 10,000 ppm males and females and 20,000 ppm stop-exposure males, were also considered secondary changes that were slightly increased over the background incidence typically seen in control rats (Tables 18, A5, and B5). In male rats the increased incidence of lymphoid hyperplasia was seen primarily in the regional mandibular lymph node of rats with Zymbal's gland carcinoma. Splenic hematopoiesis was often present in rats with multiple neoplasms, including carcinoma of the Zymbal's, mammary, or clitoral gland.

TABLE 18
Incidences of Mononuclear Cell Leukemia and Nonneoplastic Lesions in the Hematopoietic System in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
2-Year Study					
Lymph node, mandibular[b]	49	52	49	55	59
Hyperplasia[c]	4 (2.3)[d]	2 (2.0)	2 (3.5)	3 (2.7)	10** (2.8)
Spleen	51	53	51	54	60
Fibrosis, focal	15 (2.0)	10 (2.1)	22* (2.2)	24** (2.3)	28** (2.4)
Hematopoiesis	1 (3.0)	3 (3.0)	3 (2.7)	8* (2.8)	17** (2.9)
Mononuclear Cell Leukemia[e]					
Overall rate[f]	27/51 (53%)	29/53 (55%)	40/51** (78%)	34/55** (62%)	25/60** (42%)

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test.
** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test or the life table test (mononuclear cell leukemia).
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with organ examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 661/1,353 (48.9% ± 8.8%); range 32%-62%
[f] Number of animals with neoplasm per number of animals necropsied

Pancreas: The incidences of pancreatic acinar adenoma in all groups of exposed males were slightly greater than the incidence in controls, and the increase was significant in the 5,000 ppm group (Tables 19 and A3). The incidence in each group of exposed males was within the historical control range (0%-10%; Tables 19 and A4p). Acinar adenomas were discrete, nodular masses that slightly compressed or displaced surrounding pancreatic tissue. Adenomas were composed of glands or irregularly formed acini of generally well-differentiated pancreatic acinar cells, some of which varied slightly in size and shape compared to normal acinar cells.

The incidences of hyperplasia in all exposed groups of male rats were significantly greater than the incidence in the control group (Tables 19 and A5). These minimal to mild focal lesions were morphologically similar to the adenomas but were smaller and did not compress or distort surrounding tissue. There was minimal alteration of the acinar structure and minimal variation in cell size compared to the normal acinar cells.

TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Male Rats in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm[a] (Stop-Exposure)
Pancreas, Acinar Cell[b]	51	53	51	53	59
Focal Hyperplasia[c]	3 (1.7)[d]	9* (1.9)	12* (1.3)	14** (1.8)	27** (2.0)
Acinar Cell Adenoma[e]					
Overall rate[f]	1/51 (2%)	2/53 (4%)	4/51 (8%)	3/53 (6%)	3/59 (5%)
Adjusted rate[g]	3.8%	10.0%	30.8%	34.1%	8.5%
Terminal rate[h]	1/26 (4%)	2/20 (10%)	4/13 (31%)	0/1 (0%)	0/0
First incidence (days)	736 (T)	736 (T)	736 (T)	604	507
Logistic regression test[i]	P=0.005	P=0.408	P=0.033	P=0.089	P=0.447

* Significantly different ($P \leq 0.05$) from the control group by the logistic regression test
** $P \leq 0.01$
(T)Terminal sacrifice
[a] Ten male rats receiving 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed were evaluated at 3 months. The remaining 60 male rats in this group received control feed until the end of the 2-year study.
[b] Number of animals with pancreas examined microscopically
[c] Number of animals with lesion
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 24/1,340 (1.8% ± 2.3%); range 0%-10%
[f] Number of animals with neoplasm per number of animals with pancreas examined microscopically
[g] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality.
[h] Observed incidence in animals surviving until the end of the study
[i] In the control column are the P values associated with the trend test (the 20,000 ppm stop-exposure group was excluded from the trend test). In the exposure group columns are the P values corresponding to pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.

MICE

13-WEEK STUDY

Five male and four female mice, dispersed among control and exposed groups, died during the study (Table 20). The final mean body weights and body weight gains of 1,250, 2,500, 5,000, and 10,000 ppm males and females and of 625 ppm females were significantly lower than those of the controls. Feed consumption by exposed mice was generally higher than that by controls throughout the study (Table 20). Dietary levels of 625, 1,250, 2,500, 5,000, and 10,000 ppm delivered average daily doses of 100, 200, 500, 1,300, and 3,000 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight to males and 140, 300, 600, 1,200, and 2,900 mg/kg to females. Clinical findings included abnormal posture and hypoactivity in 10,000 ppm male and female mice.

At the end of the study, serum blood urea nitrogen concentrations were increased in 5,000 ppm females and 10,000 ppm males and females (Table G2). Additionally, decreased urine specific gravity occurred in 10,000 ppm females. Renal papillary necrosis with tubular regeneration and fibrosis occurred in males exposed to 2,500 ppm or greater and in females exposed to 10,000 ppm. This would be consistent with the blood urea nitrogen concentration increases (azotemia) and the isosthenuric specific gravity.

The absolute and relative weights of several organs in 5,000 and 10,000 ppm animals were lower than those in the control group (Table F4). These findings were attributed to the low body weights in these groups.

In males exposed to 5,000 or 10,000 ppm, weights of the right cauda and right epididymis were significantly lower than those of control males and decreased with increasing exposure level (Table H2). In females, estrous cycle length increased with increasing exposure level, but the differences from the controls were not statistically significant.

TABLE 20
Survival, Mean Body Weights, and Feed Consumption of Mice in the 13-Week Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 13
Male							
0	10/10	22.7 ± 0.4	33.3 ± 0.8	10.6 ± 0.7		4.1	4.6
625	8/10[d]	22.3 ± 0.3	31.7 ± 0.9	9.5 ± 0.6	95	4.4	4.5
1,250	10/10	22.7 ± 0.4	30.9 ± 0.8*	8.2 ± 0.6**	93	4.3	4.4
2,500	10/10	22.8 ± 0.3	29.4 ± 0.4**	6.6 ± 0.3**	88	5.0	5.0
5,000	10/10	22.2 ± 0.3	26.1 ± 0.3**	3.9 ± 0.4**	78	5.7	6.7
10,000	7/10[e]	22.7 ± 0.4	21.7 ± 0.5**	−0.8 ± 0.6**	65	5.5	7.5
Female							
0	9/10[f]	17.9 ± 0.3	30.2 ± 0.9	12.3 ± 0.8		4.9	4.6
625	9/10[g]	17.6 ± 0.3	28.4 ± 0.8*	10.7 ± 0.6*	94	4.8	5.6
1,250	9/10[g]	18.0 ± 0.3	27.8 ± 0.8**	9.7 ± 0.7**	92	5.3	5.7
2,500	9/10[h]	17.6 ± 0.2	25.5 ± 0.2**	7.9 ± 0.2**	85	5.3	5.7
5,000	9/10[i]	17.5 ± 0.3	22.3 ± 0.3**	4.9 ± 0.3**	74	5.0	4.4
10,000	10/10	17.8 ± 0.3	17.9 ± 0.4**	0.1 ± 0.4**	59	5.0	5.4

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Week of death: 9, 9
[e] Week of death: 2, 2, 3
[f] Week of death: 6
[g] Week of death: 8
[h] Week of death: 2
[i] Week of death: 7

There were no treatment-related gross lesions in exposed mice from the 13-week study. Treatment-related microscopic lesions were present in the kidney and urinary bladder of male and female mice (Table 21). In the kidney, there was an exposure- and treatment-related increase in the incidence of papillary necrosis. In the cortex of the kidney, there were foci of renal tubule regeneration and fibrosis. These lesions were present in 2,500, 5,000, and 10,000 ppm male mice and in the 10,000 ppm females. Papillary necrosis involved both the interstitial and renal tubule epithelium at the tip of the renal papilla. Renal tubule regeneration consisted of multiple, focal lesions in the cortex characterized by degeneration and regeneration of tubule epithelium; minimal to mild fibrosis was frequently present in the areas of regeneration. In the urinary bladder of mice from the 5,000 and 10,000 ppm groups there was mild hyperplasia of the transitional epithelium. In seven of nine female mice from the 10,000 ppm group, there was also a minimal inflammatory cell infiltration in the urinary bladder mucosa and focal necrosis of the transitional cell epithelium.

TABLE 21
Incidences of Selected Nonneoplastic Lesions in Mice in the 13-Week Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	625 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm
Male						
Kidney[a]	10	10	10	10	10	10
Necrosis, Papillary[b]	0	0	0	5* (1.2)[c]	4* (1.5)	9** (2.2)
Regeneration, Renal Tubule	0	0	0	4* (1.3)	4* (1.5)	7** (2.3)
Fibrosis	0	0	0	4* (1.3)	2 (1.5)	7** (2.1)
Urinary Bladder	10	10	10	10	10	8
Hyperplasia	0	0	0	0	4* (1.0)	7** (2.0)
Female						
Kidney	10	10	10	10	10	10
Necrosis, Papillary	0	0	0	0	0	2 (1.0)
Regeneration, Renal Tubule	0	0	0	0	0	4* (1.8)
Fibrosis	0	0	0	0	0	2 (1.5)
Urinary Bladder	10	10	10	10	10	10
Hyperplasia	0	0	0	0	10** (2.0)	9** (1.6)

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

Dose Selection Rationale: Based on lower final mean body weights and organ weights in 5,000 and 10,000 ppm males and females, and the presence of kidney (papillary necrosis) and urinary bladder lesions in the 2,500, 5,000, and 10,000 ppm males and females in the 13-week feed study, the high dose selected for the 2-year feed study in male and female mice was 1,250 ppm.

2-YEAR STUDY

Survival

Estimates of 2-year survival probabilities for male and female mice are shown in Table 22 and in the Kaplan-Meier survival curves in Figure 3. Survival of 1,250 ppm males and females was significantly lower than that of the respective controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

Mean body weights of exposed male and female mice were similar to controls throughout the study (Figure 4 and Tables 23 and 24). Final mean body weights were also generally similar to those of controls. Feed consumption by exposed male and female mice was similar to that by controls (Tables J3 and J4). Dietary levels of 312, 625, and 1,250 ppm delivered average daily doses of 35, 70, and 140 mg 2,2-bis(bromomethyl)-1,3-propanediol/kg body weight to males and 40, 80, and 170 mg/kg to females. Clinical findings included swelling, discharge, and tissue masses involving the eye in exposed mice.

TABLE 22
Survival of Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	9	10	10
Accidental deaths[a]	0	0	0	1
Missing[a]	0	0	0	1
Moribund	3	12	11	13
Natural deaths	5	3	4	5
Animals surviving to study termination	42	36	35	30
Percent probability of survival at the end of study[b]	84	71	70	63
Mean survival (days)[c]	710	675	698	684
Survival analysis[d]	P=0.054	P=0.169	P=0.174	P=0.038
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	8	10	9	10
Moribund	9	14	14	29
Natural deaths	6	6	11	10
Animals surviving to study termination	37	30	26	11
Percent probability of survival at the end of study	71	60	51	22
Mean survival (days)	690	685	691	625
Survival analysis	P<0.001	P=0.422	P=0.117	P<0.001

[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 dosed columns.

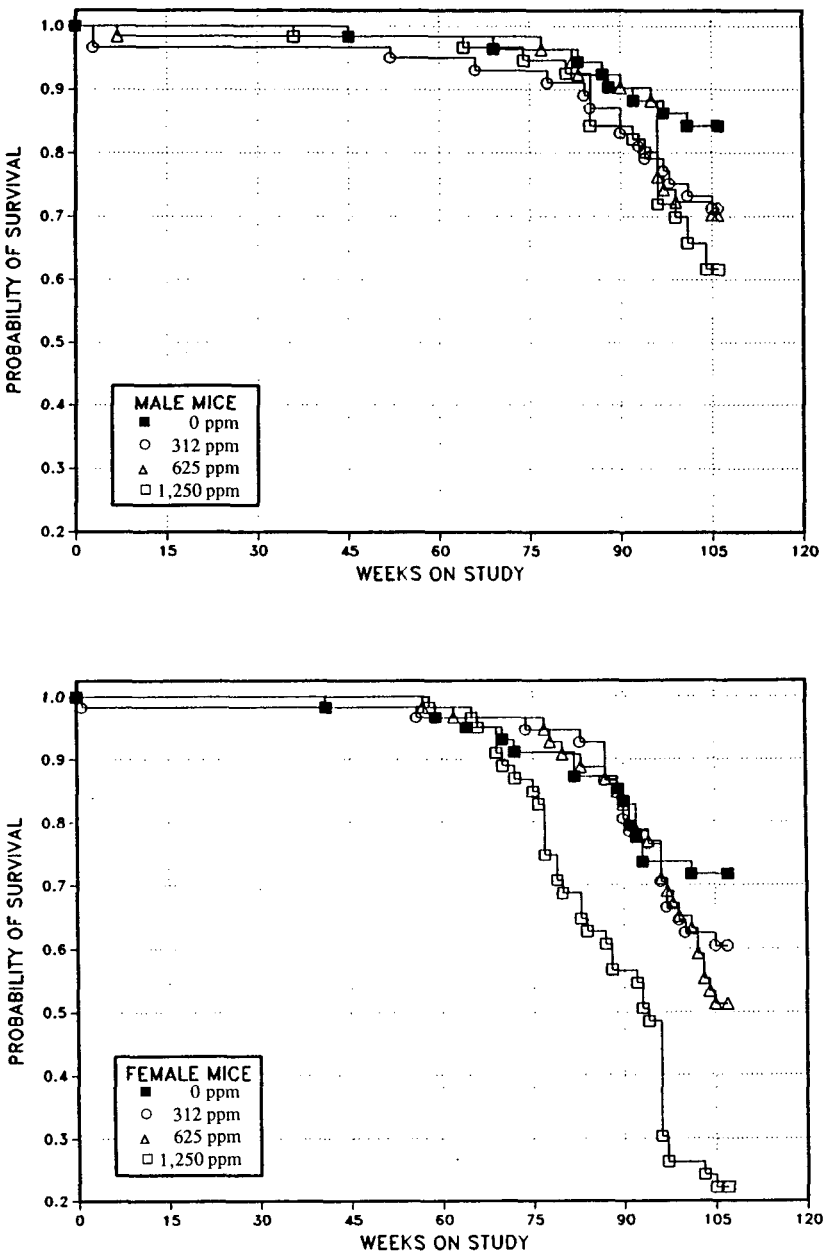


FIGURE 3
Kaplan-Meier Survival Curves for Male and Female Mice Administered 2,2-Bis(bromomethyl)-1,3-propanediol in Feed for 2 Years

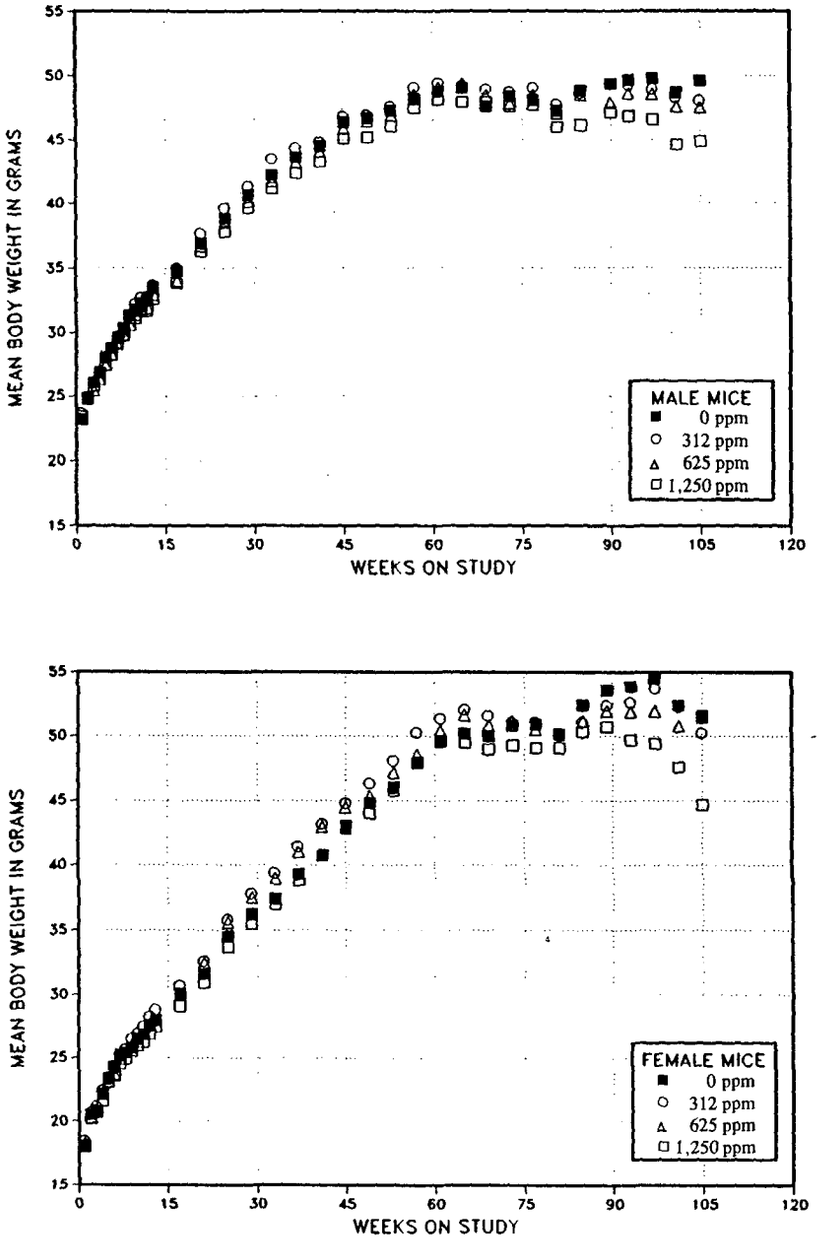


FIGURE 4
Growth Curves for Male and Female Mice Administered 2,2-Bis(bromomethyl)-1,3-propanediol in Feed for 2 Years

TABLE 23
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Weeks on Study	0 ppm		312 ppm			625 ppm			1,250 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.2	60	23.7	102	60	23.2	100	60	23.4	101	60
2	24.9	60	24.9	100	60	24.8	100	60	24.7	99	60
3	26.1	60	26.0	100	59	25.4	97	60	25.8	99	60
4	26.8	60	26.9	100	58	26.4	99	60	26.3	98	60
5	28.1	60	28.0	100	58	27.4	98	60	27.5	98	60
6	28.8	60	28.7	100	58	28.3	98	60	28.2	98	60
7	29.6	60	29.6	100	58	29.1	98	59	29.1	98	60
8	30.3	60	30.1	99	58	29.8	98	59	29.7	98	60
9	31.4	60	31.2	99	58	30.6	98	59	30.6	98	60
10	31.8	60	32.2	101	58	31.4	99	59	31.1	98	60
11	32.2	60	32.7	102	58	31.8	99	59	31.6	98	60
12	32.6	60	32.8	101	58	31.9	98	59	31.7	97	60
13	33.5	60	33.7	101	58	32.9	98	59	32.6	97	60
17	34.8	60	35.0	101	58	34.0	98	59	33.8	97	60
21	36.9	60	37.7	102	58	36.7	100	59	36.3	98	60
25	38.8	60	39.6	102	58	38.5	99	59	37.8	97	60
29	40.7	60	41.3	102	58	40.1	99	59	39.7	98	60
33	42.2	60	43.5	103	58	41.7	99	59	41.2	98	60
37	43.6	60	44.4	102	58	43.2	99	59	42.4	97	59
41	44.5	60	44.8	101	58	44.1	99	59	43.3	97	59
45	46.4	59	46.8	101	58	45.9	99	59	45.1	97	59
49	46.7	59	46.9	100	58	46.5	100	59	45.2	97	59
53	47.3	59	47.6	101	57	46.9	99	59	46.1	98	59
57	48.2	59	49.1	102	57	48.5	101	59	47.5	99	59
61	48.8	59	49.4	101	57	49.1	101	59	48.1	99	59
65	49.0	59	49.2	100	57	49.4	101	59	48.0	98	57
69[a]	47.6	49	48.9	103	47	48.5	102	49	47.9	101	47
73	48.4	48	48.7	101	47	47.8	99	49	47.6	98	47
77	48.1	48	49.1	102	47	48.5	101	49	47.7	99	46
81	47.3	48	47.8	101	46	47.1	100	48	46.0	97	46
85	48.8	47	48.7	100	45	48.5	99	46	46.2	95	44
90	49.4	45	49.3	100	42	47.9	97	46	47.2	96	41
93	49.7	44	49.3	99	41	48.6	98	45	46.9	94	40
97	49.8	43	48.9	98	40	48.6	98	38	46.6	94	35
101	48.7	43	48.3	99	38	47.6	98	36	44.6	92	33
105	49.6	42	48.1	97	36	47.5	96	36	44.9	91	30
Mean for weeks											
1-13	29.2		29.3	100		28.7	98		28.6	98	
14-52	41.6		42.2	101		41.2	99		40.5	97	
53-105	48.6		48.7	100		48.2	99		46.8	96	

[a] Interim evaluation occurred during week 66.

TABLE 24
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

Weeks on Study	0 ppm Av. Wt. (g)	0 ppm No. of Survivors	312 ppm Av. Wt. (g)	312 ppm Wt. (% of controls)	312 ppm No. of Survivors	625 ppm Av. Wt. (g)	625 ppm Wt. (% of controls)	625 ppm No. of Survivors	1,250 ppm Av. Wt. (g)	1,250 ppm Wt. (% of controls)	1,250 ppm No. of Survivors
1	18.0	60	18.5	103	60	18.3	102	60	18.1	101	60
2	20.5	60	20.7	101	60	20.3	99	60	20.2	99	60
3	20.9	60	21.2	101	60	20.9	100	60	20.7	99	60
4	22.1	60	22.4	101	60	22.2	101	60	21.6	98	60
5	23.4	60	23.5	100	60	23.1	99	60	23.0	98	60
6	24.3	60	24.1	99	60	23.7	98	60	23.5	97	60
7	25.2	60	25.2	100	60	24.9	99	60	24.5	97	60
8	25.4	60	25.7	101	60	25.4	100	60	24.9	98	60
9	25.7	60	26.5	103	60	25.9	101	60	25.5	99	60
10	26.5	60	26.9	102	60	26.5	100	60	26.0	98	60
11	26.8	60	27.5	103	60	26.9	100	60	26.2	98	60
12	27.6	60	28.3	103	60	27.6	100	60	26.8	97	60
13	28.0	60	28.8	103	60	28.0	100	60	27.5	98	60
17	30.1	60	30.7	102	60	29.9	99	60	29.1	97	60
21	31.6	60	32.6	103	60	32.5	103	60	30.9	98	60
25	34.5	60	35.8	104	60	35.6	103	60	33.7	98	60
29	36.2	60	37.8	104	60	37.5	104	60	35.5	98	60
33	37.4	60	39.4	105	60	39.0	104	60	37.0	99	60
37	39.3	60	41.5	106	60	41.1	105	60	38.9	99	60
41	40.7	60	43.2	106	60	43.0	106	60	40.7	100	60
45	42.8	59	44.8	105	60	44.5	104	60	43.0	101	60
49	44.8	59	46.3	103	60	45.4	101	60	44.0	98	60
53	46.0	59	48.1	105	60	47.2	103	60	45.8	100	60
57	48.0	59	50.3	105	59	48.6	101	60	47.9	100	60
61	49.6	58	51.4	104	59	50.4	102	59	49.6	100	59
65	50.2	57	52.1	104	59	51.7	103	58	49.5	99	58
69[a]	50.0	49	51.6	103	49	50.9	102	49	49.0	98	46
73	50.8	47	51.1	101	49	51.2	101	49	49.3	97	43
77	50.9	47	51.1	100	48	50.5	99	48	49.1	97	39
81	50.2	47	50.0	100	48	50.2	100	46	49.1	98	34
85	52.4	45	51.1	98	47	51.2	98	45	50.3	96	31
89	53.6	44	52.4	98	44	52.0	97	44	50.7	95	28
93	53.9	38	52.6	98	40	51.9	96	40	49.7	92	27
97	54.5	38	53.7	99	36	51.9	95	36	49.4	91	14
101	52.4	37	52.3	100	32	50.8	97	33	47.6	91	13
105	51.6	37	50.3	98	31	51.5	100	26	44.7	87	12
Mean for weeks											
1-13	24.2		24.6	102		24.1	100		23.7	98	
14-52	37.5		39.1	104		38.7	103		37.0	99	
53-105	51.0		51.3	101		50.7	99		48.7	96	

[a] Interim evaluation occurred during week 66.

Pathology and Statistical Analysis

This section describes statistically significant and biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the harderian gland, lung, skin, kidney, forestomach, and mammary gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of 5% in at least one exposure group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

Harderian Gland: The incidences of harderian gland adenoma in male and female mice exposed to 625 and 1,250 ppm were significantly greater than those in the control groups (Tables 25, C3, and D3). The incidence of harderian gland carcinoma in 1,250 ppm females was significantly greater than that in the control group (Tables 25 and D3). In 625 and 1,250 ppm males, and in all female exposure groups, the incidences of adenoma or carcinoma (combined) were significantly greater than those in the control groups (Tables 25, C3, and D3). In males exposed to 1,250 ppm, many of these neoplasms were bilateral (Tables 25 and C1). The incidences of adenoma and carcinoma in 625 and 1,250 ppm males and exposed females exceeded the NTP historical control range (Tables 25, C4a, and D4a). The majority of the harderian gland neoplasms were observed grossly at necropsy; in some instances these neoplasms were the primary reason for the moribund condition of the animals. Adenomas were expansile masses that had a variable growth pattern consisting of acini and cystic glands with papillary and solid areas. Carcinomas were more invasive, often with focal fibrosis. There was cellular pleomorphism, and, in some carcinomas, neoplastic cells had large cytoplasmic vacuoles. Metastases of carcinomas to the lung and other sites occurred in exposed and control groups of male and female mice (Plate 8). At the 15-month interim evaluation, the incidences of adenoma of the harderian gland in 1,250 ppm males and females were slightly greater than those in the control groups, but these differences were not statistically significant (Tables 25, C3, and D3). At 2 years, the incidences of hyperplasia in exposed male and females did not differ significantly from those in the control groups (Tables 25, C5, and D5).

TABLE 25
Incidence of Neoplasms and Nonneoplastic Lesions of the Harderian Gland in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
15-Month Interim Evaluation				
Harderian Gland[a]	4	6	5	4
Hyperplasia[b]	0	0	1 (2.0)[c]	1 (3.0)
Adenoma	0	0	1	2
2-Year Study				
Harderian Gland	22	25	28	32
Hyperplasia	0	1 (3.0)	1 (2.0)	2 (2.0)
Adenoma				
Overall rate[d]	3/50 (6%)	6/51 (12%)	12/50 (24%)	18/49 (37%)
Adjusted rate[e]	7.1%	15.6%	31.0%	47.5%
Terminal rate[f]	3/42 (7%)	4/36 (11%)	9/35 (26%)	11/30 (37%)
First incidence (days)	736 (T)	656	669	565
Logistic regression test[g]	P<0.001	P=0.213	P=0.010	P<0.001
Adenoma, Bilateral				
Overall rate	0/50 (0%)	0/51 (0%)	1/50 (2%)	8/49**(16%)
Carcinoma				
Overall rate	1/50 (2%)	1/51 (2%)	4/50 (8%)	4/49 (8%)
Adjusted rate	2.3%	2.8%	10.1%	10.9%
Terminal rate	0/42 (0%)	1/36 (3%)	2/35 (6%)	1/30 (3%)
First incidence (days)	674	736 (T)	666	589
Logistic regression test	P=0.071	P=0.762	P=0.182	P=0.187
Adenoma or Carcinoma[h]				
Overall rate	4/50 (8%)	7/51 (14%)	16/50 (32%)	22/49 (45%)
Adjusted rate	9.3%	18.2%	39.4%	54.3%
Terminal rate	3/42 (7%)	5/36 (14%)	11/35 (31%)	12/30 (40%)
First incidence (days)	674	656	666	565
Logistic regression test	P<0.001	P=0.233	P=0.003	P<0.001

(continued)

TABLE 25
Incidence of Neoplasms and Nonneoplastic Lesions of the Harderian Gland in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
15-Month Interim Evaluation				
Harderian Gland	4	5	4	7
Hyperplasia	0	0	1 (1.0)	1 (3.0)
Adenoma	1	1	0	4
2-Year Study				
Harderian Gland	18	27	27	33
Hyperplasia	1 (3.0)	1 (1.0)	2 (1.5)	0
Adenoma				
Overall rate	2/52 (4%)	6/50 (12%)	8/51 (16%)	15/50 (30%)
Adjusted rate	4.3%	17.7%	23.7%	55.7%
Terminal rate	0/37 (0%)	3/30 (10%)	4/26 (15%)	3/11 (27%)
First incidence (days)	447	669	557	551
Logistic regression test	P<0.001	P=0.125	P=0.040	P<0.001
Carcinoma				
Overall rate	1/52 (2%)	6/50 (12%)	5/51 (10%)	7/50 (14%)
Adjusted rate	2.5%	17.6%	16.1%	25.0%
Terminal rate	0/37 (0%)	4/30 (13%)	3/26 (12%)	0/11 (0%)
First incidence (days)	646	627	669	575
Logistic regression test	P=0.095	P=0.052	P=0.098	P=0.033
Adenoma or Carcinoma[i]				
Overall rate	3/52 (6%)	12/50 (24%)	13/51 (25%)	19/50 (38%)
Adjusted rate	6.7%	33.3%	37.5%	64.2%
Terminal rate	0/37 (0%)	7/30 (23%)	7/26 (27%)	3/11 (27%)
First incidence (days)	447	627	557	551
Logistic regression test	P<0.001	P=0.010	P=0.006	P=0.002

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with harderian gland examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to the pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 80/1,474 (5.4% ± 4.5%); range 0%-20%
[i] Historical incidence: 59/1,470 (4.0% ± 3.1%); range 0%-10%

Lung: The incidences of alveolar/bronchiolar adenoma and of alveolar/bronchiolar adenoma or carcinoma (combined) in 1,250 ppm males and females and 625 ppm females were significantly greater than those in the control groups (Tables 26, C3, and D3). In males exposed to 1,250 ppm, the incidences of multiple adenoma and of alveolar/bronchiolar carcinoma were significantly greater than those in the control group (Tables 26 and C1). The incidences of alveolar/bronchiolar adenoma or carcinoma (combined) in 625 and 1,250 ppm males and females exceeded the NTP historical control range (Tables 26, C4b, and D4b).

The majority of these neoplasms were visible grossly as white or gray nodules in the lung. The morphology of the lung neoplasms was similar in control and exposed groups. Carcinomas had variable growth patterns, increased cellular pleomorphism, increased numbers of mitoses and evidence of local invasion (Plate 9). In one male and one female in the 625 ppm groups and in one male in the 1,250 ppm group, carcinomas had foci of metastases in lymph nodes, liver, and other sites.

At 15 months, the incidences of alveolar/bronchiolar neoplasms and alveolar epithelial hyperplasia in exposed mice were not significantly different from those in the control groups (Tables 26, C1, C5, D1, and D5). At 2 years, the incidences of alveolar epithelial hyperplasia in 625 and 1,250 ppm females were significantly greater than that in the control group (Tables 26, C5, and D5).

TABLE 26
Incidence of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
15-Month Interim Evaluation				
Lung[a]	10	9	10	10
Alveolar Epithelium, Hyperplasia[b]	1 (1.0)[c]	0	1 (1.0)	3 (1.3)
Alveolar/bronchiolar Adenoma	2	1	4	0
Alveolar/bronchiolar Carcinoma	0	0	0	1
2-Year Study				
Lung	50	51	50	49
Alveolar Epithelium, Hyperplasia	6 (1.5)	7 (2.1)	5 (2.0)	8 (2.0)
Alveolar/bronchiolar Adenoma (Single and Multiple)				
Overall rate[d]	12/50 (24%)	4/51 (8%)	12/50 (24%)	21/49 (43%)
Adjusted rate[e]	27.1%	10.3%	30.6%	57.6%
Terminal rate[f]	10/42 (24%)	3/36 (8%)	9/35 (26%)	15/30 (50%)
First incidence (days)	478	586	536	593
Logistic regression test[g]	P=0.001	P=0.030N	P=0.589	P=0.020
Alveolar/bronchiolar Adenoma, Multiple				
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	10/49** (20%)
Alveolar/bronchiolar Carcinoma (Single and Multiple)				
Overall rate	3/50 (6%)	7/51 (14%)	8/50 (16%)	11/49 (22%)
Adjusted rate	7.1%	18.7%	19.9%	33.5%
Terminal rate	3/42 (7%)	6/36 (17%)	5/35 (14%)	9/30 (30%)
First incidence (days)	736 (T)	646	572	641
Logistic regression test	P=0.011	P=0.130	P=0.098	P=0.009
Alveolar/bronchiolar Carcinoma, Multiple				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/49 (6%)
Alveolar/bronchiolar Adenoma or Carcinoma[h]				
Overall rate	15/50 (30%)	11/51 (22%)	16/50 (32%)	25/49 (51%)
Adjusted rate	33.9%	28.4%	38.9%	66.9%
Terminal rate	13/42 (31%)	9/36 (25%)	11/35 (31%)	18/30 (60%)
First incidence (days)	478	586	536	593
Logistic regression test	P=0.003	P=0.280N	P=0.491	P=0.011

(continued)

TABLE 26
Incidence of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
15-Month Interim Evaluation				
Lung	8	10	9	10
Alveolar Epithelium, Hyperplasia	1 (1.0)	0	0	0
Alveolar/bronchiolar Adenoma	1	0	0	2
Alveolar/bronchiolar Carcinoma	1	0	0	0
2-Year Study				
Lung	52	50	51	50
Alveolar Epithelium, Hyperplasia	1 (1.0)	3 (1.3)	8** (1.5)	15** (1.9)
Alveolar/bronchiolar Adenoma (Single and Multiple)				
Overall rate	3/52 (6%)	3/50 (6%)	9/51 (18%)	17/50 (34%)
Adjusted rate	7.7%	8.8%	29.1%	64.2%
Terminal rate	2/37 (5%)	2/30 (7%)	5/26 (19%)	4/11 (36%)
First incidence (days)	640	619	669	534
Logistic regression test	P<0.001	P=0.642	P=0.048	P<0.001
Alveolar/bronchiolar Adenoma, Multiple				
Overall rate	1/52 (2%)	0/50 (0%)	2/51 (4%)	2/50 (4%)
Alveolar/bronchiolar Carcinoma (Single and Multiple)				
Overall rate	2/52 (4%)	2/50 (4%)	6/51 (12%)	5/50 (10%)
Adjusted rate	5.3%	6.7%	17.6%	33.9%
Terminal rate	1/37 (3%)	2/30 (7%)	3/26 (12%)	2/11 (18%)
First incidence (days)	705	743 (T)	428	669
Logistic regression test	P=0.048	P=0.659	P=0.125	P=0.094
Alveolar/bronchiolar Carcinoma, Multiple				
Overall rate	0/52 (0%)	0/50 (0%)	1/51 (2%)	1/50 (2%)
Alveolar/bronchiolar Adenoma or Carcinoma[i]				
Overall rate	5/52 (10%)	5/50 (10%)	15/51 (29%)	19/50 (38%)
Adjusted rate	12.7%	15.3%	43.4%	71.9%
Terminal rate	3/37 (8%)	4/30 (13%)	8/26 (31%)	5/11 (45%)
First incidence (days)	640	619	428	534
Logistic regression test	P<0.001	P=0.597	P=0.011	P<0.001

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
(T)Terminal sacrifice
[a] Number of animals with lung examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals with lung examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to the pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal. A lower incidence in an exposure group is indicated by N.
[h] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 265/1,469 (18.0% ± 7.6%); range 4%-32%
[i] Historical incidence: 110/1,469 (7.5% ± 5.0%); range 2%-26%

Skin: The incidence of subcutaneous tissue sarcoma and the combined incidences of fibrosarcoma or sarcoma in 1,250 ppm female mice were significantly greater than those in the controls (Tables 27 and D3). Malignant mesenchymal neoplasms of the skin occurred in 24% of the females exposed to 1,250 ppm. The incidences of fibrosarcoma or sarcoma (combined) in 1,250 ppm females exceeded the NTP historical control range (Tables 27 and D4c). There was variation in morphology within and between these neoplasms (Plates 10 and 11). Most consisted of spindle-shaped or pleomorphic or vacuolated cells forming irregular, interwoven patterns or areas with intercellular edema.

TABLE 27
Incidence of Neoplasms of the Subcutaneous Tissue of the Skin in Female Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	312 ppm	625 ppm	1,250 ppm
Skin[a]	52	50	51	50
Subcutaneous Tissue, Schwannoma, Malignant[b]	0	1	0	0
Subcutaneous Tissue, Fibrosarcoma	0	0	0	1
Subcutaneous Tissue, Sarcoma	0	1	4	11**
Subcutaneous Tissue, Fibrosarcoma or Sarcoma[c]	0	1	4	12**

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
[a] Number of animals with skin examined microscopically
[b] Number of animals with neoplasm
[c] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 21/1,470 (1.4% ± 2.2%); range 0%-8%

Kidney: Marginally increased in incidences of renal tubule adenoma were observed in 625 and 1,250 ppm male mice (0/49, 0/51, 3/50, 2/49; Table C1). These incidences exceeded the NTP historical control range for this neoplasm (Table C4c). In three of the five male mice with adenomas, the adenomas were observed grossly at necropsy. Adenomas were all expansile, well-differentiated tumors with tubular and glandular patterns (Plate 12). Focal renal tubule hyperplasia was present in two males from the 1,250 ppm group (Table C5).

Forestomach: The incidences of squamous cell papilloma of the forestomach in 625 and 1,250 ppm female mice were significantly greater than that in the control group (Tables 28 and D3). In 1,250 ppm males, the incidence of squamous cell papilloma or squamous cell carcinoma (combined) was significantly greater than that in the control group (Tables 28 and C3). In addition, papillomas also occurred in one male and one female in the 1,250 ppm groups at the 15-month interim evaluation. The incidences of squamous cell adenoma or squamous cell carcinoma (combined) in exposed male mice were at or slightly greater than the upper limit of the NTP historical control range (0%-6%; Table C4d). The papillomas were well-differentiated, benign neoplasms consisting of multiple fronds of a squamous epithelium supported by delicate fibrovascular stroma. Squamous cell carcinomas, which were present in two 1,250 ppm males, were invasive malignant neoplasms; one carcinoma metastasized to the lung as well as other abdominal organs (Table C1).

TABLE 28
Incidence of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male				
15-Month Interim Evaluation				
Forestomach[a]	10	9	10	10
Mucosa, Hyperplasia[b]	0	0	1 (3.0)[c]	0
Squamous Cell Papilloma	0	0	0	1
2-Year Study				
Forestomach	49	51	50	48
Mucosa, Hyperplasia	4 (1.5)	1 (1.0)	3 (2.0)	4 (2.0)
Squamous Cell Papilloma				
Overall rate[d]	0/50 (0%)	3/51 (6%)	2/50 (4%)	2/49 (4%)
Adjusted rate[e]	0.0%	8.0%	5.7%	6.7%
Terminal rate[f]	0/42 (0%)	2/36 (6%)	2/35 (6%)	2/30 (7%)
First incidence (days)	—[h]	683	736 (T)	736 (T)
Logistic regression test[g]	P=0.262	P=0.112	P=0.199	P=0.168
Squamous Cell Carcinoma				
Overall rate	0/50 (0%)	0/51 (0%)	1/50 (2%)	2/49 (4%)
Adjusted rate	0.0%	0.0%	2.9%	5.9%
Terminal rate	0/42 (0%)	0/36 (0%)	1/35 (3%)	1/30 (3%)
First incidence (days)	—	—	736 (T)	669
Logistic regression test	P=0.061	—	P=0.464	P=0.226
Squamous Cell Papilloma or Squamous Cell Carcinoma[i]				
Overall rate	0/50 (0%)	3/51 (6%)	3/50 (6%)	4/49 (8%)
Adjusted rate	0.0%	8.0%	8.6%	12.4%
Terminal rate	0/42 (0%)	2/36 (6%)	3/35 (9%)	3/30 (10%)
First incidence (days)	—	683	736 (T)	669
Logistic regression test	P=0.053	P=0.112	P=0.091	P=0.047

(continued)

TABLE 28
Incidence of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol (continued)

	0 ppm	312 ppm	625 ppm	1,250 ppm
Female				
15-Month Interim Evaluation				
Forestomach	8	10	9	10
Mucosa, Hyperplasia	1 (1.0)	0	3 (2.0)	3 (2.3)
Squamous Cell Papilloma	0	0	0	1
2-Year Study				
Forestomach	51	50	51	49
Mucosa, Hyperplasia	9 (2.0)	5 (2.2)	13 (1.8)	6 (2.0)
Squamous Cell Papilloma[j]				
Overall rate	0/52 (0%)	1/50 (2%)	5/51 (10%)	3/50 (6%)
Adjusted rate	0.0%	2.4%	16.6%	24.0%
Terminal rate	0/37 (0%)	0/30 (0%)	3/26 (12%)	2/11 (18%)
First incidence (days)	—	625	639	677
Logistic regression test	P=0.022	P=0.504	P=0.029	P=0.028

(T)Terminal sacrifice
[a] Number of animals with forestomach examined microscopically
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals necropsied
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposure group columns are the P values corresponding to the pairwise comparisons between the controls and that exposure group. The logistic regression test regards these lesions as nonfatal.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence for 2-year NTP feed studies with untreated control groups (mean ± standard deviation): 22/1,474 (1.5% ± 2.0%); range 0%-6%
[j] Historical incidence: 31/1,470 (2.1% ± 2.9%); range 0%-14%

Mammary Gland: The incidences of carcinoma of the mammary gland were slightly increased in 625 and 1,250 ppm female mice (0/50, 0/50, 1/50, 3/49; Table D1). An adenoacanthoma was also present in one 1,250 ppm female (Table D1). One of the female mice from the 1,250 ppm group had multiple carcinomas; however, the incidence of these mammary gland neoplasms was within the NTP historical control range (Table D4e).

Other: The incidence of hemangioma or hemangiosarcoma (combined) was significantly increased in 1,250 ppm female mice (1/52, 2/50, 0/51, 5/50; Tables D1 and D3) and was slightly increased in 312 and 1,250 ppm males (2/50, 6/51, 0/50, 5/49; Tables C1 and C3). In male mice there was no dose response and the highest incidence was well within the historical control range. In females, the highest incidence slightly exceeded the historical control range (Table D4f). The neoplasms occurred at various sites (bone marrow, colon, kidney, liver, mesentery, spleen, subcutis, testes, urinary bladder, and uterus) in both exposed and control mice. The morphology of benign and malignant vascular tumors was similar between exposed and control groups.

GENETIC TOXICOLOGY

2,2-Bis(bromomethyl)-1,3-propanediol was shown to be mutagenic *in vitro* and *in vivo*, but the conditions required to observe the positive responses were highly specific, and 2,2-bis(bromomethyl)-1,3-propanediol was not active in all assays. In the two *Salmonella* assays reported here (Table E1), 2,2-bis(bromomethyl)-1,3-propanediol gave a positive response only in the second assay (Zeiger *et al.*, 1992), which used a different concentration of S9 from the first assay (Mortelmans *et al.*, 1986). Metabolic activation, specifically in the form of 30% Aroclor 1254-induced male Syrian hamster liver S9, was required to obtain the mutagenic response; 10% hamster S9 was ineffective, as was 10% or 30% S9 derived from livers of pretreated rats. No other *Salmonella* strain/activation combination was responsive to the effects of 2,2-bis(bromomethyl)-1,3-propanediol.

In cytogenetic tests with cultured Chinese hamster ovary cells (Galloway *et al.*, 1987), 2,2-bis(bromomethyl)-1,3-propanediol did not induce sister chromatid exchanges, with or without S9 (Table E2), but a dose-related increase in chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated in the presence of induced rat liver S9 (Table E3). Both tests were conducted up to doses which induced marked cytotoxicity; cell confluence in the sister chromatid exchange test was reduced 75% at the top dose tested with S9 (1,200 μg/mL). A majority of the breaks which were observed in the aberration assay were located in the heterochromatic region of the long arm of the X chromosome. The reason for this preferential breakage site is not known. Also, the type of damage pattern seen with 2,2-bis(bromomethyl)-1,3-propanediol (induction of chromosomal aberrations but not sister chromatid exchanges) is unusual. Most chemicals which induce chromosomal aberrations also induce sister chromatid exchanges (Galloway *et al.*, 1987).

2,2-Bis(bromomethyl)-1,3-propanediol was also shown to be genotoxic *in vivo*. Significant increases in micronucleated normochromatic erythrocytes were observed in peripheral blood samples obtained from male and female mice exposed for 13 weeks to 2,2-bis(bromomethyl)-1,3-propanediol in feed (Table E6). These increases were observed in the two highest dose groups of male mice (5,000 and 10,000 ppm) and the three highest dose groups of female mice (2,500, 5,000, and 10,000 ppm).

In the first of two mouse bone marrow micronucleus tests performed to confirm the positive results seen in the 13-week feed study, inconsistent results were obtained between two trials which used the same dose range of 100 to 400 mg/kg 2,2-bis(bromomethyl)-1,3-propanediol, administered by gavage three times at 24-hour intervals (Table E4). Results of the first trial were negative; however, in the second trial, 2,2-bis(bromomethyl)-1,3-propanediol produced a clear, dose-related increase in micronucleated polychromatic erythrocytes. Because the positive response was not reproduced, the results were concluded to be equivocal.

In an attempt to clarify the results obtained in the first bone marrow micronucleus test, a second investigation was performed using both male and female mice. 2,2-Bis(bromomethyl)-1,3-propanediol was administered as a single intraperitoneal injection (150 to 600 mg/kg) and bone marrow samples were taken 48 hours after dosing. The results of this experiment, shown in Table E5, provide evidence of the ability of 2,2-bis(bromomethyl)-1,3-propanediol to induce micronuclei in bone marrow cells of female mice. Although male mice in all three dose groups showed a two-fold increase in the frequency of micronucleated polychromatic erythrocytes, the trend test was not significant due to the similarity in the responses, and pairwise analyses were also insignificant. The response in female mice was somewhat stronger (2.5-fold increase over background, at the highest dose) and was directly related to increasing doses of 2,2-bis(bromomethyl)-1,3-propanediol. These results were consistent with the stronger response observed in female mice in the 13-week feed study (Table E4).

In conclusion, 2,2-bis(bromomethyl)-1,3-propanediol was genotoxic *in vitro* and *in vivo*, inducing gene mutations in *Salmonella* strain TA100, chromosomal aberrations in cultured Chinese hamster ovary cells, and micronuclei in erythrocytes of male and female mice. The *in vitro* responses required S9.

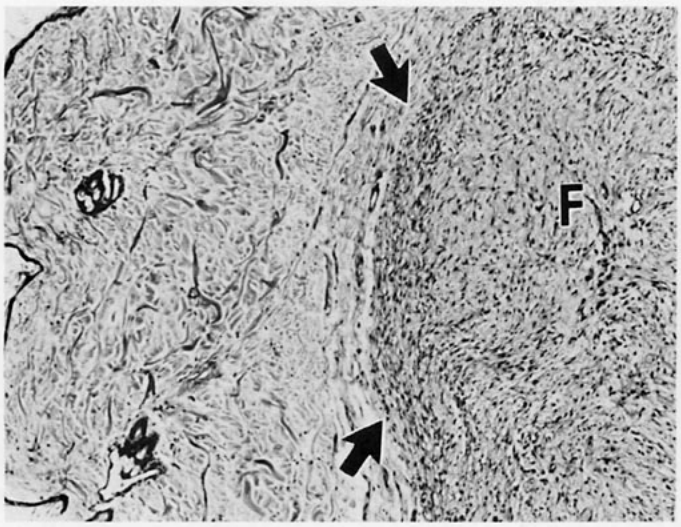


PLATE 1
Fibroma in skin of male F344/N rat administered 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 13 weeks in a stop-exposure group and necropsied at week 95. Well-differentiated fibroma (F) comprised of densely packed spindle cells is clearly demarcated (arrows) from the adjacent normal dermis; skin surface is at far left. H&E 65×

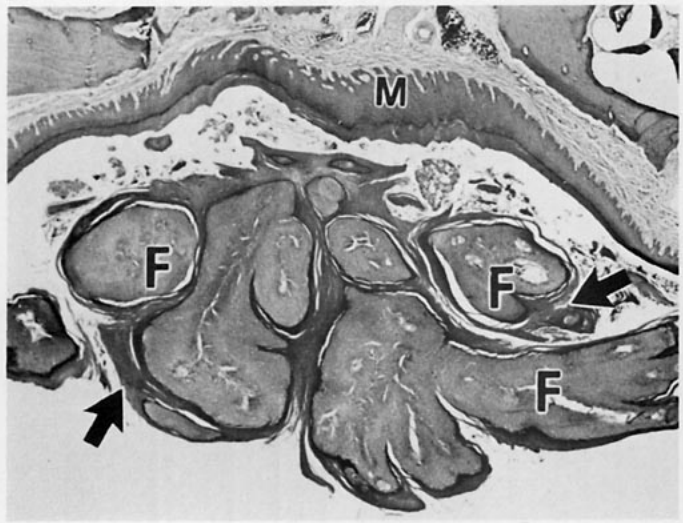


PLATE 2
Squamous cell papilloma in the dorsal posterior portion of the pharynx (hard palate) of a male F344/N rat administered 2,500 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. This exophytic mass arising from the oral cavity mucosa (M) consists of prominent papillary fronds (F) of well-differentiated squamous epithelium covered by a layer of keratin (arrows). H&E 25×

PLATE 3
Squamous cell papilloma in the forestomach of a male F344/N rat administered 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. Note the absence of inflammation or hyperplasia in adjacent forestomach mucosa (arrows). H&E 40×

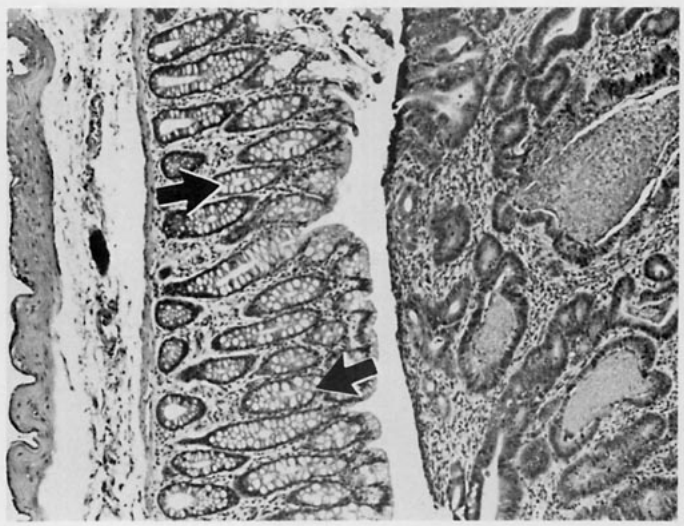


PLATE 4
Adenoma of the colon in a male F344/N rat administered 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. Large tumor mass (right) fills lumen of colon and is comprised of dilated glands lined by a closely packed tall columnar epithelium that lacks the goblet cell differentiation which is present in normal colonic mucosa (arrows). H&E 65×

PLATE 5
Mesothelioma attached to the capsular surface of the testis (arrows) in a male F344/N rat administered 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 13 weeks in a stop-exposure study and necropsied at week 75. Tumor arising from tunica vaginalis consists of densely cellular solid areas with formation of papillary structures. H&E 30×

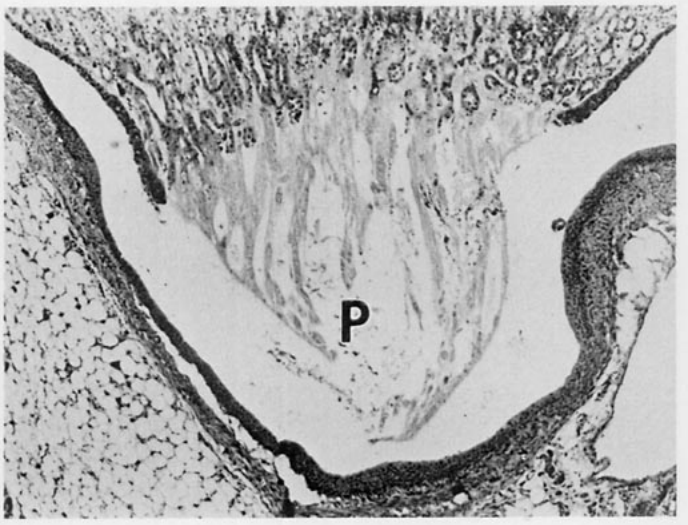


PLATE 6
Papillary degeneration and necrosis of the tip of the renal papilla (P) in a female F344/N rat administered 10,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. There is necrosis of the urothelium and stroma in the distal portion of the papilla. H&E 40×

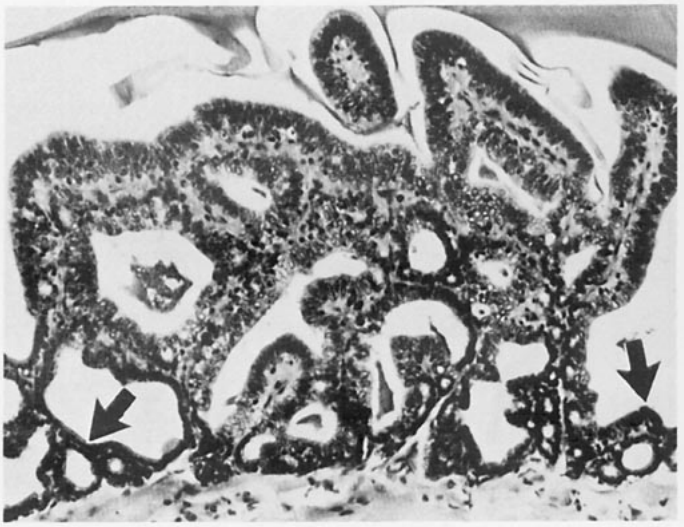


PLATE 7
Focal hyperplasia in seminal vesicle of male F344/N rat administered 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 13 weeks in a stop-exposure study and necropsied at week 75. Note increased height and crowding of hyperplastic epithelium compared to the cells in the normal adjacent mucosa (arrows). H&E 160×

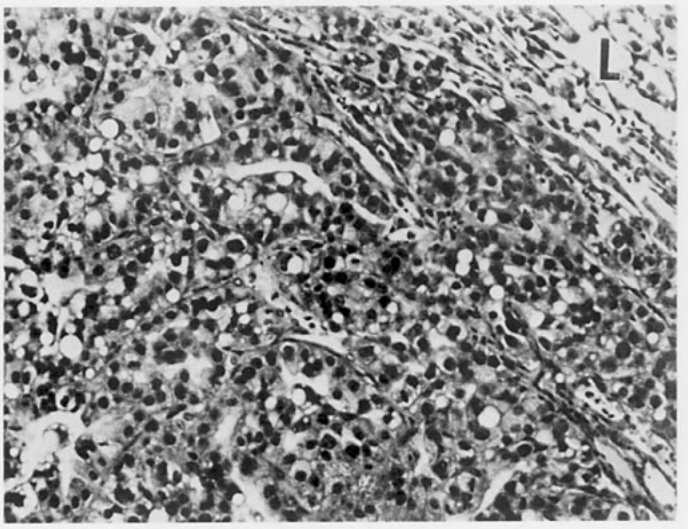


PLATE 8
Harderian gland carcinoma in a female B6C3F$_1$ mouse administered 312 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. Neoplastic cells with foamy to vacuolated cytoplasm form a glandular or acinar pattern in this tumor which has metastasized to the lung (L). H&E 160×

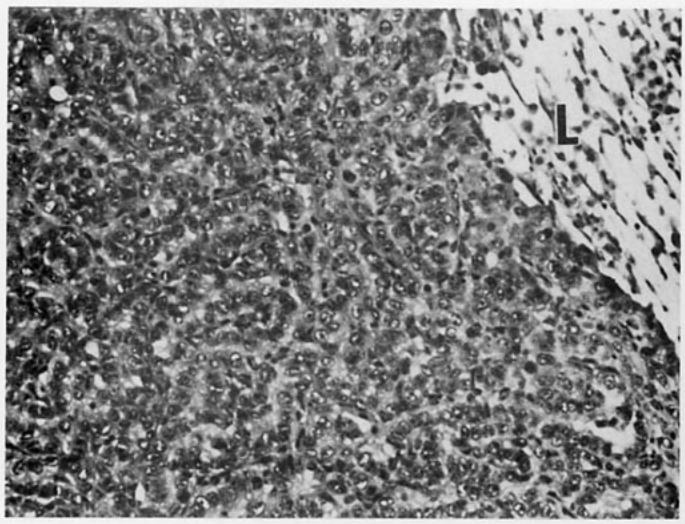


PLATE 9
Alveolar/bronchiolar carcinoma in lung of male B6C3F$_1$ mouse administered 1,250 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. Neoplastic cuboidal epithelium forms a densely packed glandular pattern that is compressing alveoli of adjacent lung (L). H&E 160×

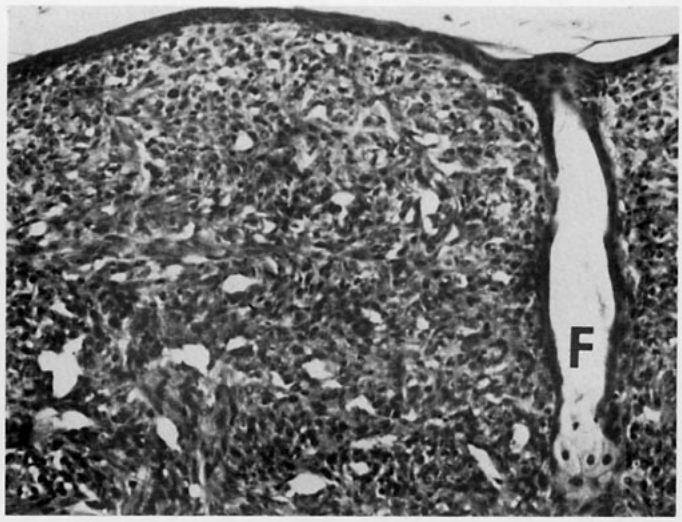


PLATE 10
Sarcoma in dermis of female B6C3F$_1$ mouse administered 1,250 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 2 years. A few remaining hair follicles (F) and sebaceous glands are present in dermis which has been replaced by neoplastic mesenchymal cells. H&E 160×

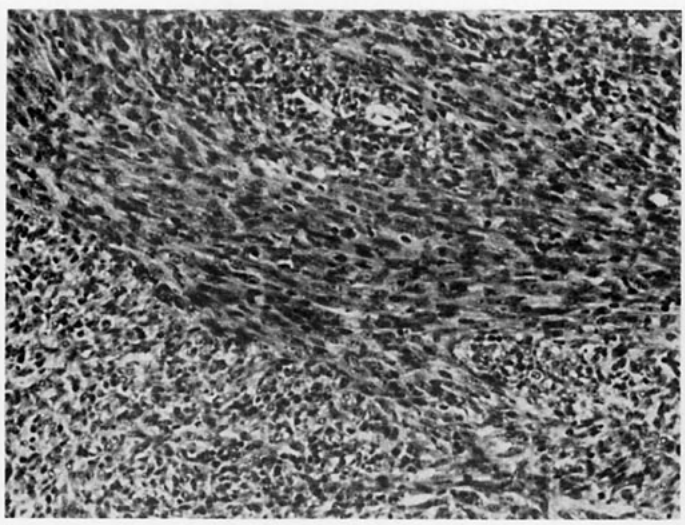


PLATE 11
Detail of another area of sarcoma shown in Plate 10 demonstrates pattern of interlacing bundles of neoplastic spindle cells. H&E 160×

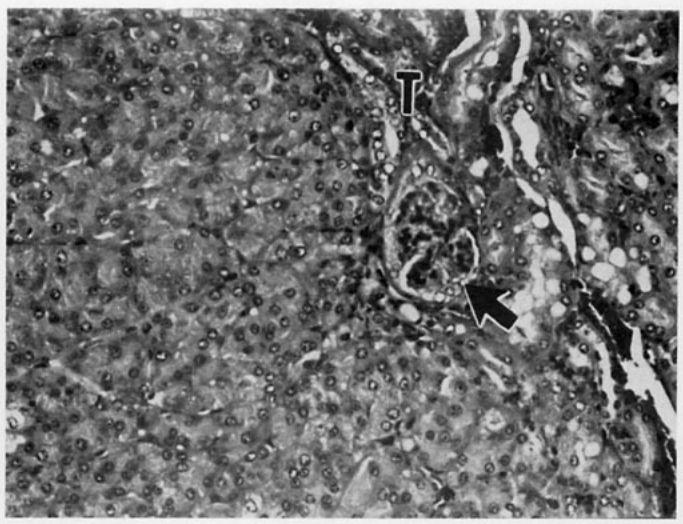


PLATE 12
Adenoma of renal tubule in male B6C3F$_1$ mouse consists of an expansile mass of well-differentiated neoplastic renal tubule epithelial cells compressing the normal cortical tubules (T) and glomerulus (arrow). H&E 160×

DISCUSSION AND CONCLUSIONS

These studies of 2,2-bis(bromomethyl)-1,3-propanediol [technical grade FR-1138®; 78.6% 2,2-bis(bromomethyl)-1,3-propanediol, 6.6% 2,2-bis(hydroxymethyl)-1-bromo-3-hydroxypropane, 6.9% 2,2-bis(bromomethyl)-1-bromo-3-hydroxypropane, 0.2% pentaerythritol, and 7.7% dimers and structural isomers] show that this flame retardant is a multi-site, multispecies carcinogen (Table 29).

In the 13-week feed studies, the high dose for rats was 20,000 ppm (estimated to deliver about 900 to 1,340 mg/kg) and for mice was 10,000 ppm (estimated to deliver 2,900 mg/kg). There were no treatment-related deaths, but mean body weights of male and female rats exposed to 5,000 ppm and above, of male mice exposed to 1,250 ppm and above, and of female mice exposed to 625 ppm and above were lower than those of the control groups.

Based on the results of clinical chemistry and histopathology, chemical related toxicity was evident in the kidney and urinary bladder of rats and mice. Urinalysis demonstrated the development of an isosthenuric polyuria in rats primarily in the 10,000 and 20,000 ppm exposure groups, indicating the kidneys had not altered the concentration of the glomerular filtrate. This change was less evident in females. The primary control of urine volume and tonicity occurs by antidiuretic hormone-influenced water resorption in the distal renal tubules and collecting ducts. There are renal and nonrenal causes of polyuria, including renal injury or disease, drugs (e.g., diuretics or aminoglycosides), increased water intake, decreased response to antidiuretic hormone, osmotic diuresis, and hyperadrenocorticism. In this study, minimal to mild renal papillary injury in rats may have contributed to altered distal tubular function resulting in the isosthenuric polyuria. However, water deprivation tests demonstrated that male and female rats were able to concentrate their urine in response to reduced water intake throughout the study. This indicates that the antidiuretic hormone-dependent pituitary-renal axis was still intact. In the mice, the clinical chemistry and urinalysis findings were slightly different from those in rats. There was no evidence of polyuria in the mice, but blood urea nitrogen was significantly increased in 10,000 ppm males and females. Blood urea nitrogen concentration is considered an insensitive biomarker of renal damage and requires approximately 75% of the nephrons to be nonfunctional before increased serum blood urea nitrogen concentration occurs (Finco, 1989). In this study, there were mild to moderate tubule and papillary changes, but the increased blood urea nitrogen may have also been secondary to increased protein catabolism related to the decreased body weight gain or body weight loss in 10,000 ppm mice.

Chemical-related lesions were observed only in the urinary bladder and kidney of rats and mice. Kidney lesions in mice (papillary necrosis and renal tubule regeneration and fibrosis) were more severe than those observed in rats (papillary degeneration). Urinary bladder lesions in the mice were also more severe than in rats. The presence of kidney and urinary bladder lesion in mice at exposure concentrations that were lower than those which caused similar but less severe lesions in rats would indicate that mice are more sensitive to the renal effects of 2,2-bis(bromomethyl)-1,3-propanediol in 13-week toxicity studies. Based on an equivalent dose per body weight, the toxic effects of 2,2-bis(bromomethyl)-1,3-propanediol on the urinary bladder and kidney of rats and mice were similar whether administered by gavage or in feed (Elwell *et al.*, 1989).

In rats and mice, no abnormalities were observed in sperm morphology, count, or motility or in the estrous cycle length. While body weights in exposed groups were lower than in the controls, diet restriction studies have shown that body weight effects alone (20% lower body weight) do not cause reproductive system toxicity in rats or mice (Chapin *et al.*, 1993).

In continuous breeding studies, 2,2-bis(bromomethyl)-1,3-propanediol has been shown to impair

fertility in female mice in the absence of an effect on reproductive organ weights or estrual cyclicity (Treinen *et al.*, 1989). The ovary may be a target for 2,2-bis(bromomethyl)-1,3-propanediol since 0.4% 2,2-bis (bromomethyl)-1,3-propanediol significantly decreased the number of primary and growing ovarian follicles in female mice (Heindel *et al.*, 1989). It should be noted that in these studies where ovarian toxicity occurred, 2,2-bis(bromomethyl)-1,3-propanediol exposure began *in utero*. In contrast, in the present 13-week and 2-year studies reported here, exposure did not begin until animals were 6 to 7 weeks of age, and ovarian toxicity was not observed.

The 2,2-bis(bromomethyl)-1,3-propanediol 2-year studies consisted of continuous-exposure studies in which the chemical was administered continuously to rats and mice in feed. In addition there was a stop-exposure study in male rats in which the animals received 20,000 ppm 2,2-bis(bromomethyl)-1,3-propanediol in feed for 3 months, and then received undosed feed for the remainder of the 2-year study.

2,2-Bis(bromomethyl)-1,3-propanediol administered in the feed caused early deaths in 10,000 ppm male and female rats, 1,250 ppm female mice, and 20,000 ppm stop-exposure male rats. These early deaths were attributed primarily to the carcinogenic effects of the chemical.

The incidences of skin tumors in male rats from the 5,000 and 10,000 ppm continuous-exposure groups and the 20,000 ppm stop-exposure group were significantly greater than those in the control group, and included increased incidences of squamous cell papilloma, keratoacanthoma, basal cell adenoma, sebaceous gland adenoma, and trichoepithelioma. The incidences of skin tumors in exposed female rats did not differ significantly from those in the control group. Other studies [e.g., benzidine congener dyes (NTP, 1990a, 1991a,b, 1992, and 1994a); 2,3-dibromo-1-propanol (NTP, 1994b)] have shown that genotoxic chemicals administered orally can cause skin tumors in rats, and the incidence for these tumors is generally greater in male rats than in female rats. The mechanism for this sex difference could not be determined from this study but may be due, in part, to metabolic differences between the sexes.

In the Zymbal's gland, a modified sebaceous gland, there was an increased incidence of neoplasms in male rats. The Zymbal's gland and skin are related epithelial tissues. In a review of NTP findings, 17 chemicals induced Zymbal's gland neoplasms, 14 induced skin neoplasms, and 11 induced neoplasms at both sites in rats. Most of the chemicals inducing Zymbal's gland and skin neoplasms also caused neoplasms at other sites. These chemicals are generally genotoxic in the *Salmonella* assay system, and chemically induced genetic damage is thought to be the underlying mechanism for development of skin and Zymbal's gland neoplasms (NTP, 1991a).

2,2-Bis(bromomethyl)-1,3-propanediol exposure increased the incidence of mammary gland neoplasms in rats. The treatment-related increase in mammary gland fibroadenoma was greater in female than in male rats. However, there was a significant increase in subcutaneous fibroma in exposed groups of male rats. Other chemicals which have caused an increase in the incidences of mammary gland neoplasms in female rats have also been associated with an increased incidences in fibroma (cytembena; NTP, 1981), fibroadenoma (glycidol; NTP, 1990b), or a combination of fibroma and fibroadenoma (methylene chloride; NTP, 1986c) in male rats. The chemicals that cause mammary gland neoplasms in rats are frequently genotoxic chemicals suggesting that genetic damage may contribute to this neoplastic response. Recent epidemiology studies have found an association between exposure to halogenated hydrocarbons and breast cancer in certain subsets of populations examined (Wolff *et al.*, 1993; Kreiger *et al.*, 1994).

There were treatment-related increased incidences of squamous cell neoplasms in the oral cavity (tongue and pharynx) and esophagus in male and female rats. In addition, there were treatment-related squamous cell neoplasms of the forestomach and adenoma and carcinoma of the small and large intestine in male rats. There was no evidence for toxicity at these sites in the 13-week studies or at the 15-month interim evaluation of the 2-year study. The presence of neoplasms in the gastrointestinal tract of exposed rats suggests that the chemical may interact directly with the mucosal epithelium. Although the increased incidence in intestinal neoplasms was limited to male rats, this effect was seen primarily in the stop-exposure group, which did not include females.

Other chemicals which have been found to cause oral cavity neoplasms in rats [including benzene (NTP, 1986a), benzidene-congener chemicals or dyes (NTP, 1990a, 1991a,b, 1992, and 1994a), glycidol (NTP, 1990b), trichloropropane (NTP, 1994c), 1,2-dibromo-3-chloropropane (NTP, 1982a), 2,3-dibromo-1-propanol (NTP, 1994b), and dimethylvinyl chloride (NTP, 1986b)] are also genotoxic chemicals. Rats are more susceptible than mice to the formation of oral cavity neoplasms, and oral cavity neoplasms have previously been reported only in the 1,2,3-trichloropropane mouse study (NTP, 1994c). Chemical-related esophageal neoplasms have previously been observed in rats in only two other studies [2,3-dibromo-1-propanol (NTP, 1994b) and dimethylvinyl chloride (NTP, 1986b)].

An increased incidence in benign and malignant neoplasms of the small and large intestine was seen in male rats. There was no increase in the incidence of intestinal neoplasms in female rats, but most of the neoplasms observed in males occurred in the stop-exposure group above the highest exposure level for females. In previous NTP studies where intestinal neoplasms have resulted from chemical administration, the number of neoplasms has been slightly greater in males than in females (bromoform; NTP, 1989a, bromodichloroethane; NTP, 1988a; 3,3-dimethylbenzidine; NTP, 1991b). Although the number of neoplasms in the small intestine were increased in the stop-exposure group, the response was much less than that observed for the large intestine. In previous NTP studies, a smaller number of neoplasms have been observed in the small intestine compared to the large intestine (3,3-dimethoxybenzidine; NCI, 1979a; dimethylhydrazine; Ward, 1974). In several instances there has been a marked increase in the incidence of neoplasms of the large intestine with no effect on the small intestine (bromoform, bromodichloromethane). In this study, most of the neoplasms of the large intestine were benign and were morphologically similar to the adenomas of the colon that rarely occur in controls. Six of the seven neoplasms of the small intestine were malignant and contained cystic areas as well as foci of osseous metaplasia. Morphologic features were similar to those that have been described for spontaneous and chemically induced neoplasms of the small intestine (Ward, 1974). Two gross lesions diagnosed as cystic hyperplasia and focal hyperplasia with osseous metaplasia in two other dosed rats are rare spontaneous lesions of the small intestine which may be preneoplastic. Other brominated chemicals also cause intestinal neoplasms in rats [bromodichloromethane (NTP, 1988a), tribromomethane (NTP, 1989a), 2,3-dibromo-1-propanol (NTP, 1994b), 1-amino-2,4-dibromoanthraquinone (NTP, 1996)], suggesting that these brominated chemicals may be acting by a similar mechanism.

There were increased incidences of urinary bladder transitional cell neoplasms in male rats at 15 months and 2 years. While these incidences were low, these neoplasms rarely occur in untreated animals (mean: 0.2%), and these neoplasms were considered to be related to treatment. Only 10 chemicals studied by the NTP have caused treatment-related urinary bladder neoplasms in male rats. It has been suggested that some of these chemicals caused the urinary bladder neoplasms by formation of calculi, subsequent irritation, and tumor formation (e.g., melamine; NTP, 1983), but this does not appear to be the mechanism for the development of urinary bladder neoplasms observed in the present study. The early occurrence of transitional cell hyperplasia suggests that 2,2-bis(bromomethyl)-1,3-propanediol or its metabolites have a direct toxic effect on the urinary bladder in male rats.

2,2-Bis(bromomethyl)-1,3-propanediol caused renal tubule degeneration and hyperplasia in male and female rats at 15 months and 2 years. Four renal tubule adenomas (one in the 5,000 ppm group and three in the 10,000 ppm group) occurred in male rats. These neoplasms are uncommon in males (mean: 2%) and may have been related to chemical administration. There was no evidence for a carcinogenic response in the kidney of the female rat.

2,2-Bis(bromomethyl)-1,3-propanediol exposure caused neoplasms of the thyroid gland in male and female rats. The occurrence of these neoplasms in the absence of diffuse thyroid gland hyperplasia supports the hypothesis that 2,2-bis(bromomethyl)-1,3-propanediol causes a direct thyroid response that is not likely secondary to sustained high concentrations of thyroid stimulating hormone.

There was a treatment-related increased incidence of mesothelioma in male rats. Mesothelioma typically

arises in the abdominal peritoneal cavity of F344 rats and is seen almost exclusively in males. Treatment-related increases of mesothelioma observed in previous NTP studies have also been in male rats. Other chemicals which have caused a marked increase in the incidence of mesotheliomas in male rats have also caused increases in mammary gland neoplasms in females (cytembena; NTP, 1981; glycidol; NTP, 1990b; *o*-toluidine; NCI, 1979b).

A marginal increase in the incidence of acinar cell adenoma of the pancreas was observed in exposed groups of male rats. Focal acinar cell hyperplasia was significantly increased in all exposure groups. Because there was no dose-related increase in the incidence of adenomas, and all incidences were within the NTP historical control range, it was uncertain if these neoplasms were related to treatment.

The stop-exposure study in male rats showed that 2,2-bis(bromomethyl)-1,3-propanediol administered for only 3 months was carcinogenic at all the sites where carcinogenic activity was observed in the 2-year continuous-exposure male rat study. The incidences of neoplasms were greater in the stop-exposure study male rats than in continuous-exposure male rats at the following sites: oral cavity, forestomach, small intestine, large intestine, lung, Zymbal's gland, thyroid gland, and mesothelium.

In the male stop-exposure group, there was an adenoma and a carcinoma of the seminal vesicle. The spontaneous development of these neoplasms is extremely rare in control rats, but treatment-related increases in hyperplasia and neoplasms have been reported in other strains of rats administered N-nitroso-N-methylurea (Slayter *et al.*, 1994) or 3,2'-dimethy-4-aminobiphenyl (Bosland *et al.*, 1990) followed by treatment with testosterone propionate or cyproterone acetate, respectively. Because of the rarity of these neoplasms in control rats and the presence of a dose-related increase in hyperplasia, the neoplasms in the stop-exposure group were considered to be related to treatment.

Based on the findings from this stop-exposure study, genetic damage appears to occur within the first few months of exposure. This genetic damage is irreversible, and neoplasms develop in the absence of a toxic response.

In a previous study of 2,2-bis(bromomethyl)-1,3-propanediol [(FR-1138®) containing approximately the same components of parent compound and impurities as used in these NTP studies], there were no clear carcinogenic effects in male or female Sprague-Dawley rats administered doses in the feed that were reported to deliver 5 or 100 mg/kg per day for 2 years (Keyes *et al.*, 1979).

In the NTP F344/N rat study, 2,2-bis(bromomethyl)-1,3-propanediol was administered at 2,500, 5,000, or 10,000 ppm, delivering approximately 100, 200, or 400 mg/kg of the chemical per day throughout most of the study. The low dose delivered in the present study was approximately the same as the higher dose in the Keyes *et al.* (1979) study, and at this dose, treatment-related neoplasms occurred in the subcutaneous tissues and oral cavity of male rats and mammary gland of female rats. As the dose was increased, a wider spectrum of carcinogenic responses occurred. The variance in the results for the two studies in rats may have been related to metabolic differences in the strains or differences in the incidence of spontaneous neoplasms in control animals. The Sprague-Dawley rat has a very high background incidence and multiplicity of mammary gland neoplasms, which could have masked this neoplastic effect of the chemical. In the male F344 rat, the small increase in the incidence of oral cavity neoplasms at the lowest dose (2,500 ppm) was significantly greater than the incidence in the control group, but the treatment-related increased incidences were more apparent at higher exposure levels than those used in the Keyes *et al.* (1979) study.

The incidences of harderian gland neoplasms were increased in exposed male and female mice. Other chemicals causing these neoplasms are usually multispecies/site carcinogens [benzene (NTP, 1986a), cupferron (NCI, 1978a), ethylene oxide (NTP, 1988b), glycidol (NTP, 1990b), *n*-methylolacryamide (NTP, 1989b), 4,4'oxydianiline (NCI, 1980), 1,2,3-trichloropropane (NTP, 1994c), and, 1,3-butadiene (NTP, 1993)].

The incidences of lung neoplasms were increased in exposed male and female mice. Lung neoplasms have been observed in mice (but not in rats) in studies of ozone (NTP, 1994d), benzene (NTP, 1986a), benzofuran (NTP, 1989c) as well as other

halogenated hydrocarbons [1,2-dibromo-3-chloropropane (NTP, 1982a), 1,2-dibromoethane (NCI, 1978b; NTP, 1982b), 2,3-dibromo-1-propanol (NTP, 1994b), 1,2-dichloroethane (NCI, 1978c), and tris(2,3-dibromopropyl)phosphate (NCI, 1978d)]. It is not known why the mouse lung is particularly responsive to the effects from these halogenated hydrocarbons, but this response could be due to differences in metabolism between species.

The toxicity observed in the urinary bladder and kidney of mice in the 13-week study was not seen in the 2-year study, but the highest dose (1,250 ppm) was below the level at which these lesions were seen in the 13-week study where there was renal toxicity characterized by papillary necrosis and increased tubule regeneration. Although the highest dose in the 2-year study was half the dose causing these lesions in the 13-week study, there was a small increase in the incidence of renal tubule adenoma in male mice. In NTP studies of approximately 450 chemicals, only seven other chemicals have been identified as causing kidney neoplasms in the male mouse. Two of these were brominated chemicals [bromodichloromethane (NTP, 1988a) and tris(2,3-dibromopropyl)phosphate (NCI, 1978d)].

Other neoplastic responses occurred in the forestomach of exposed male and female mice and the mammary gland and circulatory system in exposed female mice. Minimal increases in the incidences of neoplasms of the forestomach were seen in male and female mice. There was no treatment-related increase in the incidence of hyperplasia of the forestomach squamous epithelium. Because the number of forestomach neoplasms was within or just above the historical control range, it was uncertain if this increase was related to treatment.

In female mice, there was a significant increase in hemangiosarcoma and hemangioma (combined) in the 1,250 ppm group. Two of the hemangiosarcomas were in the subcutis, which was also a site for treatment-related sarcomas in female mice. Since the combined total number of neoplasms marginally exceeded the historical control range, it is uncertain if the increase in the incidence of these neoplasms was related to treatment.

Although 2,2-bis(bromomethyl)-1,3-propanediol caused mammary gland neoplasms in male and female rats, in exposed groups of female mice there were only four mammary gland carcinomas (one in the 625 ppm group and three in the 1,250 ppm group). Because the incidences for these neoplasms were within the historical range, it was uncertain if the increase was related to chemical administration.

2,2-Bis(bromomethyl)-1,3-propanediol and other brominated chemicals have been shown to be genotoxic in a spectrum of tests. It is hypothesized that the carcinogenic activity of brominated chemicals is due to genotoxic mechanisms, although at this time we have not identified the genotoxic metabolite or characterized the spectrum of genetic changes on a molecular level.

Of the 11 aliphatic and three aromatic brominated chemicals studied by the NTP in 2-year rodent studies, 13 of 14 chemicals were carcinogenic (Table 30). It would be expected that C-Br bonds in 2,2-bis(bromomethyl)-1,3-propanediol would be cleaved more readily than C-Cl bonds in halogenated compounds because of a lower bond energy (bond strengths: C-Cl, 95 kCal; C-Br, 67 kCal; Weast and Astle, 1978). Once the C-Br bond is broken, a free radical is available that can participate in various chemical reactions. Weiss *et al.* (1986) showed that eosinophils contain a lysosomal peroxidase that oxidizes halides to highly reactive and toxic hypohalous acids. Even though chloride is found at 1,000 times the concentration of bromide, the eosinophils used bromide preferentially to form the hypobromous acid. Bromide was shown to bind more readily to cellular proteins and macromolecules than other halide ions.

Two hypotheses for the carcinogenic activity of brominated chemicals are: 1) bromine causes oxidative damage to DNA and other cellular constituents and 2) the C-Br bond is broken and the remaining carbon-containing electrophilic group forms DNA adducts with subsequent DNA damage.

Studies with potassium bromate (Kurokawa *et al.*, 1983) have shown that this chemical administered in drinking water at 250 or 500 ppm to F344 rats caused renal and intestinal neoplasms in male and female rats and mesotheliomas of the peritoneum in

male rats. Following oral administration of $KBrO_3$ a significant increase of 8-hydroxydeoxyguanosine was observed in DNA. 8-Hydroxydeoxyguanosine is one of the DNA-damage products formed by oxygen radicals, and this is thought to be one of the DNA lesions involved in $KBrO_3$ carcinogenesis (Kasai *et al.* 1987; Sai *et al.*, 1992).

These NTP studies found that the flame retardant 2,2-bis(bromomethyl)-1,3-propanediol (FR-1138®) was carcinogenic in rodents causing a wide spectrum of organ carcinogenic responses. Other brominated flame retardants have also been shown to be carcinogenic in rodents [2,3-dibromo-1-propanol, polybrominated biphenyl, tris (2,3-dibromopropyl)phosphate, and bis(2,3-dibromopropyl)-phosphate (Takada *et al.*, 1991; IARC, 1990)]. Of the 10 aliphatic and three aromatic brominated chemicals studied by the NTP in 2-year rodent studies, 12 were carcinogenic (Table 30).

Common sites for carcinogenic activity from the brominated chemicals studied by the NTP (Table 31) include oral cavity, forestomach, intestine, lung, and kidney. Treatment-related lesions are generally not seen at these sites early in the study, but develop with time. In the 2,2-bis(bromomethyl)-1,3-propanediol stop-exposure study, neoplasm development in the male rat requires only 3 months of exposure, and while lesions were not seen in the target organ at the end of this 3-month exposure period, the essential damage to the cell had been done, and carcinogenic lesions developed with time. Nonneoplastic lesions were observed in the pancreas, seminal vesicles, thyroid gland, lung, kidney, and urinary bladder in male rats; in the kidney of female rats; and in the lung of female mice. A carcinogenic response was observed in some of these organs; however, there were many sites where a carcinogenic response was observed in the absence of nonneoplastic lesions.

CONCLUSIONS

Under the conditions of these 2-year feed studies, there was *clear evidence of carcinogenic activity** of 2,2-bis-(bromomethyl)-1,3-propanediol (FR-1138®) in male F344/N rats based on increased incidences of neoplasms of the skin, subcutaneous tissue, mammary gland, Zymbal's gland, oral cavity, esophagus, forestomach, small and large intestines, mesothelium, urinary bladder, lung, thyroid gland, and seminal vesicle, and the increased incidence of mononuclear cell leukemia.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in female F344/N rats based on increased incidences of neoplasms of the oral cavity, esophagus, mammary gland, and thyroid gland.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in male $B6C3F_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and kidney.

There was *clear evidence of carcinogenic activity* of 2,2-bis(bromomethyl)-1,3-propanediol in female $B6C3F_1$ mice based on increased incidences of neoplasms of the harderian gland, lung, and subcutaneous tissue.

Slight increases in the incidences of neoplasms of the pancreas and kidney in male rats; forestomach in male mice; and forestomach, mammary gland, and circulatory system in female mice may have also been related to treatment.

Exposure of male and female rats to 2,2-bis(bromomethyl)-1,3-propanediol was associated with alveolar/bronchiolar hyperplasia in the lung (males only); focal atrophy, papillary degeneration, transitional epithelial hyperplasia (pelvis), and papillary epithelial hyperplasia in the kidney; follicular cell hyperplasia in the thyroid gland (males only); hyperplasia in the seminal vesicle and pancreas (males only); mucosal hyperplasia in the forestomach (males only); and urinary bladder hyperplasia (males only). Exposure of mice to 2,2-bis(bromomethyl)-1,3-propanediol was associated with hyperplasia of the alveolar epithelium in females.

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

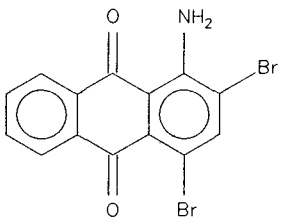
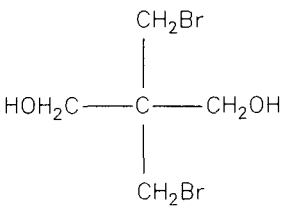
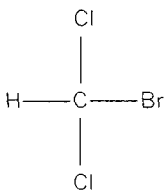
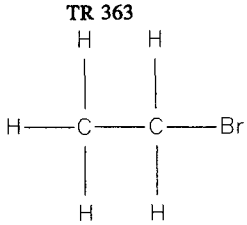
TABLE 29
Incidences of Selected Treatment-related Neoplasms in F344/N Rats and B6C3F$_1$ Mice in the 2-Year Feed Study of 2,2-Bis(bromomethyl)-1,3-propanediol

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm (Stop-Exposure)
Male Rats[a]	51	53	51	55	60
Skin Tumors (all types)[b]	4	6	14 **	24 **	21 **
Subcutaneous Tissue	2	9 *	13 **	16 **	10 **
Mammary Gland (fibroadenoma)	0	4 *	6 **	6 **	5 **
Zymbal's Gland	2	1	4	5	15 **
Oral Cavity	0	4 *	9 **	10 **	13 **
Esophagus	0	0	1	5 *	0
Forestomach	0	0	0	1	5 *
Small Intestine	0	0	0	2	5 *
Large Intestine	0	0	3	4	10 **
Mesothelioma	0	3	8 **	9 **	26 **
Kidney (renal tubule adenoma)	0	0	1	3 **	1
Urinary Bladder	0	0	1	3	2
Lung	1	1	3	4 *	7 *
Thyroid Gland, Follicular Cell	0	2	6 *	3	9 **
Seminal Vesicle	0	0	0	0	2
All Organs, Mononuclear Cell Leukemia	27	29	40 **	34 **	25 **
Pancreas	1	2	4 *	3	3
Female Rats	50	51	53	52	
Oral Cavity	2	3	5	6	
Esophagus	0	0	1	10 **	
Mammary Gland (fibroadenoma)	25	45 **	46 **	45 **	
Thyroid Gland, Follicular Cell	0	0	2	4 **	

	0 ppm	312 ppm	625 ppm	1,250 ppm
Male Mice	50	51	50	49
Harderian Gland	4	7	16 **	22 **
Lung	15	11	16	25 *
Kidney	0	0	3	2
Forestomach	0	3	3	4 *
Female Mice	52	50	51	50
Harderian Gland	3	12 *	13 **	19 **
Lung	5	5	15 *	19 **
Subcutaneous Tissue	0	1	4	12 **
Forestomach	0	1	5 *	3 *
Mammary Gland	0	0	1	3
Circulatory System	1	2	0	5 *

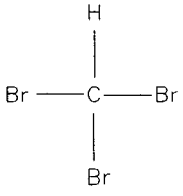
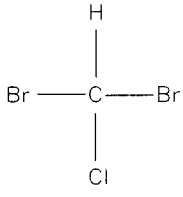
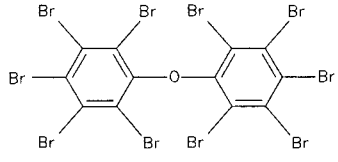
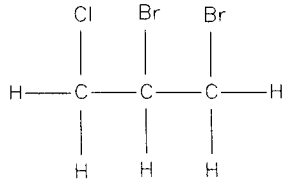
* Significantly different ($P \leq 0.05$) from controls by the life table test (Zymbal's gland or subcutaneous tissue neoplasms and mononuclear cell leukemia) or the logistic regression test (all other neoplasms)
** $P \leq 0.01$
[a] Number of animals necropsied
[b] Number of animals with neoplasms

TABLE 30
Results of Carcinogenicity and Mutagenicity Tests of Selected Brominated Chemicals in Male and Female F344/N Rats and Male and Female B6C3F$_1$ Mice[a]

Chemical and Route	Carcinogenicity[b] Male Rat	Female Rat	Male Mouse	Female Mouse	*Salmonella* Test Result
1-Amino-2,4-dibromoanthraquinone (feed) TR 383 (in press) 	+ L, I, K, Ub	+ L, I, K, Ub	+ L, F, Lu	+ L, F, Lu	+[c]
2,2-Bis(bromomethyl)-1,3-propanediol (feed) TR 452 	+ Sk, S, Oc, E, F, I, Ma, Lu, P, K, Ub, Sv, Z, Ty, Me	+ Oc, E, Ma, Lu, Ty, Z	+ F, Lu, K, Ha	+ S, F, Ma, Lu, H, Ci	+[d]
Bromodichloromethane (gavage) TR 321 	+ K, I	+ K	+ K	+ L	+[e]
Bromoethane (inhalation) TR 363 	+ A, Br, Lu	+/− Br, Lu	+/− Lu	+ U	+[f]

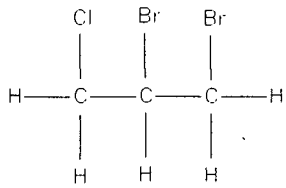
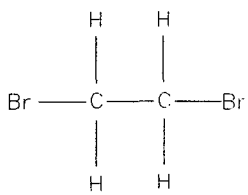
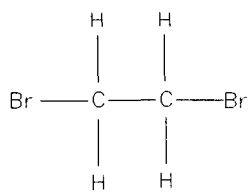
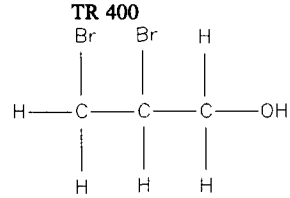
(continued)

TABLE 30
Results of Carcinogenicity and Mutagenicity Tests of Selected Brominated Chemicals in Male and Female F344/N Rats and Male and Female B6C3F$_1$ Mice (continued)

Chemical and Route	Carcinogenicity				*Salmonella* Test Result
	Male Rat	Female Rat	Male Mouse	Female Mouse	
Bromoform (tribromomethane; gavage) TR 350 	+ I	+ I	–	–	+[g]
Chlorodibromomethane (gavage) TR 282 	–	–	+/– L	+ L	+[e]
Decabromodiphenyl Oxide (feed) TR 309 	+ L	+ L	+/– L, Ty	–	–[c]
1,2-Dibromo-3-chloropropane (gavage) TR 28 	+ F	+ F, Ma	+ F	+ F	+[h]

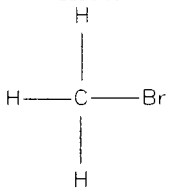
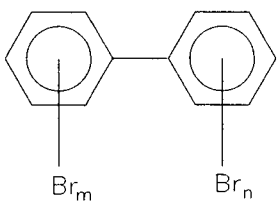
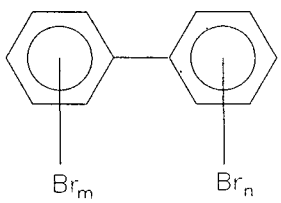
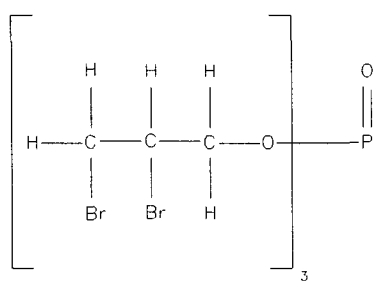
(continued)

TABLE 30
Results of Carcinogenicity and Mutagenicity Tests of Selected Brominated Chemicals in Male and Female F344/N Rats and Male and Female B6C3F$_1$ Mice (continued)

Chemical and Route	Carcinogenicity				*Salmonella* Test Result
	Male Rat	Female Rat	Male Mouse	Female Mouse	
1,2-Dibromo-3-chloropropane (inhalation) TR 206 	+ N, Oc	+ N, Oc, A	+ N, Lu	+ N, Lu	+[h]
1,2-Dibromoethane (gavage) TR 86 	+ F, Ci	+ F, L	+ F, Lu	+ F, Lu	+[i]
1,2-Dibromoethane (inhalation) TR 210 	+ N, Ci, Me	+ N, Ci, Ma, Lu	+ Lu	+ Lu, Ci, S, N, Ma	+[i]
2,3-Dibromo-1-propanol (dermal) TR 400 	+ N, Me, Sp, Sk, Oc, E, F, I, L, K, Z	+ Sk, Z, Cl, Ma, N, Oc, I, F, I, L, K	+ Sk, F, L, Lu	+ Sk, F	+[c]

(continued)

TABLE 30
Results of Carcinogenicity and Mutagenicity Tests of Selected Brominated Chemicals in Male and Female F344/N Rats and Male and Female B6C3F$_1$ Mice (continued)

Chemical and Route	Carcinogenicity				*Salmonella* Test Result
	Male Rat	Female Rat	Male Mouse	Female Mouse	
Methyl Bromide (inhalation) TR 385 	NT	NT	–	–	+[j]
Polybrominated Biphenyls[k] (gavage) TR 244 	+ L	+ L	+ L	+ L	NT
Polybrominated Biphenyls[k] (feed) TR 398 	+ L	+ L	+ L	+ L	NT
Tris (2,3-Dibromopropyl) Phosphate (feed) TR 76 	+ K	+ K, F, Lu	+ K, F, Lu	+ F, L, Lu	+[l]

(continued)

TABLE 30
Results of Carcinogenicity and Mutagenicity Tests of Selected Brominated Chemicals in Male and Female F344/N Rats and Male and Female B6C3F$_1$ Mice (continued)

[a] Carcinogenic response: + = some or clear evidence of carcinogenic activity; − = no evidence of carcinogenic activity; +/− = equivocal evidence of carcinogenic activity; NT = not tested
[b] Site of carcinogenic activity: A = adrenal gland; Br = brain; Ci = circulatory system; Cl = clitoral gland; E = esophagus; F = forestomach; H = harderian gland; He = hemangiosarcoma; I = intestine; K = kidney; L = liver; Lu = lung; Ma = mammary gland; Me = mesothelium; N = nasal cavity; Oc = oral cavity; P = pancreas; S = subcutaneous tissue; Sk = skin; Sp = spleen; Sv = seminal vesicle; Ty = thyroid gland; U = uterus; Ub = urinary bladder; and Z = Zymbal's gland
[c] Haworth *et al.*, 1983
[d] Mortelmans *et al.*, 1986; Zeiger *et al.*, 1992
[e] Simmon *et al.*, 1977; Simmon, 1978; Simmon and Kauhanen, 1978; Simmon and Tardiff, 1978
[f] Haworth *et al.*, 1983; Zeiger *et al.*, 1992
[g] Haworth *et al.*, 1983; Zeiger, 1990
[h] Zeiger *et al.*, 1988
[i] Zeiger *et al.*, 1992
[j] Unpublished
[k] Sum of m and n ranges from 2 to 7
[l] Dunkel *et al.*, 1985

TABLE 31
Summary of Selected Neoplasms in NTP Studies of Brominated Chemicals

	1-Amino-2,4-dibromoanthraquinone (feed)	2,2-Bis-(bromomethyl)-1,3-propanediol (feed)	Bromodichloromethane (gavage)	Bromoform (gavage)	Bromoethane (inhalation)
Alimentary System[a]					
Forestomach	MM, FM	MR	—	—	—
Intestine	MR, FR	MR	MR	MR, FR	—
Liver	MR, FR, MM, FM	—	FM	—	—
Oral Cavity	—	MR, FR	—	—	—
Circulatory System	—	—	—	—	—
Endocrine System					
Adrenal Gland	—	—	—	—	MR
Thyroid Gland	—	MR,FR	—	—	—
Hematopoietic System					
Spleen	—	—	—	—	—
Integumentary System					
Skin	—	MR	—	—	—
Mammary Gland	—	MR, FR	—	—	—
Mesothelium	—	MR	—	—	—
Nervous System					
Brain	—	—	—	—	MR, FR
Respiratory System					
Lung	MM, FM	MR	MR, FR, MM	—	MM
Nasal Cavity	—	—	—	—	—
Urinary System					
Kidney	MR, FR	MR	MR, FR, MM	—	—
Urinary Bladder	MR, FR	—	—	—	—
Other	—	MR, FR, MM, FM	—	—	FM

(continued)

TABLE 31
Summary of Selected Neoplasms in NTP Studies of Brominated Chemicals (continued)

	Chlorodibromomethane (gavage)	Decabromo-diphenyl Oxide (feed)	1,2-Dibromo-3-chloro-propane (gavage)	1,2-Dibromo-3-chloro-propane (inhalation)	1,2-Dibromoethane (gavage)
Alimentary System					
Forestomach	—	—	MR, FR, MM, FM	—	MR, FR, MM, FM
Intestine	—	—	—	—	—
Liver	MM, FM	MR, FR	—	—	FR
Oral Cavity	—	—	—	MR, FR	—
Circulatory System	—	—	—	—	MR
Endocrine System					
Adrenal Gland	—	—	—	—	—
Thyroid Gland	—	FM	—	—	—
Hematopoietic System					
Spleen	—	—	—	—	—
Integumentary System					
Skin	—	—	—	—	—
Mammary Gland	—	—	FR	—	—
Mesothelium	—	—	—	—	—
Nervous System					
Brain	—	—	—	—	—
Respiratory System					
Lung	—	—	—	MM, FM	MM, FM
Nasal Cavity	—	—	—	MR, FR, MM, FM	—
Urinary System					
Kidney	—	—	—	—	—
Urinary Bladder	—	—	—	—	—
Other	—	—	—	—	—

(continued)

TABLE 31
Summary of Selected Neoplasms in NTP Studies of Brominated Chemicals (continued)

	1,2-Dibromoethane (inhalation)	2,3-Dibromo-1-propanol (dermal)	Methyl Bromide[b] (inhalation)	Polybrominated Biphenyls (gavage/feed)	tris(2,3-Dibromopropyl) Phosphate (feed)
Alimentary System					
Forestomach	—	MR, FR, MM, FM	—	—	FR, MM, FM
Intestine	—	MR, FR	—	—	—
Liver	—	MR, FR, MM	—	MR, FR, MM, FM	FM
Oral Cavity	—	MR	—	—	—
Circulatory System	MR, FR, FM	—	—	—	—
Endocrine System					
Adrenal Gland	FR	—	—	—	—
Thyroid Gland	—	—	—	—	—
Hematopoietic System					
Spleen	MM	—	—	—	—
Integumentary System					
Skin	—	MR, MM, FM	—	—	—
Mammary Gland	FR, FM	FR	—	—	—
Mesothelium	MR	MR	—	—	—
Nervous System					
Brain	—	—	—	—	—
Respiratory System					
Lung	FR, MM, FM	MM	—	—	FR, MM, FM
Nasal Cavity	MR, FR, FM	MR, FR	—	—	—
Urinary System					
Kidney	—	MR, FR	—	—	MR, FR, MM
Urinary Bladder	—	—	—	—	—
Other	FM	MR, FR	—	—	—

[a] MR = male rats; FR = female rats; MM = male mice; FM = female mice
[b] Study conducted only in mice

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Appendix B: Keyes, D.G., R.J. Kociba, R.W. Schwetz, C.E. Wade, D.A. Dittenber, T. Quinn, S.J. Gorzinski, E.A. Hermann, J.J. Momany, and B.A. Schwetz. (1980). Results of a two-year toxicity and oncongenic study of rats ingesting diets containing dibromoneopentyl glycol (FR-1138®) J Comb Toxicol 7, pp B-1 – B-22.

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RESULTS OF A TWO-YEAR TOXICITY AND ONCONGENIC STUDY OF RATS INGESTING DIETS CONTAINING DIBROMONEOPENTYL GLYCOL (FR-1138)

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ABSTRACT: FR-1138 is being developed as a reactive flame retardant for unsaturated polyester resins and polyurethane foams. FR-1138 primarily consists of 80% dibromoneopentyl glycol, 8% tribromoneopentyl alcohol and 6% monobromoneopentyl triol. A lifetime toxicity dietary study of FR-1138 was conducted in rats to assess the potential for chronic toxicity and possible oncogenesis.

Rats ingesting the lower dietary level of 5 mg FR-1138/kg/day had no adverse effects related to the lifetime treatment. Rats ingesting a higher dietary level of 100 mg FR-1138/kg/day had some evidence of toxicity, including degenerative changes in the liver, eye and possibly thyroid gland; however, there was no oncogenic response, even when FR-1138 was administered at a sufficiently high dosage to induce some toxicity.

Analysis of selected tissues during the course of the study indicated a slight increase in bromide content in the tissues of rats ingesting the higher dose level of 100 mg FR-1138/kg/day. At the lower dose level of 5 mg FR-1138/kg/day, there was only a marginal increase in bromide content of some of the tissues, with most values in the same range as the controls.

INTRODUCTION

DIBROMONEOPENTYL GLYCOL (FR-1138) is being developed as a flame-retardant chemical. The primary market is in unsaturated polyester resins. It is also used as a reactive flame retardant for flexible and rigid polyurethane foams, and polymeric plasticizers. It differs from other flame retardants proposed for similar applications because it is bonded to the polyester polymers by ester linkage.

The acute oral LD_{50} in male rats was found to be 3458 mg/kg, with 95% confidence limits of 2810—4257 mg/kg (unpublished data, the Dow Chemical Com-

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pany, Midland, MI). Other acute toxicity studies showed that it was nonirritating to the eye and intact skin, not likely to be absorbed in toxic amounts, and slightly irritating to abraded skin.

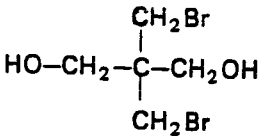
In a 30-day toxicity study conducted in this laboratory (unpublished data, The Dow Chemical Company, Midland, MI), male and female Sprague-Dawley Spartan substrain rats were given dose levels of 0, 10, 30, 100, or 300 mg/kg/day. The parameters evaluated included appearance, demeanor, body weight, food consumption, hematology, urinalysis, and clinical chemistry parameters including serum levels of urea nitrogen, alkaline phosphatase and glutamic pyruvic transaminase, organ weight and organ-to-body weight ratios, and gross and histopathologic examination. Effects attributed to treatment included a trend towards decreased body weight of male rats ingesting 300 mg/kg/day, and a statistical increase in liver weight of both males and females given the 300 mg/kg/day dose level. At 100 mg/kg/day, there was a trend towards increased liver weights. No untoward effects were observed at the lower dose levels of 30 and 10 mg FR-1138/kg/day.

Since the toxicologic effects associated with lifetime ingestion of FR-1138 have not been evaluated, the study reported herein was conducted to assess the toxicological and carcinogenic potential of FR-1138 when administered to rats in the diet throughout their lifetime. In addition, liver, kidney, fat and serum were saved for analysis for bromine content to determine if chronic ingestion of the test material is associated with an elevation of bromine levels in these tissues of the body.

EXPERIMENTAL

Test Material

The test material used in this study was supplied by the Halogens Research Laboratory, The Dow Chemical Company, Midland, MI. The analytical description of this lot of FR-1138 indicates that it contained approximately 6% monobromoneopentyl triol, 80% dibromoneopentyl glycol, 8% tribromoneopentyl alcohol and 3% other impurities. This analysis represents a recovery of approximately 97% of the original sample and was considered to be typical of normal production material. The chemical formula for dibromoneopentyl glycol (FR-1138) is as follows:



Experimental Design

Groups of male and female Sprague-Dawley (Spartan substrain) SPF-derived rats (Spartan Research Animals, Haslett, MI), 7–8 weeks of age were housed in suspended wire-bottom cages with 2 rats/cage, food (Purina Laboratory Chow, Ralston-

Purina Company, St. Louis, MO), and water were accessible at all times. The rats were randomized into test groups using a table of random numbers and were allowed to acclimate for a period of one week prior to placement on diets containing the test material. Individual rats were identified by metal ear tags and rats of each dose level were also identified by toe-clipping. Three groups consisting of 49–50 rats of each sex, plus 5/sex/group for the one year interim kill, and 10/sex/group for tissue analysis, were placed on diets supplying 0, 5 or 100 mg FR-1138/kg/day.

Diet Preparation and Analysis

The test diets were prepared by mixing FR-1138 with ground Purina Laboratory Chow to make a 5% premix. The concentration of the test material was adjusted on a weekly basis for the first 3 months, and quarterly thereafter to maintain the designated dose levels on a mg/kg of body weight/day basis according to the mean food consumption and body weight data. Control rats were supplied with untreated ground laboratory chow. Diet samples collected on 8 different dates during the study were submitted for neutron activation analysis for bromide.

Body Weight-Food Consumption

Body weights were recorded weekly for the first three months of the study on the first 20 rats/sex/dose level, and on all rats at monthly intervals. Food consumption was measured on 20 rats/sex/dose level during each week for the first three months and during one week of each month throughout the remainder of the study.

Clinical Observations

Rats were observed for general health status and possible toxicologic response during the study with observations recorded at least weekly for the first year of the study, and almost daily for the second year of the study.

Hematological determinations were conducted on blood samples collected from the tail veins from 10 rats/sex/group after 90–91 days and 356–357 days of treatment. Also, blood samples were collected from 6–10 rats/sex/group after 713–714 days, and additional bleedings of 10/group of the female rats after 725 and 731 days. Hematological parameters monitored included total erythrocyte count (RBC), total and differential leukocyte counts (WBC), packed cell volume (PCV), and hemoglobin (Hgb) concentration using automated (Coulter Counter Model ZBI and Hemoglobinometer, Coulter Electronics, Hialeah, FL), or manual procedures.

Urine samples were collected from identical numbers of rats as had been used for hematological determinations after 90–91, 356–357 and 713–714 days of treatment. Parameters measured (Ames Multilabstix, Ames Company, Elkhart, IN, and

TS Meter, AO Optical, Buffalo, NY) included urine specific gravity, pH, and the presence or absence of glucose, protein, ketones, bilirubin, occult blood and urobilinogen.

Clinical chemistry parameters were monitored from blood samples collected by orbital eye puncture after 94 days of treatment from 10 rats/sex/group. Blood samples from the rats killed after 1 year were collected by decapitation from 5 rats/sex/group. At the termination of the study after two years, blood samples were collected from a maximum of 10 rats/sex/group. Clinical chemistry parameters included blood urea nitrogen (BUN), serum glutamic pyruvic transaminase (SGPT), and serum alkaline phosphatase (AP). In addition, glucose values were measured on serum samples from rats which were part of the two-year terminal kill. Automated procedures (Technicon AutoAnalyzer, Technicon Corporation, Rye, NY (94 Days), Centrifichem System 400, Methods File, Union Carbide Corporation, Rye, NY (1-year and 2-year), were used for these determinations.

Bromide Analysis on Selected Tissues

Samples of adipose tissue, liver, kidney and serum were collected and the weights of liver and kidneys were determined from 3 or 4 rats/sex/group on days 10, 31 and 90 of the study. Tissues for analysis were also saved from 3 rats/sex/group at the 1-year interim kill and at termination of the study. Neutron activation analysis (Radio Chemical Research, The Dow Chemical Company, Midland, MI) was used to detect total bromide content of these tissue specimens.

Necropsy Examination

An interim necropsy examination was conducted after 1 year of treatment on 5 rats/sex/group that had been predesignated for this purpose. All rats were deprived of food overnight prior to killing by decapitation. The eyes of all rats were examined by gently pressing a glass slide against the cornea under bright fluorescent illumination. Any observations on the eyes were recorded as part of the gross necropsy observation records. The eyes from 5 rats/sex/group were preserved in Zenker's fixative. A complete gross pathologic examination was performed by a veterinary pathologist. Representative sections of all major organs and tissues were preserved in formalin fixative. These tissues included liver, kidneys, heart, pancreas, spleen, brain (cerebrum, cerebellum and brain stem), vertebrae with spinal cord, peripheral (sciatic) nerve, pituitary gland, stomach, small intestine, large intestine, cecum, mesenteric lymph node(s), skeletal (thigh) muscle, salivary gland, testes, epididymides, accessory male sex glands, urinary bladder, urerus, ovary trachea, esophagus, aorta, thoracic lymph node(s), thymus, lungs, integument, thyroid gland, parathyroid glands, adipose tissue, adrenal gland(s), sternum, tongue, mandible and any other grossly observed lesion. The weights of the liver, kidneys, brain, heart and testes (males) were recorded on all 5 rats/sex/group.

All rats which died or were culled during the course of the two-year study were

also subjected to a gross pathologic examination. Representative portions of all major organs and tissues as listed above, plus mandible, skull (including nasal turbinates, ear canal) along with any gross lesions suggestive of a significant pathologic process or tumor formation were collected from each rat and preserved in formalin fixative.

Terminal necropsy examination was conducted on all survivors at the end of 2 years of treatment, using procedures similar to those described for the 1-year interim necropsy to weigh and preserve in fixative all those organs and tissues listed above. Also, at the time of terminal necropsy, a femoral bone marrow smear was prepared from all female rats, and filed for future reference, if indicated.

Histologic Examination of Tissues

Histologic examination was conducted on paraffin embedded sections of tissues which were stained with hematoxylin and eosin (H&E). All rats from the control and treated groups killed after 1 year of treatment were subjected to histologic examination of an extensive list of tissues. All rats from all treatment and control groups of the 2-year study, regardless of whether they died, or were culled during the study, or were killed at the termination, were subjected to histologic examination of H&E stained sections of an extensive list of tissues from all organ systems of the body. Additional sections of livers from male and female rats from the control and treated groups of the terminal kill were stained with Oil Red O, for lipid content.

Statistical Evaluation of Data

Hematology, urinary and clinical chemistry parameters, body weights, organ weights, and organ/body weight ratio data were statistically analyzed by a one-way analysis of variance followed by Dunnett's Test, $p<0.05$ [1]. Food consumption data were analyzed using the sequential outlier's test [2], $P = 0.02$ (two-sided) to identify outlying data points. These data points were not included in the subsequent analysis of data using a one-way analysis of variance followed by Dunnett's Test, $p<0.05$. Data on mortality, palpable masses, gross pathology, histopathology and tumor incidences of the rats of the 2-year study were analyzed using Fisher's Exact Probability Test, $p<0.05$, one-sided test [3]. For gross pathology observations, statistical evaluation of the cumulative data for the entire study compared the data of each of the treatment groups against the data of the control group of that sex. The data were visually inspected, and those cases suggestive of a possible statistical difference from control were analyzed. For histopathology observations and tumor incidence, this statistical evaluation compared the cumulative data of each dose group against the data of the control group of that sex. The exact number of tissues examined was used as the total group size in each analysis performed. The data were visually inspected and those cases suggestive of a possible statistical difference from control were analyzed.

RESULTS AND DISCUSSION

Due to the voluminous nature of the data generated during this two-year toxicity study, most of the actual data can not be included in this publication; however, these are on file with the authors.

Dietary Content of FR-1138

Results of the analyses of diet samples collected on 8 different dates during the study, overall showed generally good agreement during the course of the study between the analytically-observed dietary content and the desired nominal content for each sex and dose level.

The results of 8 different analyses conducted on samples collected during months 5, 8, 13, 17, 18, 19, 20, and 22 indicated the following analytical content of diets (expressed as percentage of analytically observed/desired nominal content of test material):

Control	Group	None Detected
5 mg/kg/day	Female	95.5 ± 24.7%
5 mg/kg/day	Male	113.0 ± 46.0%
100 mg/kg/day	Female	97.8 ± 7.2%
100 mg/kg/day	Male	97.8 ± 4.4%

Body Weights

Although body weight data indicated a trend towards lower body weights of both groups of males given FR-1138 after 100 days on test, the only statistical decrease in body weight was noted on Day 272 in the group of males ingesting 100 mg of FR-1138/kg/day (Summary Table 1). In view of the relatively higher than normal male control body weights noted in the study when compared to historical control data from two previous 2-year dietary studies [4, 5], using rats of the same age and strain, this isolated statistical decrease was probably not related to treatment with 100 mg of FR-1138/kg/day. Female rats given 5 or 100 mg of FR-1138/kg/day showed no difference in body weights when compared to the controls.

Food Consumption

There were no consistent deviations in the food consumption of males or females at either of the 2 dose levels during the course of the 2-year study. The sporadic cases in which there was a statistical increase or decrease between the control and various treatment groups followed no consistent trend, and were considered of no toxicological significance.

Cumulative Mortality

Male rats given 5 or 100 mg/kg/day showed no differences in mortality when

Table 1. Summary of Major Observations in Male and Female Rats Maintained on Diets Containing FR-1138 (Dibromoneopentyl Glycol) For Up to Two Years. (Including selected data extracted from entire data base for the purpose of depicting effects considered related to treatment.)

Sex	Males			Females		
Dose (mg/kg/day)	0	5	100	0	5	100
Number of Rats in 2-Year Study	50	50	50	48	50	50
Number of Rats Killed After 1 Year	5	5	5	5	5	5
PARAMETER						
BODY WEIGHT (grams)	616±59	598±58	591[a]±53	340±33	337±23	337±25
FOOD CONSUMPTION	-	-	-	-	-	-
MORTALITY	-	-	-	-	-	-
PALPABLE MASSES	-	-	-	-	-	-
CLINICAL OBSERVATIONS	-	-	-	-	-	-
HEMATOLOGY (RBC, PCV, Hgb, WBC, differential WBC)	-	-	-	-	-	-
URINALYSIS (specific gravity, pH, protein, glucose, ketones, bilirubin, occult blood, urobilonogen)	-	-	-	-	-	-
CLINICAL CHEMISTRY (SGPT, BUN, AP, glucose)	-	-	-	-	-	-
ORGAN WEIGHTS						
Brain, heart, liver, testes	-	-	-	-	-	-
Kidneys (90-Day Kill Only) (gram/100 gram body weight)	0.65±0.03	0.67±0.02	0.70±0.03	0.64±0.04	0.68±0.02	0.71[a]±0.04
Liver (1-Year Kill Only) (gram/100 gram body weight)	2.74±0.41	3.11±0.25	3.30[a]±0.34	2.63±0.20	2.70±0.50	2.39±0.12
TISSUES FOR BROMINE ANALYSIS (elevated at all time periods measured) (ppm)						
Liver (1 year)	4.6±0.6	6.0±0.3	20[a]±3	4.7±0.6	4.6±0.3	10.1[a]±0.9
Kidney (1 year)	10±2	12±1	66[a]±34	10±1	12[a]±1	22[a]±1
Serum (1 year)	10±2	13±1	51[a]±5	10±0	10±2	27[a]±3
Fat (1 year)	2.7±0.3	2.7±0.5	8.3±0.7	4.2±1.5	3.2±0.4	6.6[a]±0.3

[a]Statistically different from control values(s) when analyzed using analysis of variance and Dunnett's Test, $p<0.05$ or Fisher's Exact Probability Test, $p<0.05$.

– Indicates parameter was considered unaffected at any level of treatment. For parameters with multiple observations, only selected representative values are listed in summary table.

Table 1. (Concluded).

GROSS AND HISTOPATHOLOGIC EXAMINATION (No. effected)						
1-YEAR KILL						
Liver - Increased hepatocellular centrilobular cytoplasmic homogeneity	0	0	5	0	0	0
2-YEAR STUDY						
Thyroid						
Thyroid retention cyst formation	1	0	7[a]	1	1	2
Liver						
Increased hepatocellular cytoplasmic homogeneity	0	0	0	1	0	14[a]
Several foci of hepatocellular alteration	12	8	18	7	7	13
Single area of hepatocellular alteration	5	4	5	5	9	13[a]
Eyes						
Bilateral diffuse opacity of lenses	0	0	2	0	0	6[a]
Bilateral lenticular degeneration, anterior cortex - moderate	0	0	0	0	0	6[a]
Bilateral lenticular degeneration, posterior cortex - moderate	0	0	0	0	1	3
Basophilic staining material, posterior cortex	0	0	0	0	0	5
Tumors (all organ systems)	-	-	-	-	-	-

[a]Statistically different from control value(s) when analyzed using analysis of variance and Dunnett's Test, $p<0.05$ or Fisher's Exact Probability Test, $p<0.05$.

— Indicates parameter was considered unaffected at any level of treatment. For parameters with multiple observations, only selected representative values are listed in summary table.

compared to that of the control group. Female rats receiving 100 mg/kg/day had statistically increased mortality rates for months 16 and 17. In view of the isolated occurrence, this observation was considered to be of questionable toxicological significance. Mortality data on female rats given 5 mg/kg/day showed no differences from control data.

Palpable Masses

There were no statistical differences between control and treated rats over the course of the two-year study and, thus, the incidence of palpable masses was considered to be unaffected by treatment. After approximately 1 year, the females given the higher dose level had a slight transient upward trend in the number of rats with palpable masses when compared to concurrent controls. However, this apparent transient upward trend was not considered related to treatment in view of the somewhat lower incidence noted in this concurrent control group when compared to historical control data.

Clinical Observations

There were no changes in appearance or demeanor of the rats during the course of the study that could be attributed to ingestion of FR-1138 in the diet.

Hematology

Repetitive hematological parameters monitored after approximately 90–91, 356–357, 713–714, 725, and 731 days showed no effects which were considered to be related to treatment. Isolated occurrences of statistical differences between the treated and control groups were encountered; however, due to either the lack of a dose response and/or comparison with historical control data, these were not considered to be of any toxicological significance.

Urinalysis

Routine examination of urinary parameters after approximately 90–91, 356–357 and 713–714 days revealed no observations for either males or females given 5 or 100 mg/kg/day which were considered to be the result of treatment.

Clinical Chemistry

Analyses of serum samples from rats killed after 94 days, 1 year, 2 years, measuring levels of BUN, SGPT, and AP showed no alterations considered to be the result of treatment. There was a statistical decrease in SGPT of male rats given 100 mg/kg/day. The slight decrease in SGPT was not considered to be of any toxicological significance. Glucose values of serum samples from rats terminated after two years showed no effects which could be considered to be the result of treatment.

female rats given 5 mg/kg/day. Microscopically, the group of female rats given 100 mg/kg/day showed six of eleven rats with bilateral lenticular degeneration of the anterior cortex graded as moderate in degree (Figure 2). In addition, some of the rats of this high dose level showed bilateral degeneration of the posterior cortex and basophilic staining material within this region of the lens (Figure 3). These changes in the posterior cortex of the lens were probably also a result of treatment with 100 mg/kg/day of FR-1138.

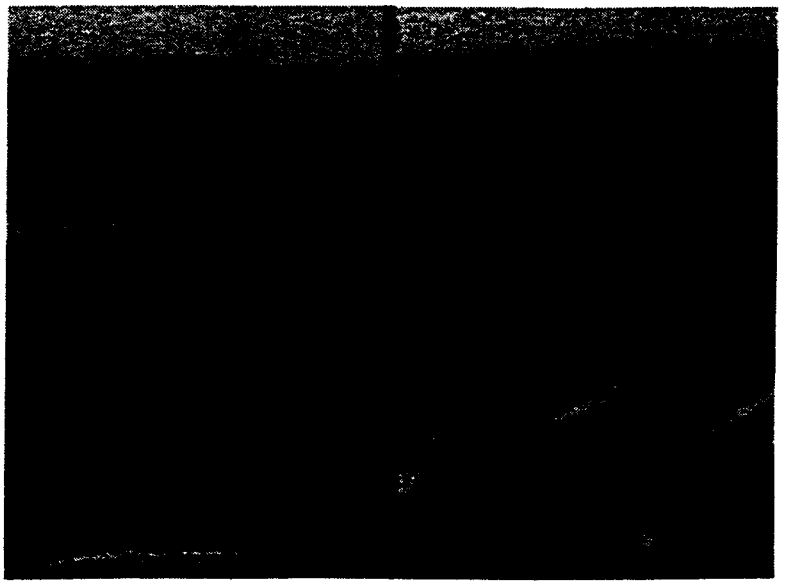


Figure 2. Note degeneration of anterior lenticular fibers of lens of female rat given 100 mg/kg/day of FR-1138 for 2 years (B) compared to control (A). Hematoxylin and eosin x 400.

These lenticular changes were similar in morphology and location to those experimentally induced by a number of agents, including galactose, xylose, lactose, corticosteroids or catecholamines [7]. The formation of cataracts, by high levels of the three sugars listed previously, had been proposed to be related to the formation and accumulation of sugar alcohols in the lens. In the lens, the enzyme aldose reductase converts sugar to the sugar alcohol form. The enzyme polyol dehydrogenase metabolizes some sugar alcohols, but not others including galactose. This explains the more severe cataractogenic activity of this sugar. When sugar alcohol concentrations increase within the lens, an osmotic change results with increased amounts of water being drawn into the lens to maintain an osmotic equilibrium. The increased fluid levels in the lens cause swelling and eventual rupture of the lens fibers leaving areas of degenerate protein. Once these degenerate fibers occur, they will persist and only progress if continued high sugar alcohol levels are maintained [7].

Organ Weights

Female rats given 100 mg/kg/day which were killed after 90 days showed a statistical increase in the relative kidney weight (Summary Table 1). This was not evident at any of the other time periods monitored. Due to its isolated occurrence, this alteration may or may not have been related to treatment. However, if it was related to treatment, it was only a transient phenomenon and was not seen after one or two years. Male rats given 100 mg/kg/day killed after 1 year of treatment showed a statistically significant increase in relative liver weights. This was considered to be the result of treatment. Male rats given 5 mg/kg/day which were killed after one year showed a statistical increase in the relative heart weight; this was not considered of any toxicological significance due to its isolated occurrence, lack of dose response, and absence at the termination of the 2-year study. There were no alterations or consistent trends in the weights of brain, or testes when compared to control values at any of the time periods.

Tissue Levels of Bromide

Results of tissue analysis for bromide content of male rats are summarized in Figure 1. Rats ingesting the high dose level of 100 mg/kg/day of FR-1138 had statistically increased level of bromide in liver, kidney, fat and serum. However these increased bromide levels, less than a 10-fold increase over the controls, were achieved relatively early and appeared to plateau during the remainder of the study, with the possible exception of the kidney in both sexes and fat in females, in which there was a slight upward trend which appeared to continue to some degree throughout the duration of the study. The concentration of bromide in the liver and fat never exceeded that in the serum any time during the study at either dose level of FR-1138 or in either sex. The concentration in the kidney exceeded that in the serum only in the rats killed at one and two years. The highest kidney to serum ratio, 2.3, was seen in the 100 mg/kg/day males at the termination of the study. In the group of rats ingesting 5 mg/kg/day of FR-1138, there was only a marginal increase in bromide content of some of the tissues measured, with most values in the same range as the controls. Overall, these data are interpreted to indicate that the compound FR-1138 does not have significant potential to bioaccumulate in mammalian tissues.

Gross and Histopathologic Observations

The voluminous data compiled during gross necropsy and histopathological examination of rats from the 1-year interim kill, spontaneous deaths and termination after 2 years are on file with the authors. Examples of the major effects considered to be related to treatment have been included in the attached Summary Table 1, and these pathology results are discussed below.

After 1 year of treatment, there were no gross observations which were consid-

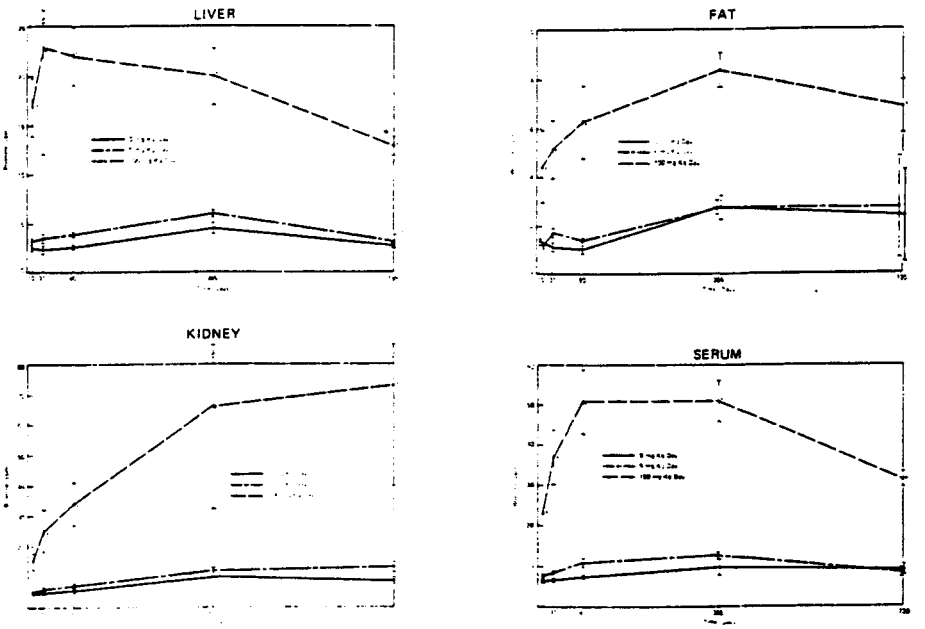


*Figure 1. Bromine levels (PPM) in male rats maintained on diets containing dibromoneopentyl glycol (FR-1138) for up to two years. (*Statistically different from control mean using analysis of variance and Dunnett's Test, $p<0.05$.*

ered to be related to treatment. Histopathologic examination indicated a slight liver alteration which was only seen in the group of male rats given 100 mg/kg/day. All of the rats within this group showed an increased centrilobular homogeneity of the hepatocellular cytoplasm. All other histopathologic observations were considered to be spontaneous in nature and unrelated to treatment.

The gross necropsy and histopathologic observations for rats that died, were culled or survived to the termination as part of the 2-year study indicated certain observations which were considered to be related to treatment. Histopathologic examination showed a statistical increase in the incidence of thyroid retention cyst formation in the group of male rats given 100 mg/kg/day. This observation may or may not have been the result of treatment, but there was no increase in follicular hypertrophy or hyperplasia. Male rats given 5 mg/kg/day showed no evidence of this alteration. A previously published subchronic study of sodium bromine attributed thyroid hyperplasia to treatment with relatively high levels of sodium bromide [6].

Gross necropsy and histopathologic examination of the lenses of eyes of female rats which were terminated after receiving 100 mg/kg/day for two years, indicated alterations which were considered to be the result of treatment. Gross necropsy examination revealed six of eleven female rats given 100 mg/kg/day with bilateral diffuse opacity of the lenses. This was not noted in the controls or the group of

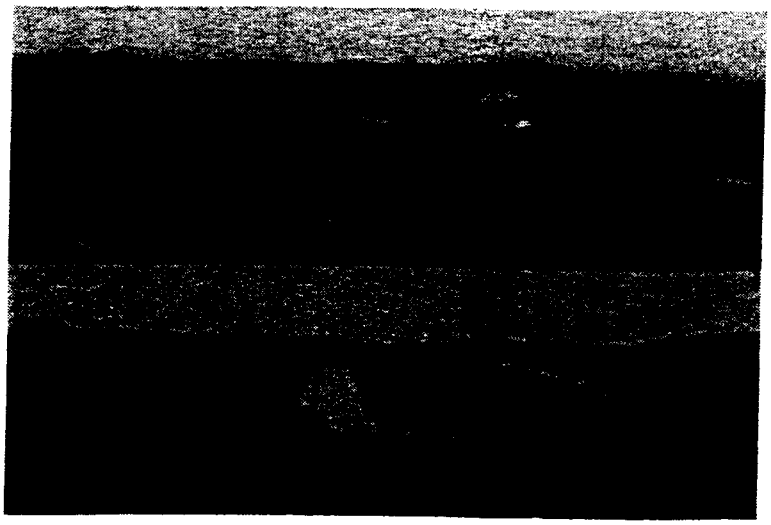


Figure 3. Note degeneration of posterior lenticular fibers and accumulation of basophilic staining material in lens of female rat given 100 mg/kg/day of FR-1138 for 2 years (B) compared to control (A). Hematoxylin and eosin x 400.

One possible explanation of the alterations noted in the lenses of female rats given 100 mg FR-1138/kg/day could be that metabolism of dibromoneopentyl glycol eliminated bromine and left a compound structurally similar to a sugar alcohol. This might explain the mechanism by which the lenticular degeneration occurred in this study.

Gross necropsy observations of female rats given 100 mg/kg/day showed a statistical increase in the number of rats bearing 3 subcutaneous masses in the mammary or midcervical region. This observation is not considered to be related to treatment due to the somewhat lower incidence of this observation in this control group when compared to historical control data [4, 5] and the absence of any increase in the number of rats of this group bearing 1, 2, 4, 5 or 6 subcutaneous masses in the mammary or midcervical region.

The livers of female rats given 100 mg/kg/day showed no gross evidence of toxicity; however, upon microscopic examination there were several observations noted primarily in the group of rats terminated after 2 years which were considered to be related to treatment. These included hepatocellular degenerative changes consisting of increased eosinophilic cytoplasmic homogeneity (Figure 4) which was accompanied by a slight increase in the incidence of livers having several foci or a single area of hepatocellular alteration. The trend towards a slight increased incidence of individual hepatocellular necrosis noted in this group may or may not have

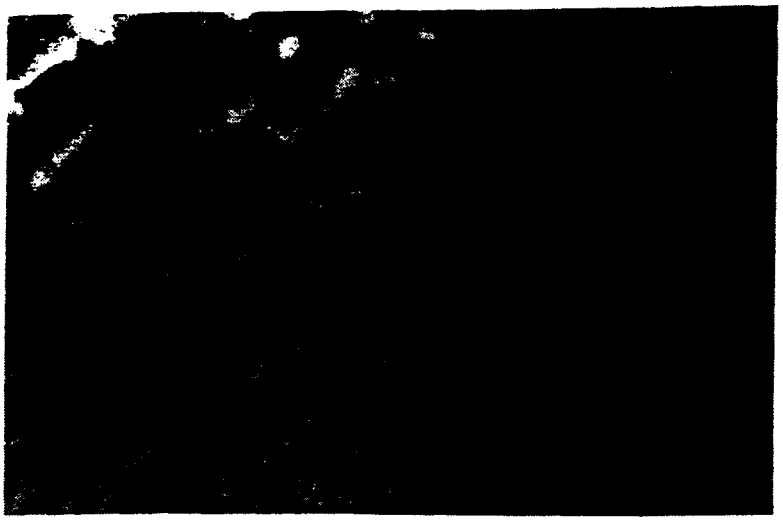


Figure 4. Note increased eosinophilic homogeneity of hepatocellular cytoplasm of female rat given 100 mg/kg/day of FR-1138 for 2 years (B) compared to control (A). Hematoxylin and eosin x 400.

been the result of treatment. Oil Red O stain for lipid revealed no discernible difference between control and treated groups.

Gross and histopathologic examination of tissues of the urinary tract, cardiovascular system, respiratory tract, reproductive system, endocrine organs (except thyroid), gastrointestinal tract, tongue, mesenteric tissue, pancreas, salivary glands, musculoskeletal system, lymphoreticular system, central nervous system, peripheral nerve, subcutaneous tissues, integument and mammary glands revealed various geriatric and inflammatory processes in all control and experimental groups which were of the type and severity historically encountered in rats of this strain and age, and were considered as spontaneous in origin and unrelated to ingestion of these dose levels of FR-1138.

Tumor Incidence

Tumor incidence rates for male and female rats are tabulated in Tables 2 and 3, respectively. These data are listed for each of the sequential 6-month periods as well as the terminal kill. The total results for each category of tumor type are also included. The spectrum of tumors that have been historically observed in rats of this strain were noted in the liver, nasal turbinates/hard palate, lungs, pancreas, kidney, urinary bladder, testes, ovary, uterus, musculoskeletal tissue, oral cavity, tongue, salivary glands, stomach, small intestine, large intestine, subcutaneous tis-

Table 2. Tumor Incidence in Male Rats Maintained on Diets Containing FR-1138 (Dibromoneopentyl Glycol) For Up to Two Years.

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	13	12	12	25	26	26	9	7	6	50	50	50
Tumor/Tumor-like Lesions												
Hepatocellular hyperplastic nodule(s)	0	0	0	0	0	0	1	0	0	1	0	0
Hepatocellular carcinoma(s)	0	0	0	1	1	0	0	0	0	1	1	0
Renal tubular adenocarcinoma	0	0	0	1	0	1	0	0	0	1	0	1
Osteoma of nasal turbinates	0	0	0	0	1	0	0	0	0	0	1	0
Interstitial cell tumor of testis	0	0	0	0	0	1	0	0	0	0	0	1
Interstitial cell tumor - malignant of testis	0	0	0	1	0	0	0	0	0	1	0	0
Total interstitial cell tumors of testis	0	0	0	1	0	1	0	0	0	1	0	1
Stratified squamous cell carcinoma of tongue	1	0	0	0	0	2	0	0	3	1	0	2
Squamous papilloma of tongue	0	0	0	0	1	0	0	0	0	0	1	0
Squamous polyp of stomach	1	0	0	0	0	1	0	0	0	1	0	1
Mucocystadenocarcinoma of stomach	0	0	0	0	1	0	0	0	0	0	1	0
Squamous cell carcinoma of stomach	0	0	0	0	0	1	0	0	0	0	0	1
Mucocystadenocarcinoma of small intestine	1	0	0	0	1	1	0	0	0	1	1	1
Lymphosarcoma of small intestine	0	0	0	0	1	0	0	0	0	0	1	0
Leiomyosarcoma of small intestine	0	0	0	0	1	0	0	0	0	0	1	0
Mucocystadenoma of small intestine	0	0	0	0	1	0	0	0	0	0	1	0
Lymphosarcoma of large intestine with metastasis	0	0	0	0	1	0	0	0	0	0	1	0
Intraabdominal malignant neoplasm with metastasis	0	0	0	0	1	0	0	0	0	0	1	0
Astrocytoma of brain	0	0	0	1	1	0	0	0	0	1	1	0
Granular cell myoblastoma of brain	0	0	0	0	0	1	0	0	0	0	0	1
Malignant tumor of pineal gland	0	0	0	0	0	1	0	0	0	0	0	1
Mixed glioma of spinal cord	0	0	0	1	0	0	0	1	0	1	0	0
Oligodendroglioma of spinal cord	0	0	0	0	0	1	0	0	0	0	0	1
Malignant schwannoma involving epididymis	0	0	1	0	0	0	0	0	0	0	0	1
Subauricular malignant schwannoma	0	0	0	0	0	1	0	0	0	0	0	1
Generalized lymphosarcoma with widespread metastasis	0	0	1	0	0	0	0	0	0	0	0	1
Myelomonocytic leukemia (generalized)	0	0	0	0	2	1	0	0	0	0	2	1
Hemangioma of mesenteric lymph node	0	0	0	1	0	0	0	0	0	1	0	0

During Months 1-6, there was only one tumor (subcutaneous carcinosarcoma) noted in the control group. Tumors occurring during Months 7-12 included one adrenal pheochromocytoma - unilateral (control), one subcutaneous fibrosarcoma with metastasis (control), one mixed glioma of the spinal cord (5 mg/kg/day), one adrenal pheochromocytoma - unilateral (5 mg/kg/day), one mammary gland fibroadenoma/adenofibroma (5 mg/kg/day), one pancreatic islet cell adenoma (100 mg/kg/day), and one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day). All of these tumors have been included in the above total tabulation.

There were no statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

Table 2. (Continued).

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	13	12	12	25	26	26	9	7	6	50	50	50
Tumor/Tumor-like Lesions (cont'd)												
Pituitary adenoma formation(s)	1	0	0	2	6	6	0	2	2	3	8	8
Pituitary adenocarcinoma without extension or invasion of brain	0	0	0	0	0	0	0	1	1	0	1	1
Pituitary adenocarcinoma with invasion of brain	0	1	0	0	0	0	0	0	0	0	1	0
Unclassified tumor of pituitary	1	0	0	0	0	0	0	0	0	1	0	0
Adrenal adenoma - unilateral	0	0	1	3	0	2	0	1	1	3	1	4
Adrenal adenoma - bilateral	0	0	1	0	0	0	0	0	0	0	0	1
Adrenal adenocarcinoma	1	0	0	0	0	1	0	0	0	1	0	1
Adrenal adenocarcinoma with metastasis	1	0	0	0	0	0	0	0	0	1	0	0
Adrenal pheochromocytoma - unilateral	0	1	1	8	5	7	1	1	0	10	8	8
Adrenal pheochromocytoma - bilateral	0	1	0	1	0	2	0	0	0	1	1	2
Adrenal pheochromocytoma - malignant, no metastasis - unilateral	0	0	0	1	1	0	1	0	2	2	1	2
Adrenal pheochromocytoma - malignant, metastasis - unilateral	0	0	0	0	0	0	1	0	0	1	0	0
Thyroid interfollicular C-cell adenoma(s)	0	0	1	1	1	2	2	2	1	3	3	4
Thyroid interfollicular C-cell adenocarcinoma without metastasis	0	0	0	3	1	1	2	0	0	5	1	1
Pancreatic acinar adenoma	2/1*	2/2	5/3	16/6	15/11	28/7	2/2	6/4	3/1	20/9	23/17	36/11
Pancreatic acinar adenocarcinoma	0	0	0	1/1	0	0	0	0	0	1/1	0	0
Pancreatic islet cell adenoma	0	0	0	4/3	4/4	4/4	1/1	1/1	0/0	5/4	5/5	5/5
Pancreatic islet cell adenocarcinoma	0	0	0	0	0	2/2	0	0	0	0	0	2/2
Subcutaneous fibroma	0	0	1/1	1/1	2/2	5/4	1/1	0/0	2/2	2/2	2/2	8/7
Subcutaneous fibrosarcoma with metastasis	0	0	0	0	0	0	0	0	0	1/1	0	0
Subcutaneous carcinosarcoma	0	0	0	0	0	0	0	0	0	1/1	0	0
Subcutaneous malignant fibrous histiocytoma with metastasis	0	0	0	0	0	1/1	0	0	0	0	0	1/1
Subcutaneous neurofibrosarcoma with metastasis	0	0	0	0	0	1/1	0	0	0	0	0	1/1

*In those cases where multiple tumors of the same classification occurred in individual rats, data entry listed as total number of this tumor/number of rats bearing this type tumor.

During Months 1-6, there was only one tumor (subcutaneous carcinosarcoma) noted in the control group. Tumors occurring during Months 7-12 included one adrenal pheochromocytoma - unilateral (control), one subcutaneous fibrosarcoma with metastasis (control), one mixed glioma of the spinal cord (5 mg/kg/day), one adrenal pheochromocytoma - unilateral (5 mg/kg/day), one mammary gland fibroadenoma/adenofibroma (5 mg/kg/day), one pancreatic islet cell adenoma (100 mg/kg/day), and one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day). All of these tumors have been included in the above total tabulation.

There were no statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

Table 2. (Concluded).

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	13	12	12	25	26	26	9	7	6	50	50	50
Tumor/Tumor-like Lesions (cont'd)												
Mammary gland fibroadenoma/adenofibroma	0	1/1	0	0	1/1	0	1/1	0	0	1/1	3/1	1/1
Mammary gland adenocarcinoma without metastasis	0	0	0	0	1/1	0	0	0	0	0	1/1	0
Squamous epithelial polyp	0	0	0	0	0	0	1/1	0	0	1/1	0	0
Squamous cell carcinoma	0	1/1	0	0	0	1/1	0	0	0	0	1/1	1/1
Squamous papilloma	1/1	1/1	0	0	0	0	0	0	2/2	1/1	1/1	2/2
Basal cell carcinoma	0	0	0	0	0	0	1/1	0	0	1/1	0	0
Keratoacanthoma	0	0	0	0	0	0	1/1	0	0	1/1	0	0
Squamous cell carcinoma of prepuce	0	0	0	0	0	1/1	0	0	0	0	0	1/1
Sebaceous cystadenoma	0	0	0	0	0	1/1	0	0	0	0	0	1/1
Basal/squamous cell carcinoma	0	0	0	0	0	0	0	0	1/1	0	0	1/1
Hemangiopericytoma	0	0	0	0	1/1	0	0	0	0	0	1/1	0
Zymbal gland carcinoma	0	0	1/1	1/1	1/1	1/1	0	0	0	1/1	1/1	2/2
Rhabdomyosarcoma with metastasis	0	1	0	0	0	0	0	0	0	0	1	0
Chondrosarcoma of mandible	0	0	0	1	0	0	0	0	0	1	0	0

During Months 1-6, there was only one tumor (subcutaneous carcinosarcoma) noted in the control group. Tumors occurring during Months 7-12 included one adrenal pheochromocytoma - unilateral (control), one subcutaneous fibrosarcoma with metastasis (control), one mixed glioma of the spinal cord (5 mg/kg/day), one adrenal pheochromocytoma - unilateral (5 mg/kg/day), one mammary gland fibroadenoma/adenofibroma (5 mg/kg/day), one pancreatic islet cell adenoma (100 mg/kg/day), and one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day). All of these tumors have been included in the above total tabulation.

There were o statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

Table 3. Tumor Incidence in Female Rats Maintained on Diets Containing FR-1138 (Dibromoneopentyl Glycol) For Up to Two Years.

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	6	8	8	28	23	27	12	19	11	48	50	50
Tumor/Tumor-like Lesions												
Hepatocellular hyperplastic nodule(s)	0	0	0	1	0	1	2	0	2	3	0	3
Hepatocellular carcinoma(s)	0	0	0	1	0	0	0	0	0	1	0	0
Squamous cell carcinoma of hard palate	0	0	0	0	0	1	0	0	0	0	0	1
Keratinizing squamous cell carcinoma of lung	0	0	0	0	0	1	0	0	0	0	0	1
Granulosal cell neoplasm of ovary - unilateral	0	0	0	0	0	2	1	0	0	1	0	2
Granulosal cell neoplasm of ovaries - bilateral	0	0	0	1	0	0	0	0	0	1	0	0
Adenomatous polyp of uterus	0	0	0	0	0	0	0	0	1/1	0	0	1/1
Stromal polyp of uterus	0	0	1/1*	3/3	2/2	5/5	2/2	9/5	4/2	5/5	11/7	10/8
Cystic polyp of uterus	0	0	0	1/1	0	0	0	0	0	1/1	0	0
Adenoma or papillary adenoma of uterus	0	0	0	1	0	0	0	1	0	1	1	0
Malignant schwannoma of vagina	0	0	0	0	2	0	0	0	0	0	2	0
Malignant schwannoma of uterus with metastasis	1	0	0	0	1	0	0	0	1	2	1	1
Myxosarcoma of uterus	0	0	0	1	0	0	0	0	0	1	0	0
Hemangioma of uterus	0	0	0	1	0	0	0	0	0	1	0	0
Sclerosing carcinoma of uterus with extension	0	0	0	0	0	1	0	0	0	0	0	1
Fibrosarcoma of vagina	0	0	0	0	0	0	0	0	1	0	0	1
Malignant schwannoma of vagina or proximal uterus with local invasion	0	0	1	0	0	0	0	0	0	0	0	1
Stratified squamous cell carcinoma of tongue	0	0	0	0	0	0	1	0	0	1	0	0
Squamous polyp of stomach	0	0	0	1	0	0	3	2	0	4	2	0
Squamous papilloma of stomach	0	0	0	0	1	0	0	0	0	0	1	0
Leiomyoma of small intestine	0	0	0	0	1	0	0	0	0	0	1	0
Leiomyosarcoma of small intestine	0	0	0	0	0	1	0	0	0	0	0	1
Adenocarcinoma of large intestine	0	0	0	1	0	0	0	0	0	1	0	0
Intrasbdominal carcinosarcoma	0	0	0	0	1	0	0	0	0	0	1	0
Glioma of spinal cord	0	0	0	1	0	0	0	0	0	1	0	0

*In those cases where multiple tumors of the same classification occurred in individual rats, data entry listed as total number of this type tumor/number of rats bearing this type tumor.

Only one tumor occurred during Months 1-6, a mammary gland adenocarcinoma without metastasis (100 mg/kg/day). Tumors occurring during Months 7-12 included one malignant schwannoma of the uterus (control), one mammary gland fibroadenoma/adenofibroma (control), one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day), and one generalized myeloid leukemia (control). These tumors are included in the above total tabulation.

There were no statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

Table 3. (Continued).

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	6	8	8	28	23	27	12	19	11	48	50	50
Tumor/Tumor-like Lesions (cont'd)												
Benign schwannoma of abdominal peripheral nerve	0	0	0	0	0	0	0	0	1	0	0	1
Neurofibrosarcoma of cranial cavity	0	0	0	0	1	0	0	0	0	0	1	0
Generalized lymphosarcoma	0	0	0	0	0	0	1	0	1	1	0	1
Generalized myelomonocytic leukemia	1	0	0	1	0	0	0	0	0	2	0	0
Thymic lymphosarcoma	0	0	0	0	0	1	0	0	0	0	0	1
Thymic myxosarcoma	0	0	0	0	0	0	0	0	1	0	0	1
Pituitary adenoma formation(s)	1	0	5	13	13	8	6	11	3	20	24	16
Pituitary adenocarcinoma without extension or invasion of brain	1	2	0	4	3	4	1	3	3	6	8	7
Pituitary adenocarcinoma with invasion of brain	0	1	0	3	1	2	0	0	0	3	2	2
Unclassified tumor of pituitary	0	0	0	1	0	0	0	0	0	1	0	0
Adrenal adenoma - unilateral	0	0	0	2	1	0	1	1	1	3	2	1
Adrenal adenocarcinoma	0	0	0	0	0	0	0	0	1	0	0	1
Adrenal pheochromocytoma - unilateral	0	0	0	1	1	1	0	2	1	1	3	2
Adrenal pheochromocytoma - bilateral	0	0	0	0	0	1	1	1	1	1	1	2
Adrenal pheochromocytoma, malignant, metastasis - unilateral	0	0	0	0	0	0	0	1	0	0	1	0
Thyroid interfollicular C-cell adenoma(s)	0	0	0	3	0	4	1	4	2	4	4	6
Thyroid interfollicular C-cell adenocarcinoma without metastasis	0	1	0	1	1	0	0	1	1	1	3	1
Thyroid interfollicular C-cell adenocarcinoma with metastasis	0	0	0	0	0	0	0	1	0	0	1	0
Parathyroid adenoma	0	0	0	0	1	0	0	0	0	0	1	0
Pancreatic acinar adenoma(s)	1/1	0	0	0	0	0	0	0	0	1/1	0	0
Pancreatic islet cell adenoma(s)	0	0	0	1/1*	0	3/3	4/4	1/1	2/2	5/5	1/1	5/5
Subcutaneous neurofibrosarcoma with metastasis	0	1/1	0	0	0	0	0	0	0	0	1/1	0

*In those cases where multiple tumors of the same classification occurred in individual rats, data entry listed as total number of this type tumor/number of rats bearing this type tumor.

Only one tumor occurred during Months 1-6, a mammary gland adenocarcinoma without metastasis (100 mg/kg/day). Tumors occurring during Months 7-12 included one malignant schwannoma of the uterus (control), one mammary gland fibroadenoma/adenofibroma (control), one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day), and one generalized myeloid leukemia (control). These tumors are included in the above total tabulation.

There were no statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

Table 3. (Concluded).

Time Interval	Months 13-18			Months 19-24			Terminal Kill			Total		
Dose Level (mg/kg/day)	0	5	100	0	5	100	0	5	100	0	5	100
Number of Rats Examined	6	8	8	28	23	27	12	19	11	48	50	50
Tumor/Tumor-like Lesions (cont'd)												
Subcutaneous undifferentiated sarcoma with metastasis	0	0	0	1/1	0	0	0	0	0	1/1	0	0
Mammary gland fibroadenoma/adenofibroma	3/3*	2/2	8/5	43/21	36/17	63/26	17/9	23/14	22/8	64/34	61/33	94/40
Mammary gland adenoma	0	1/1	1/1	1/1	1/1	2/2	3/2	4/2	4/2	4/3	6/4	7/5
Mammary gland adenocarcinoma without metastasis	1/1	0	1/1	5/5	2/2	0	0	2/2	0	6/6	4/4	2/2
Mammary gland adenocarcinoma with metastasis	0	0	0	0	1/1	0	0	0	0	0	1/1	0
Mammary gland cystadenoma	1/1	0	0	3/1	1/1	0	0	3/2	2/2	4/2	4/3	2/2
Mammary gland fibroma	0	1/1	0	1/1	0	3/3	0	1/1	0	1/1	2/2	3/3
Mammary gland cystfibroadenoma/cystadenofibroma	0	0	0	3/2	3/3	10/8	4/3	7/5	1/1	7/5	10/8	11/9
Trichoepithelioma	0	0	0	0	1/1	0	0	0	0	0	1/1	0
Fibroadenoma	0	0	0	0	0	1/1	0	0	0	0	0	1/1
Zymbal gland carcinoma	0	0	0	0	1/1	0	0	0	0	0	1/1	0
Generalized myeloid leukemia	0	0	0	0	0	0	0	0	0	1	0	0

*In those cases where multiple tumors of the same classification occurred in individual rats, data entry listed as total number of this type tumor/number of rats bearing this type tumor.

Only one tumor occurred during Months 1-6, a mammary gland adenocarcinoma without metastasis (100 mg/kg/day). Tumors occurring during Months 7-12 included one malignant schwannoma of the uterus (control), one mammary gland fibroadenoma/adenofibroma (control), one mammary gland fibroadenoma/adenofibroma (100 mg/kg/day), and one generalized myeloid leukemia (control). These tumors are included in the above total tabulation.

There were no statistical differences from control values when analyzed using Fisher's Exact Probability Test, $p<0.05$.

sues, integument, mammary gland, ear canal, brain, peripheral nerves, pituitary gland, cranial cavity, adrenal glands, eye, lymph nodes, thymus, spleen, mesentery, thyroid and parathyroid glands. Upon statistical anslyses of these tumor data, the incidence rates for all categories of tumor types occurring in any of the groups given 5 or 100 mg FR-1138/kg/day were comparable to the control group.

Thus, none of the tumors listed above were considered related to treatment with either of those dose levels of FR-1138. Table 4 summarizes the total numbers of tumors per group, the average number of tumors per rat, and the time of necropsy of rats with tumors for each group. None of these parameters were considered to be affected by any of these dose levels of FR-1138 when compared to the control data and historical control data [5].

Table 4. Tumor Incidence Data in Male and Female Rats Maintained on Diets Containing FR-1138 (Dibromoneopentyl Glycol) For Up to Two Years.

Sex	Historical	Males			Historical	Females		
Dose in mg/kg/day	Control	0	5	100	Control	0	5	100
Number of Rats in Group	Data	50	50	50	Data	48	50	50
TOTAL NUMBER OF TUMORS IN GROUP		83	79	112		162	163	193
AVERAGE NUMBER OF TUMORS PER RAT	2.0	1.7	1.6	2.2	3.6	3.4	3.3	3.9
NUMBER OF TUMOR BEARING RATS/NUMBER OF RATS IN GROUP								
(These data listed in time intervals in months):								
1-6		1/1	0/0	0/1		0/0	0/0	1/2
7-12		1/2	3/5	2/5		2/2	0/0	1/2
13-18		6/13	5/12	9/12		5/6	7/8	8/8
19-24		24/25	25/26	22/26		28/28	23/23	27/27
Terminal Kill		7/9	7/7	6/6		12/12	19/19	11/11
Total[a]		39/50	40/50	39/50		47/48	49/50	48/50
Percentage	88%	78%	80%	78%	97%	98%	98%	96%

[a]No statistical differences from control data when analyzed using Fisher's Exact Probability Test, $p<0.05$.

KEY WORD INDEX

Carcinogenicity
Chronic Toxicity
Dibromoneopentyl Glycol
Eye (lens) Toxicity
Flame Retardant
FR-1138
Liver Toxicity
Monobromoneopentyl Triol
Oncogenicity
Thyroid Gland Toxicity
Tribromoneopentyl Alcohol

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