FINAL

Report on Carcinogens Background Document for

2,3-Dibromo-1-propanol

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

Prepared for the:
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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

2,3-Dibromo-1-propanol

CASRN 96-13-9

Carcinogenicity

2,3-Dibromo-1-propanol is *reasonably anticipated to be a human carcinogen* based on sufficient evidence of carcinogenicity in rats and mice (NTP 1993).

2,3-Dibromo-1-propanol administered to rats by skin painting for up to 55 weeks induced increased incidences of tumors of the skin, nasal mucosa, digestive tract, Zymbal gland, liver, kidney, tunica vaginalis, and spleen. Mice similarly exposed for up to 42 weeks exhibited increased incidences of tumors of skin, forestomach, liver, and lung.

No case reports or epidemiological studies of the occurrence of human cancer and exposure to 2,3-dibromo-1-propanol were found.

Other Information Relating to Carcinogenesis or Possible Mechanisms of Carcinogenesis

2,3-Dibromo-1-propanol demonstrated genotoxicity in *in vitro* and *in vivo* systems including *Salmonella typhimurium*, *Escherichia coli*, V79 hamster cell, and mouse lymphoma cell mutation assays. It also induced sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster*. Chromosomal aberrations were induced in Chinese hamster ovary cells *in vitro*, but micronuclei were not induced in the bone marrow of mice administered 2,3-dibromo-1-propanol by injection.

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

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1 Introduction

2,3-Dibromo-1-propanol (DBP) was nominated for listing in the Report on Carcinogens (RoC) by the National Institute of Environmental Health Sciences (NIEHS) RoC Review Group (RG1) based on the results of a skin painting study reported in a 1993 National Toxicology Program (NTP) bioassay technical report that indicated clear evidence of carcinogenicity in rats and mice (NTP 1993).

1.1 Chemical identification

DBP ($C_3H_6Br_2O$, mol wt 217.89, CASRN 96-13-9) also is known by the following names:

glycerol 1,2-dibromohydrin
beta-dibromohydrin
dibromopropanol
allyl alcohol dibromide.

DBP is a halogenated aliphatic alcohol used as a chemical intermediate in the synthesis of flame retardants, insecticides, and pharmaceuticals. It is a clear, colorless, viscous liquid. The structure of DBP is illustrated in Figure 1-1.

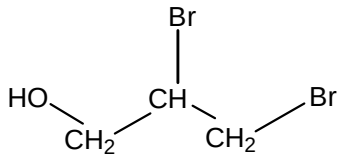

Figure 1-1. Structure of DBP

1.2 Physical-chemical properties

DBP is incompatible with strong oxidizers (Radian 1991). Its RTECS number is UB0175000. The physical and chemical properties of DBP are summarized in Table 1-1.

Table 1-1. Physical and chemical properties of DBP

Property	Information	Reference
Molecular weight	217.89	CRC (1998)
Color	colorless	CRC (1998)
Physical state	viscous liquid	CRC (1998)
Boiling point (°C)	219	CRC (1998
Flash point (°C)	112	Lenga (1985)
Specific gravity	2.12	Chemfinder (1999); Radian (1991)
Density (g/mL)	2.12	Lenga (1985); Aldrich (1988)

Property	Information	Reference
Solubility at 20°C in:		
Water	50 - 100 mg/mL	Radian (1991)
Dimethylsulfoxide	≥ 100 mg/mL	Radian (1991)
95% Ethanol	≥ 100 mg/mL	Radian (1991)
Acetone	≥ 100 mg/mL	Radian (1991)

1.3 Identification of metabolites

DBP is a metabolite of the flame retardant tris(2,3-dibromopropyl)phosphate (TRIS-BP) ($C_9H_{15}Br_6O_4P$, mol wt 697.61, CASRN 126-72-7). TRIS-BP is a viscous, pale yellow liquid that is slightly soluble in water and is hydrolyzed by acids and bases. The RTECS number for TRIS-BP is UB0350000 and the U.S. Environmental Protection Agency (EPA) hazardous waste number is U235 (HSDB 1998). The structure of TRIS-BP is illustrated in Figure 1-2.

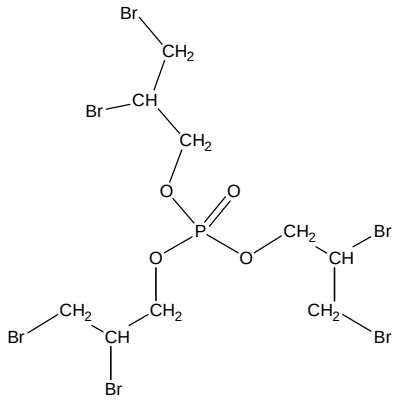

Figure 1-2. Structure of TRIS-BP

DBP undergoes oxidation, followed by dehydrohalogenation, to yield 2-bromoacrolein, an unstable intermediate leading to the formation of bromoacrylic acid (Marsden and Casida 1982, cited in NTP 1993). The structure of 2-bromoacrolein (C_3H_3BrO, mol wt 134.96) is shown in Figure 1-3 and the structure of bromoacrylic acid ($C_3H_3BrO_2$, mol wt 150.95) is shown in Figure 1-4.

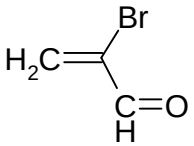

Figure 1-3. Structure of 2-bromoacrolein

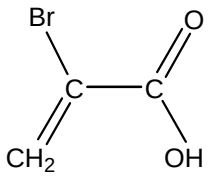

Figure 1-4. Structure of bromoacrylic acid

DBP also may be oxidized to 3-bromo-1,2-propane epoxide (Jones and Fakhouri 1979, cited in NTP 1993). The structure of this epoxybromopropane (C_3H_5BrO mol wt 136.97) is shown in Figure 1-5.

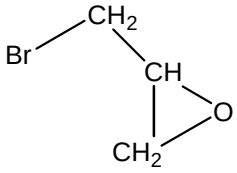

Figure 1-5. Structure of 3-bromo-1,2-propane epoxide

2 Human Exposure

2.1 Use

The major use of DBP is as an intermediate in the production of flame retardants, insecticides, and pharmaceuticals. DBP was previously used in the production of TRIS-BP, a flame retardant used in children's clothing and other products (NTP 1998). All domestic production of TRIS-BP was banned in 1978 (Radian 1991 and HSDB 1998).

2.2 Production

Only one U.S. producer of DBP, Great Lakes Chemical Corporation was identified, but production levels were not given (SRI 1989, cited by HSDB 1998). U.S. production of DBP was greater than 10 million lb in 1976 (Fishbein 1979). Sleepwear containing DBP and TRIS-BP was banned in 1977, after studies showed that DBP was mutagenic in bacteria and TRIS-BP was mutagenic and carcinogenic in rats. Production of DBP decreased drastically after the ban. Current production values have not been reported (NTP 1993). U.S. EPA, in 1994, reported the U.S. production of DBP to be < 1 million lb, and it was not listed in the high production volume (HPV) chemical list (U.S. EPA 1994).

2.3 Analysis

DBP is detected in urine via a negative ion mass spectrometry method (Blum *et al.* 1978). In air, it is detectable with a gas-liquid chromatographic analytical procedure (Choudhary 1987).

2.4 Environmental occurrence

DBP is not found naturally in the atmosphere, and most environmental exposures to DBP occur through industrial release. Based on a model of gas/particle partitioning of semivolatile organic compounds in the atmosphere, DBP (with a vapor pressure of 0.09 mm Hg at 25°C) is expected to remain almost entirely in the vapor phase when released into the ambient atmosphere (HSDB 1998). The Toxic Release Inventory (TRI 1996) did not identify DBP as being released by industry into the environment.

2.5 Environmental fate

2.5.1 Air

DBP released into the atmosphere is in vapor form. DBP has an estimated half-life of eight days in the vapor phase of the atmosphere, as a result of reaction with photochemically produced hydroxyl radicals. The estimated eight-day half-life is based on the assumption that the atmospheric concentration of hydroxyl radicals is $5x10^5$ (HSDB 1998).

2.5.2 Water

DBP is not expected to adsorb to suspended solids and sediments in water, and volatilization is not expected to be an important process of elimination from water. The estimated bioconcentration factor (BCF) for DBP is 3; it s potential for bioconcentration is extremely low, because a BCF greater than 1,000 is required for significant

bioaccumulation in aquatic organisms. The volatilization half-life of DBP in a model river (1 m deep, flowing 1 m/sec, and wind velocity of 3 m/sec) is estimated at 107 days. The volatilization half-life of DBP in a model lake (1 m deep, flowing 0.05 m/sec, and wind velocity of 0.5 m/sec) is estimated at 780 days (HSDB 1998).

2.5.3 Soil

Based on a classification scheme by Swann *et al.* (1983, cited in HSDB 1998) and an estimated K_{oc} value of 4, determined via a structure estimation method, DBP is expected to have very high mobility in soil. Volatilization is not expected to be an important process of elimination for DBP in wet or dry soils. In addition, limited data show that DBP may biodegrade under aerobic conditions (HSDB 1998).

2.6 Environmental exposure

The primary modes of potential human exposure to DBP are inhalation and dermal contact. Over 50 million children wearing TRIS-BP-treated sleepwear may have been exposed to DBP (as a metabolite of TRIS-BP) before the 1977 Consumer Product Safety Commission banned the use of TRIS-BP in children's clothing (Blum *et al.* 1978). No consumer exposure or manufacture information was found for DBP.

2.7 Occupational exposure

Occupational exposure to DBP may occur through inhalation and dermal contact in those industries where DBP is used as an intermediate for the production of flame-retardant materials, pharmaceuticals, insecticides, and TRIS-BP. Information on estimated occupational exposures to DBP is not available (HSDB 1998).

2.8 Biological indices of exposure

DBP was found in urine samples from 10 children who were wearing TRIS-BP-treated sleepwear. By mass spectrometry, urine levels were found to range from 0.4 to 29 ng/mL. Low values come from children who were wearing previously washed pajamas, and higher values came from children wearing new, unwashed pajamas. Washing, however, was not considered to diminish the risk of DBP exposure, because guidelines at the time required sleepwear to be flame-resistant for more than 50 washes (Blum *et al.* 1978).

2.9 Regulations

U.S. EPA regulates DBP under the Toxic Substances Control Act (TSCA), requiring manufacturers and processors to report production, use, and any exposure-related information for chemicals with toxic or dangerous characteristics. These regulations are summarized in Table 2-1. No FDA or OSHA regulations were found for DBP and TRIS-BP.

Table 2-1. U.S. EPA regulations

U.S. EPA Regulations	
Regulatory action	Effect of regulation and other comments
40 CFR 712 – PART 712–CHEMICAL INFORMATION RULES. Promulgated: 47 FR 26998, 06/22/82. U.S. Codes: 15 U.S.C. 2607(a). 2,3-Dibromo-1-propanol has an effective date of 10/29/90 and a sunset date of 12/27/90.	This part establishes procedures for chemical manufacturers and processors to report production, use, and exposure-related information on listed chemical substances. Subpart A establishes requirements that apply to all reporting under this part. Subpart B covers manufacturers' and processors' reporting.
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). 2,3-Dibromo-1-propanol has an effective date of 10/29/90 and a sunset date of 12/27/95.	The provisions of this part require 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 chemicals for which U.S. EPA requires health and safety information in fulfilling the purposes of TSCA.

Source: These regulations have been updated through the 1998 Code of Federal Regulations 40 CFR, July 1, 1998.

3 Human Cancer Studies

No case reports or epidemiological studies of the occurrence of human cancer and exposure to 2,3-dibromo-1-propanol were found.

4 Studies of Cancer in Experimental Animals

4.1 Carcinogenesis studies of DBP

4.1.1 Carcinogenicity study in rats

The carcinogenic potential of dermally applied DBP (98% pure) was assessed in male and female F344/N rats (NTP 1993; Eustis *et al.* 1995). Dose levels for the long-term study were selected on the basis of the results of a 13-week range-finding study during which groups of 10 animals of each sex received five applications per week of 0, 44, 88, 177, 375, or 750-mg/kg of DBP dissolved in 95% ethanol. Doses were applied to the shaved interscapular skin five days/week for 13 weeks. After 13 weeks, male rats in the 375-, and 750-mg/kg groups exhibited nephropathy, and female rats in the 750-mg/kg group exhibited hepatocellular necrosis.

In the long-term carcinogenicity study, groups of rats (50/sex) received daily dermal applications of 0, 188, or 375 mg/kg of DBP five days per week. The study was originally planned to last for 104 weeks. The study was terminated, however, at 48 to 51 weeks for males and 52 to 55 weeks for females because of reduced survival in high-dose groups. Early deaths and sacrifices of moribund animals were attributable to treatment-associated neoplasms. Dermal application of DBP caused a variety of dermal and systemic neoplasms in both sexes. Tumor incidences are summarized in Tables 4-1, 4-2, 4-3, and 4-4 (NTP 1993).

Table 4-1. Incidences of skin neoplasms in F344/N rats dosed with DBP for 48 to 55 weeks

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Males			
Squamous cell papilloma	1/50	3/50	0/50
Squamous cell carcinoma	0/50	5/50*	8/50**
Basal cell tumor	0/50	13/50**	21/50**
Sebaceous adenoma	0/50	5/50*	5/50*
Keratoacanthoma	0/50	4/50	12/50**
Neoplasms, any type	1/50	22/50**	33/50**
Females			
Squamous cell papilloma	0/50	0/50	2/50
Squamous cell carcinoma	0/50	0/50	1/50
Basal cell tumor	0/50	3/50	12/50**
Sebaceous adenoma	0/50	0/50	2/50
Keratoacanthoma	0/50	0/50	5/50*
Neoplasms, any type	0/50	3/50	18/50**

Source: NTP (1993).

[a]Statistically significant by Fisher exact test: *$P \leq 0.05$; **$P \leq 0.01$

Table 4-2. Incidences of digestive tract neoplasms in F344/N rats dosed with DBP for 48 to 55 weeks

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Males			
Oral mucosa			
Squamous cell papilloma	0/50	40/50*	33/50*
Squamous cell carcinoma	0/50	16/50*	25/50*
Esophagus			
Squamous cell papilloma	0/50	19/50*	33/50*
Squamous cell carcinoma	0/50	1/50	0/50
Forestomach			
Squamous cell papilloma	0/50	1/50	17/50*
Small intestine			
Adenomatous polyp	0/50	1/50	3/50
Adenocarcinoma	0/50	8/50*	11/50*
Large intestine			
Adenomatous polyp	1/50	13/50*	29/50*
Adenocarcinoma	1/50	1/50	2/50
Females			
Oral mucosa			
Squamous cell papilloma	0/50	27/50*	41/50*
Squamous cell carcinoma	0/50	15/50*	27/50*
Esophagus			
Squamous cell papilloma	0/50	9/50*	38/50*
Squamous cell carcinoma	0/50	0/50	1/50
Forestomach			
Squamous cell papilloma	1/50	3/50	23/50*
Small intestine			
Adenomatous polyp	0/50	1/50	0/49
Adenocarcinoma	0/50	3/50	4/49
Large intestine			
Adenomatous polyp	0/50	12/50*	37/50*
Adenocarcinoma	0/50	0/50	0/50

Source: NTP (1993).

[a]Statistically significant by Fisher exact test: *$P \leq 0.001$.

Table 4-3. Incidences of neoplastic and nonneoplastic lesions of the nasal mucosa, Zymbal gland, liver, and kidney in F344/N rats dosed for 48 to 55 weeks with DBP

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Males			
Nasal mucosa			
Adenoma	0/50	48/50**	48/50**
Adenocarcinoma	0/50	2/50	1/50
Zymbal gland			
Adenoma	0/50	1/50	7/50*
Adenocarcinoma	0/50	8/50*	29/50**
Liver			
Neoplastic nodule	0/49	3/50	2/50
Carcinoma	0/49	1/50	3/50
Kidney			
Adenoma	0/50	0/50	4/50
Females			
Nasal mucosa			
Adenoma	1/50	44/50**	49/50**
Zymbal gland			
Adenoma	0/50	7/50*	3/50
Adenocarcinoma	1/50	2/50	19/50**
Liver			
Neoplastic nodule	0/50	10/50**	11/50**
Carcinoma	0/50	2/50	6/50*
Kidney			
Adenoma	0/50	1/50	4/50

Source: NTP (1993).

[a]Statistically significant by Fisher exact test: *$P \leq 0.05$; **$P \leq 0.001$.

Table 4-4. Incidences of other neoplasms in Fischer 344/N rats dosed with DBP for 48 to 55 weeks

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Males			
Peritoneum (tunica vaginalis)			
Mesothelioma	0/50	1/50	4/50
Spleen			
Hemangioma	0/50	0/50	3/50
Hemangiosarcoma	0/50	0/50	1/50
Females			
Clitoral gland			
Adenoma	0/50	1/50	3/50
Adenocarcinoma	0/50	0/50	3/50
Mammary gland			
Adenocarcinoma	0/50	0/50	5/50*

Source: NTP (1993).
[a]Statistically significant by Fisher exact test: *$P \leq 0.05$.

4.1.2 Carcinogenicity study in mice

The carcinogenic potential of dermally applied DBP (98% pure) was assessed in male and female $B6C3F_1$ mice (NTP 1993; Eustis *et al.* 1995). Dose levels for the long-term carcinogenicity study were selected on the basis of the results of a 13-week range-finding study during which groups of 10 animals of each sex received five applications per week of 0, 44, 88, 177, 375, or 750 mg/kg of DBP dissolved in 95% ethanol. Doses were applied to the shaved interscapular skin five days/week for 13 weeks. Dosed mice had treatment-associated bronchiole pleomorphism after administration of DBP at 88, 177, 375 or 750 mg/kg to males or 375 or 750 mg/kg to females. The incidence of centrilobular hepatocellular necrosis also was increased among males in the 750-mg/kg group, and among female in 177-, 375-, or 750-mg/kg groups.

During the long-term carcinogenicity study, groups of 50 male and 50 female mice received daily dermal applications of 0, 88, or 177 mg/kg of DBP five days per week. The planned two-year study was terminated at 36 to 39 weeks for males and 39 to 42 weeks for females because antibodies to lymphocytic choriomeningitis (LCM) virus were detected in sentinel animals housed in the same room. Although LCM depresses humoral and cellular immunity, it is unlikely that the LCM infection affected the outcome of this long-term study as it relates to the carcinogenic potential of DBP. The infection occurred in only 13% of both control and treated mice and the incidence of neoplasms in the control mice was comparable to historical controls. Thus, carcinogenic response was comparable between LCM-infected and LCM-uninfected mice of the same dose group and the induced neoplasms in exposed mice occurred with very high incidences and short

latency (NTP 1993). Despite the study's abbreviated duration, it provides unequivocal evidence of carcinogenicity of DBP in $B6C3F_1$ mice. Dermal application of DBP caused a variety of dermal and forestomach neoplasms in both sexes, pulmonary tumors in both sexes, and liver tumors in males. The incidences of tumors in male and female mice are summarized in Table 4-5.

Table 4-5. Incidences of neoplasms in $B6C3F_1$ mice dosed with DBP for 36 to 42 weeks

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Males			
Skin			
Squamous cell papilloma	0/50	3/50	9/50**
Squamous cell carcinoma	0/50	0/50	2/50
Sebaceous gland adenoma	0/50	1/50	8/50*
Neoplasms, any type	0/50	4/50	18/50**
Forestomach			
Squamous papilloma	0/50	12/50**	20/49**
Squamous carcinoma	0/50	2/50	1/49
Lung			
Alveolar/bronchiolar adenoma	1/50	1/50	6/50
Alveolar/bronchiolar carcinoma	0/50	0/50	0/50
Liver			
Hepatocellular adenoma	1/50	2/50	9/50*
Hepatocellular carcinoma	0/50	0/50	3/50
Females			
Skin			
Squamous cell papilloma	0/50	1/50	5/50*
Squamous cell carcinoma	0/50	0/50	1/50
Sebaceous gland adenoma	0/50	3/50	2/50
Neoplasms, any type	0/50	4/50	9/50**
Forestomach			
Squamous papilloma	0/50	12/49**	17/50**
Squamous carcinoma	0/50	7/49*	6/50*

Tumor type	Daily dose (mg/kg)		
	0	188	375
	Tumor incidence/number examined[a]		
Lung			
Alveolar/bronchiolar adenoma	0/50	3/50	4/50
Alveolar/bronchiolar carcinoma	1/50	0/50	0/50
Liver			
Hepatocellular adenoma	0/50	0/50	1/50
Hepatocellular carcinoma	1/50	0/50	0/50

Source: NTP (1993).
[a]Statistically significant by Fisher exact test: *$P \leq 0.05$; **$P \leq 0.001$.

4.1.3 Nonneoplastic changes observed during carcinogenesis studies of DBP

In addition to the multiple-organ carcinogenic responses of rats and mice to dermal application of DBP, administration of the chemical caused a variety of nonneoplastic responses. In rats, DBP caused increased incidences of hyperkeratosis in the skin, forestomach, and esophagus; epithelial dysplasia in the nose; pleomorphism and basophilic and clear cell changes in the liver; and nuclear enlargement in the kidney. Other observations included DBP-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver, and renal hyperplasia in male rats and epithelial dysplasia of the forestomach and bile duct hyperplasia in the liver of female rats (NTP 1993).

In mice, which received DBP for a shorter time than rats, observations included DBP-related hyperplasia in the skin, epithelial dysplasia of the forestomach, and bronchiolar epithelial pleomorphism and hyperplasia in both sexes. The incidence of eosinophilic cytoplasmic change was increased in the livers of male mice (NTP 1993).

4.2 Studies with TRIS-BP

DBP is a metabolite of the flame retardant, TRIS-BP. Dietary administration of TRIS-BP to Fischer 344 rats (50 or 100 ppm) and B6C3F$_1$ mice (500 or 1000 ppm) for 103 weeks caused multiple organ carcinogenesis in both sexes of both species. In male and female rats, TRIS-BP administration was associated with significantly increased incidences of tubular cell neoplasms of the kidney. TRIS-BP-dosed mice exhibited increased incidences of neoplasms of the forestomach and lung in both male and female mice, kidney in male mice, and liver in female mice (NCI 1978, cited in IARC 1979, NTP 1993; Reznik *et al.* 1979, cited in NTP 1993). In another study, the dermal application of 10 or 30 mg of TRIS-BP three times a week for 67 to 71 weeks to the dorsal skin of ICR/Ha Swiss mice resulted in increased incidences of neoplasm of the skin, forestomach, oral cavity, and lung (Van Duuren *et al.* 1978, cited in IARC 1979, NTP 1993). A third study observed adenomas of the colon in F344/N rats administered gavage doses of 100 mg/kg TRIS-BP in corn oil, five days a week for 52 weeks (Reznik *et al.* 1981, cited in IARC 1999; NTP 1993).

Based on these observations, the IARC considers TRIS-BP to be *probably carcinogenic to humans* (*Group 2A*) (IARC 1999) and the NTP considers TRIS-BP to be *reasonably anticipated to be a human carcinogen* (NTP 1993).

4.3 Summary

DBP is carcinogenic to both sexes of rats and mice, causing increased incidences of benign and malignant tumors in multiple organs.

5 Genotoxicity

5.1 Prokaryotic systems

5.1.1 Induction of mutation in Salmonella typhimurium

DBP was mutagenic in *Salmonella typhimurium* strain TA100 both when incubated at a concentration of 0.05 mM with metabolic activation by S9 liver homogenate from phenobarbital-treated rats and when co-cultured with hepatocytes isolated from untreated rats (Holme *et al*. 1983).

NTP (1993) tested DBP for mutagenicity in various strains of *S. typhimurium. In vitro* assays were conducted in *S. typhimurium* strains TA100, TA1535, TA1537, and TA98 at DBP concentrations ranging from 0.0 to 2000.0 μg/plate in the presence or absence of a 10% hamster or rat S9 metabolic activating system. DBP was mutagenic in *S. typhimurium* strains TA100, TA1535, and TA98 in the presence or absence of hamster or rat S9 metabolic activation. In strain TA1537, DBP was nonmutagenic without S9 activation or with hamster S9 metabolic activation, and gave equivocal results with rat S9 metabolic activation. The highest concentration of DBP tested (2000 μg/plate) was toxic to *S. typhimurium* strains TA1537 and TA98. At 1,000 μg/plate, DBP was slightly toxic to all *S. typhimurium* strains tested.

DBP gave positive results in assays conducted to evaluate its oxidative and crosslinking potential. In these assays, DBP was tested in *S. typhimurium* strains TA102 (*his*G428, *rfa*, pKM101) and TA2638 (*hisG428*, *rfa*, pKM101) at concentrations ranging from 0 to 5,000 μg/plate, by the plate incorporation method, in the presence or absence of liver S9 metabolic activation. Both strains TA102 and TA2638 are sensitive in the detection of oxidative agents and crosslinking agents, because they contain AT base pairs at the hotspot (*his*G428) (Watanabe *et al*. 1998).

5.1.2 Induction of mutation in Escherichia coli

The mutagenicity of DBP was evaluated in two *Escherichia coli* strains, WP2/pKM101 (*trpE*56, pKM101) and WP2 uvrA/pKM101 (*trpE*56, *uvra*, pKM101), sensitive in detecting oxidative agents and crosslinking agents. DBP was tested at concentrations ranging from 0 to 5,000 μg/plate, by the plate incorporation method, in the presence or absence of liver S9 metabolic activation. DBP was mutagenic with or without activation in both strains, although the WP2 *uvr*A/pKM10 strain of *E. coli* was more sensitive in detecting mutations induced by oxidative and crosslinking agents. DBP was toxic to both *E. coli* strains at the highest concentration tested (Watanabe *et al*. 1998).

5.2 Eukaryotic systems

5.2.1 Mutagenicity in Drosophila melanogaster

5.2.1.1 Sex-linked recessive lethal assay

DBP caused sex-linked recessive lethal mutations in the germ cells of *Drosophila melanogaster* in three mating trails when adult males received DBP at a concentration of 500 ppm in feed. In three mating trials, 51% of flies died and 9% became sterile. A

combined total of 53 lethal mutations per three broods tested was observed (Yoon *et al*. 1985, cited in NTP 1993).

5.2.1.2 Reciprocal translocation test

When administered to adult *D. melanogaster* in feed at a concentration of 400 ppm, DBP induced a significant increase in reciprocal translocations ($P < 0.01$). The increase in frequency of reciprocal translocations was 36-fold relative to concurrent controls and 18-fold relative to historical controls (Yoon *et al*. 1985, cited in NTP 1993).

The recombinagenic potential of DBP was tested in another study using the *D. melanogaster* w/w+ eye mosaic assay, an *in vivo* short-term test measuring genetic damage in somatic cells of *Drosophila* after treatment of larvae. DBP was clearly recombinagenic at concentrations of 0.10 and 0.25 nM under the conditions of this assay (Vogel and Nivard 1993).

5.3 Mammalian Systems

5.3.1 In vitro *assays*

5.3.1.1 Mouse lymphoma assay

DBP was tested for its potential to induce trifluorothymidine resistance in L5178Y mouse lymphoma cells at concentrations ranging from 0.0625 to 0.75 μg/mL in two trials without metabolic activation (NTP 1993). In the first trial, DBP significantly increased induction of trifluorothymidine resistance at a concentration of ≥ 0.125 μg/mL, with an average mutation frequency of 69%. In the second trial, DBP significantly increased the induction of trifluorothymidine resistance at a concentration of ≥ 0.0625 μg/mL, with an average mutation frequency of 88%. The highest concentration (0.75 μg/mL) was lethal to the mouse lymphoma cells.

DBP was mutagenic in Chinese hamster V79 lung cells at a concentration of 0.02 mM with metabolic activation by S9 liver homogenate from phenobarbital-treated rats. The number of 6-thioguanine-resistant mutants was increased by a factor of 4.5 (15.8 mutations/10^6 cells, compared with 3.5 mutations/10^6 cells in dimethylsulfoxide controls) (Holme *et al*. 1983).

5.3.1.2 Sister chromatid exchanges and chromosomal aberrations

Increases in sister chromatid exchanges (SCE) were observed when DBP was incubated with Chinese hamster ovary (CHO) cells at concentrations ranging from 50.9 to 1,700.0 μg/mL with or without metabolic activation by S9 liver homogenate from Aroclor 1254-induced male Sprague-Dawley rats (NTP 1993). DBP induced SCE (20% increase over solvent control, $P < 0.001$) in a concentration-dependent manner at DBP concentrations up to 508.80 μg/mL with or without activation. DBP also was tested at concentrations ranging from 110.7 to 507.1 μg/mL without S9 metabolic activation. DBP induced SCE in a concentration-dependent manner at concentrations up to 253.6 μg/mL.

In a test of its potential to induce chromosomal aberrations, DBP was incubated with CHO cells at concentrations ranging from 626.4 to 2,493.1 μg/mL with or without

metabolic activation by S9 liver homogenate from Aroclor 1254-induced male Sprague-Dawley rats (NTP 1993). Chromosomal aberrations were significantly increased ($P < 0.001$) at concentrations up to 1,869.8 µg/mL with or without metabolic activation. DBP also was tested at concentrations ranging from 620.6 to 2,238.7 µg/mL without S9 metabolic activation. Chromosomal aberrations were significantly increased at concentrations up to 1,880.5 µg/mL.

5.3.2 In vivo *assays*

5.3.2.1 Mouse bone marrow micronucleus test

In a test of DBP's potential to induce micronuclei *in vivo*, male $B6C3F_1$ mice were given 0, 25, 50, or 100 mg/kg DBP by intraperitoneal injection, three times over a 24-hour period. DBP failed to increase the frequency of micronucleated cells ($P = 0.763$) or of polychromatic micronucleated erythrocytes ($P = 0.363$) in the bone marrow of mice. DBP was nontoxic to bone marrow over the dose range tested (NTP 1993).

5.4 Summary

DBP is a direct-acting mutagen, causing gene mutation in *S. typhimurium* with or without metabolic activation and inducing sex-linked recessive lethal mutations and reciprocal translocations in *D. melanogaster*.

6 Other Relevant Data

6.1 Absorption of DBP

Dermal absorption of DBP was measured in male Fischer 344/N rats (six weeks old) and male $B6C3F_1$ mice (eight weeks old) and blood concentrations following dermal and oral (gavage) exposure were compared (NTP 1993).

Animals were fasted (rats, overnight; mice, four hours) before dermal or gavage administration of DBP. For dermal application, DBP was dissolved in ethanol; for gavage administration, it was mixed with corn oil. Twenty-eight rats received 500 mg/kg of DBP and 28 mice received 177 mg/kg of DBP applied to their shaved interscapular skin. Twenty-eight rats and 28 mice received 177 mg/kg of DBP by gavage. The concentration of DBP in blood was determined at 0.25, 0.5, 1, 2, 4, 12, and 24 hours after dosing. Blood was analyzed by a gas chromatography method. The results are summarized in Tables 6-1 and 6-2 (NTP 1993).

Table 6-1. Concentration of DBP (10^{-7} g/mL) in the blood of rats following gavage or dermal administration

Time after dosing (h)	Gavage		Dermal	
	Vehicle Control[a]	177 mg/kg[b]	Vehicle Control[a]	500 mg/kg[b]
0.25	1.0	65.6 ± 14.7	21.6	126.2 ± 29.9
0.5	2.6	47.2 ± 27.4	2.6	45.3 ± 17.7
1	1.9	10.3 ± 2.5	1.5	116.6 ± 37.1
2	4.9	10.0 ± 2.7	4.9	8.1 ± 1.3
4	2.8	6.6 ± 2.2	2.4	2.0 ± 0.3
12	0.9	7.5 ± 3.5	1.3	1.5 ± 0.3
24	0.5	6.5 ± 2.7[c]	1.3	1.3 ± 0.4

Source: NTP (1993)

[a] Results of triplicate analyses of samples taken from a single vehicle control animal per time period.

[b] Mean ± SE for groups of four animals unless otherwise specified. Results of triplicate analyses.

[c] N=3

Table 6-2. Concentration of DBP (10^{-7} g/mL) in the blood of mice following gavage or dermal administration

Time after dosing (h)	Gavage		Dermal	
	Vehicle Control[a]	177 mg/kg[b]	Vehicle Control	177 mg/kg[b]
0.25	1.7	21.3 ± 3.1	2.1	17.9 ± 2.2
0.5	1.3	35.2 ± 7.3	1.6	19.1 ± 4.7
1	0.7	19.4 ± 8.4	0.6	4.8 ± 1.1
2	0.9	51.6 ± 15.1	1.2	1.8 ± 0.4
4	1.0	19.7 ± 5.5	0.9	0.9 ± 0.3
12	0.5	2.2 ± 0.5	0.6	0.6 ± 0.1
24	1.5	1.6 ± 0.4	2.2	1.2 ± 0.3

Source: NTP (1993)
[a] Results of triplicate analyses of samples taken from a single vehicle control animal per time period.
[b] Mean ± SE for groups of four animals. Results of triplicate analyses.

DBP was absorbed rapidly and extensively after either dermal or oral administration. The highest concentrations of DBP in blood were found 15 minutes after dermal or gavage administration in rats and 30 minutes after dermal administration or 2 hours after gavage administration in mice. The efficiency of dermal absorption, relative to oral absorption was estimated to be 68% for rats and 37% for mice. The chemical was consistently cleared from blood in less than 4 hours, except in the case of gavage administration in mice, where clearance was complete in less than 12 hours.

6.2 Metabolism of DBP

DBP is a metabolite of TRIS-BP. Current understanding of the metabolism of DBP stems from studies of metabolism of the parent compound. The probable metabolism of DBP was reviewed by NTP (1993) and is shown in Figure 6-1.

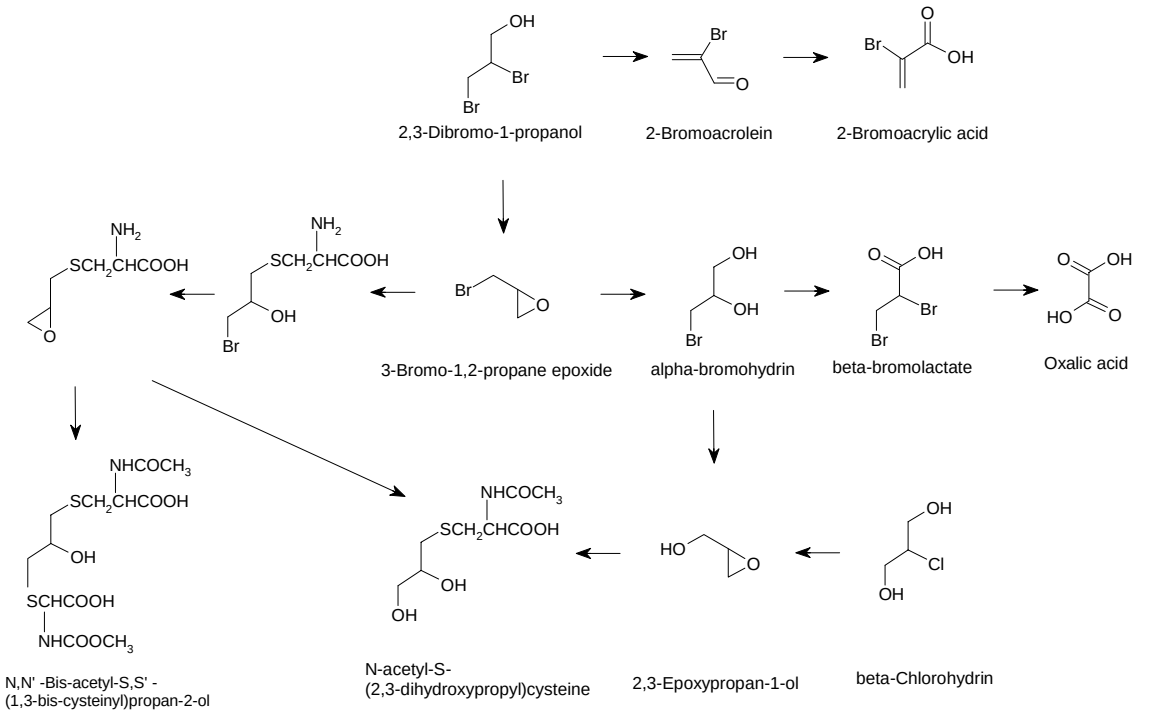

Figure 6-1. Proposed metabolic pathway for DBP

Source: Jones and Fakhouri (1979), Marsden and Casida (1982), both cited in NTP (1993).

Marsden and Casida (1982, cited in NTP 1993) suggested that DBP undergoes oxidation and dehydrohalogenation to form an unstable intermediate, 2-bromoacrolein, which results in the formation of 2-bromoacrylic acid. The researchers identified haloacrylic acids in the urine of rats administered either dibromo- or dichloro-propanol.

DBP also may undergo oxidation to 3-bromo-1,2-propane epoxide, which may react with glutathione to form *N*,*N*′-bis-acetyl-*S*,*S*′-(1,3-bis-cysteinyl)propan-2-ol and *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine. Both of these thio metabolites have been identified in the urine of rats administered dihalopropanols (Jones and Fakhouri 1979, cited in NTP 1993). These investigators also identified β-bromolactate in the urine of rats given DBP. This observation suggests that, in addition to conjugation with glutathione, 3-bromo-1,2-propane epoxide may also undergo hydrolysis to form α-bromohydrin which is then oxidized to form β-bromolactate.

Jones and Fakhouri (1979, cited in NTP 1993) also reported that when β-chlorohydrin was administered to rats, it resulted in the formation of *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine. The same metabolite was found in the urine of rats dosed with α-chlorohydrin (Jones 1973, cited in NTP 1993). In order for the metabolite to be produced from α- and β-chlorohydrin, they both must be converted to the epoxide, 2,3-epoxypropan-1-ol. This observation was considered additional evidence of the obligatory formation of epoxides as intermediate metabolites during the metabolism of DBP (NTP 1993).

6.3 Genotoxicity of putative metabolites of DBP

DBP is mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA1535 (see Section 5.1.1), and its mutagenic activity is enhanced by the presence of metabolic activating systems. 2-Bromoacrolein, identified in the urine of rats dosed with TRIS-BP and a probable reactive metabolite of DBP, is mutagenic in *S. typhimurium* strain TA100 with and without metabolic activation. This metabolite also causes DNA damage in cultured hepatoma cells and induces morphological transformation of Syrian hamster embryo cells (Gordon *et al*. 1985).

6.4 Metabolism of TRIS-BP to DBP

DBP is a metabolite of TRIS-BP in humans. DBP was detected in the urine of a seven year old female child who wore TRIS-BP-treated sleepwear for 12 consecutive nights. Urinary DBP concentrations ranged from 0.4 to 29 ng/mL (Blum *et al.* 1978).

In animal studies, DBP was identified among the urinary and biliary products in rats were intravenously and orally administered TRIS-BP. Urinary hydrolysis products, free and conjugated forms of DBP, also were found in rats administered 100 mg of TRIS by dermal application (St. John *et al.* 1976, cited in IARC 1979). *In vitro*, DBP is formed following addition of reduced nicotinamide adenine dinucleotide-dependent microsomal enzymes from rat liver to TRIS-BP (Nomeir and Matthews 1983).

6.5 Summary

DBP is readily absorbed from the gastrointestinal tract and through intact skin of rats and mice. In humans and rats, TRIS-BP is metabolized to DBP. Indirect evidence, largely generated during studies of the metabolism of the carcinogenic flame retardant TRIS-BP, indicates that DBP undergoes biotransformation in rats. The putative metabolic pathways probably produce at least three epoxide intermediates. DBP *per se* has been shown to be a mutagen, and its mutagenic activity is enhanced by the presence of hepatic microsomal metabolizing systems.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF 2,3-DIBROMO-1-PROPANOL

(CAS NO. 96-13-9)

IN F344/N RATS AND B6C3F$_1$ MICE

(DERMAL STUDIES)

NATIONAL TOXICOLOGY PROGRAM
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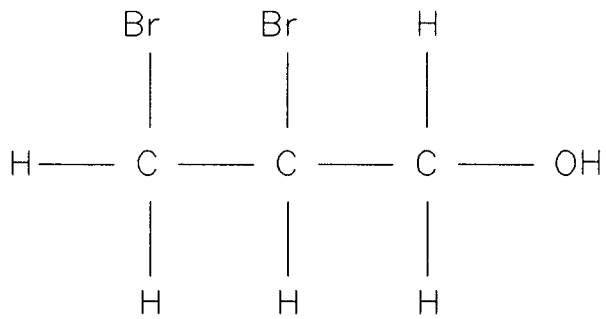
CONTENTS

ABSTRACT



2,3-DIBROMO-1-PROPANOL

CAS No. 96-13-9

Chemical Formula: $C_3H_6Br_2O$ Molecular Weight: 217.9

Synonyms: 2,3-dibromopropanol; 2,3-dibromopropyl alcohol

2,3-Dibromo-1-propanol, a colorless liquid, has been used as a flame retardant, as an intermediate in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate, and as an intermediate in the manufacture of pesticides and pharmaceutical preparations. Toxicology and carcinogenicity studies were conducted by applying 2,3-dibromo-1-propanol (approximately 98% pure) in ethanol to the subscapular area of the skin of male and female F344/N rats and B6C3F$_1$ mice 5 days per week for 16 days, 13 weeks, 48 to 51 weeks (male rats), 52 to 55 weeks (female rats), 36 to 39 weeks (male mice), or 39 to 42 weeks (female mice). Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, *Drosophila melanogaster*, mouse lymphoma cells, and mouse bone marrow cells.

16-DAY STUDY IN RATS

Groups of five male and five female rats received dermal applications of 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol 5 days per week for 16 days. One male and one female receiving 750 mg/kg died before the end of the study. The mean body weight gains and final mean body weights of dosed rats were similar to those of the controls. There were no clinical findings or gross lesions associated with chemical application.

16-DAY STUDY IN MICE

Groups of five male and five female mice received dermal applications of 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol 5 days per week for 16 days. Four males and one female receiving 750 mg/kg died before the end of the study. The mean body weight gains and final mean body weights of dosed mice were similar to those of the controls. There were no clinical findings or gross lesions associated with chemical application.

13-WEEK STUDY IN RATS

Groups of 10 male and 10 female rats received dermal applications of 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol 5 days per week for 13 weeks. All rats survived until the end of the study. For rats in the 750 mg/kg groups, the mean body weight gain was 11% lower than that of the

controls for males and 13% lower for females. The mean liver weights and liver-weight-to-body-weight ratios of males receiving 375 or 750 mg/kg and of females receiving 750 mg/kg were increased.

Chemical-related lesions occurred in the kidney of male rats and in the liver of female rats. The average severity of nephropathy was slightly increased in males receiving dermal applications of 750 mg/kg, while individual cell necrosis was observed in the liver of all female rats in the 750 mg/kg group.

13-Week Study in Mice

Groups of 10 male and 10 female mice received dermal applications of 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol 5 days per week for 13 weeks. Eight male mice receiving 750 mg/kg died during the study, while all female mice survived. The final mean body weights of dosed and control mice were similar. The mean liver weights and liver-weight-to-body-weight ratios of males receiving 375 or 750 mg/kg and of females receiving 750 mg/kg were increased.

Chemical-related lesions occurred in the liver and lung of mice. Centrilobular hepatocellular necrosis occurred in all males in the 750 mg/kg group that died during the study, while individual cell necrosis was observed in the liver of females receiving 177, 375, or 750 mg/kg. Pleomorphism of the epithelium in pulmonary bronchioles occurred with a dose-related increased incidence in males and females. Necrosis of the bronchiolar epithelium was observed in males receiving 750 mg/kg.

Long-Term Study in Rats

Originally planned to last for 2 years, the chronic study in rats was terminated early because of reduced survival in the high-dose groups related to chemical-induced neoplasms and because of the detection of antibodies to lymphocytic choriomeningitis virus in sentinel mice. Groups of 50 male and 50 female rats received dermal applications of 0, 188, or 375 mg/kg 2,3-dibromo-1-propanol 5 days per week for 48 to 51 weeks (males) or 52 to 55 weeks (females).

Survival, Body Weights, and Clinical Findings

The survival of 375 mg/kg male and female rats was significantly lower than that of the controls (males: 50/50, 41/50, 16/50; females: 48/50, 38/50, 24/50). In the 375 mg/kg groups, the final mean body weight was 23% lower than that of the controls for males and 14% lower for females. There were no chemical-related clinical findings.

Pathology Findings

Application of 2,3-dibromo-1-propanol to the skin produced significant dose-related increases in the incidences of neoplasms at numerous sites in male and female rats. Almost all dosed rats had malignant neoplasms; only one control male and one control female had malignant neoplasms. In male rats, the incidences of benign or malignant neoplasms of the skin, nose, Zymbal's gland, oral mucosa, esophagus, and small and large intestines were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the forestomach and liver were significantly increased only in the high-dose group. Neoplasms of the kidney, vascular neoplasms of the spleen, and mesotheliomas in males occurred with a significant positive trend. In female rats, the incidences of benign or malignant neoplasms of the nose, Zymbal's gland, oral mucosa, esophagus, large intestine, and liver were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the skin, forestomach, small intestine, mammary gland, and clitoral gland were significantly increased in the high-dose group only. Neoplasms of the kidney in females occurred with a significant positive trend.

Long-Term Study in Mice

Originally planned to last for 2 years, the chronic study in mice was terminated early because of the detection of antibodies to lymphocytic choriomeningitis virus in sentinel mice. Groups of 50 male and 50 female mice received dermal applications of 0, 88, or 177 mg/kg 2,3-dibromo-1-propanol 5 days per week for 36 to 39 weeks (males) or 39 to 42 weeks (females).

Survival, Body Weights, and Clinical Findings

All mice (except two low-dose females) survived until study termination. Mean body weights of control and

dosed mice were similar throughout the study, and there were no clinical findings attributed to 2,3-dibromo-1-propanol.

Pathology Findings

Application of 2,3-dibromo-1-propanol to the skin produced significant dose-related increases in the incidences of neoplasms at several sites in male and female mice. Benign or malignant neoplasms were observed in 40% of the low-dose males, 66% of the high-dose males, 52% of the low-dose females, and 56% of the high-dose females. In control groups, neoplasms occurred in 6% of the males and 10% of the females. In male and female mice, the incidences of benign or malignant neoplasms of the forestomach were significantly increased in the low- and high-dose groups, while the incidences of neoplasms of the skin were significantly increased only in the high-dose groups. The incidences of liver and lung neoplasms were increased in high-dose males.

GENETIC TOXICOLOGY

2,3-Dibromo-1-propanol was mutagenic in a variety of short-term tests, independent of exogenous metabolic activation (S9). It induced gene mutations in three strains of *Salmonella typhimurium* (TA98, TA100, and TA1535) and was positive in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells. 2,3-Dibromo-1-propanol induced sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells. In germ cells of male *Drosophila melanogaster*, 2,3-dibromo-1-propanol induced sex-linked recessive lethal mutations and reciprocal translocations. Results of an *in vivo* bone marrow micronucleus assay in male mice treated with 2,3-dibromo-1-propanol were negative.

CONCLUSIONS

Under the conditions of these long-term dermal studies, there was *clear evidence of carcinogenic activity** of 2,3-dibromo-1-propanol in male F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, kidney, tunica vaginalis, and spleen. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in female F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, kidney, clitoral gland, and mammary gland. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in male B6C3F_1 mice based on increased incidences of neoplasms of the skin, forestomach, liver, and lung. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in female B6C3F_1 mice based on increased incidences of neoplasms of the skin and the forestomach. The increased incidences of alveolar/bronchiolar adenomas in female mice may have been related to chemical administration.

In rats, 2,3-dibromo-1-propanol caused increased incidences of hyperkeratosis in the skin, forestomach, and esophagus, epithelial dysplasia in the nose, pleomorphism and basophilic and clear cell changes in the liver, and nuclear enlargement in the kidney. There were also chemical-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver, and renal hyperplasia in male rats and epithelial dysplasia of the forestomach and bile duct hyperplasia in the liver in female rats. Chemical-related increases occurred in the incidences of hyperplasia in the skin, epithelial dysplasia of the forestomach, and bronchiolar epithelial pleomorphism and hyperplasia in male and female mice and in the incidence of eosinophilic cytoplasmic change in the liver in males.

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

Summary of the Long-Term Carcinogenesis and Genetic Toxicology Studies of 2,3-Dibromo-1-propanol

Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Doses			
0, 188, or 375 mg/kg	0, 188, or 375 mg/kg	0, 88, or 177 mg/kg	0, 88, or 177 mg/kg
Final body weights			
High-dose group lower than controls	High-dose group lower than controls	Dosed groups similar to controls	Dosed groups similar to controls
Survival rates[a]			
50/50, 41/50, 16/50	48/50, 38/50, 24/50	50/50, 50/50, 50/50	50/50, 48/50, 50/50
Nonneoplastic effects			
Skin: hyperkeratosis (0/50, 1/50, 23/50)	Skin: hyperkeratosis (0/50, 0/50, 24/50)	Skin: hyperplasia (0/50, 7/50, 12/50)	Skin: hyperplasia (0/50, 8/50, 5/50)
Nose: epithelial dysplasia (0/50, 33/50, 49/50)	Nose: epithelial dysplasia (1/50, 49/50, 50/50)	Forestomach: epithelial dysplasia (0/50, 14/50, 33/49)	Forestomach: epithelial dysplasia (0/50, 16/49, 41/50)
Esophagus: hyperkeratosis (0/50, 18/50, 48/50)	Esophagus: hyperkeratosis (1/50, 20/50, 49/50)	Lung/bronchioles: epithelial pleomorphism (0/50, 50/50, 50/50); focal hyperplasia (0/50, 1/50, 6/50)	Lung/bronchioles: epithelial pleomorphism (0/50, 46/50, 50/50); focal hyperplasia (0/50, 6/50, 5/50)
Forestomach: hyperkeratosis (2/50, 6/50, 32/50); ulcer (0/50, 3/50, 5/50); acanthosis (0/50, 1/50, 6/50)	Forestomach: hyperkeratosis (0/50, 6/50, 30/50); epithelial dysplasia (0/50, 1/50, 8/50)	Liver: eosinophilic cytoplasmic change (0/50, 0/50, 11/50)	
Liver: pleomorphism (0/49, 0/50, 37/50); basophilic change (2/49, 28/50, 16/50); clear cell change (2/49, 15/50, 5/50); angiectasis (2/49, 27/50, 46/50)	Liver: pleomorphism (0/50, 0/50, 44/50); basophilic change (5/50, 27/50, 19/50); clear cell change (1/50, 8/50, 7/50); bile duct hyperplasia (1/50, 6/50, 37/50)		
Kidney: nuclear enlargement (0/50, 0/50, 41/50); hyperplasia (0/50, 1/50, 5/50)	Kidney: nuclear enlargement (0/50, 6/50, 47/50)		
Neoplastic effects			
Skin: squamous cell papilloma or carcinoma (1/50, 8/50, 8/50); basal cell tumor, sebaceous gland adenoma, or keratoacanthoma (0/50, 20/50, 31/50)	Skin: squamous cell papilloma or carcinoma (0/50, 0/50, 3/50); basal cell tumor, sebaceous gland adenoma, or keratoacanthoma (0/50, 3/50, 18/50)	Skin: squamous cell papilloma or carcinoma (0/50, 3/50, 11/50); sebaceous gland adenoma (0/50, 1/50, 8/50)	Skin: squamous cell papilloma or carcinoma (0/50, 1/50, 6/50); sebaceous gland adenoma (0/50, 3/50, 2/50)
Nose: adenoma (0/50, 48/50, 48/50)	Mammary gland: adeno-carcinoma (0/50, 0/50, 5/50)	Forestomach: squamous cell papilloma or carcinoma (0/50, 14/50, 21/49)	Forestomach: squamous cell papilloma or carcinoma (0/50, 18/49, 19/50)

Summary of the Long-Term Carcinogenesis and Genetic Toxicology Studies of 2,3-Dibromo-1-propanol (continued)

Male F344/N Rats	Female F344/N Rats	Male $B6C3F_1$ Mice	Female $B6C3F_1$ Mice
Neoplastic effects (continued)			
Oral mucosa: squamous cell papilloma or carcinoma (0/50, 47/50, 48/50)	Nose: adenoma (0/50, 44/50, 49/50)	Liver: hepatocellular adenoma or carcinoma (1/50, 2/50, 11/50)	
Esophagus: squamous cell papilloma (0/50, 19/50, 33/50)	Oral mucosa: squamous cell papilloma or carcinoma (0/50, 39/50, 49/50)	Lung: alveolar/bronchiolar adenoma (1/50, 1/50, 6/50)	
Forestomach: squamous cell papilloma (0/50, 1/50, 17/50)	Esophagus: squamous cell papilloma (0/50, 9/50, 38/50)		
Small intestine: adeno-carcinoma (0/50, 8/50, 11/50)	Forestomach: squamous cell papilloma (1/50, 3/50, 23/50)		
Large intestine: adenomatous polyp (1/50, 13/50, 29/50)	Small intestine: adeno-carcinoma (0/50, 3/50, 4/49)		
Liver: neoplastic nodules or carcinoma (0/49, 4/50, 5/50)	Large intestine: adenomatous polyp (0/50, 12/50, 37/50)		
Kidney: tubule cell adenoma (0/50, 0/50, 4/50)	Liver: neoplastic nodules or carcinoma (0/50, 11/50, 14/50)		
Zymbal's gland: adenoma or adenocarcinoma (0/50, 9/50, 35/50)	Kidney: tubule cell adenoma (0/50, 1/50, 4/50)		
Tunica vaginalis: mesothelioma (0/50, 1/50, 4/50)	Zymbal's gland: adenoma or adenocarcinoma (1/50, 9/50, 22/50)		
Spleen: hemangioma or hemangiosarcoma (0/50, 0/50, 4/50)	Clitoral gland: adenoma or adenocarcinoma (0/50, 1/50, 6/50)		
Uncertain findings			
None	None	None	Lung: alveolar/bronchiolar adenoma or carcinoma (1/50, 3/50, 4/50)
Level of evidence of carcinogenic activity			
Clear evidence	Clear evidence	Clear evidence	Clear evidence

Summary of the Long-Term Carcinogenesis and Genetic Toxicology Studies of 2,3-Dibromo-1-propanol
(continued)

Genetic toxicology	
Salmonella typhimurium gene mutations:	Positive with and without S9 in strains TA98, TA100, and TA1535 Negative with and without S9 in strain TA1537
L5178Y mouse lymphoma gene mutations:	Positive without S9
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Chromosomal aberrations	
Chinese hamster ovary cells *in vitro*:	Positive with and without S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Positive
Reciprocal translations	
Drosophila melanogaster:	Positive
Micronucleated erythrocytes	
Mouse bone marrow cells:	Negative

[a] The studies were terminated during the following weeks: male rats, weeks 48-51; female rats, weeks 52-55; male mice, weeks 36-39; female mice, weeks 39-42.

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 2,3-dibromo-1-propanol on June 23, 1992, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel 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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*Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On June 23, 1992, the draft Technical Report on the toxicology and carcinogenesis studies of 2,3-dibromo-1-propanol 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. K.M. Abdo, NIEHS, introduced the toxicology and carcinogenesis studies of 2,3-dibromo-1-propanol by discussing the uses and rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on compound-related neoplasms and nonneoplastic lesions in rats and mice. The proposed conclusions were *clear evidence of carcinogenic activity* in male and female F344/N rats and B6C3F_1 mice.

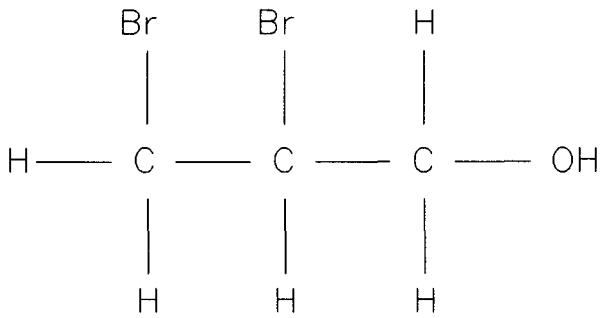
Dr. Zeise, a principal reviewer, agreed with the proposed conclusions. She asked if information including statistics could be provided for neoplasm sites that might have been of borderline significance, but were not discussed. Dr. J.K. Haseman, NIEHS, responded that because the study was terminated early there were few neoplasms occurring spontaneously. However, all neoplasms that occurred in sufficient numbers for meaningful analysis could be included in a table along with P values. (Editor's note: These values have been included in the tables in the results section.) Dr. Zeise said she would like to see an indication in the study rationale as to why the dermal route was selected. Dr. Abdo said the most common routes of human exposure were dermal and, to a lesser extent, inhalation.

Because Mr. Beliczky, the second principal reviewer, was unable to attend the meeting, Dr. L.G. Hart, NIEHS, read his review into the record. Mr. Beliczky agreed with the proposed conclusions. He noted the early termination of the chronic studies because of the presence of antibodies against lymphocytic choriomeningitis virus (LCM) in sentinel animals. Since the LCM virus also puts humans at risk, this action verifies the usefulness of the Sentinel Animal Program and the priority NTP places on the safety of laboratory personnel. Mr. Beliczky stated that since some carcinogenicity data on the chemical has been available since 1983, there should have been efforts by NTP and other Federal agencies to notify the public, industry, and workers.

Dr. Hayden asked whether it was usual NTP policy to terminate a long-term study when sentinel animals were diagnosed to be serologically positive for a potential human pathogen. Dr. S.L. Eustis, NIEHS, said this was the first such instance in his experience at NIEHS; however, in any future situation where there was a viral disease present that could be a hazard to humans the same action would be taken.

Dr. Zeise moved that the Technical Report on 2,3-dibromo-1-propanol 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. Davis seconded the motion, which was accepted unanimously with seven votes.

INTRODUCTION



2,3-DIBROMO-1-PROPANOL

CAS No. 96-13-9

Chemical Formula: $C_3H_6Br_2O$ Molecular Weight: 217.9

Synonyms: 2,3-dibromopropanol; 2,3-dibromopropyl alcohol

CHEMICAL AND PHYSICAL PROPERTIES

2,3-Dibromo-1-propanol is a colorless liquid with a melting point of 6° C, a boiling point of 219° C, and a density of 2.12 g/mL. It is slightly soluble in water and is soluble in acetone, benzene, and diethyl ether (CRC, 1983). 2,3-Dibromo-1-propanol is prepared by a reaction of allyl alcohol with bromine in carbon tetrachloride (TOXNET, 1991).

PRODUCTION, USE, AND HUMAN EXPOSURE

2,3-Dibromo-1-propanol has been used as an intermediate in the preparation of the flame retardant tris(2,3-dibromopropyl) phosphate, as an active flame retardant itself, and as a chemical intermediate for insecticide and pharmaceutical preparations (Fishbein, 1979). The U.S. Environmental Protection Agency has detected 2,3-dibromo-1-propanol in industrial effluent discharges at a concentration of 0.5×10^{-3} g/L (Webb *et al.*, 1973; CEC, 1976). 2,3-Dibromo-1-propanol has been identified as a metabolite of tris(2,3-dibromopropyl) phosphate in humans wearing treated fabrics (Blum *et al.*, 1978). Prior to 1977, tris(2,3-dibromopropyl) phosphate was the most widely used flame retardant in synthetic fabrics, particularly polyester materials used in children's sleepwear. After studies showed that this flame retardant was mutagenic in bacteria (Prival *et al.*, 1977) and carcinogenic in rats and mice (NCI, 1978; Van Duuren *et al.*, 1978; Reznik *et al.*, 1979), the Consumer Product Safety Commission (CPSC) banned the sale of sleepwear containing this compound (CPSC, 1977a,b). In 1976, the production volume of 2,3-dibromo-1-propanol in the United States was greater than 10 million pounds (Fishbein, 1979). However, as a result of the ban of tris(2,3-dibromopropyl) phosphate by the CPSC, the production volume of 2,3-dibromo-1-propanol decreased drastically. Information on the current production level is not available.

ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

Experimental Animals

2,3-Dibromo-1-propanol has been detected in the urine of rats after absorption of tris(2,3-dibromopropyl) phosphate through the skin (St. John *et al.*, 1976) and after oral or intravenous injection (Lynn *et al.*, 1982; Nomeir and Matthews, 1983).

The possible metabolic pathways for tris(2,3-dibromopropyl) phosphate and 2,3-dibromo-1-propanol are shown in Figure 1. Marsden and Casida (1982) suggested that 2,3-dibromo-1-propanol and other haloalkanols undergo oxidation and dehydrohalogenation, with the formation of 2-haloacrolein as an unstable intermediate. Consistent with this hypothesis, these investigators identified haloacrylic acids in the urine of rats given dibromopropanol or dichloropropanol (10 μmol/kg body weight by intraperitoneal injection) at levels of 10% and 50% of the amounts resulting from direct haloacrylic acid administration.

2,3-Dibromo-1-propanol may also undergo oxidation to an epoxyhalopropane intermediate, 3-bromo-1,2-propane epoxide (Figure 1), which reacts with glutathione to form mercapturic acids. Jones and Fakhouri (1979) identified two acetylated cysteine-containing compounds in the urine of rats given each of four dihalopropanols. The two compounds were *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine and *N,N'*-bis-acetyl-*S,S'*-(1,3-bis-cysteinyl)propan-2-ol. Moreover, these investigators also identified a β-halolactate (β-bromolactate or β-chlorolactate, depending on the dihalopropanol) in the urine of rats given the dihalopropanols; this finding suggests that the epoxyhalopropane may also undergo hydrolysis to the α-halohydrin and further oxidation to the β-halolactate and oxalic acid, in addition to direct conjugation with glutathione, to form the mercapturic acids.

β-Chlorohydrin administered to rats (Jones and Fakhouri, 1979) produced one mercapturic acid, *N*-acetyl-*S*-(2,3-dihydroxypropyl)cysteine, as a urinary metabolite; this is the same metabolite produced from α-chlorohydrin (Jones, 1973). To produce the same metabolite, both α- and β-chlorohydrin must be converted to the epoxide 2,3-epoxypropan-1-ol, providing further evidence of the obligatory formation of epoxides as intermediary metabolites.

Although 2,3-dibromo-1-propanol has been identified in the urine and tissues of rats given tris(2,3-dibromopropyl) phosphate, the major metabolite of this flame retardant compound in the plasma and bile of dosed rats is bis(2,3-dibromopropyl) phosphate (Lynn *et al.*, 1982). Nelson *et al.* (1984) have shown that 2-bromoacrolein and bis(2,3-dibromopropyl) phosphate are formed by oxidation of tris(2,3-dibromopropyl) phosphate, primarily at the C-1 position in an NADPH-dependent reaction by rat liver microsomes (see Figure 1). Consistent with these findings, 2-bromoacrylic acid has been identified in the urine of rats given tris(2,3-dibromopropyl) phosphate (Marsden and Casida, 1982).

Humans

2,3-Dibromo-1-propanol, a metabolite of tris(2,3-dibromopropyl) phosphate, has been identified in the urine of children wearing sleepwear treated with the flame retardant (Blum *et al.*, 1978).

TOXICITY

Experimental Animals

Little is known about the toxic effects of 2,3-dibromo-1-propanol. It is more acutely toxic in rats and mice than is tris(2,3-dibromopropyl) phosphate. A single intraperitoneal injection of 200 mg/kg 2,3-dibromo-1-propanol to male Wistar rats killed all animals within 24 hours, whereas a single intraperitoneal injection of 750 mg/kg tris(2,3-dibromopropyl) phosphate was not lethal (Søderlund *et al.*, 1980). The intraperitoneal LD_{50} of 2,3-dibromo-1-propanol in mice is 125 mg/kg (NIOSH, 1987), while the LD_{50} of tris(2,3-dibromopropyl) phosphate in mice is 1.15 g/kg (Salamone and Katz, 1981). The oral LD_{50} of tris(2,3-dibromopropyl) phosphate in Sprague-Dawley rats is 1.88 g/kg (Seabaugh *et al.*, 1981).

Although 2,3-dibromo-1-propanol is a urinary metabolite of tris(2,3-dibromopropyl) phosphate, it apparently is not responsible for the renal toxicity associated with the administration of tris(2,3-dibromopropyl) phosphate. Søderlund *et al.* (1980) demonstrated that intraperitoneal injection of single doses of 250 mg/kg or higher tris(2,3-dibromopropyl)

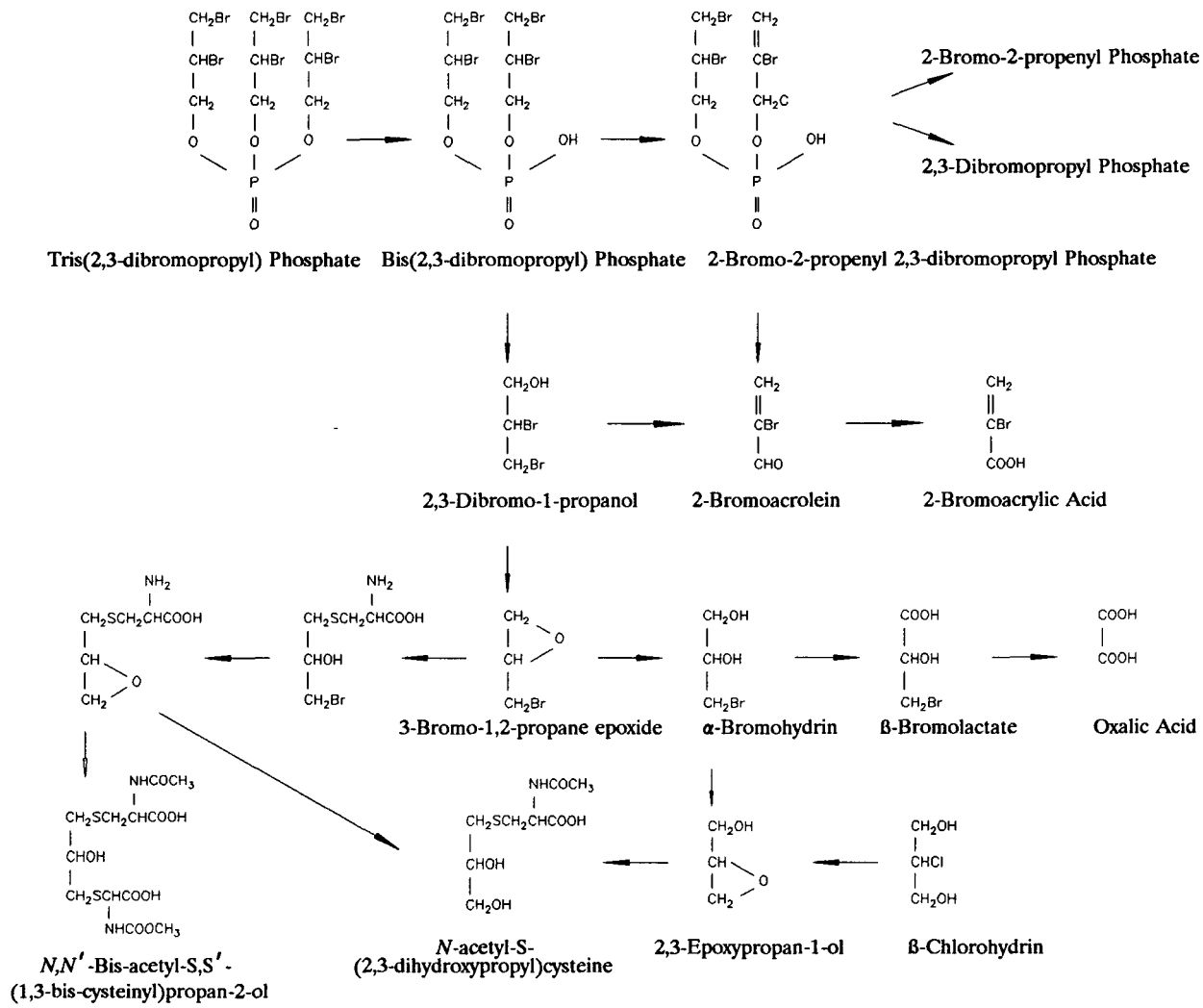


FIGURE 1
Proposed Metabolic Pathways for 2,3-Dibromo-1-propanol and Relationship to Tris(2,3-dibromopropyl) Phosphate (Adapted from Jones and Fakhouri, 1979, and Marsden and Casida, 1982)

phosphate to male Wistar rats resulted in necrosis of the proximal tubule epithelium and elevation of plasma urea and creatinine. Further, these investigators also showed that pretreatment of male rats with cobalt chloride, an agent that decreases cytochrome P-450 levels and increases tissue glutathione levels, reduced the extent of renal tubule necrosis caused by tris(2,3-dibromopropyl) phosphate. In contrast, single intraperitoneal doses of 100 mg 2,3-dibromo-1-propanol/kg body weight (one-half the lethal dose) did not produce renal tubule necrosis (Søderlund *et al.*, 1980). Similarly, Elliot *et al.* (1982) showed that an intraperitoneal dose of 61 mg 2,3-dibromo-1-propanol/kg body weight was not nephrotoxic in Sprague-Dawley rats, whereas an approximately equimolar dose of 154 mg tris(2,3-dibromopropyl) phosphate/kg body weight caused acute proximal tubule necrosis accompanied by elevated levels of serum urea and creatinine. Elliot *et al.* (1982) also demonstrated that bis(2,3-dibromopropyl) phosphate, another metabolite of tris(2,3-dibromopropyl) phosphate, caused more severe renal damage than did the parent compound and may be primarily responsible for the renal toxicity. In a follow-up study of their earlier work, Søderlund *et al.* (1982) showed that bis(2,3-dibromopropyl) phosphate and (2,3-dibromopropyl) phosphate caused more extensive renal lesions and higher levels of plasma urea and creatinine than did the parent compound.

Humans

No information was available on the toxicity of 2,3-dibromo-1-propanol in humans.

Reproductive and Developmental Toxicity

Experimental Animals

Sperm morphology and vaginal cytology studies were conducted on F344/N rats exposed to 0, 188, or 375 mg/kg 2,3-dibromo-1-propanol for 13 weeks in the present NTP studies (Environmental Health Research and Testing, Inc., report dated April 1983, on file at NIEHS). Caudal, testicular, and epididymal weights of treated rats were significantly decreased. Sperm motility was not affected, but sperm density was reduced. 2,3-Dibromo-1-propanol did not alter the length of estrus or the relative frequency of various estrous stages. No other reproductive and developmental toxicity studies of 2,3-dibromo-1-propanol were found.

Dermal application of 2.27 g/kg tris(2,3-dibromopropyl) phosphate to the backs of male and female New Zealand albino rabbits once weekly for 3 months caused testicular atrophy in males, but no adverse effect in females (Osterberg *et al.*, 1977). Testicular atrophy and decreased epididymal sperm counts were dose-related effects observed in Sprague-Dawley rats that had been administered tris(2,3-dibromopropyl) phosphate by intraperitoneal injection three times per week for 72 days (Cochran and Wiedow, 1986). Whether the effects observed in these two studies were caused by the parent compound, bis(2,3-dibromopropyl) phosphate, or 2,3-dibromo-1-propanol is unknown.

No teratogenic effects were observed in Sprague-Dawley rats that were administered 0, 5, 25, or 125 mg/kg tris(2,3-dibromopropyl) phosphate by gavage daily on days 6 through 15 of gestation (Seabaugh *et al.*, 1981). Maternal body weight gain was marginally decreased during gestation in the 125 mg/kg dose group. No teratogenic effects were observed in Wistar rats after daily gavage administration of 25, 50, 100, or 200 mg/kg tris(2,3-dibromopropyl) phosphate on days 7 through 15 of gestation (Kawashima *et al.*, 1981).

Humans

No information was available on the reproductive toxicity of 2,3-dibromo-1-propanol in humans.

Carcinogenicity

Experimental Animals

No studies have been reported on the carcinogenicity of 2,3-dibromo-1-propanol in laboratory animals. However, tris(2,3-dibromopropyl) phosphate given to F344/N rats at doses of 50 or 100 ppm and to B6C3F$_1$ mice at doses of 500 or 1,000 ppm in feed for 2 years induced renal tubule cell adenomas and carcinomas in both species (NCI, 1978a; Reznik *et al.*, 1979). These neoplasms appeared to originate from the proximal convoluted tubule epithelium. In addition, the incidences of benign and malignant neoplasms of the forestomach, lung, and liver were increased in dosed mice (NCI, 1978a). Dermal application of 10 or 30 mg of tris(2,3-dibromopropyl)

phosphate three times weekly for 67 to 71 weeks to the dorsal skin of ICR/Ha Swiss mice caused increased incidences of neoplasms of the skin, forestomach, oral cavity, and lung (Van Duuren *et al.*, 1978). Reznik *et al.* (1981) observed adenomas of the colon in F344/N rats administered 100 mg/kg tris(2,3-dibromopropyl) phosphate in corn oil by gavage 5 days a week for 52 weeks. Thus, tris(2,3-dibromopropyl) phosphate, the parent compound of 2,3-dibromo-1-propanol, is carcinogenic at multiple organ sites in laboratory animals. Whether the proximate carcinogen in these studies was tris(2,3-dibromopropyl) phosphate or one of its metabolites is unknown.

In NTP/National Cancer Institute (NCI) 2-year studies, neoplasms of the kidney in rats were most frequently produced by alkyl or alkenyl halide compounds (Kluwe *et al.*, 1984). These chemical-induced neoplasms occurred more commonly in rats than in mice and more commonly in males than in females. All of the halogenated three-carbon compounds previously tested by NTP/NCI were found to be carcinogenic. 1,2-Dibromo-3-chloropropane was carcinogenic in male and female rats and mice, causing increased incidences of forestomach squamous cell carcinomas. In female rats, it caused significantly increased incidences of mammary gland adenocarcinomas (NCI, 1978b). 1,2-Dichloropropane was not carcinogenic in male rats but caused marginally increased incidences of adenocarcinomas of the mammary gland in female rats. It was carcinogenic in male and female mice, causing increased incidences of hepatocellular neoplasms, primarily adenomas (NTP, 1986). 1,3-Dichloropropene was carcinogenic in male and female rats, causing increased incidences of squamous cell papillomas and carcinomas of the forestomach and increased incidences of neoplastic nodules in the liver of male rats. The study in male mice was considered inadequate because of poor survival in the control group. 1,3-Dichloropropene was carcinogenic in female mice, causing increased incidences of transitional cell carcinomas of the urinary bladder, alveolar/bronchiolar adenomas of the lung, and squamous cell papillomas and carcinomas of the forestomach (NTP, 1985).

Humans

No epidemiology studies on the relationship between exposure to 2,3-dibromo-1-propanol and the incidence of cancer in humans have been reported. A mortality analysis of 628 male workers potentially exposed to tris(2,3-dibromo-propyl) phosphate at two manufacturing plants did not detect any significant, cause-specific, excessive mortality (Wong *et al.*, 1984). However, the exposure data were inadequate and the number of mortalities (36 deaths, with 7 due to cancer) was too small to draw definitive conclusions.

GENETIC TOXICITY

2,3-Dibromo-1-propanol is mutagenic in *Salmonella typhimurium* strains TA98, TA100, and TA1535. The base-pair substitution strains, TA100 and TA1535, show a greater mutagenic response than does the frameshift strain, TA98 (Blum and Ames, 1977; Prival *et al.*, 1977; Carr and Rosenkranz, 1978; Nakamura *et al.*, 1979; Søderlund *et al.*, 1979; Lynn *et al.*, 1982; Haworth *et al.*, 1983; Holme *et al.*, 1983). The mutagenic activity of 2,3-dibromo-1-propanol was enhanced by the addition of S9 or microsomal metabolic activation systems. 2,3-Dibromo-1-propanol was also mutagenic in V79 Chinese hamster lung cells (Holme *et al.*, 1983) and germ cells of male *Drosophila melanogaster* (Yoon *et al.*, 1985). It induced unscheduled DNA repair synthesis in cultured rat hepatocytes (Holme *et al.*, 1983), elicited preferential growth inhibition in a DNA repair-deficient strain of *Escherichia coli* ($polA_1^-$) compared to the nondeficient strain ($polA^+$) (Hyman *et al.*, 1980), and caused reciprocal translocation (Yoon *et al.*, 1985) and chromosomal breakage (Zimmering, 1983) in germ cells of male *D. melanogaster*.

2-Bromoacrolein and 2,3-dibromopropanal, two related compounds and potential reactive metabolites of tris(2,3-dibromopropyl) phosphate and 2,3-dibromo-1-propanol, were mutagenic in *S. typhimurium* strain TA100 with and without rat liver microsomes, caused single strand breaks in the DNA of cultured hepatoma cells, and induced morphological transformation of Syrian hamster embryo cells (Gordon *et al.*, 1985).

2,3-Dibromo-1-propanol was a less potent mutagen than the parent compound, tris(2,3-dibromopropyl) phosphate, which is mutagenic in *S. typhimurium* strains TA100 and TA1535 when incubated with S9 or microsomal activation systems (Blum and Ames, 1977; Prival *et al.*, 1977; Nakamura *et al.*, 1979; Søderlund *et al.*, 1979; Brusick *et al.*, 1980; Lynn *et al.*, 1982; Zeiger *et al.*, 1982; Holme *et al.*, 1983). At concentrations of approximately 250 nmol/plate and higher, tris(2,3-dibromopropyl) phosphate does not require exogenous metabolic activation for its mutagenicity in *S. typhimurium* (Zeiger *et al.*, 1982). Tris(2,3-dibromopropyl) phosphate also induced mutations in V79 Chinese hamster lung cells and in L5178Y mouse lymphoma cells, morphological transformation in Syrian hamster embryo cells and in Balb/3T3 cells, and unscheduled DNA repair synthesis in cultured rat hepatocytes (Brusick *et al.*, 1980; Holme *et al.*, 1983; Søderlund *et al.*, 1985). In addition, tris(2,3-dibromopropyl) phosphate induced sister chromatid exchanges in Chinese hamster V79 cells and in L5178Y mouse lymphoma cells (Furukawa *et al.*, 1978; Nakanishi and Schneider, 1979; Brusick *et al.*, 1980), induced chromosomal aberrations in mouse bone marrow cells and L5178Y mouse lymphoma cells (Nakanishi and Schneider, 1979; Brusick *et al.*, 1980), increased the frequencies of abnormal sperm morphology and micronucleated polychromatic erythrocytes in $B6C3F_1$ mice (Salamone and Katz, 1981), caused strand breaks in DNA of cultured human cells (Gutter and Rosenkranz, 1977), and induced sex-linked recessive lethal mutations in *D. melanogaster* (Brusick *et al.*, 1980).

STUDY RATIONALE

2,3-Dibromo-1-propanol was selected for long-term dermal toxicology and carcinogenesis studies as part of an organohalide class evaluation and because this compound is a metabolite of the flame retardant tris(2,3-dibromopropyl) phosphate, a known carcinogen in animals. Because the primary route of human exposure to flame retardants is through the skin, the dermal route of administration was chosen for the studies.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

2,3-Dibromo-1-propanol was obtained from Great Lakes Chemical Corporation (Bayport, TX) in two lots. Lot 4-44-726 was used during the 16-day, 13-week, and a portion of the long-term studies, until it was depleted; thereafter, lot H1P was used. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix G). Both lots of the bulk chemical, a clear, colorless, viscous liquid, were identified as 2,3-dibromo-1-propanol by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

The purity of lots 4-44-726 and H1P was approximately 98%, as determined by elemental analyses, Karl Fischer water analysis, titration of acidic components with sodium hydroxide, thin-layer chromatography, and gas chromatography. For both lots, elemental analyses for carbon, hydrogen, and bromine were in agreement with the theoretical values. Karl Fischer water analysis indicated no more than 0.07% water. Titration for acidic components indicated less than 25 ppm acid (hydrogen bromide). Thin-layer chromatography indicated only one trace impurity for each lot. Gas chromatography indicated up to five impurities which were present at a total area of approximately 1% and seven additional impurities with areas less than 0.1% for lot 4-44-726; up to five impurities with a total area of approximately 2% and 11 additional impurities with areas less than 0.1% for lot H1P.

Stability studies performed by the analytical chemistry laboratory using gas chromatography indicated that 2,3-dibromo-1-propanol is stable as a bulk chemical for at least 2 weeks at temperatures up to 60° C. Throughout the studies, the bulk chemical was stored in amber glass bottles at 0° to 6° C. The stability of the bulk chemical was monitored periodically by the study laboratory using gas chromatography. No degradation of the study material was observed throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing 2,3-dibromo-1-propanol with ethanol (Table G1). Stability studies of the dose formulations conducted by the analytical chemistry laboratory using gas chromatography confirmed that the solutions were stable for at least 7 days when stored at room temperature. An additional stability study performed by the study laboratory using the same gas chromatographic system used by the analytical chemistry laboratory indicated that the dose formulations were stable for up to 8 weeks when stored at 0° to 8° C. During the studies, the dose formulations were stored at 2° to 6° C for up to 16 days; after the fourth month of the long-term studies, the dose formulations were stored protected from light.

The study laboratory conducted periodic analyses of the formulations using gas chromatography. Dose formulations were analyzed twice during the 13-week studies and approximately every 4 weeks during the long-term studies. During the 13-week studies, three of four dose formulations were within 10% of the target concentrations (Table G2). During the long-term studies, all samples were within 10% of the target concentrations (Table G3). Results of the periodic referee analyses performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table G4).

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Portage, MI and Kingston, NY) and observed for 19 days before the studies began. Rats were 52 days old and mice were 59 days old at the beginning of the studies. Groups of five male and five female rats and mice were administered 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol in 95% ethanol applied to the subscapular skin (Table 1). The area of skin receiving the dose application was shaved

4 days prior to the first dose; rats were reshaved 8 days later. All groups were treated for 16 days, excluding weekends, for a total of 12 exposure days. Animals were housed five per cage; water and feed were available *ad libitum*. Animals were observed twice daily for signs of toxicity. Clinical observations were recorded on the day of necropsy. Animals were weighed initially, weekly, and at necropsy. Complete necropsies were performed on all animals. Further experimental details are presented in Table 1.

13-WEEK STUDIES

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to 2,3-dibromo-1-propanol and to determine appropriate concentrations for use in the long-term studies. Male and female F344/N rats and $B6C3F_1$ mice were obtained from Harlan Industries (Indianapolis, IN) and were observed for 16 days (rats) or 9 to 16 days (mice) before the studies began. Rats were 48 days old and mice were 60 days old when the studies began. Groups of 10 male and 10 female rats and mice were administered 0, 44, 88, 177, 375, or 750 mg/kg 2,3-dibromo-1-propanol in 95% ethanol applied to the subscapular skin 5 days a week for 13 weeks. Animals were clipped initially at the site of dose application and were reclipped once or twice weekly. Rats and mice were housed five per cage; water and feed were available *ad libitum*. Animals were observed twice daily and clinical observations were recorded daily. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I). Animals were weighed initially and weekly thereafter. Further experimental details are presented in Table 1.

Necropsies were performed on all animals. The liver of animals surviving to the end of the study was weighed at necropsy. Complete histopathology was performed on all control animals, all rats that received 750 mg/kg, all mice that received 375 mg/kg, and all mice receiving 750 mg/kg that survived to the end of the study. Tissues examined for rats in the 44, 88, 177, and 375 mg/kg groups were the kidney of males and liver of females. The liver and lung of all mice in the 44, 88, and 177 mg/kg groups and female mice in the 375 mg/kg group were also examined. Additional information is provided in Table 1.

LONG-TERM STUDIES

Study Design

The long-term studies were originally designed for 2 years. Groups of 50 male and 50 female rats and mice were administered 2,3-dibromo-1-propanol in 95% ethanol applied to the subscapular skin 5 days a week. Rats were administered 0, 188, or 375 mg/kg; mice were administered 0, 88, or 177 mg/kg. The studies were terminated early (rats at study weeks 48 to 51 for males and 52 to 55 for females; mice at study weeks 36 to 39 for males and 39 to 42 for females) to protect the health of workers performing these studies. Antibodies against lymphocytic choriomeningitis virus were detected in the sera of sentinel mice at 6 months and later in the sera of all groups of male mice. Lymphocytic choriomeningitis virus is a human pathogen and has been reported to cause serious illness (meningitis and death).

Source and Specification of Animals

Male and female F344/N rats and $B6C3F_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the long-term studies. Rats were quarantined 24 days and mice were quarantined 19 days. Five male and five female rats and mice were randomly selected and killed for parasite evaluation and gross observation of disease. Blood samples were collected for viral screens. Rats and mice were approximately 56 days old when the studies began. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program.

Animal Maintenance

Rats and mice were housed five per cage. Feed and water were available *ad libitum*. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix H.

Clinical Examinations and Pathology

All animals were observed twice daily and findings were recorded monthly or as necessary. Animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter.

Necropsies were performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and

preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathology was performed on all animals. Tissues examined are listed in Table 1.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Carcinogenesis Bioassay Data System. The microscope 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 sent to an independent pathology quality assessment laboratory. The clitoral and preputial glands, esophagus, kidney, large intestine, liver, nasal cavity, oral cavity, forestomach, small intestine, skin, and Zymbal's gland of male and female rats; the mammary gland of female rats; the lung, stomach, skin, and uterus of mice; and the liver of male mice were reviewed microscopically by the quality assessment pathologist for neoplasms or 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 there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnosis between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chair to the PWG for review. These included examples of neoplasms of the skin, oral cavity, esophagus, forestomach, intestine, nasal cavity, preputial and clitoral glands, liver, and kidney in rats and skin, forestomach, liver, and lung in mice. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that 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 by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are 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 were censored from the survival analyses at the time they were found dead of other than natural causes; animals dying from natural causes were not censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) 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, A4, B1, B4, C1, C4, D1, and D4 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 and all nonneoplastic lesions are given as the ratio of the number of affected animals to the number of animals with the site examined microscopically. However, when macroscopic examination was required to detect lesions 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.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences

Because of infection with lymphocytic choriomeningitis virus, as well as chemical-related neoplasms at multiple sites in rats and mice, dosed and control animals were killed early and generally within a 4-week time frame. Mean survival differences among groups were generally only 0 to 2 weeks, with the largest difference being 6 weeks. Because of the similarity in survival times, it was deemed unnecessary to employ survival-adjusted analyses of neoplasm rates. Consequently, pairwise comparisons

were made by the Fisher exact test, and dose-response trends were assessed by the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979, Haseman, 1984). These same analyses were used to evaluate the incidences of selected non-neoplastic lesions.

Analysis of Continuous Variables

Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's test). Average nephropathy and necrosis severity values for the 13-week studies were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973).

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of neoplasm incidence (Haseman *et al.*, 1984, 1985). However, the NTP has no historical data for ethanol vehicle control dermal application studies for comparison with the treated groups in the 2,3-dibromo-1-propanol studies. Moreover, the 2,3-dibromo-1-propanol studies were terminated between 36 and 55 weeks. Nevertheless, historical control data for untreated F344/N rats and $B6C3F_1$ mice terminated between 35 and 62 weeks are provided in this report (Tables A3, B3, C3, and D3). These historical control data are from studies conducted during the same general period as the 2,3-dibromo-1-propanol studies. These historical control data are provided to give the reader perspective on the spontaneous rate of neoplasms in rats and mice at approximately 1 year of age.

QUALITY ASSURANCE METHODS

The 13-week and long-term were conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they 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 preliminary review draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so all had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICOLOGY

The genetic toxicity of 2,3-dibromo-1-propanol 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, sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster*, and micronucleated erythrocytes in mouse bone marrow cells. The protocols for these studies and the test results are given in Appendix E.

The genetic toxicity studies of 2,3-dibromo-1-propanol 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 of the chemical and its responses 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 Dermal Studies of 2,3-Dibromo-1-propanol

16-Day Studies	13-Week Studies	Long-Term Studies
Study Laboratory Papanicolaou Cancer Research Institute at Miami, Inc. (Miami, FL)	Papanicolaou Cancer Research Institute at Miami, Inc. (Miami, FL)	Papanicolaou Cancer Research Institute at Miami, Inc. (Miami, FL)
Strain and Species Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source Charles River Breeding Laboratories (Portage, MI - rats; Kingston, NY - mice)	Harlan Industries (Indianapolis, IN)	Frederick Cancer Research Facility (Frederick, MD)
Size of Study Groups 5 males and 5 females	10 males and 10 females	50 males and 50 females
Doses 0, 44, 88, 177, 375, and 750 mg/kg 2,3-dibromo-1-propanol in 95% ethanol, applied to the subscapular skin	0, 44, 88, 177, 375, and 750 mg/kg 2,3-dibromo-1-propanol in 95% ethanol, applied to the subscapular skin	Rats: 0, 188, and 375 mg/kg 2,3-dibromo-1-propanol in 95% ethanol, applied to the subscapular skin Mice: 0, 88, and 177 mg/kg 2,3-dibromo-1-propanol in 95% ethanol, applied to the subscapular skin
Time Held Before Study 19 days	Rats: 16 days Mice: 9-16 days	Rats: 24 days Mice: 19 days
Age When Placed on Study Rats: 52 days Mice: 59 days	Rats: 48 days Mice: 60 days	Rats: 56 days Mice: 56 days
Date of First Dose 7 July 1980	22 September 1980	Rats: 14 December 1981 Mice: 25 January 1982
Duration of Dosing 16 days, excluding weekends (total of 12 dosing days)	13 weeks (5 days/week)	Male rats: 48-51 weeks (5 days/week) Female rats: 52-55 weeks (5 days/week) Male mice: 36-39 weeks (5 days/week) Female mice: 39-42 weeks (5 days/week)
Date of Last Dose 22 July 1980	19 December 1980	Male rats: 9 December 1982 Female rats: 4 January 1983 Male mice: 26 October 1982 Female mice: 15 November 1982

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of 2,3-Dibromo-1-propanol
(continued)

16-Day Studies	13-Week Studies	Long-Term Studies
Method of Sacrifice CO_2	CO_2	CO_2
Necropsy Date 30 July 1980	22-24 December 1980	Male rats: 17 November 1982 - 10 December 1982 Female rats: 13 December 1982 - 6 January 1983 Male mice: 4-27 October 1982 Female mice: 27 October 1982 - 16 November 1982
Average Age at Necropsy Rats: 11 weeks Mice: 12 weeks	Rats: 20-21 weeks Mice: 22-23 weeks	Rats: 56-64 weeks Mice: 44-50 weeks
Method of Animal Distribution Animals were randomized by weight with a computer-generated randomization chart.	Animals were randomized by weight with a computer printout generated by Tracor-Jitco, Inc. (Rockville, MD).	Rats and female mice: same as 13-week studies. Male mice were randomized with a standard random number table.
Animals per Cage 5	5	5
Method of Animal Identification Ear notch and india ink injection	Same as 16-day studies	Ear notch and toe notch
Diet NIH-07 Rat and Mouse Ration (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Water Tap water (city of Miami water supply) via outside-the-cage automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Cages Polycarbonate cages (Lab Products, Inc., Rochelle Park, NJ)	Same as 16-day studies	Same as 16-day studies
Bedding Sani-Chip hardwood chips (P.J. Murphy, Forest Product Corp., Montville, NJ), changed twice weekly	Same as 16-day studies	BetaChips hardwood chips (Northeastern Products Corp., Warrensburg, NY); changed twice weekly

TABLE 1
Experimental Design and Materials and Methods in the Dermal Studies of 2,3-Dibromo-1-propanol
(continued)

16-Day Studies	13-Week Studies	Long-Term Studies
Cage Filters		
Cerex spun nylon filters (Florida Filters, Miami, FL), changed every 2 weeks	Same as 16-day studies	Cerex spunbonded nylon filters (Monsanto Co., St. Louis, MO), changed every 2 weeks
Racks		
Stainless steel racks (Lab Products, Inc., Rochelle Park, NJ), changed every 10 days	Stainless steel racks (Lab Products, Inc., Rochelle Park, NJ), changed every 2 weeks	Same as 13-week studies
Animal Room Environment		
Temperature: 72°-76° F Relative humidity: 40%-60% Fluorescent light: 12 hours/day Room air changes: 10-15/hour	Temperature: 72°-77° F Relative humidity: 52%-67% Fluorescent light: 12 hours/day Room air changes: >15/hour	Temperature: 73.4° ± 1.8° F Relative humidity: 59.9% ± 7.0% Fluorescent light: 12 hours/day Room air changes: 10-15/hour
Type and Frequency of Observation		
Observed twice daily; weighed initially, weekly and at necropsy; clinical observations recorded at necropsy	Observed twice daily; weighed initially and weekly; clinical observations recorded daily	Observed twice daily; weighed initially, weekly for 13 weeks, and monthly thereafter; clinical observations recorded monthly
Necropsy		
Necropsy was performed on all animals.	Necropsy was performed on all animals. The liver was weighed for all animals surviving to necropsy.	Necropsy was performed on all animals.
Histopathology		
None	Complete histopathology on all control animals, all rats that received 750 mg/kg, all male mice that received 375 mg/kg, and all mice receiving 750 mg/kg that survived to the end of the studies. Tissues that were routinely examined microscopically included: adrenal gland, bile duct, bone marrow, brain, esophagus, heart, kidney, large intestine, liver, lung and bronchi, lymph nodes, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. The kidney of male rats and the liver of female rats in the 44, 88, 177, and 375 mg/kg groups were examined. The liver and lung of all mice receiving 44, 88, or 177 mg/kg and female mice receiving 375 mg/kg were examined.	Complete histopathology performed on all animals. Tissues that were routinely examined microscopically included: adrenal gland, bile duct, bone marrow, brain, esophagus, heart, kidney, large intestine, liver, lung and bronchi, lymph nodes, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

RESULTS

RATS

16-DAY STUDY

One male and one female rat in the 750 mg/kg groups died on day 2 of the study (Table 2). There were no significant differences in final mean body weights or body weight gains in dosed or control male and female rats. No treatment-related clinical findings or gross observations were noted.

TABLE 2
Survival and Mean Body Weights of Rats in the 16-Day Dermal Study of 2,3-Dibromo-1-propanol

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	126 ± 7	219 ± 5	93 ± 3	
44	5/5	124 ± 6	219 ± 6	95 ± 2	100
88	4/4	130 ± 4	221 ± 7	91 ± 4	101
177	5/5	124 ± 6	221 ± 7	97 ± 4	101
375	5/5	127 ± 7	214 ± 5	88 ± 1	98
750	4/5[c]	125 ± 7	221 ± 4	90 ± 3	101
Female					
0	5/5	100 ± 5	145 ± 5	45 ± 3	
44	5/5	101 ± 6	146 ± 4	44 ± 2	101
88	5/5	99 ± 4	148 ± 5	49 ± 3	102
177	5/5	99 ± 3	151 ± 4	52 ± 2	104
375	5/5	99 ± 4	144 ± 5	45 ± 2	99
750	4/5[c]	102 ± 4	151 ± 4	49 ± 2	104

[a] Number of animals surviving at 16 days/number of animals initially in group. Differences from the control group were not significant by Williams' or Dunnett's test.
[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] Day of death: 2

13-WEEK STUDY

All rats survived to the end of the study (Table 3). Final mean body weights of male and female rats receiving 750 mg/kg were 94% of the control values. Mean body weight gains of males in the 750 mg/kg group and females in the 375 and 750 mg/kg groups were 89%, 92%, and 87% of control values, respectively. No biologically significant differences in these parameters were noted in other dose groups. Absolute and relative liver weights were increased in male rats receiving 375 mg/kg and male and female rats receiving 750 mg/kg (Table F1).

Dosed rats, especially in the 375 and 750 mg/kg groups, exhibited an unusual behavior pattern that was not observed in controls. After chemical application, dosed rats separated themselves from cagemates instead of huddling together as would be expected for group-housed rats. Recongregation typically occurred several hours later. No dose-related gross observations were noted at necropsy.

Lesions associated with dermal exposure to 2,3-dibromo-1-propanol were observed in the kidney of males and in the liver of females. Nephropathy was observed in most male rats, but the average severity was significantly increased in the 750 mg/kg group (Table 4). Nephropathy was characterized by a few scattered clusters of cortical tubules with slightly thickened basement membranes and basophilic epithelial cells (Plate 1). The interstitium surrounding these clusters of tubules generally contained increased collagen and occasional inflammatory cells. The nephropathy observed in control males was generally minimal in extent; only one or two foci were observed in the kidney sections. In the 750 mg/kg group, the average severity of nephropathy was mild, with three to five foci observed.

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Dermal Study of 2,3-Dibromo-1-propanol

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	124 ± 3	310 ± 5	187 ± 5	
44	10/10	123 ± 4	299 ± 9	176 ± 6	96
88	10/10	125 ± 4	310 ± 7	185 ± 6	100
177	10/10	122 ± 4	307 ± 10	185 ± 7	99
375	10/10	122 ± 4	301 ± 7	178 ± 4	97
750	10/10	125 ± 3	292 ± 3	167 ± 3*	94
Female					
0	10/10	105 ± 2	181 ± 2	75 ± 2	
44	10/10	105 ± 2	181 ± 2	76 ± 2	100
88	10/10	105 ± 2	179 ± 2	73 ± 3	99
177	10/10	105 ± 2	184 ± 3	79 ± 3	102
375	10/10	105 ± 2	174 ± 4	69 ± 4	96
750	10/10	105 ± 2	170 ± 3*	65 ± 2**	94

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** ($P \leq 0.01$)
[a] Number of animals surviving at 13 weeks/number of animals initially in group
[b] Weights and weight changes are given as mean ± standard error

TABLE 4
Incidences of Selected Nonneoplastic Lesions in Rats in the 13-Week Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	44 mg/kg	88 mg/kg	177 mg/kg	375 mg/kg	750 mg/kg
Male						
Kidney[a]	10	10	10	10	10	10
Nephropathy[b]	6 (0.7)[c]	6 (0.6)	8 (0.9)	10* (1.1)	10* (1.4)*	10* (1.8)**
Female						
Liver	10	0	0	0	10	10
Hepatocellular necrosis	0 (0.0)	–	–	–	0 (0.0)	10** (1.7)**

* Significantly different (P≤0.05) from the control group by the Fisher exact test (rates) or Mann-Whitney U test (severity)
** P≤0.01
[a] Number of animals with organ examined microscopically
[b] Number of animals with lesion
[c] Group average severity of lesion where 0=no lesion, 1=minimal, 2=mild

Individual hepatocyte necrosis was observed in all female rats in the 750 mg/kg group. In each of the 750 mg/kg female rats, there were from one to several randomly distributed necrotic hepatocytes that were sometimes surrounded by a few neutrophils, macrophages, or both (Plate 2).

Dose Selection Rationale: In rats receiving 750 mg/kg during the 13-week studies, hepatocellular lesions were seen in females, while the severity of nephropathy, a progressive, potentially life-threatening lesion, was increased in males. Therefore, dose levels selected for the long-term study were 188 and 375 mg/kg.

LONG-TERM STUDY

Survival

Estimates of survival probability for male and female rats are shown in Table 5 and in the Kaplan-Meier curves in Figure 2. The study was terminated early (weeks 48 to 51 for males and weeks 52 to 55 for females) because of the reduced survival of high-dose rats and because sentinel mice housed in the same room as the rats tested positive for lymphocytic choriomeningitis virus. Serum samples taken at necropsy from all rats were negative for the virus by complement fixation. Survival of male groups at week 48 was: control, 50/50; low-dose, 41/50; high-dose, 16/50 (Table 5 and Figure 2). Survival of female groups at week 52 was: 48/50, 38/50, 24/50. Most of the rats dying early were killed moribund because of the presence of large neoplasms, particularly of the oral cavity, Zymbal's gland, and mammary gland.

Body Weights and Clinical Findings

The mean body weight of high-dose male rats was similar to that of the controls until week 28 (Table 6 and Figure 3). Thereafter, the mean body weight of high-dose males was lower than that of the controls, and at week 44, the last weighing period, the mean body weight of high-dose males was 23% lower. The mean body weight of high-dose female rats was within 10% of that of the controls until week 48, when it was 48% lower (Table 7).

There were no clinical findings directly attributable to 2,3-dibromo-1-propanol administration. Emaciation, dyspnea, and lethargy, which were observed in some treated rats, especially high-dose males, occurred as a result of neoplasms associated with the application of the chemical.

TABLE 5
Survival in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	188 mg/kg	375 mg/kg
Male			
Animals initially in study	50	50	50
Natural deaths or moribund kills	0	9	34
Animals surviving until study termination	50	41	16
Percent probability of survival at end of study[a]	100	82	32
Mean survival (weeks)[b]	50	49	44
Survival analysis[c]	P<0.001	P=0.018	P<0.001
Female			
Animals initially in study	50	50	50
Natural deaths or moribund kills	2	12	26
Animals surviving until study termination	48	38	24[d]
Percent probability of survival at end of study[a]	96	76	48
Mean survival (weeks)[b]	53	51	49
Survival analysis[c]	P<0.001	P=0.011	P<0.001

[a] Kaplan-Meier determinations
[b] Mean of all deaths (uncensored, censored, terminal sacrifice)
[c] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.
[d] Includes three animals that died or were killed moribund during the terminal sacrifice period

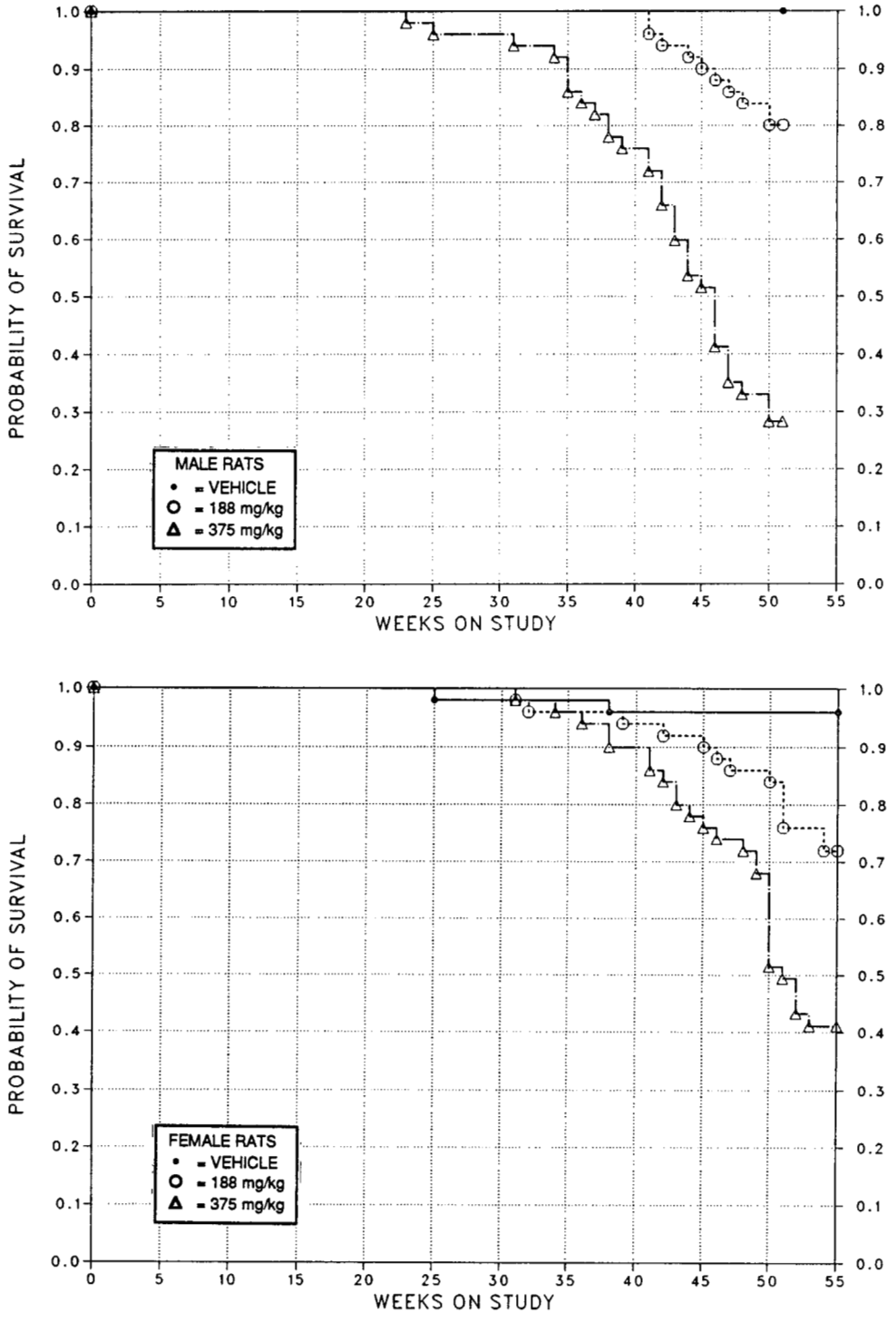


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered 2,3-Dibromo-1-propanol by Dermal Application for 51 or 55 Weeks

TABLE 6
Mean Body Weights and Survival of Male Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

Week on Study	Vehicle Control		188 mg/kg			375 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls	Number of Survivors
0	189	50	164	87	50	183	97	50
1	219	50	219	100	50	214	98	50
2	239	50	240	100	50	235	98	50
3	262	50	263	100	50	257	98	50
4	277	50	282	102	50	276	100	50
5	295	50	297	101	50	291	99	50
6	310	50	311	100	50	304	98	50
7	324	50	324	100	50	318	98	50
8	333	50	334	100	50	327	98	50
9	345	50	342	99	50	335	97	50
10	354	50	352	99	50	343	97	50
11	355	50	355	100	50	348	98	50
12	362	50	365	101	50	356	98	50
16	389	50	389	100	50	375	96	50
20	403	50	397	99	50	387	96	50
24	400	50	416	104	50	400	100	49
28	428	50	419	98	50	390	91	48
32	439	50	427	97	50	392	89	47
36	445	50	433	97	50	390	88	43
40	456	50	43ᵥ	94	50	378	83	38
44	462	50	433	94	46	358	77	28
48	467	50	423	91	41	346	74	16
Mean for weeks								
1-13	306		307	100		300	98	
14-44	428		418	98		384	90	

TABLE 7
Mean Body Weights and Survival of Female Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

Week on Study	Vehicle Control		188 mg/kg			375 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls	Number of Survivors
0	138	50	140	101	50	139	101	50
1	152	50	152	100	50	153	101	50
2	160	50	161	101	50	162	101	50
3	170	50	172	101	50	171	101	50
4	175	50	178	102	50	179	102	50
5	182	50	183	101	50	183	101	50
6	188	50	189	101	50	189	101	50
7	192	50	193	101	50	192	100	50
8	198	50	197	99	50	196	99	50
9	201	50	200	100	50	200	100	50
10	205	50	204	100	50	205	100	50
11	207	50	206	100	50	208	100	50
12	210	50	209	100	50	210	100	50
16	221	50	220	100	50	219	99	50
20	225	50	224	100	50	223	99	50
24	234	50	238	102	50	235	100	50
28	241	49	243	101	50	238	99	50
32	244	49	251	103	49	239	98	49
36	253	49	263	104	48	247	98	48
40	267	48	273	102	47	256	96	45
44	276	48	279	101	46	259	94	40
48	286	48	286	100	43	245	86	38
Mean for weeks								
1-13	187		187	100		187	100	
14-48	250		253	101		240	96	

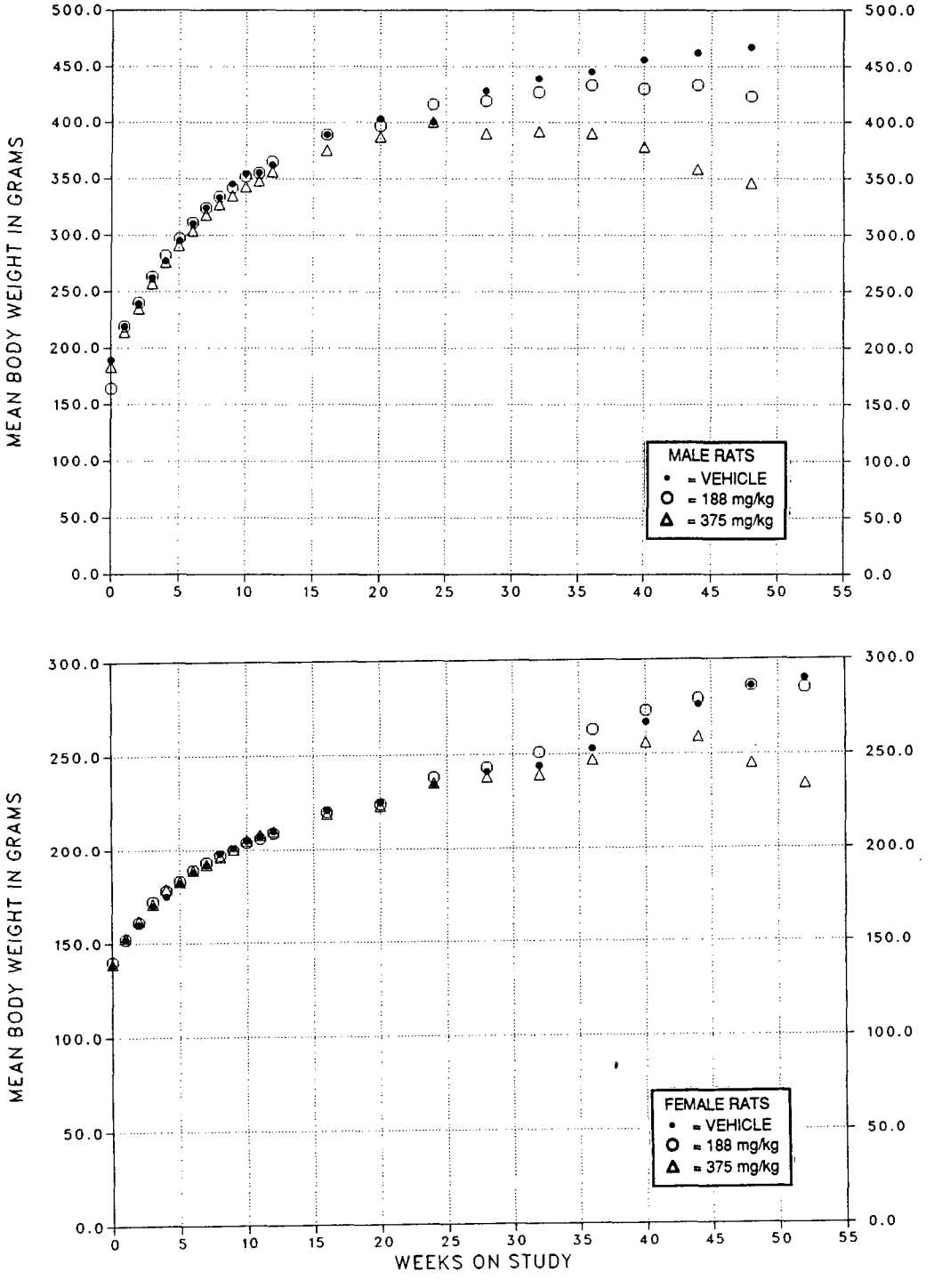


FIGURE 3
Growth Curves for Rats Administered 2,3-Dibromo-1-propanol by Dermal Application for 51 or 55 Weeks

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the skin (application site), gastrointestinal tract, nose, Zymbal's gland, kidney, liver, preputial and clitoral glands, mammary gland, mesothelium, and spleen. Summaries of the incidences of neoplasms and nonneoplastic lesions and individual animal tumor diagnoses are presented in Appendix A for male rats and Appendix B for female rats.

Skin: Hyperkeratosis of the skin at the site of 2,3-dibromo-1-propanol administration was observed in 2% of the low-dose male rats, 46% of the high-dose males, and 48% of the high-dose females, but not in the controls. Epithelial neoplasms of various histologic types were observed in the skin at or near the site of application in 44% of the low-dose males, 66% of the high-dose males, 6% of the low-dose females, and 36% of the high-dose females (Table 8). A squamous cell papilloma was observed in a single control male. The neoplasms in dosed rats frequently exhibited divergent differentiation, with varying numbers of basal cells, sebaceous cells, and/or squamous cells within an individual neoplasm, and the diagnosis was based on the predominant growth pattern and cell type.

The squamous cell papillomas were exophytic masses composed of irregular papillary fronds with fibrovascular cores covered by well-differentiated squamous epithelium and thick layers of keratin. Squamous cell carcinomas were poorly circumscribed, locally invasive masses composed of cords of squamous epithelium exhibiting dysplasia, atypical keratinization (keratin pearl formation), and numerous mitoses (Plate 3). The basal cell tumors were well-circumscribed endophytic masses consisting of cords and solid lobules of basal cells separated by dense collagenous connective tissue. The basal cells were relatively uniform in size and shape, with round nuclei and basophilic cytoplasm (Plate 4). In some basal cell tumors, small clusters of cells exhibited squamous or sebaceous differentiation; neoplasms exhibiting extensive sebaceous differentiation were designated sebaceous adenomas. Keratoacanthomas were invaginated, crateriform masses of stratified squamous epithelium continuous with the surface epithelium (Plate 5). The stratified epithelium formed papillary or nodular masses covered by thick layers of keratin which often filled the central cavity of the mass (Plate 6).

Oral Mucosa (Tongue, Lip, Gum, Palate, Pharynx), Esophagus, and Forestomach: Squamous cell papillomas or carcinomas of the oral mucosa occurred in nearly all low- and high-dose males and in almost all high-dose and most low-dose females; none were seen in the controls (Table 9). Moreover, many of the dosed rats had multiple oral neoplasms. The majority of neoplasms occurred on the dorsum of the tongue or on the pharynx, but a few were observed arising from the mucosa of the lip, palate, or gingiva.

Squamous cell papillomas of the esophagus and forestomach were also observed in many dosed rats (Table 9). In contrast to the frequent occurrence of squamous cell carcinomas of the tongue and pharynx, esophageal carcinomas were observed in only one low-dose male and one high-dose female, and no forestomach carcinomas occurred. Further, the incidence of squamous cell papillomas of the forestomach in each group was less than the incidence of squamous cell papillomas of the esophagus. The histologic appearance of the squamous cell papillomas (Plate 7) and carcinomas of the upper gastrointestinal tract was generally similar to those arising from the skin.

The incidences of hyperkeratosis of the esophageal epithelium were increased in low- and high-dose rats, while the incidences of hyperkeratosis of the forestomach epithelium were increased primarily in the high-dose groups (Table 9). Hyperkeratosis was characterized by a slight to moderate increase in the thickness of the keratin layer. While such an increase can be the result of increased keratin formation associated with hyperplasia, it may also result from a decrease in keratin loss because of reduced food intake and reduction in mechanical debridement.

Dysplasia of the forestomach epithelium was seen in a number of dosed male and female rats, but not in the controls. In females, the highest incidence was in the high-dose group, while in males the highest incidence was in the low-dose group (males: 0/50, 6/50, 1/50; females: 0/50, 1/50, 8/50). The dysplasia

TABLE 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Hyperkeratosis						
Overall rate[a]	0/50 (0%)	1/50 (2%)	23/50 (46%)	0/50 (0%)	0/50 (0%)	24/50 (48%)
Cochran-Armitage test[b]	P<0.001			P<0.001		
Fisher exact test[b]		P=0.500	P<0.001		–[c]	P<0.001
Squamous Cell Papilloma						
Overall rate	1/50 (2%)	3/50[d] (6%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Cochran-Armitage test	P=0.378N			P=0.110		
Fisher exact test		P=0.309	P=0.500N		–	P=0.247
Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	5/50 (10%)	8/50 (16%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Cochran-Armitage test	P=0.004			P=0.333		
Fisher exact test		P=0.028	P=0.003		–	P=0.500
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	1/50 (2%)	8/50 (16%)	8/50 (16%)	0/50 (0%)	0/50 (0%)	3/50 (6%)
Cochran-Armitage test	P=0.020			P=0.036		
Fisher exact test		P=0.015	P=0.015		–	P=0.121
Basal Cell Tumor						
Overall rate	0/50 (0%)	13/50 (26%)	21/50 (42%)	0/50 (0%)	3/50 (6%)	12/50 (24%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P=0.121	P<0.001
Sebaceous Adenoma						
Overall rate	0/50 (0%)	5/50 (10%)	5/50 (10%)	0/50 (0%)	0/50 (0%)	2/50 (4%)
Cochran-Armitage test	P=0.036			P=0.110		
Fisher exact test		P=0.028	P=0.028		–	P=0.247
Keratoacanthoma						
Overall rate	0/50 (0%)	4/50 (8%)	12/50 (24%)	0/50 (0%)	0/50 (0%)	5/50 (10%)
Cochran-Armitage test	P<0.001			P=0.004		
Fisher exact test		P=0.059	P<0.001		–	P=0.028
Basal Cell Tumor, Sebaceous Adenoma, or Keratoacanthoma						
Overall rate	0/50 (0%)	20/50 (40%)	31/50 (62%)	0/50 (0%)	3/50 (6%)	18/50 (36%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P=0.121	P<0.001
Epithelial Neoplasms (all types)						
Overall rate	1/50 (2%)	22/50 (44%)	33/50 (66%)	0/50 (0%)	3/50 (6%)	18/50 (36%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P=0.121	P<0.001

[a] Number of lesion-bearing animals/number of animals necropsied
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[c] Not applicable; no lesions in animal group
[d] Multiple occurrence of morphology in the same organ tissue is counted only once.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Digestive System in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Oral Mucosa						
Squamous Cell Papilloma						
Overall rate[a]	0/50 (0%)	40/50 (80%)	33/50 (66%)	0/50 (0%)	27/50 (54%)	41/50 (82%)
Cochran-Armitage test[b]	P<0.001			P<0.001		
Fisher exact test[b]		P<0.001	P<0.001		P<0.001	P<0.001
Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	16/50 (32%)	25/50 (50%)	0/50 (0%)	15/50 (30%)	27/50 (54%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	47/50 (94%)	48/50 (96%)	0/50 (0%)	39/50 (78%)	49/50 (98%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Esophagus						
Hyperkeratosis						
Overall rate[c]	0/50 (0%)	18/50 (36%)	48/50 (96%)	1/50 (2%)	20/50 (40%)	49/50 (98%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Squamous Cell Papilloma						
Overall rate[c]	0/50 (0%)	19/50 (38%)	33/50 (66%)	0/50 (0%)	9/50 (18%)	38/50 (76%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P=0.001	P<0.001
Squamous Cell Carcinoma						
Overall rate[c]	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Cochran-Armitage test	P=0.667			P=0.333		
Fisher exact test		P=0.500	–[d]		–	P=0.500
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate[c]	0/50 (0%)	20/50 (40%)	33/50 (66%)	0/50 (0%)	9/50 (18%)	38/50 (76%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P=0.001	P<0.001
Forestomach						
Hyperkeratosis						
Overall rate[c]	2/50 (4%)	6/50 (12%)	32/50 (64%)	0/50 (0%)	6/50 (12%)	30/50 (60%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P=0.134	P<0.001		P=0.013	P<0.001
Epithelial Dysplasia						
Overall rate[c]	0/50 (0%)	6/50 (12%)	1/50 (2%)	0/50 (0%)	1/50 (2%)	8/50 (16%)
Cochran-Armitage test	P=0.406			P<0.001		
Fisher exact test		P=0.013	P=0.500		P=0.500	P=0.003

(continued)

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Digestive System in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol (continued)

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Forestomach (continued)						
Acanthosis						
Overall rate[c]	0/50 (0%)	1/50 (2%)	6/50 (12%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Cochran-Armitage test	P=0.005			P=0.333		
Fisher exact test		P=0.500	P=0.013		–	P=0.500
Squamous Cell Papilloma						
Overall rate[c]	0/50 (0%)	1/50 (2%)	17/50 (34%)	1/50 (2%)	3/50 (6%)	23/50 (46%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P=0.500	P<0.001		P=0.309	P<0.001

[a] Number of lesion-bearing animals/number of animals necropsied, unless otherwise specified
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[d] Not applicable; no lesions in animal group

denoted intraepithelial foci of cellular atypia associated with increased thickness of the epithelium, often located near the junction of the glandular stomach and forestomach. Increased incidences of hyperplasia (diagnosed as acanthosis in males), characterized by focal to diffuse thickening of the stratum spinosum, and ulcers were also observed in dosed rats (Tables A4 and B4). The incidences of hyperplasia were 0/50, 1/50, and 6/50 in males and 0/50, 2/50, and 4/50 in females. The incidences of ulcers were 0/50, 3/50, and 5/50 in males and 0/50, 0/50, and 2/50 in females.

Small Intestine: The incidences of adenomatous polyps and adenocarcinomas of the small intestine in low- and high-dose males were significantly greater than those of the controls (Table 10). Adenocarcinomas of the small intestine also occurred in two low-dose and four high-dose females, and an adenomatous polyp occurred in another low-dose female. Although the incidences of these neoplasms in dosed groups of female rats were not significantly greater than those in the controls, they were considered to be chemical related because similar neoplasms occurred in the large intestine and a similar effect was observed in the small intestine of males.

Adenomatous polyps and adenocarcinomas constitute a morphologic continuum. The adenomatous polyps were focally elevated, pedunculated masses composed of a disordered array of irregular and often dilated glands. The glandular structures were lined by cuboidal to columnar epithelial cells which failed to show normal differentiation from the base of the crypts to the surface. The epithelial cells had large, round, hyperchromatic nuclei and basophilic cytoplasm. The adenocarcinomas were distinguished from polyps primarily on the basis of invasion of the submucosa or muscularis (Plate 8), although some also exhibited a cellular atypia and a scirrhous reaction. Formation of large, mucus-filled, cyst-like structures was also noted in some adenocarcinomas.

Large Intestine: The incidences of adenomatous polyps of the large intestine in low- and high-dose male and female rats were significantly greater than those in the controls (Table 10). Despite the high incidences of these benign neoplasms in the dosed groups, adenocarcinomas were observed in only one low-dose male and two high-dose males; none were observed in dosed or control females. The neoplasms of the large intestine were morphologically similar to those of the small intestine (Plates 9 and 10).

TABLE 10
Incidences of Selected Neoplasms of the Small and Large Intestine in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Small Intestine						
Adenomatous Polyp						
Overall rate[a]	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/49 (0%)
Cochran-Armitage test[b]	P=0.060			P=0.667		
Fisher exact test[b]		P=0.500	P=0.121		P=0.500	–[c]
Adenocarcinoma						
Overall rate	0/50 (0%)	8/50 (16%)	11/50 (22%)	0/50 (0%)	3/50 (6%)	4/49 (8%)
Cochran-Armitage test	P<0.001			P=0.027		
Fisher exact test		P=0.003	P<0.001		P=0.121	P=0.056
Adenomatous Polyp or Adenocarcinoma						
Overall rate	0/50 (0%)	9/50 (18%)	12/50 (24%)	0/50 (0%)	4/50 (8%)	4/49 (8%)
Cochran-Armitage test	P<0.001			P=0.035		
Fisher exact test		P=0.001	P<0.001		P=0.059	P=0.056
Large Intestine						
Adenomatous Polyp						
Overall rate[d]	1/50 (2%)	13/50 (26%)	29/50 (58%)	0/50 (0%)	12/50 (24%)	37/50 (74%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Adenocarcinoma						
Overall rate[d]	1/50 (2%)	1/50 (2%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Cochran-Armitage test	P=0.267			–		
Fisher exact test		P=0.753	P=0.500		–	–
Adenomatous Polyp or Adenocarcinoma						
Overall rate[d]	2/50 (4%)	14/50 (28%)	30/50 (60%)	0/50 (0%)	12/50 (24%)	37/50 (74%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Not applicable; no neoplasms in animal group
[d] Number of lesion-bearing animals/number of animals necropsied or number of animals with tissue examined microscopically

Nose: Epithelial dysplasia occurred in almost all dosed male and female rats (males: 0/50, 33/50, 49/50; females: 1/50, 49/50, 50/50) (Tables A4 and B4). The term "dysplasia" encompasses several related lesions of the respiratory and olfactory epithelium, including hyperplasia (increased number of epithelial cell layers), squamous metaplasia of the respiratory epithelium, respiratory metaplasia of the olfactory epithelium, and loss of normal cellular and nuclear orientation (Plates 11-13).

Adenomas of the nasal mucosa also occurred in nearly all dosed male and female rats, while none were observed in the controls (Table 11).

TABLE 11
Incidences of Neoplasms of the Nose in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	188 mg/kg	375 mg/kg
Male			
Adenoma			
Overall rate[a]	0/50 (0%)	48/50 (96%)	48/50 (96%)
Cochran-Armitage test[b]	P<0.001		
Fisher exact test[b]		P<0.001	P<0.001
Adenocarcinoma			
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)
Cochran-Armitage test	P=0.369		
Fisher exact test		P=0.247	P=0.500
Adenoma or Adenocarcinoma			
Overall rate	0/50 (0%)	49/50 (98%)	49/50 (98%)
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001
Female			
Adenoma			
Overall rate	0/50 (0%)	44/50 (88%)	49/50 (98%)
Cochran-Armitage test	P<0.001		
Fisher exact test		P<0.001	P<0.001

[a] Number of lesion-bearing animals/number of animals necropsied
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

Adenocarcinomas were seen in two low-dose males and one high-dose male; none were observed in the females.The adenomas (Plates 14 and 15) and adenocarcinomas were sessile exophytic masses arising from the respiratory epithelium of the turbinates and septum and consisted of cuboidal to short columnar epithelium arranged in gland-like or tubular structures or cords of epithelium separated by a scant vascular stroma. The few adenocarcinomas exhibited greater heterogeneity in growth pattern, cellular pleomorphism and atypia, and invasion of the submucosa.

Zymbal's Gland: Adenomas or adenocarcinomas of the Zymbal's gland occurred in 18% of low-dose and 70% of high-dose males, and in 18% of low-dose and 44% of high-dose females (Table 12). The majority of the neoplasms in dosed rats were malignant (adenocarcinomas). One control female rat also had an adenocarcinoma, but none were observed in the control males. The combined incidence of adenoma or adenocarcinoma in each of the dosed groups was significantly greater than that in the controls.

Adenomas were well-circumscribed masses lacking normal lobular architecture. They consisted of well-differentiated sebaceous cells arranged in irregular, solid clusters, sometimes admixed with thick cords or islands of squamous epithelium. The adenocarcinomas were less well circumscribed and invaded surrounding soft tissues. They consisted of irregular acini or solid sheets of cells and frequently

TABLE 12
Incidences of Neoplasms of the Zymbal's Gland in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Adenoma						
Overall rate[a]	0/50 (0%)	1/50 (2%)	7/50 (14%)	0/50 (0%)	7/50 (14%)	3/50 (6%)
Cochran-Armitage test[b]	P=0.002			P=0.158		
Fisher exact test[b]		P=0.500	P=0.006		P=0.006	P=0.121
Adenocarcinoma						
Overall rate	0/50 (0%)	8/50 (16%)	29/50 (58%)	1/50 (2%)	2/50 (4%)	19/50 (38%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P=0.003	P<0.001		P=0.247	P<0.001
Adenoma or Adenocarcinoma						
Overall rate	0/50 (0%)	9/50 (18%)	35/50 (70%)	1/50 (2%)	9/50 (18%)	22/50 (44%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P=0.001	P<0.001		P=0.001	P<0.001

[a] Number of neoplasm-bearing animals/number of animals necropsied
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

contained cystic cavities filled with debris (sebum, keratin, and necrotic cells). The neoplastic cells varied from moderately well-differentiated sebaceous cells or squamous cells to pleomorphic, undifferentiated cells.

Kidney: Nuclear enlargement (karyomegaly) of renal tubule epithelial cells was observed in most high-dose rats and a few low-dose females (males: 0/50, 0/50, 41/50; females: 0/50, 6/50, 47/50) (Tables A4 and B4). Only a few scattered cells in the inner cortex and outer stripe of the outer medulla were affected in each section of kidney.

Renal tubule adenomas occurred in four high-dose males, one low-dose female, and four high-dose females, while none occurred in control rats (Table 13). The renal tubule adenomas occurred with significant positive trends in both males and females, but the incidence in each of the dosed groups was not significantly greater than that in the controls by pairwise comparisons. The adenomas in dosed rats are considered to be chemical related because a similar effect was observed in males and females and because the incidences of focal hyperplasia of the tubule epithelium were also increased, particularly in males.

Renal tubule cell hyperplasia and adenoma constitute a morphologic continuum. Hyperplasia was characterized by one or several adjacent tubule cross-sections with multiple layers of epithelium which partially or completely filled the tubule lumen(s). The adenomas were generally larger, greater than five tubules in diameter, and consisted of solid clusters or compact nests of cells.

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Kidney in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	188 mg/kg	375 mg/kg
Male			
Hyperplasia			
Overall rate[a]	0/50 (0%)	1/50 (2%)	5/50 (10%)
Cochran-Armitage test[b]	P=0.011		
Fisher exact test[b]		P=0.500	P=0.028
Renal Tubule Adenoma			
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)
Cochran-Armitage test	P=0.011		
Fisher exact test		–[c]	P=0.059
Female			
Hyperplasia			
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)
Cochran-Armitage test	P=0.147		
Fisher exact test		P=0.500	P=0.247
Renal Tubule Adenoma			
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)
Cochran-Armitage test	P=0.023		
Fisher exact test		P=0.500	P=0.059

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Not applicable; no neoplasms in animal group

Liver: Neoplastic nodules occurred with a significant positive trend in female rats, and the incidence in each of the dose groups was significantly greater than that in the controls (Table 14). Although hepatocellular carcinomas also occurred with a significant trend, only the incidence in the high-dose group was significantly greater than that in the controls. In male rats, there was a marginal increase in the incidence of neoplastic nodules or hepatocellular carcinomas (combined), but the incidence of neither neoplastic nodules nor hepatocellular carcinomas in the high-dose group was significantly greater than that in the controls (Table 14). Nevertheless, the hepatocellular neoplasms in males and females were considered chemical related because of their infrequent occurrence in historical control rats.

At the time the studies were performed, the diagnostic term "neoplastic nodule" was applied to proliferative lesions currently classified as hepatocellular adenoma. The neoplastic nodules were discrete masses usually larger than several hepatic lobules which slightly compressed the surrounding parenchyma. Normal lobular architecture was not apparent within the lesion, and the hepatic plates merged at abnormal angles with those of the normal adjacent tissue. The hepatocytes within neoplastic nodules were well-differentiated but were often larger than normal. Hepatocellular carcinomas were considerably larger than the neoplastic nodules and exhibited heterogenous growth patterns with the hepatocytes arranged in trabeculae or gland-like structures.

TABLE 14
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	188 mg/kg	375 mg/kg	Vehicle Control	188 mg/kg	375 mg/kg
Basophilic Cytoplasmic Change						
Overall rate[a]	2/49 (4%)	28/50 (56%)	16/50 (32%)	5/50 (10%)	27/50 (54%)	19/50 (38%)
Cochran-Armitage test[b]	P=0.002			P=0.002		
Fisher exact test[b]		P<0.001	P<0.001		P<0.001	P<0.001
Clear Cell Cytoplasmic Change						
Overall rate	2/49 (4%)	15/50 (30%)	5/50 (10%)	1/50 (2%)	8/50 (16%)	7/50 (14%)
Cochran-Armitage test	P=0.253			P=0.037		
Fisher exact test		P<0.001	P=0.226		P=0.015	P=0.030
Eosinophilic Cytoplasmic Change						
Overall rate	0/49 (0%)	2/50 (10%)	4/50 (8%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Cochran-Armitage test	P=0.038			P=0.060		
Fisher exact test		P=0.253	P=0.061		P=0.500	P=0.121
Cellular Pleomorphism						
Overall rate	0/49 (0%)	0/50 (0%)	37/50 (74%)	0/50 (0%)	0/50 (0%)	44/50 (88%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		–[c]	P<0.001		–	P<0.001
Angiectasis						
Overall rate	2/49 (4%)	26/50 (52%)	46/50 (92%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Cochran-Armitage test	P<0.001			P=0.060		
Fisher exact test		P<0.001	P<0.001		P=0.500	P=0.121
Periportal Bile Duct: Hyperplasia						
Overall rate	20/49 (40%)	13/50 (26%)	10/50 (20%)	1/50 (2%)	6/50 (12%)	37/50 (74%)
Cochran-Armitage test	P=0.015N			P<0.001		
Fisher exact test		P=0.088N	P=0.021N		P=0.056	P<0.001
Neoplastic Nodule						
Overall rate	0/49 (0%)	3/50 (6%)	2/50 (4%)	0/50 (0%)	10/50 (20%)	11/50 (22%)
Cochran-Armitage test	P=0.207			P=0.001		
Fisher exact test		P=0.125	P=0.253		P<0.001	P<0.001
Hepatocellular Carcinoma						
Overall rate	0/49 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)	2/50 (4%)	6/50 (12%)
Cochran-Armitage test	P=0.061			P=0.007		
Fisher exact test		P=0.505	P=0.125		P=0.247	P=0.013
Neoplastic Nodule or Hepatocellular Carcinoma						
Overall rate	0/49 (0%)	4/50 (8%)	5/50 (10%)	0/50 (0%)	11/50 (22%)	14/50 (28%)
Cochran-Armitage test	P=0.031			P<0.001		
Fisher exact test		P=0.061	P=0.030		P<0.001	P<0.001

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[c] Not applicable; no lesions in animal group

Increased incidences of foci of cytoplasmic change (basophilic, clear cell, and eosinophilic) and cellular pleomorphism were observed in dosed male and female rats. Dosed male rats also exhibited an increased incidence of angiectasis (Table 14). The incidence of bile duct hyperplasia was increased in dosed females, but not in males. Foci of cytoplasmic change were characterized by altered staining of the hepatocyte cytoplasm. The foci were generally smaller than three hepatic lobules in size, and the affected hepatocytes retained normal lobular arrangement. Pleomorphism consisted of panlobular cytomegaly (cellular enlargement) with some variation in nuclear size. "Angiectasis" referred to a change also known as cystic degeneration or spongiosis hepatis, and was characterized by focally dilated sinusoids forming cystic spaces filled with granular or flocculent material (protein) and variable numbers of erythrocytes. The bile duct hyperplasia in female rats was characterized by increased numbers of small, well-differentiated bile ducts in the portal areas, often accompanied by increased fibrous stroma.

Preputial and Clitoral Glands: Preputial glands in male rats and clitoral glands in female rats are specialized holocrine glands which function as homologous accessory sex organs. In dosed female rats, clitoral gland neoplasms (adenomas or adenocarcinomas) occurred with a significant positive trend, and the incidence in the high-dose group was significantly greater than that in the control group by pairwise comparison (Table 15). The incidence of preputial gland adenomas in low-dose males was slightly greater than that in the controls, but the difference was not significant.

Mammary Gland: Adenocarcinomas of the mammary gland occurred in five high-dose females; none were seen in the low-dose or control groups (Table 15). The Cochran-Armitage trend test was significant and the incidence in the high-dose group was significantly greater than that in the controls by pairwise comparison.

Mesothelium: Mesotheliomas of the testicular tunica vaginalis occurred in one low-dose male and four high-dose males; none were observed in the controls (Table 15). The trend test was significant, but the pairwise comparisons were not. Nevertheless, because of the low spontaneous occurrence of mesotheliomas in NTP historical control rats, these neoplasms are considered chemical related.

Spleen: Hemangiomas of the spleen occurred in three high-dose male rats and a hemangiosarcoma occurred in another high-dose male; none were seen in the controls (Table 15). Although the trend test was significant, the incidence of vascular neoplasms (benign or malignant, combined) in the high-dose group was not significantly greater than that in the controls.

TABLE 15
Incidences of Selected Neoplasms of the Preputial or Clitoral Gland, Spleen, Mesothelium, and Mammary Gland in Rats in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	188 mg/kg	375 mg/kg
Male			
Preputial Gland			
Adenoma			
Overall rate[a]	2/50 (4%)	6/50 (12%)	3/50 (6%)
Cochran-Armitage test[b]	P=0.424		
Fisher exact test[b]		P=0.134	P=0.500
Tunica Vaginalis			
Mesothelioma			
Overall rate	0/50 (0%)	1/50 (2%)	4/50 (8%)
Cochran-Armitage test	P=0.023		
Fisher exact test		P=0.500	P=0.059
Spleen			
Hemangioma			
Overall rate[c]	0/50 (0%)	0/50 (0%)	3/50 (6%)
Cochran-Armitage test	P=0.036		
Fisher exact test			P=0.121
Hemangiosarcoma			
Overall rate[c]	0/50 (0%)	0/50 (0%)	1/50 (2%)
Cochran-Armitage test	P=0.333		
Fisher exact test			P=0.500
Hemangioma or Hemangiosarcoma			
Overall rate[c]	0/50 (0%)	0/50 (0%)	4/50 (8%)
Cochran-Armitage test	P=0.011		
Fisher exact test		–[d]	P=0.059
Female			
Clitoral Gland			
Adenoma			
Overall rate[a]	0/50 (0%)	1/50 (2%)	3/50 (6%)
Cochran-Armitage test	P=0.060		
Fisher exact test		P=0.500	P=0.121
Adenocarcinoma			
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)
Cochran-Armitage test	P=0.036		
Fisher exact test		–	P=0.121
Adenoma or Adenocarcinoma			
Overall rate	0/50 (0%)	1/50 (2%)	6/50 (12%)
Cochran-Armitage test	P=0.005		
Fisher exact test		P=0.500	P=0.013
Mammary Gland			
Adenocarcinoma			
Overall rate	0/50 (0%)	0/50 (0%)	5/50 (10%)
Cochran-Armitage test	P=0.004		
Fisher exact test		–	P=0.028

[a] Number of neoplasm-bearing animals/number of animals necropsied, unless otherwise specified
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[d] Not applicable; no neoplasms in animal group

MICE

16-DAY STUDY

Four male mice in the 750 mg/kg group died on day 2 and one female in the 750 mg/kg group died on day 3 of the study (Table 16). There were no biologically significant differences in final mean body weights or body weight gains in dosed males or females. No chemical-related clinical findings or gross observations were noted in dosed male or female mice.

TABLE 16
Survival and Mean Body Weights of Mice in the 16-Day Dermal Study of 2,3-Dibromo-1-propanol

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	25.7 ± 0.7	29.8 ± 0.8	4.2 ± 1.0	
44	5/5	25.9 ± 0.6	28.5 ± 0.6	2.6 ± 0.3	96
88	5/5	25.9 ± 0.9	29.2 ± 0.6	3.3 ± 1.1	98
177	5/5	25.5 ± 0.9	30.2 ± 0.5	4.7 ± 0.6	101
375	5/5	25.6 ± 1.1	30.0 ± 0.7	4.5 ± 0.7	101
750	1/5[c]	26.0 ± 1.1	30.5	5.4	102
Female					
0	5/5	17.8 ± 0.7	19.6 ± 0.5	1.8 ± 0.3	
44	5/5	17.6 ± 0.6	19.8 ± 0.1	2.2 ± 0.5	101
88	5/5	17.9 ± 0.5	20.0 ± 0.3	2.1 ± 0.6	102
177	5/5	17.6 ± 0.8	19.8 ± 0.2	2.2 ± 0.7	101
375	5/5	17.8 ± 0.8	21.0 ± 0.7	3.2 ± 0.9	107
750	4/5[d]	18.2 ± 0.9	21.8 ± 0.7**	3.7 ± 0.4	111

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 16 days/number of animals 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. No standard error was calculated for groups with high mortality.
[c] Day of death: all deaths occurred on day 2
[d] Day of death: 3

13-WEEK STUDY

Eight male mice receiving 750 mg/kg died during the first four days of the study (Table 17). There were no other deaths. Final mean body weights of dosed mice were similar to those of the controls. Final mean body weight gains of male mice that received 177 mg/kg were 85% of control values. Final mean body weight gains of females in the 177, 375, and 750 mg/kg groups were 88%, 75% and 88% of control values, respectively. Final mean body weight gains of other dosed groups were similar to those of the controls. Absolute and relative liver weights of males receiving 375 or 750 mg/kg and females receiving 750 mg/kg were higher than those of the controls (Table F2). Male mice that received 750 mg/kg, especially those that died, were observed to be lethargic and weak. The post-exposure "separation" behavior previously described for rats in the 13-week

study occurred sporadically in 750 mg/kg males and 375 or 750 mg/kg females. No treatment-related gross observations were noted at necropsy.

Necrosis of the pulmonary bronchial and bronchiolar epithelium (Plate 16) and centrilobular hepatocellular necrosis (Plate 17) were observed in many male mice receiving 750 mg/kg that died at the beginning of the study. Pleomorphism of the bronchial and bronchiolar epithelium also occurred with dose-related increased incidences in males and females, and was considered to be directly related to chemical administration (Table 18). The decreased incidence noted in males that received 750 mg/kg was probably related to the high mortality. Bronchiolar epithelial pleomorphism was characterized by a loss of nuclear and cellular polarity, cytomegaly with karyomegaly, and atypia and syncytia formation (Plate 18). An alveolar/bronchiolar adenoma was noted in a 375 mg/kg female. Hepatocellular necrosis occurred with increased incidences in the liver of dosed female mice, and was also observed in one male receiving 750 mg/kg that survived to the end of the study. This lesion consisted of coagulative necrosis of scattered individual hepatocytes or small clusters of hepatocytes, with or without accumulation of a few mononuclear cells, neutrophils, or both.

Dose Selection Rationale: Based on lung and liver lesions observed during the 13-week studies, dose levels selected for the long-term study were 88 and 177 mg/kg.

TABLE 17
Survival and Mean Body Weights of Mice in the 13-Week Dermal Study of 2,3-Dibromo-1-propanol

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	22.3 ± 0.5	27.6 ± 0.6	5.3 ± 0.5	
44	10/10	22.2 ± 0.4	28.1 ± 0.4	5.9 ± 0.2	102
88	10/10	22.4 ± 0.4	27.6 ± 0.7	5.3 ± 0.4	100
177	10/10	22.5 ± 0.5	27.0 ± 0.6	4.5 ± 0.6	98
375	10/10	22.1 ± 0.4	27.9 ± 0.7	5.8 ± 0.6	101
750	2/10[c]	22.4 ± 0.5	27.9 ± 0.9	6.4 ± 0.7	101
Female					
0	10/10	18.9 ± 0.4	24.5 ± 0.5	5.6 ± 0.4	
44	10/10	19.1 ± 0.5	24.5 ± 0.7	5.4 ± 0.3	100
88	10/10	19.0 ± 0.5	25.0 ± 0.8	6.0 ± 0.4	102
177	10/10	18.7 ± 0.4	23.6 ± 0.5	4.9 ± 0.4	96
375	10/10	18.9 ± 0.5	23.1 ± 0.8	4.2 ± 0.4	94
750	10/10	19.1 ± 0.4	24.1 ± 0.5	4.9 ± 0.3	98

[a] Number of animals surviving at 13 weeks/number of animals 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] Week of death: all deaths occurred during week 1

TABLE 18
Incidences of Selected Lesions in Mice in the 13-Week Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	44 mg/kg	88 mg/kg	177 mg/kg	375 mg/kg	750 mg/kg
Male						
Lung[a]	9	10	10	10	10	9
Bronchiole pleomorphism[b]	0 (0.0)[c]	2 (0.2)	6** (0.6)	10** (1.0)	8** (1.0)	2 (0.4)
Necrosis	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5* (2.0)
Liver	9	0	0	0	0	10
Hepatocellular necrosis	0 (0.0)	–	–	–	–	1 (0.1)
Centrilobular necrosis	0 (0.0)	–	–	–	–	8** (2.4)
Female						
Lung	10	10	10	10	10	10
Bronchiole pleomorphism[b]	0 (0.0)	1 (0.1)	1 (0.2)	2 (0.2)	5* (0.6)	10** (1.7)
Alveolar/bronchiolar adenoma	0	0	0	0	1	0
Liver	10	10	10	10	10	10
Hepatocellular necrosis	0 (0.0)	2 (0.2)	1 (0.2)	7** (1.0)	5* (0.6)	5* (0.8)

* 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; the diagnostic term used by the study pathologist for bronchiole pleomorphism was "metaplasia, NOS"
[c] Group average severity of lesion, where 0=no lesion, 1=minimal, 2=mild, 3=moderate, 4=marked

LONG-TERM STUDY

Survival

At 6 months, sera from sentinel mice housed in the same room as the study animals were found to be positive for antibodies to lymphocytic choriomeningitis virus by complement fixation and immunofluorescent antibody tests. Because of the potential for workers at the laboratory to contract the virus, the study was terminated early (weeks 36 to 39 for male mice and weeks 39 to 42 for female mice). All male mice in each of the groups survived until week 36, while all but two low-dose female mice survived until week 39 (Table 19). Serum samples taken from mice in the study at necropsy or moribund sacrifice were also tested for antibodies to lymphocytic choriomeningitis using complement fixation. Although none of the samples from female mice were clearly positive, samples from 9 of 49 control, 7 of 50 low-dose, and 24 of 50 high-dose males were positive. Despite the serological evidence of infection with lymphocytic choriomeningitis virus, no clinical signs of illness or histological lesions were observed.

Body Weights and Clinical Findings

Mean body weights of control and dosed mice were similar throughout the study (Tables 20 and 21 and Figure 4). No treatment-related clinical findings were observed.

TABLE 19
Survival in Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	88 mg/kg	177 mg/kg
Male			
Animals initially in study	50	50	50
Animals surviving until study termination	50	50[a]	50
Percent probability of survival at end of study[b]	100	100	100
Mean survival (weeks)[c]	38	38	38
Female			
Animals initially in study	50	50	50
Natural deaths or moribund kills	0	2	0
Animals surviving until study termination	50	48	50
Percent probability of survival at end of study[b]	100	96	100
Mean survival (weeks)[c]	41	40	41
Survival analysis[d]	P=1.000	P=0.475	P=1.000

[a] Includes one animal that was killed moribund during the terminal sacrifice period
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, 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.

TABLE 20
Mean Body Weights and Survival of Male Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

Week on Study	Vehicle Control		88 mg/kg			177 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls	Number of Survivors
0	23.6	50	24.3	103	50	24.7	104	50
1	25.8	50	25.3	98	50	26.8	104	50
2	26.9	50	26.9	100	50	27.4	102	50
3	27.9	50	27.7	99	50	28.7	103	50
4	29.0	50	28.9	100	50	29.7	102	50
5	30.3	50	30.0	99	50	30.8	102	50
6	30.3	50	30.5	101	50	31.7	105	50
7	31.5	50	30.9	98	50	31.9	101	50
8	32.3	50	32.0	99	50	32.8	101	50
9	32.8	50	32.6	99	50	33.6	102	50
10	33.4	50	32.9	98	50	34.1	102	50
11	33.5	50	33.6	100	50	35.0	104	50
12	33.7	50	33.6	100	50	35.5	105	50
16	36.5	50	34.7	95	50	37.2	102	50
20	39.1	50	38.2	98	50	39.3	101	50
24	40.7	50	39.9	98	50	40.5	100	50
28	43.0	50	41.7	97	50	41.2	96	50
32	43.4	50	42.1	97	50	42.6	98	50
Mean for weeks								
1-13	30.6		30.4	99		31.5	103	
14-32	40.5		39.3	97		40.2	99	

TABLE 21
Mean Body Weights and Survival of Female Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

Week on Study	Vehicle Control		88 mg/kg			177 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls	Number of Survivors
0	17.8	50	18.2	102	50	18.2	102	50
1	19.1	50	19.3	101	50	19.9	104	50
2	20.4	50	20.5	101	50	20.2	99	50
3	21.2	50	20.9	99	49	21.6	102	50
4	21.8	50	21.8	100	49	22.0	101	50
5	22.3	50	22.8	102	49	22.6	101	50
6	23.0	50	22.9	100	49	23.3	101	50
7	23.5	50	23.3	99	49	23.2	99	50
8	24.0	50	23.6	98	49	23.9	100	50
9	24.2	50	24.2	100	49	25.1	104	50
10	24.5	50	24.2	99	49	24.6	100	50
11	24.8	50	24.6	99	49	25.1	101	50
12	25.3	50	25.3	100	49	25.0	99	50
16	26.3	50	25.5	97	49	26.1	99	50
20	28.2	50	27.5	97	48	27.9	99	50
24	29.1	50	28.3	97	48	29.0	99	50
28	30.8	50	29.8	97	48	30.5	99	50
32	32.3	50	30.3	94	48	31.6	98	50
36	34.0	50	33.0	97	48	33.5	99	50
Mean for weeks								
1-13	22.8		22.8	100		23.0	101	
14-36	30.1		29.1	97		29.8	99	

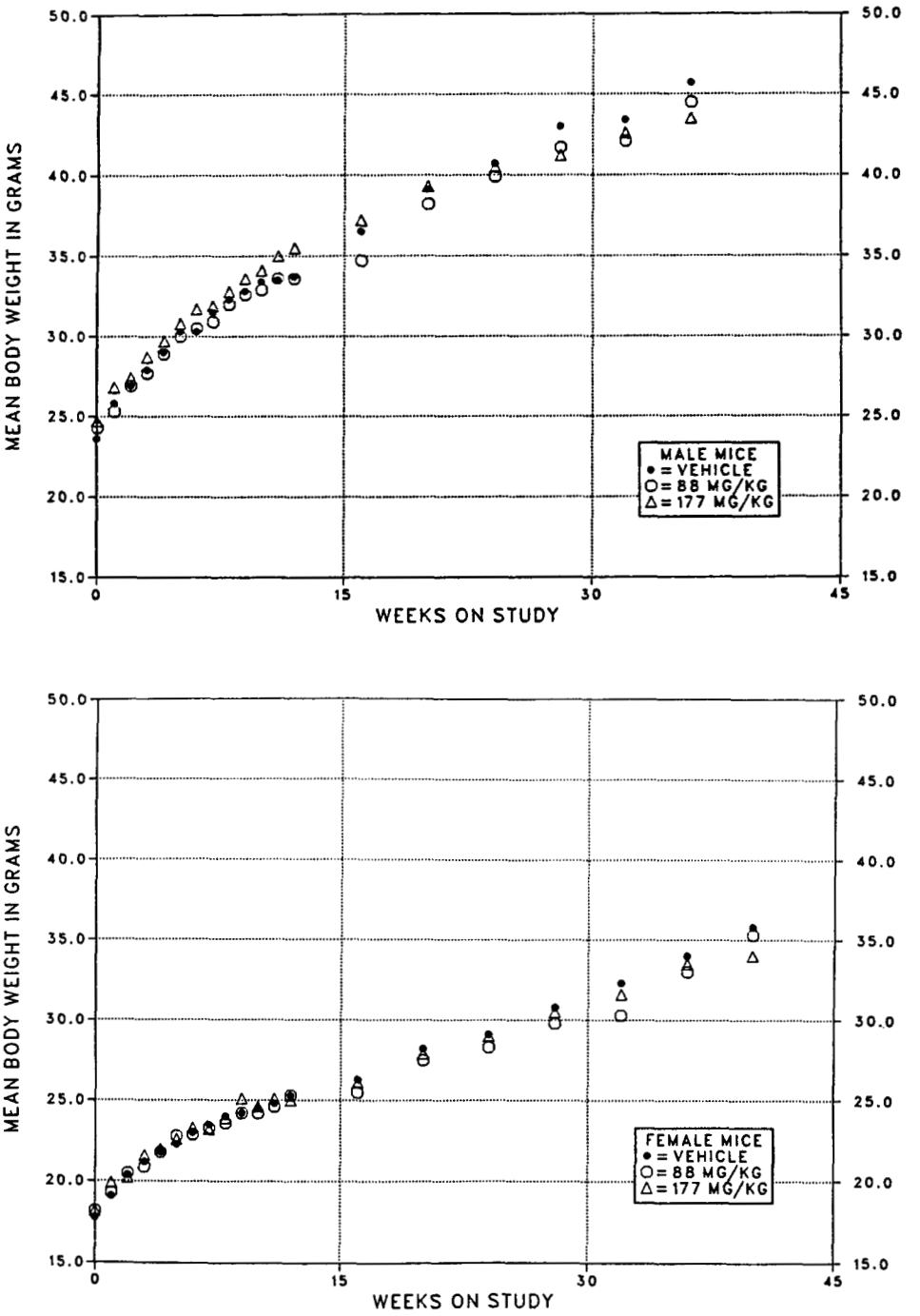


FIGURE 4
Growth Curves for Mice Administered 2,3-Dibromo-1-propanol by Dermal Application for 39 or 42 Weeks

Pathology and Statistical Evaluation

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and nonneoplastic lesions of the skin (site of application), forestomach, lung, and liver. Summaries of the incidences of neoplasms and nonneoplastic lesions and individual animal tumor diagnoses are presented in Appendix C for male mice and Appendix D for female mice.

Skin: Epithelial neoplasms were observed in the skin at or near the site of application in 8% of the low-dose male and female mice, 36% of the high-dose males, and 18% of the high-dose females (Table 22). No neoplasms of the skin occurred in the controls. Although the incidences of some individual histologic types in the low- and high-dose groups were not significantly greater than those in the controls, they were considered chemical related because they were all epithelial in origin, exhibited histologic similarities, and were supported by increased incidences of hyperplasia (Table 22).

Epithelial hyperplasia was characterized by focal thickening and folding of the stratified squamous epithelium due to increased cell layers, whereas sebaceous hyperplasia consisted of enlargement and increased cellularity of the sebaceous glands. The squamous cell papillomas and carcinomas and sebaceous gland adenomas (Plate 19) were morphologically similar to those observed in dosed rats. Some of the neoplasms were complex, with exophytic papillary structures lined by stratified squamous epithelium as well as small lobules of sebaceous cells extending into the dermis. The diagnosis applied to these neoplasms was based on the predominant histological component.

Forestomach: Dysplasia of the forestomach epithelium occurred with dose-related increases in male and female mice, and the incidence in each of the dose groups was significantly greater than that in the controls (Table 23). Squamous cell papillomas or carcinomas were seen in 28% of the low-dose males, 43% of the high-dose males, 37% of the low-dose females, and 38% of the high-dose females; none were seen in the controls (Table 23). The incidence of squamous cell neoplasms in each of the dose groups was significantly greater than that in the controls. Although the majority of these neoplasms were benign (papillomas), a larger proportion of the neoplasms in females were malignant than in males.

Epithelial dysplasia denoted thickening of the epithelium, due primarily to increased numbers of basal cells, with slight cellular atypia, loss of cellular orientation, and formation of blunt rete peglike downgrowths. The squamous cell neoplasms (Plate 20) were morphologically similar to those in rats.

Lung: Pleomorphism of the bronchiolar epithelium occurred in nearly all dosed mice, but not in the controls (Table 24). Further, the incidences of focal hyperplasia of the alveolar epithelium in high-dose males and low- and high-dose females were significantly greater than those in the controls.

Alveolar/bronchiolar neoplasms occurred more frequently in the dosed groups, but the incidences were not significantly greater than those in the controls (Table 24). Nevertheless, the chemical-related increased incidences of hyperplasia suggest that the marginal increase in alveolar/bronchiolar neoplasms is also chemical related.

Pleomorphism occurred primarily in the bronchi and bronchioles and was characterized by variation in cell and nuclear size and shape, karyomegaly, cytoplasmic vacuolation, nuclear hyperchromasia, and in a few mice, formation of papillary fronds. Focal hyperplasia of the alveolar/bronchiolar epithelium, alveolar/bronchiolar adenoma, and alveolar/bronchiolar carcinoma constitute a morphologic continuum. Hyperplasia was characterized by foci of alveolar septa lined by increased numbers of low cuboidal epithelial cells; normal alveolar architecture was maintained. The alveolar/bronchiolar adenomas were generally larger and exhibited distortion and loss of normal alveolar architecture. They consisted of single layers of uniform cuboidal to columnar epithelial cells overlying a delicate vascular stroma and arranged in irregular glandular or papillary structures. The carcinomas usually exhibited heterogeneous growth patterns and cellular atypia and pleomorphism.

TABLE 22
Incidences of Neoplasms and Nonneoplastic Lesions of the Skin in Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	88 mg/kg	177 mg/kg	Vehicle Control	88 mg/kg	177 mg/kg
Sebaceous Gland Hyperplasia						
Overall rate[a]	0/50 (0%)	1/50 (2%)	9/50 (18%)	0/50 (0%)	0/50 (0%)	0/50 (0%)
Cochran-Armitage test[b]	P<0.001			–[c]		
Fisher exact test[b]		P=0.500	P=0.001		–	–
Epithelial Hyperplasia						
Overall rate	0/50 (0%)	6/50 (12%)	3/50 (6%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Cochran-Armitage test	P=0.146			P=0.203		
Fisher exact test		P=0.013	P=0.121		P=0.121	P=0.247
Hyperplasia, NOS						
Overall rate	0/50 (0%)	1/50 (2%)	9/50 (18%)	0/50 (0%)	5/50 (10%)	3/50 (6%)
Cochran-Armitage test	P<0.001			P=0.133		
Fisher exact test		P=0.500	P=0.001		P=0.028	P=0.121
Squamous Cell Papilloma						
Overall rate	0/50 (0%)	3/50 (6%)	9/50 (18%)	0/50 (0%)	1/50 (2%)	5/50 (10%)
Cochran-Armitage test	P<0.001			P=0.011		
Fisher exact test		P=0.121	P=0.001		P=0.500	P=0.028
Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Cochran-Armitage test	P=0.110			P=0.333		
Fisher exact test		–	P=0.247		–	P=0.500
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	3/50 (6%)	11/50 (22%)	0/50 (0%)	1/50 (2%)	6/50 (12%)
Cochran-Armitage test	P<0.001			P=0.005		
Fisher exact test		P=0.121	P<0.001		P=0.500	P=0.013
Sebaceous Gland Adenoma						
Overall rate	0/50 (0%)	1/50 (2%)	8/50 (16%)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Cochran-Armitage test	P<0.001			P=0.203		
Fisher exact test		P=0.500	P=0.003		P=0.121	P=0.247
Epithelial Neoplasms (all types)						
Overall rate	0/50 (0%)	4/50 (8%)	18/50 (36%)	0/50 (0%)	4/50 (8%)	9/50 (18%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P=0.059	P<0.001		P=0.059	P=0.001

[a] Number of lesion-bearing animals/number of animals necropsied
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Not applicable; no lesions in animal group

TABLE 23
Incidences of Neoplasms and Nonneoplastic Lesions of the Forestomach in Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	88 mg/kg	177 mg/kg	Vehicle Control	88 mg/kg	177 mg/kg
Epithelial Dysplasia						
Overall rate[a]	0/50 (0%)	14/50 (28%)	33/49 (67%)	0/50 (0%)	16/49 (32%)	41/50 (82%)
Cochran-Armitage test[b]	P<0.001			P<0.001		
Fisher exact test[b]		P<0.001	P<0.001		P<0.001	P<0.001
Squamous Cell Papilloma						
Overall rate	0/50 (0%)	12/50 (24%)	20/49 (41%)	0/50 (0%)	12/49 (24%)	17/50 (34%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	2/50 (4%)	1/49 (2%)	0/50 (0%)	7/49 (14%)	6/50 (12%)
Cochran-Armitage test	P=0.357			P=0.026		
Fisher exact test		P=0.247	P=0.495		P=0.006	P=0.013
Squamous Cell Papilloma or Squamous Cell Carcinoma						
Overall rate	0/50 (0%)	14/50 (28%)	21/49 (43%)	0/50 (0%)	18/49 (37%)	19/50 (38%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.

TABLE 24
Incidences of Neoplasms and Nonneoplastic Lesions of the Lung in Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Male			Female		
	Vehicle Control	88 mg/kg	177 mg/kg	Vehicle Control	88 mg/kg	177 mg/kg
Focal Hyperplasia						
Overall rate[a]	0/50 (0%)	1/50 (2%)	6/50 (12%)	0/50 (0%)	6/50 (12%)	5/50 (10%)
Cochran-Armitage test[b]	P=0.004			P=0.042		
Fisher exact test[b]		P=0.500	P=0.013		P=0.013	P=0.028
Pleomorphism (Lung/bronchiole)						
Overall rate	0/50 (0%)	50/50 (100%)	50/50 (100%)	0/50 (0%)	46/50 (92%)	50/50 (100%)
Cochran-Armitage test	P<0.001			P<0.001		
Fisher exact test		P<0.001	P<0.001		P<0.001	P<0.001
Alveolar/bronchiolar Adenoma						
Overall rate	1/50 (2%)	1/50 (2%)	6/50 (12%)	0/50 (0%)	3/50 (6%)	4/50 (8%)
Cochran-Armitage test	P=0.022			P=0.049		
Fisher exact test		P=0.753	P=0.056		P=0.121	P=0.059
Alveolar/bronchiolar Carcinoma						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	0/50 (0%)	0/50 (0%)
Cochran-Armitage test	–[c]			P=0.333N		
Fisher exact test		–	–		P=0.500N	P=0.500N
Alveolar/bronchiolar Adenoma or Carcinoma						
Overall rate	1/50 (2%)	1/50 (2%)	6/50 (12%)	1/50 (2%)	3/50 (6%)	4/50 (8%)
Cochran-Armitage test	P=0.022			P=0.133		
Fisher exact test		P=0.753	P=0.056		P=0.309	P=0.181

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[c] Not applicable; no lesions in animal group

Liver: The incidence of eosinophilic cytoplasmic change in high-dose male mice was significantly greater than that in the controls (Table 25). Moreover, basophilic cytoplasmic change occurred in one low-dose and two high-dose males, but not in the controls (Table C4). The incidence of hepatocellular adenoma or carcinoma (combined) in high-dose males was also significantly greater than that in the controls (Table 25). Chemical-related increases in the incidences of foci of cytoplasmic change or hepatocellular neoplasms did not occur in female mice (Tables D1 and D4).

TABLE 25
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Male Mice in the Long-Term Dermal Study of 2,3-Dibromo-1-propanol

	Vehicle Control	88 mg/kg	177 mg/kg
Eosinophilic Cytoplasmic Change			
Overall rate[a]	0/50 (0%)	0/50 (0%)	11/50 (22%)
Cochran-Armitage test[b]	P<0.001		
Fisher exact test[b]		–[c]	P<0.001
Hepatocellular Adenoma			
Overall rate	1/50 (2%)	2/50 (4%)	9/50 (18%)
Cochran-Armitage test[b]	P=0.003		
Fisher exact test[b]		P=0.500	P=0.008
Hepatocellular Carcinoma			
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)
Cochran-Armitage test	P=0.036		
Fisher exact test		–	P=0.121
Hepatocellular Adenoma or Carcinoma			
Overall rate	1/50 (2%)	2/50 (4%)	11/50 (22%)
Cochran-Armitage test	P<0.001		
Fisher exact test		P=0.500	P=0.002

[a] Number of lesion-bearing animals/number of animals with tissue examined microscopically
[b] Beneath the control incidence are the P values associated with the trend test. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates.
[c] Not applicable; no lesions in animal group

GENETIC TOXICOLOGY

2,3-Dibromo-1-propanol was mutagenic in all but one of the short-term tests conducted by the NTP. It induced gene mutations in three strains of *Salmonella typhimurium* (TA98, TA100, and TA1535) when tested in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no clearly positive response was observed in strain TA1537 (Table E1; Haworth *et al.*, 1983). 2,3-Dibromo-1-propanol produced a positive response in the absence of S9 activation in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells; it was not tested with S9 (Table E2). Increases in sister chromatid exchanges and chromosomal aberrations were induced in cultured Chinese hamster ovary cells both with and without Aroclor 1254-induced male Sprague-Dawley rat liver S9 (Tables E3 and E4). 2,3-Dibromo-1-propanol induced significant increases in sex-linked recessive lethal mutations and reciprocal translocations in male germ cells of *Drosophila melanogaster* (Tables E5 and E6; Yoon *et al.*, 1985). Intraperitoneal injection (25 to 100 mg/kg) of 2,3-dibromo-1-propanol, administered three times at 24-hour intervals, did not increase the frequency of micronucleated polychromatic erythrocytes in the bone marrow of male B6C3F$_1$ mice sampled 24 hours after the third injection. Also, the percentages of polychromatic erythrocytes among the total erythrocyte population were not affected by 2,3-dibromo-1-propanol administration, indicating no toxicity in the bone marrow.

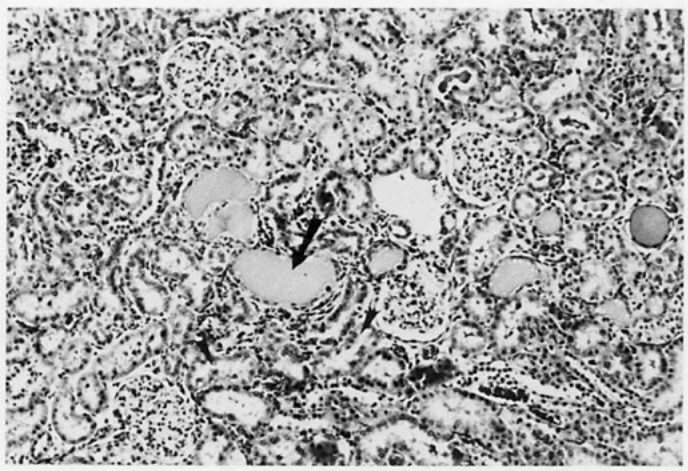


PLATE 1
Nephropathy of the kidney in a male F344/N rat receiving 750 mg/kg 2,3-dibromo-1-propanol in the 13-week dermal study. Note the hyaline casts (arrow), tubules lined by small basophilic epithelial cells (arrow head), and interstitial cellular infiltrate. H&E, 120X

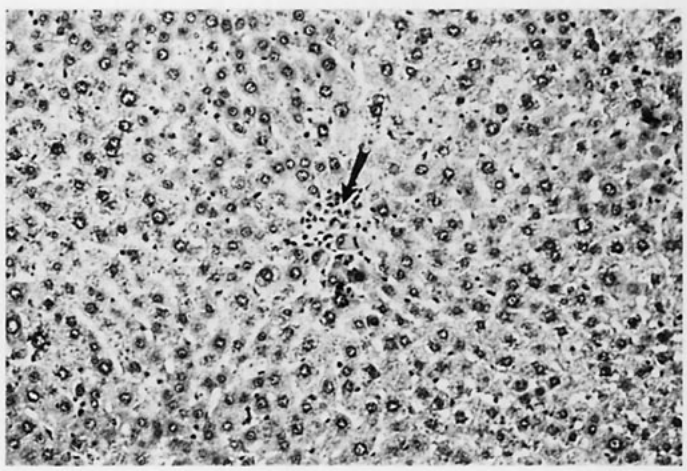


PLATE 2
Liver of a female F344/N rat receiving 750 mg/kg 2,3-dibromo-1-propanol in the 13-week dermal study. Note the focal accumulation of inflammatory cells surrounding individual necrotic hepatocytes (arrow). H&E, 25X

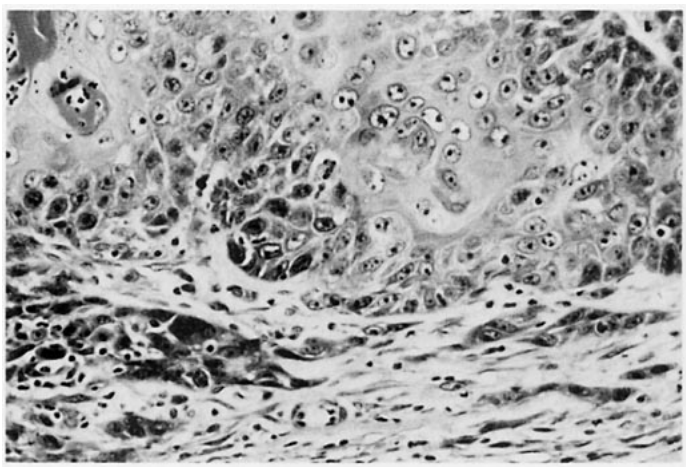


PLATE 3
Squamous cell carcinoma of the skin in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Note the cellular atypia, disordered pattern of differentiation, and invasion by anaplastic cells. H&E, 80X

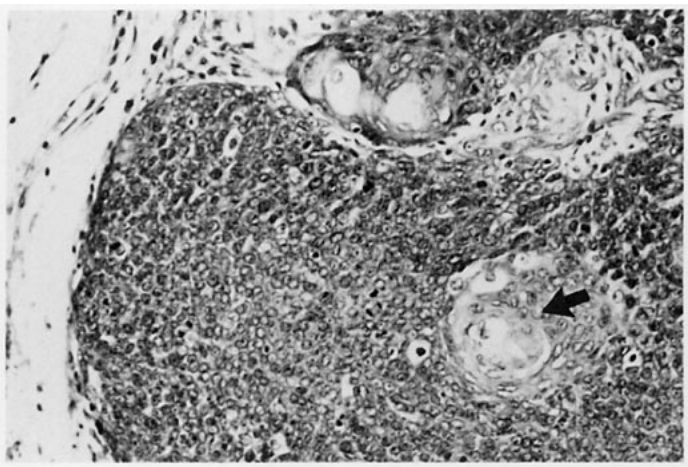


PLATE 4
Basal cell tumor of the skin in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. The neoplastic cells typically have scant, basophilic cytoplasm although small foci of squamous differentiation may be present (arrow). H&E, 80X

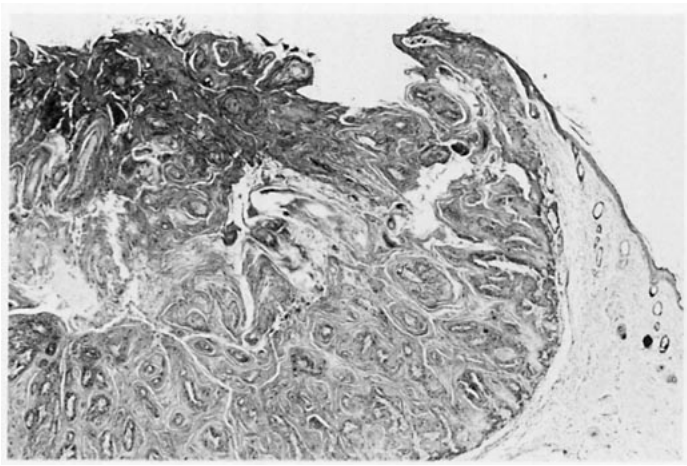


PLATE 5
Keratoacanthoma of the skin in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Note how the tumor extends below the surface of the skin to form a crateriform mass within the dermis and subcutis. H&E, 10X

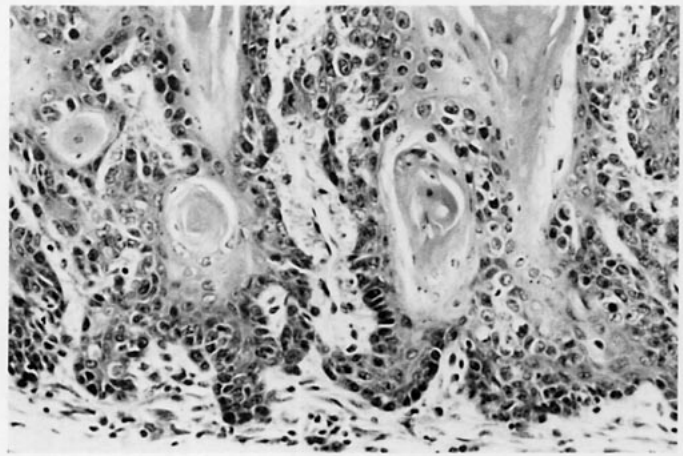


PLATE 6
High magnification of the keratoacanthoma in Plate 5 showing the keratinized, stratified squamous epithelium comprising the wall of the neoplasm. H&E, 80X

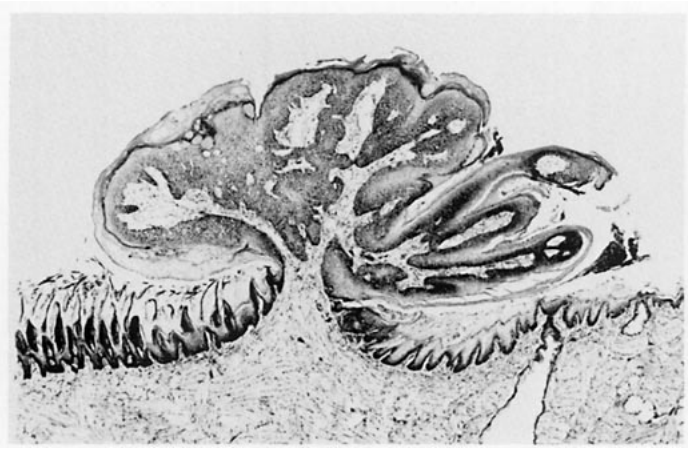


PLATE 7
Squamous cell papilloma of the tongue in a male F344/N rat receiving 188 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 30X

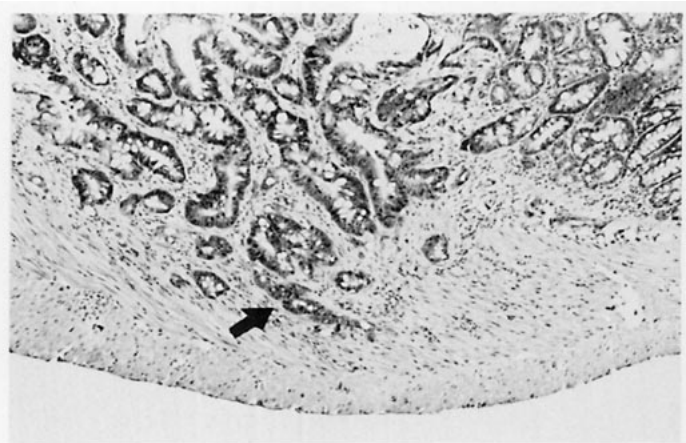


PLATE 8
Adenocarcinoma of the jejunum in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Note the invasion of the submucosa and tunica muscularis (arrow). H&E, 80X

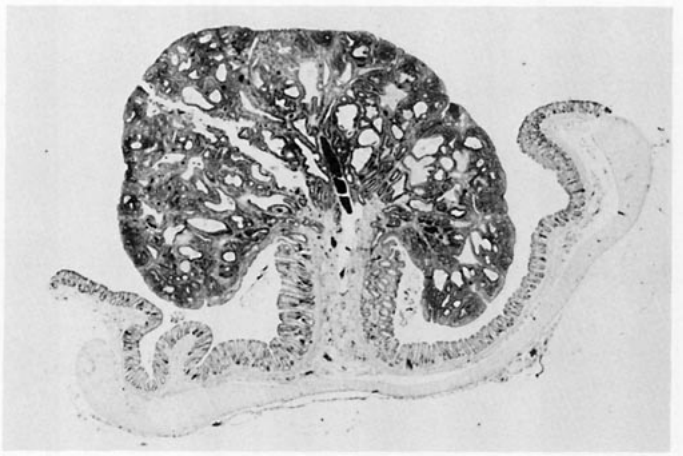


PLATE 9
Adenomatous polyp of the colon in a male F344/N rat receiving 188 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 12X

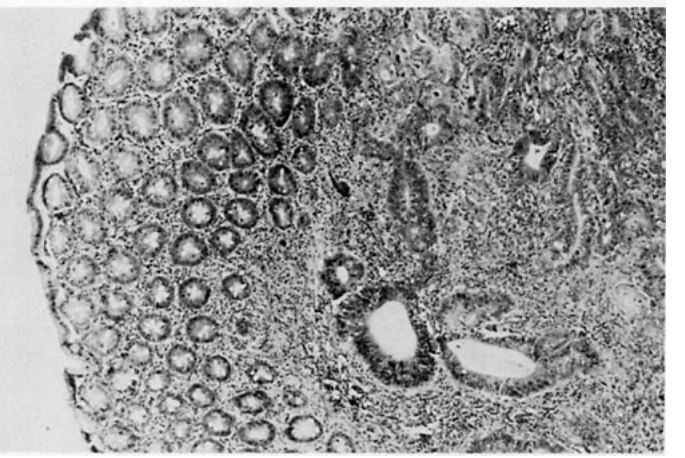


PLATE 10
Adenocarcinoma of the cecum in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Irregular glands and tubules lined by poorly differentiated epithelium invade the submucosa. H&E, 25X

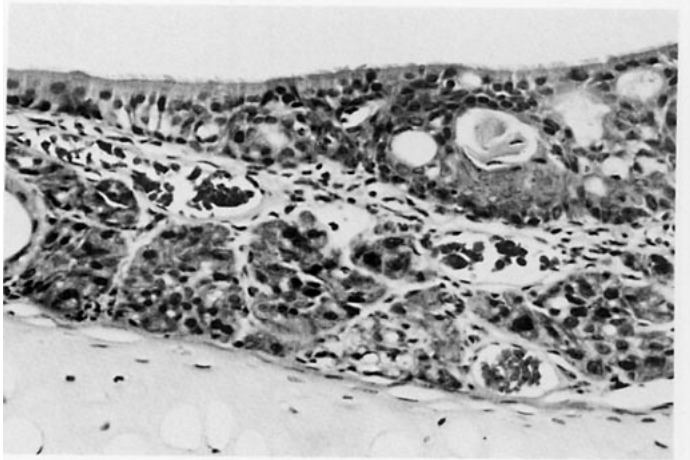


PLATE 11
Dysplasia of the respiratory epithelium in the dorsal aspect of the nasal septum in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Note the proliferation of basal cells and squamous metaplasia. H&E, 80X

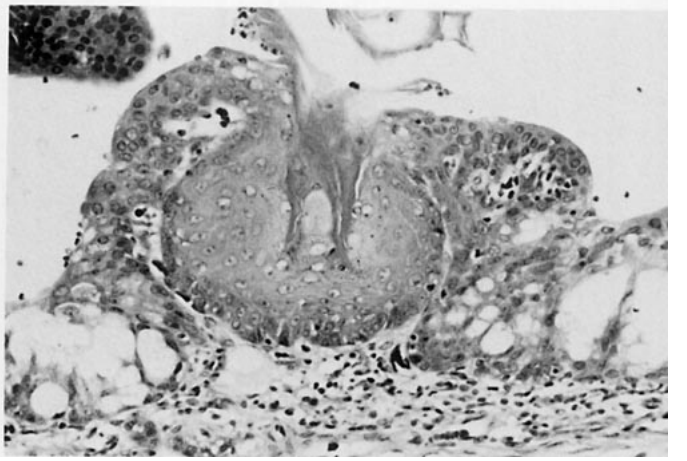


PLATE 12
Prominent focus of squamous metaplasia of the respiratory epithelium on the nasal septum in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 80X

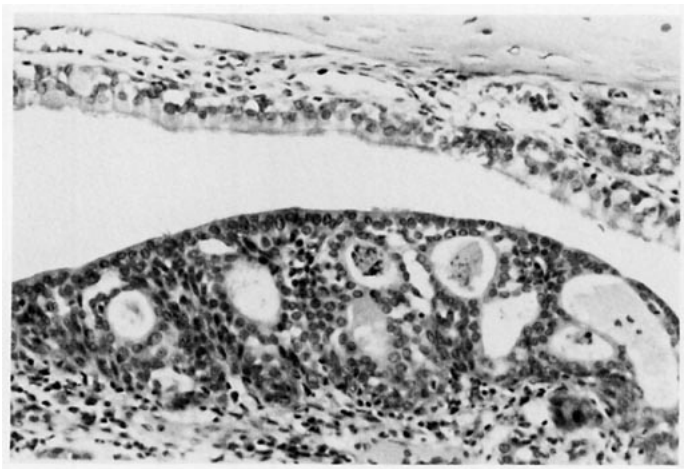


PLATE 13
Proliferation of basal cells on the lateral aspect of nasoturbinate near junction of respiratory and olfactory epithelium in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 80X

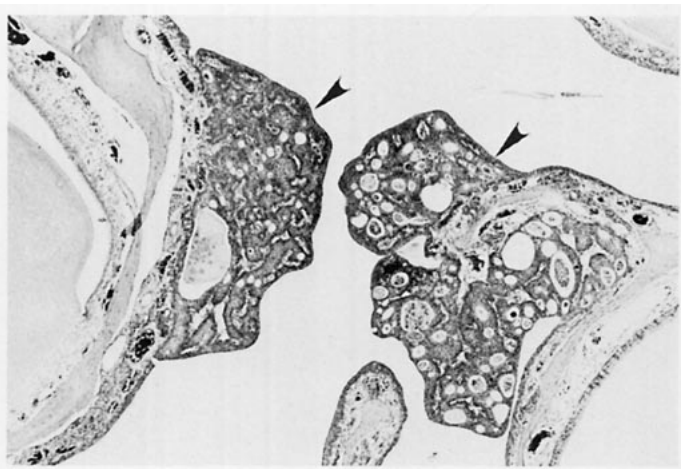


PLATE 14
Adenomas (arrowheads) of the nasal cavity in a male F344/N rat receiving 188 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 40X

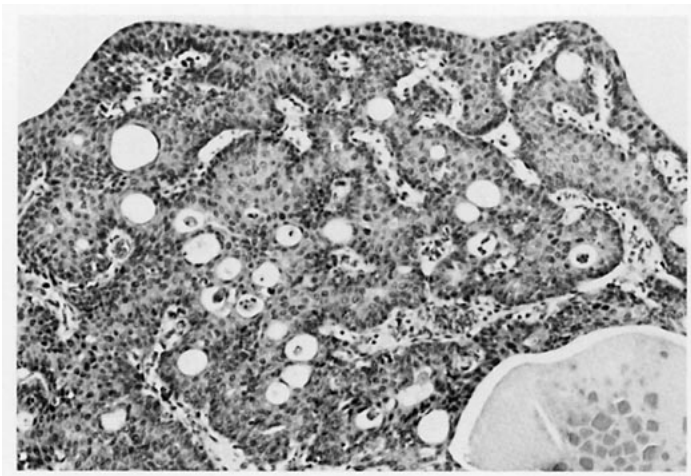


PLATE 15
Adenoma of the nasal epithelium in a male F344/N rat receiving 375 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 50X

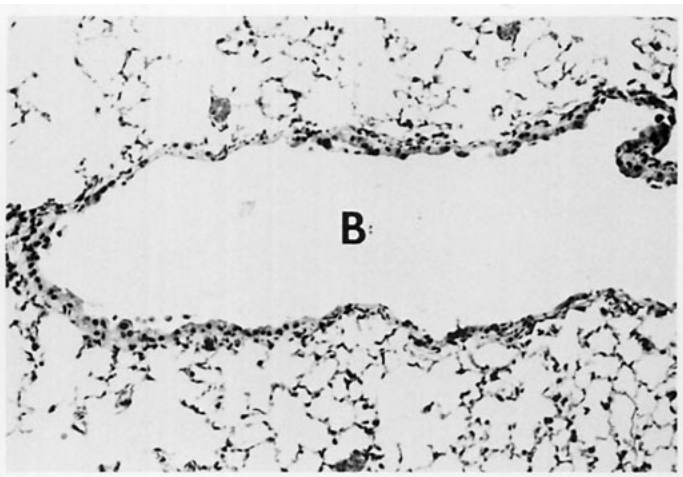


PLATE 16
Lung of a male B6C3F$_1$ mouse receiving 750 mg/kg 2,3-dibromo-1-propanol in the 13-week dermal study. The epithelium of the bronchiole (B) is reduced in cellularity and consists of flattened cells of varying size as a result of necrosis. H&E, 25X

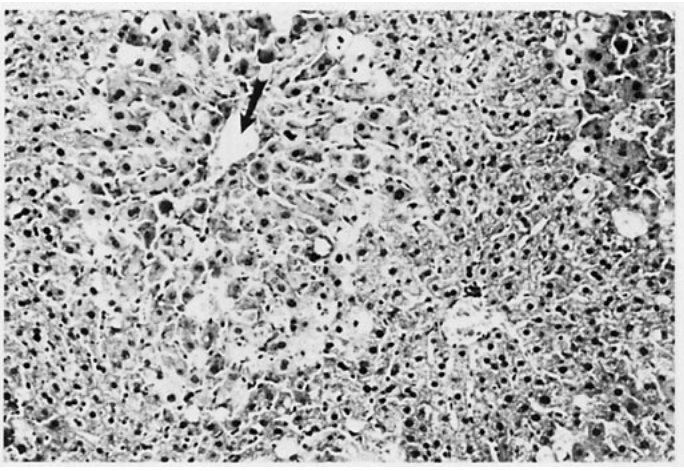


PLATE 17
Centrilobular hepatocellular necrosis of the liver in a male B6C3F$_1$ mouse that died after receiving 750 mg/kg 2,3-dibromo-1-propanol during the 13-week dermal study. The necrotic hepatocytes surrounding the central venule (arrow) have hyaline, eosinophilic cytoplasm, and pyknotic nuclei. H&E, 25X

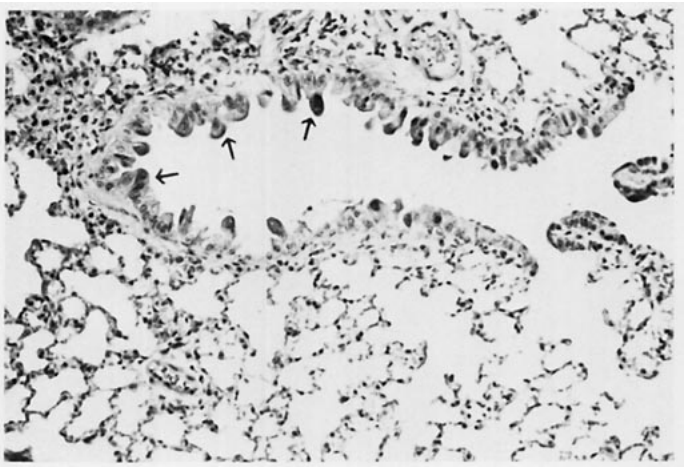


PLATE 18
Lung of a female B6C3F$_1$ mouse receiving 750 mg/kg 2,3-dibromo-1-propanol in the 13-week dermal study. The bronchiolar epithelium (arrows) consists of enlarged, pleomorphic cells with hyperchromatic, karyomegalic nuclei. H&E, 25X

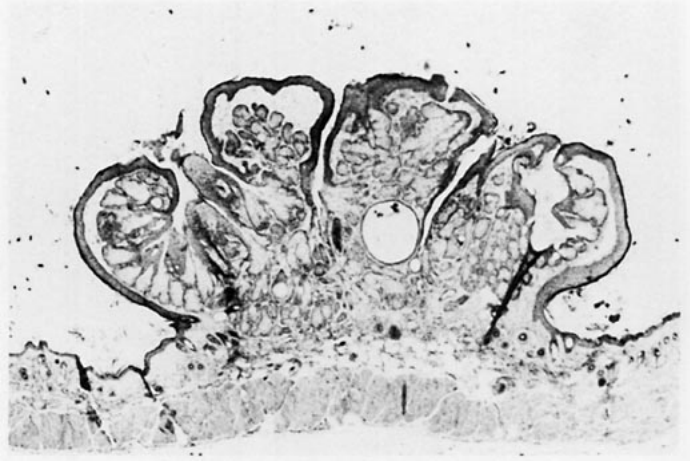


PLATE 19
Sebaceous gland adenoma of the skin (site of application) in a male B6C3F$_1$ mouse receiving 177 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. H&E, 30X

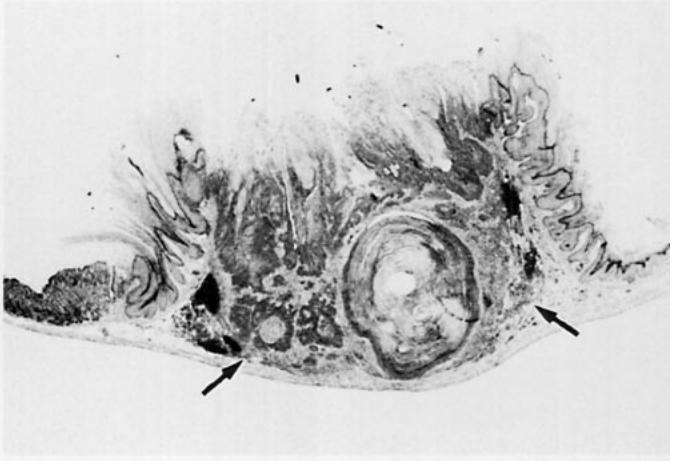


PLATE 20
Squamous cell carcinoma of the forestomach in a female B6C3F$_1$ mouse receiving 177 mg/kg 2,3-dibromo-1-propanol in the long-term dermal study. Note the invasion (arrows) into underlying mucosa. H&E, 15X

DISCUSSION AND CONCLUSIONS

2,3-Dibromo-1-propanol has been used as a flame retardant, as an intermediate in the preparation of other flame retardants including tris(2,3-dibromopropyl) phosphate, and as an intermediate in the preparation of insecticides and pharmaceutical preparations (Fishbein, 1979). 2,3-Dibromo-1-propanol was nominated by the National Cancer Institute (NCI) for toxicology and carcinogenicity testing as part of organohalide class studies and because it is a metabolite of tris(2,3-dibromopropyl) phosphate, previously shown to be a mutagen and a carcinogen in animals (NCI, 1978a). Toxicology and carcinogenicity studies were conducted by applying the chemical to the skin of male and female F344/N rats and B6C3F$_1$ mice. Comparative single-dose gavage and skin-paint studies showed that 2,3-dibromo-1-propanol, at doses ranging from 88 to 1,500 mg/kg body weight in ethanol, was well absorbed from the skin of rats and mice (Appendix J). The absorption efficiency for skin relative to gavage was 68% for rats and 37% for mice. Because the primary route of human exposure to flame retardants is through the skin, the dermal route of administration was chosen for the studies.

Male mice were more sensitive to the acute toxic effects of this chemical than were rats or female mice. Eight of 10 male mice receiving dermal applications of 750 mg/kg died during the 13-week study, but there were no deaths in rats or female mice receiving up to 750 mg/kg 2,3-dibromo-1-propanol for 13 weeks. Male mice dying as a result of treatment with 2,3-dibromo-1-propanol had generalized centrilobular necrosis of the liver. The regional specificity of the necrosis in male mice is consistent with the zonal differences in hepatic enzymes and the postulated metabolic pathway of 2,3-dibromo-1-propanol. The centrilobular region of the liver lobule (zone 3 of the liver acinus) is believed to be the region most susceptible to injury by certain chemicals because of its higher content of cytochrome P-450, epoxide hydrolase, and glutathione transferase (Gumucio and Miller, 1982). The centrilobular hepatocellular necrosis produced by bromobenzene is believed to be determined, in part, by the relative rates of conversion to a reactive epoxide intermediate by microsomal enzymes and subsequent reaction with glutathione transferase and epoxide hydrolase (Mitchell *et al.*, 1976). Similar to bromobenzene, 2,3-dibromo-1-propanol is believed to be metabolized by microsomal cytochrome P-450 to reactive intermediates (Jones and Fakhouri, 1979; Marsden and Casida, 1982). Whether the postulated metabolites of 2,3-dibromo-1-propanol, 2-bromoacrolein and 3-bromo-1,2-propane epoxide, are the direct cause of cellular injury leading to hepatocellular necrosis is unknown.

In contrast to male mice, female mice and female rats receiving dermal applications of 750 mg/kg 2,3-dibromo-1-propanol exhibited slight individual cell necrosis in the liver. The lesion consisted of a very small number of necrotic hepatocytes, sometimes associated with small numbers of inflammatory cells, in the liver sections. The differences in the distribution and severity of the liver lesions between male mice and those in female rats and female mice may be determined, in part, by the effective dose of the critical metabolites (possibly 3-bromo-1,2-propane epoxide or 2-bromoacrolein) at the target site(s) within the hepatocyte. Thus, generalized centrilobular hepatocellular necrosis might have occurred in female rats and female mice, and perhaps male rats, at higher dose levels of 2,3-dibromo-1-propanol.

Liver lesions have been observed in humans exposed to 1,2-dichloropropane (Larcan *et al.*, 1977) and in laboratory animals, including rats and mice, exposed to short-chain halogenated hydrocarbons such as carbon tetrachloride, chloroform, trichloroethylene, 1,1,2-trichloroethane (Plaa, 1986), 1,2,3-trichloropropane, and perchloroethylene (Sidorenko *et al.*, 1976). Necrosis has been observed in the lung and liver of rats exposed to 1,3-dichloropropene by inhalation (Torkelson and Oyen, 1977) and in the liver of female F344/N rats and B6C3F$_1$ mice administered 1,2-dichloropropane by gavage (NTP, 1986).

Lung lesions also occured in mice in the 13-week study. Five of the eight male mice receiving 750 mg/kg that died during the 13-week study had necrosis of the bronchial and bronchiolar epithelium, while males and females exhibited cytologic alterations (pleomorphism) in the distal airway epithelium. Because there may have been some volatilization of 2,3-dibromo-1-propanol after dermal application, inhalation exposure in the group-housed mice may have contributed to the lesions in the pulmonary airways. It is unknown why the intrapulmonary airways were more sensitive to 2,3-dibromo-1-propanol than the nasal and tracheal epithelium, but the secondary bronchi and bronchioles contain fewer goblet cells and a higher proportion of Clara cells, which are known to contain microsomal cytochrome P-450. The differences in cell population and in airflow pattern and velocity are thought to contribute to the regional specificity of airway lesions caused by chemicals.

The cytologic alterations in the epithelium of distal airways of male and female mice receiving dermal applications of 2,3-dibromo-1-propanol consisted of changes in cell size and shape (pleomorphism) and nuclear enlargement (karyomegaly). Whether this lesion is also caused by the formation of an epoxide intermediate or 2-bromoacrolein merits further study. Similar cytologic alterations have been observed in male and female mice exposed by inhalation to 1,2-dibromo-3-chloropropane (NTP, 1982a), 1,2-dibromoethane (NTP, 1982b), and 1,2-dichloropropane (NTP, 1986), and by gavage to 1,2,3-trichloropropane in NTP 13-week studies (NTP, 1993).

Although the body weight gain of rats receiving dermal applications of 750 mg/kg was 11% lower than that of the controls for males and 13% lower for females in the 13-week study, only slight histopathologic effects were observed. In male rats there was a slight increase in the severity of nephropathy, primarily in the 750 mg/kg group. Although it is apparent that 2,3-dibromo-1-propanol has some effect on the kidneys, these findings confirm previous studies indicating that 2,3-dibromo-1-propanol is not the primary metabolite responsible for the acute renal tubule necrosis associated with the administration of tris(2,3-dibromopropyl) phosphate to rats (Søderlund *et al.*, 1980). Chemical-induced nephrotoxicity in rats and mice in NTP/NCI studies has been associated with exposure to many short-chain halogenated hydrocarbons, but no consistent sex- or species-related differences in response were found (Kluwe *et al.*, 1984). In general, however, rats seem to be more susceptible to the nephrotoxic effects of these compounds than mice, and male rats appear to be more susceptible than female rats.

The highest dose selected for the planned 2-year dermal study in rats was 375 mg/kg because of the significant reduction in body weight gain and slight histopathologic effects observed in rats receiving 750 mg/kg in the 13-week study. Primarily because of the high mortality and hepatocellular necrosis in male mice receiving dermal applications of 750 mg/kg in the 13-week study, the highest dose selected for the planned 2-year study in mice was 177 mg/kg. The planned 2-year studies were terminated early after serological evidence of infection with lymphocytic choriomeningitis (LCM) virus was found in mice. This virus can infect humans, occasionally producing severe meningitis and death, thus posing a hazard to laboratory workers. Moreover, the early mortality in male rats receiving dermal applications of 350 mg/kg was an indication that the chemical is a potent carcinogen.

The viral infection in mice in the NTP long-term study was asymptomatic and no histologic evidence of disease was observed. The source of the infection is unknown, but could possibly have resulted from exposure to feral mice (Lehmann-Grube, 1982). Although *in utero* or perinatal infection can be a source of persistently infected mice, there was no evidence of infection in the breeding colony from which the study animals were obtained. The LCM virus has been shown to depress humoral and/or cellular immunity and to inhibit neoplasm induction by viruses such as polyoma virus and mammary tumor virus in mice (National Research Council, 1991). Nevertheless, it is unlikely that the LCM infection had any influence on the outcome of these long-term studies as it relates to the carcinogenic potential of this chemical, because a) the infection occurred in only 13% of the mice tested, b) the infection occurred in both dosed and control mice, c) the incidence of neoplasms in the control mice was very low and was within the expected range for historical controls of that age, d) the induced neoplasms in dosed mice occurred with very high incidences and short latency, and e) a

similar strong carcinogenic response occurred in the rats, which were not infected with LCM virus.

In the long-term study, the decreased final mean body weights of rats receiving 2,3-dibromo-1-propanol, particularly the high-dose groups, were largely due to impaired feed consumption resulting from chemical-related neoplasms of the oral mucosa. The significantly reduced survival of dosed rats was the result of aggressive moribund sacrifices necessitated by these neoplasms in dosed rats. In contrast, neither the final mean body weights nor the survival of mice was affected by 2,3-dibromo-1-propanol administration.

2,3-Dibromo-1-propanol caused significant dose-related increases in the incidences of neoplasms at numerous sites in male and female rats and, to a lesser extent, in mice in the long-term dermal studies. The total numbers of male and female rats with benign and malignant neoplasms, as well as the total numbers of these neoplasms, were significantly greater in the low- and high-dose groups than in the controls. Nearly all dosed rats, but only 2% of the control rats, had malignant neoplasms. The organs or tissues with significantly increased incidences of neoplasms included the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, liver, and Zymbal's gland of male and female rats as well as the mammary gland and clitoral gland of females. Marginally increased incidences of neoplasms also occurred in the kidney of male and female rats and in the tunica vaginalis mesothelium and spleen of male rats. No neoplasms were observed in control rats at any of these sites, with the exception of one control male that had a squamous cell papilloma of the skin. Accordingly, the increased incidence of neoplasms observed at various sites was considered to be clearly related to 2,3-dibromo-1-propanol administration.

The pattern of neoplasm response in the stratified squamous epithelium of the upper gastrointestinal tract of rats suggests that the chemical induction of neoplasms in the oral mucosa, esophagus, and forestomach may be related to oral exposure through grooming behavior rather than from dermal absorption. The incidences of squamous cell neoplasms and the proportion of malignant to benign neoplasms decreased as the distance from the oral cavity increased (Figure 5). Of these three sites, the incidence of squamous cell neoplasms and the proportion of carcinomas was highest in the oral mucosa. The incidence of squamous cell neoplasms in the esophagus was intermediate between those of the oral cavity and forestomach, and few carcinomas were observed. The lowest incidence of neoplasms occurred in the forestomach, and no carcinomas were observed. Exposure by inhalation may also have contributed to the induction of neoplasms of the nasal mucosa.

There were some differences between male and female rats in response to the carcinogenic activity of 2,3-dibromo-1-propanol, including lower incidences of liver neoplasms and higher incidences of adenocarcinomas of the small intestine in dosed males than in dosed females. Although there was a small difference in the duration of treatment between sexes (4 weeks), it seems unlikely that this difference contributed substantively to the differences in neoplasm incidence at these sites. The lower incidences of adenocarcinomas of the small intestine in exposed female rats than in males may be related to the later appearance of this neoplasm in females. The first adenocarcinoma was observed at week 25 for males and at week 48 for females.

Dermal exposure of mice to a concentration of 2,3-dibromo-1-propanol similar to that received by rats induced neoplasms at fewer sites and lower overall incidences. Chemical-induced neoplasms were observed at the site of application (the skin) as well as the forestomach, liver, and lung of males and the forestomach of females. A slight increase in lung neoplasms in female mice may also have been chemical induced. The duration of exposure in mice was about 12 weeks less than in rats; this shorter duration may have contributed to the differences in carcinogenic response between rats and mice. Nevertheless, based on the greater number of neoplasm sites observed in dosed rats compared to dosed mice (11 sites for male rats, 12 for female rats, 4 for male mice, and 2 for female mice), rats appear to be more sensitive to the carcinogenic effect of 2,3-dibromo-1-propanol.

The results of these studies showed that 2,3-dibromo-1-propanol is a multiple-organ carcinogen in rats and mice, as are its parent compound tris(2,3-dibromopropyl) phosphate (NCI, 1978a; Reznick *et al.*,

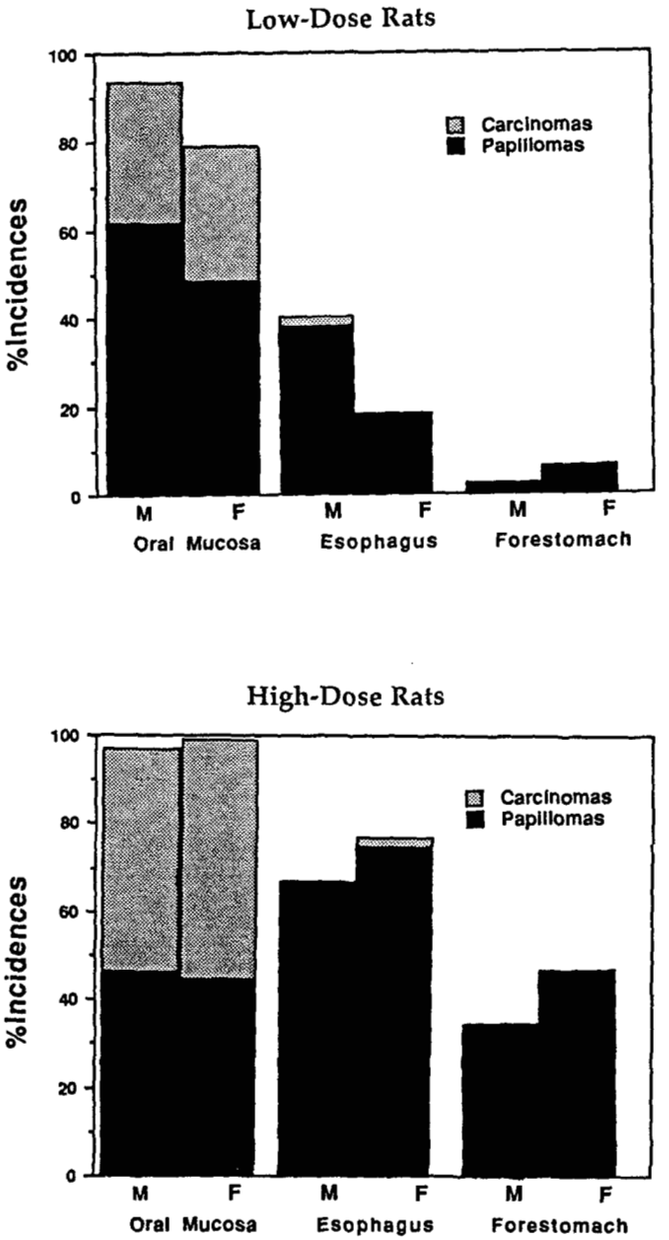


FIGURE 5
Neoplasms of the Upper Gastrointestinal Tract in Rats Administered 2,3-Dibromo-1-propanol by Dermal Application for 51 or 55 Weeks

1981) and the structurally related halogenated three-carbon compounds, 1,2-dibromo-3-chloropropane (NCI, 1978b; NTP, 1982a), 1,2-dichloropropane (NTP, 1986), and 1,3-dichloropropene (NTP, 1985). However, the number of sites affected by the dermal application of 2,3-dibromo-1-propanol was greater than the number of sites affected by the dosed feed or gavage administration of tris(2,3-dibromopropyl) phosphate and other halogenated three-carbon compounds (Tables 26 and 27). Although differences in dose level, strains of animals, route of administration, and duration of dose employed in the various studies could have contributed to the variation in response to these chemicals, the results suggest that 2,3-dibromo-1-propanol is the most potent carcinogen of these chemicals.

Among the short-chain hydrocarbons, including the halogenated hydrocarbons, are chemicals that are direct-acting carcinogens, such as epoxides and halo ethers, and others that are considered indirect-acting carcinogens, which require metabolic activation to the ultimate carcinogen in tissues such as the liver, stomach, lung, or kidney (Van Duuren, 1977). Epoxide intermediates are demonstrated metabolites of trichloroethylene (epoxy-1,1,2-trichloroethane), allyl chloride (epichlorohydrin and glycidaldehyde) (Van Duuren, 1977), and 1,2-dibromo-3-chloropropane (1,2-epoxypropane) (Jones and Gibson, 1980). 2,3-Dibromo-1-propanol is a direct-acting mutagen, producing gene mutations in *Salmonella typhimurium* and gene mutation and chromosomal damage in cultured mammalian cells (Appendix E). It also produced sex-linked recessive lethal mutations and reciprocal translocations in germ cells of *Drosophila melanogaster.* Moreover, the metabolism of 2,3-dibromo-1-propanol also appears to involve the formation of reactive intermediates including 2-bromoacrolein, 2,3-dibromopropanal (Marsden and Casida, 1982), and 3-bromo-1,2-propane epoxide (Jones and Fakhouri, 1979). The first two intermediates are direct mutagens in *S. typhimurium* and are potent inducers of DNA single-strand breaks in rat hepatoma cells (Gordon *et al.*, 1985). This mutagenic and chemical profile is consistent with the pattern of carcinogenic activity observed in these studies, that is, the induction of an early onset of neoplasms at multiple sites.

TABLE 26
Comparison of Neoplasm Sites in Rats Exposed to 2,3-Dibromo-1-propanol and Structurally Related Compounds

Compound/ Technical Report	Strain	Exposure Route and Duration	Dose	Neoplasm Site	
				Male	Female
Tris(2,3-dibromopropyl) phosphate NTP TR 76	F344/N	Dosed feed for 2 years	50 or 100 ppm (2.5 or 5 mg/kg per day)	Kidney	Kidney
1,2-Dibromo-3-chloropropane NTP TR 28	Osborne-Mendel	Corn oil gavage for 2 years	15 or 29 mg/kg per day	Forestomach	Forestomach Mammary gland
1,2-Dibromo-3-chloropropane NTP TR 206	F344/N	Inhalation for 76-103 weeks (6 hours/day, 5 days/week)	0.6 or 3.0 ppm	Nose Tongue	Nose Tongue Adrenal gland Mammary gland
1,3-Dichloropropene NTP TR 269	F344/N	Corn oil gavage for 2 years	25 or 50 mg/kg per day	Forestomach Liver	Forestomach
1,2-Dichloropropane NTP TR 263	F344/N	Corn oil gavage for 2 years	Males 125 or 250 mg/kg per day; females 62 or 125 mg/kg per day	None	None
2,3-Dibromo-1-propanol NTP TR 400	F344/N	Dermal for 48-55 weeks	188 or 375 mg/kg per day	Skin Nose Oral mucosa Esophagus Forestomach Small intestine Large intestine Liver Kidney Tunica vaginalis Zymbal's gland	Skin Nose Oral mucosa Esophagus Forestomach Small intestine Large intestine Liver Kidney Mammary gland Clitoral gland Zymbal's gland

TABLE 27
Comparison of Neoplasm Sites in Mice Exposed to 2,3-Dibromo-1-propanol and Structurally Related Compounds

Compound/ Technical Report	Strain	Exposure Route and Duration	Dose	Neoplasm Site	
				Male	Female
Tris(2,3-dibromopropyl) phosphate NCI TR 76	B6C3F$_1$	Dosed feed for 2 years	500 or 1,000 ppm (65 or 130 mg/kg per day)	Kidney Forestomach Lung	Kidney Forestomach Lung Liver
Tris(2,3-dibromopropyl) phosphate Van Duuren *et al.*, 1978	ICR/Ha Swiss	Dermal for 67-71 weeks	10 or 30 mg/kg per day	Skin Forestomach Oral cavity Lung	Skin Forestomach Oral cavity Lung
1,2-Dibromo-3-chloropropane NCI TR 28	B6C3F$_1$	Corn oil gavage for 2 years	110 or 220 mg/kg per day	Forestomach	Forestomach
1,2-Dibromo-3-chloropropane NTP TR 206	B6C3F$_1$	Inhalation for 76-103 weeks (6 hours/day, 5 days/week)	0.6 or 3.0 ppm	Nose Lung	Nose Lung
1,3-Dichloropropene[a] NTP TR 269	B6C3F$_1$	Corn oil gavage for 2 years	50 or 100 mg/kg per day	Inadequate study	Urinary bladder Forestomach Lung
1,2-Dichloropropane NTP TR 263	B6C3F$_1$	Corn oil gavage for 2 years	125 or 250 mg/kg per day	Liver	Liver
2,3-Dibromo-1-propanol NTP TR 400	B6C3F$_1$	Dermal for 36-42 weeks	88 or 177 mg/kg per day	Skin Forestomach Liver Lung	Skin Forestomach

[a] Low survival in male control group

CONCLUSIONS

Under the conditions of these long-term dermal studies, there was *clear evidence of carcinogenic activity** of 2,3-dibromo-1-propanol in male F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, kidney, tunica vaginalis, and spleen. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in female F344/N rats based on increased incidences of neoplasms of the skin, nose, oral mucosa, esophagus, forestomach, small and large intestine, Zymbal's gland, liver, kidney, clitoral gland, and mammary gland. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in male B6C3F$_1$ mice based on increased incidences of neoplasms of the skin, forestomach, liver, and lung. There was *clear evidence of carcinogenic activity* of 2,3-dibromo-1-propanol in female B6C3F$_1$ mice based on increased incidences of neoplasms of the skin and the forestomach. The increased incidences of alveolar/bronchiolar adenomas in female mice may have been related to chemical administration.

In rats, 2,3-dibromo-1-propanol caused increased incidences of hyperkeratosis in the skin, forestomach, and esophagus, epithelial dysplasia in the nose, pleomorphism and basophilic and clear cell changes in the liver, and nuclear enlargement in the kidney. There were also chemical-related increases in the incidences of forestomach ulcers and acanthosis, angiectasis in the liver, and renal hyperplasia in male rats and epithelial dysplasia of the forestomach and bile duct hyperplasia in the liver in female rats. Chemical-related increases occurred in the incidences of hyperplasia in the skin, epithelial dysplasia of the forestomach, and bronchiolar epithelial pleomorphism and hyperplasia in male and female mice and in the incidence of eosinophilic cytoplasmic change in the liver in males.

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

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TRIS(2,3-DIBROMOPROPYL) PHOSPHATE

1. Chemical and Physical Data

1.1 Synonyms and trade names

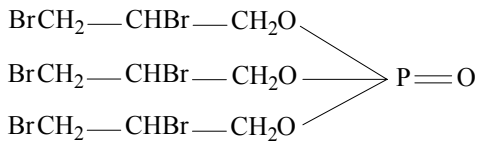
Chem. Abstr. Services Reg. No.: 126-72-7

Chem. Abstr. Name: 2,3-Dibromo-l-propanol phosphate (3:1)

Synonyms: 2,3-Dibromo-l-propanol phosphate; (2,3-dibromopropyl) phosphate; tris(2,3-dibromopropyl) phosphoric acid ester; Tris

Trade names: Anfram 3PB; Apex 462-5; Bromkal P 67-6HP; ES 685; Firemaster LV-T 23P; Firemaster T 23P; Flacavon R; Flamex T 23P; Flammex AP; Flammex T 23P; T 23P; Zetofex ZN

1.2 Structural and molecular formulae and molecular weight



$C_9H_{15}Br_6O_4P$ Mol. wt: 697.7

1.3 Chemical and physical properties of the pure substance

From Lande *et al.* (1976), unless otherwise specified

(a) Description: Viscous, pale-yellow liquid (Hawley, 1977)

(b) Freezing-point: 5.5°C

(c) Density: d^{25} 2.27

(d) Spectroscopy data: Infra-red and nuclear magnetic resonance spectral data have been tabulated (Grasselli & Ritchey, 1975).

(e) Refractive index: n_D^{20} 1.5772 (Hawley, 1977)

(f) Solubility: Insoluble in water; miscible with carbon tetrachloride, chloroform and methylene chloride (Stauffer Chemical Co., 1972)

(g) Volatility: Vapour pressure is 0.00019 mm at 25°C.

(h) Stability: Stable to 200-250°C; major decomposition begins at 308°C; stable in sunlight

(i) Reactivity: Hydrolysed by acids and bases

1.4 Technical products and impurities

Tris(2,3-dibromopropyl) phosphate is available in the US in at least two grades. The high-purity grade has the following typical properties: a clear, pale-yellow viscous liquid; density at 25°C, 2.20-2.26; refractive index at 25°C, 1.576-1.577; viscosity at 25°C, 3900-4200 centistokes; acid number (mg KOH/g), 0.05 max; volatiles, 1.5%; max; bromine content, 68.7%; and phosphorus content, 4.4%. Typical properties for a lower grade are as follows: density at 25°C, 2.2-2.3; viscosity at 25°C, 1400-1700 centistokes; acid number (mg KOH/g), 0.05 max; and volatiles, 10% max.

Impurities in tris(2,3-dibromopropyl) phosphate include 2,3-dibromopropanol, 1,2,3-tribromopropane and 1,2-dibromo-3-chloropropane (see monograph, p. 83) (Blum & Ames, 1977).

2. Production, Use, Occurrence and Analysis

A review on haloalkyl phosphates has been published (US Environmental Protection Agency, 1976a).

2.1 Production and use

(a) Production

The first described preparation of tris(2,3-dibromopropyl) phosphate is believed to have been in 1950, when it was made by the addition of bromine to a solution of triallyl phosphate in benzene. It is prepared commercially in the US by a two-step process in which bromine is added to allyl alcohol and the resultant 2,3-dibromopropanol is reacted with phosphorous oxychloride (Overbeek & Namety, 1962) (possibly in the presence of an aluminium chloride catalyst).

Commercial production of tris(2,3-dibromopropyl) phosphate in the US was first reported in 1959 (US Tariff Commission, 1960). In 1976, 2 US companies reported

production of an undisclosed amount (US International Trade Commission, 1977). US production in 1975 has been estimated to have been 4.1-5.4 million kg (US Environmental Protection Agency, 1976a).

No data on its production in Europe were available.

Japanese production of tris(2,3-dibromopropyl) phosphate is estimated to have been 100 thousand kg in 1976, the last year in which the single manufacturer made it; it is not imported.

(b) Use

Tris(2,3-dibromopropyl) phosphate is used primarily as a flame retardant additive for synthetic textiles and plastics (US Environmental Protection Agency, 1976a). It has also been recommended for use in phenolic resins, paints, paper coatings and rubber (Agranoff, 1976).

Tris(2,3-dibromopropyl) phosphate is used mainly in polyester and cellulosic acetate fabrics, but it has also been used in acrylic fabrics; twice as much is used in polyester fabrics as in cellulosic acetate fabrics. About 65% of the tris(2,3-dibromopropyl) phosphate used in the US in 1975 was applied to fabrics for children's clothing (US Environmental Protection Agency, 1976b). It may be added to textiles by the producer, although addition by dyers and finishers is believed to be more usual, at a level of 6-10% by weight.

Its addition to polyurethane foams (see IARC, 1979a) is the major use in plastics; relatively small amounts are believed to be used as an additive to polystyrene foam (see IARC, 1979b). It is added to rigid foams and to a lesser extent to flexible polyurethane foams. It has been estimated that fire-retarded polyurethane requires approximately 0.5% phosphorus and 4-7% bromine; this is equivalent to about 10% tris-(2,3-dibromopropyl) phosphate (by weight) in the product (US Environmental Protection Agency, 1976a).

Flexible polyurethane foams are used primarily for cushioning. Cushioning treated with haloalkyl phosphates is found in automotive and aircraft interiors, institutional bedding, cushions and upholstered furniture. Rigid foams containing tris(2,3-dibromopropyl) phosphate are used in insulation, furniture, automobile interior parts and water flotation devices. Less expensive fire retardants are used for rigid foams used in building insulation (US Environmental Protection Agency, 1976a).

As a result of actions taken on 8 April and 1 June 1977, the US Consumer Product Safety Commission banned children's clothing treated with tris(2,3-dibromopropyl) phosphate, the chemical itself when used or intended to be used in children's clothing and fabric, yarn or

fibre containing it when intended for use in such clothing (US Consumer Product Safety Commission, 1977a,b). However, children's clothing containing tris(2,3-dibromopropyl) phosphate is still available because the ban has not yet been fully enforced (Anon., 1978; US Consumer Product Safety Commission, 1978a,b).

In March 1978, the Consumer Product Safety Commission listed 22 products that contain tris(2,3-dibromopropyl) phosphate and are available to US consumers. These included children's clothing, industrial uniforms, draperies, tent fabric, automobile headliners, epoxy resins for the electronics industry, Christmas decorations and polyester thread (Anon., 1978).

No data on its use in Europe were available. Until 1977, it was used in Japan primarily as a fire retardant in polyester fibres and polyurethane plastics.

2.2 Occurrence

Tris(2,3-dibromopropyl) phosphate is not known to occur as a natural product.

Although environmental levels of tris(2,3-dibromopropyl) phosphate have not been measured, it has been estimated that as much as 10% of US production reaches the environment from textile finishing plants and laundries and that most of the rest will reach the environment as solid waste (US Environmental Protection Agency, 1976b). Several experimental studies conducted on polyester and cellulose acetate fabrics treated with tris(2,3-dibromopropyl) phosphate have shown that it can leach into wash and rinse water during laundering (Lande *et al*., 1976). When sheets treated with tris(2,3-dibromopropyl) phosphate were washed, up to 6 mg/L were found in the combined wash- and rinse-water (Gutenmann & Lisk, 1975).

Approximately 180 μg/day (9 μg/kg bw) tris(2,3-dibromopropyl) phosphate is absorbed through the skin of children wearing polyester pyjamas (Blum *et al*., 1978).

A 1974 National Occupational Hazard Survey indicated that workers primarily exposed to tris(2,3-dibromopropyl) phosphate are those in the telephone communication industry (National Institute for Occupational Safety & Health, 1977)

2.3 Analysis

Methods used for the analysis of tris(2,3-dibromopropyl) phosphate are listed in Table 1.

TABLE 1. METHODS FOR THE ANALYSIS OF TRIS(2,3-DIBROMOPROPYL) PHOSPHATE

ANALYTICAL METHOD			
SAMPLE TYPE	EXTRACTION/CLEAN-UP	DETECTION	REFERENCE
Textiles	Pyrolysis	GC/flame photometry	Cope (1973)
Polyester flannel	Heat in distilled water; evaporate to dryness; hydrolyse by refluxing with hydrobromic acid; complex with molybdenum blue	Spectrophotometry	Gutenmann & Lisk (1975)

Abbreviation: GC – gas chromatography

3. Biological Data Relevant to the Evaluation of Carcinogenic Risk to Humans

3.1 Carcinogenicity studies in animals

(a) Oral administration

Mouse: Groups of 50 male and 50 female B6C3F$_1$ hybrid mice, 6 weeks of age, were fed various concentrations of technical-grade tris(2,3-dibromopropyl) phosphate (containing no detectable 1,2-dibromo-3-chloropropane) in the diet for 103 weeks followed by a 1-week observation period. The experimental design of the study is shown in Table 2. Of the males, 43/50 high-dose, 38/50 low-dose and 44/55 matched control mice survived until the end of the study; of the females, 38/50 high-dose, 37/50 low-dose and 44/55 control mice survived. The compound increased the incidence of squamous-cell carcinomas and papillomas of the forestomach and of adenomas and carcinomas of the lungs in both male and female treated animals as compared with controls; there was also an increased incidence of renal tubular-cell adenomas and adenocarcinomas in treated male mice and of liver-cell adenomas and carcinomas in treated female mice. Neoplastic lesions associated with the administration of tris(2,3-dibromopropyl) phosphate are summarized in Table 2. Renal tubular dysplasia was observed in 30/49 high-dose males, 37/50 low-dose males, 12/46 high-dose females and 1/50 low-dose females but in none of the controls (National Cancer Institute, 1978).

Rat: Groups of 55 male and 55 female Fischer 344 rats, 6 weeks old, were fed diets containing various concentrations of technical-grade tris(2,3-dibromopropyl) phosphate for 103 weeks, followed by a 1- or 2-week observation period. The experimental design of the study is shown in Table 2. Of the males, 40/55 high-dose, 35/55 low-dose and 39/55 control rats survived until the end of the study; of the females, 36/55 high-dose, 44/55 low-dose and 36/55 control rats survived. The compound increased the incidence of renal tubular-cell adenomas in rats of both sexes and of tubular-cell adenocarcinomas in high-dose males. Neoplastic lesions associated with the administration of tris(2,3-dibromopropyl) phosphate are summarized in Table 2. Renal tubular dysplasia was observed in 6/54 high-dose males and in 35/54 high-dose females, but not in the control or low-dose groups (National Cancer Institute, 1978).

(b) Skin application

Mouse: Female ICR/Ha Swiss mice, 6-8 weeks old, were treated thrice weekly with tris(2,3-dibromopropyl) phosphate (97% pure) in 0.2 mL acetone applied to the shaved dorsal skin for 474-496 days. Most of the animals survived to the end of the study. The compound

TABLE 2. TUMOUR INCIDENCES IN MICE AND RATS FED TRIS(2,3-DIBROMOPROPYL) PHOSPHATE

SPECIES	SEX	NO. OF ANIMALS TREATED	CONCENTRATION (mg/kg OF DIET	DURATION (WEEKS)	NUMBER OF TUMOUR-BEARING ANIMALS/NUMBER OF ANIMALS EXAMINED			
					FORESTOMACH (SQUAMOUS-CELL CARCINOMAS OR PAPILLOMAS)	LUNG (ADENOMAS OR CARCINOMAS)	KIDNEY(TUBULAR-CELL ADENOMAS OR ADENOCARCINOMAS)	LIVER (ADENOMAS OR CARCINOMAS)
Mouse	M	55	0	105	0/51	12/54	0/54	28/54
	M	50	500	103	10/47[a]	18/44[c]	4/50	31/49
	M	50	1000	103	13/48[b]	25/50[d]	14/49[e]	23/49
	F	55	0	105	2/53	4/55	0/55	11/54
	F	50	500	103	14/48[b]	9/50	2/50	23/50[f]
	F	50	1000	103	22/44[b]	17/50[d]	2/46	35/49[f]
Rat	M	55	0	107	-	0/54	0/53	0/54
	M	55	50	103	-	3/55	26/54[e]	1/55
	M	55	100	103	-	0/55	29/54[e]	4/54
	F	55	0	107	-	-	0/52	-
	F	55	50	103	-	-	4/54	-
	F	55	100	103	-	-	10/54[e]	-

Fisher analysis of treated group *versus* control:
[a]Squamous-cell papillomas; $P < 0.01$
[b]Squamous-cell carcinomas & papillomas; $P < 0.01$
[c]Alveolar/bronchiolar adenomas & carcinomas; $P < 0.05$
[d] Alveolar/bronchiolar adenomas & carcinomas; $P < 0.01$
[e] Tubular-cell adenomas & adenocarcinomas; $P < 0.01$
[f] Hepatocellular adenomas & carcinomas; $P < 0.01$

increased the incidence of tumours of the skin, lung, forestomach and oral cavity in treated mice as compared with controls. The experimental design and the neoplastic lesions associated with the dermal application of tris(2,3-dibromopropyl) phosphate are summarized in Table 3 (Van Duuren *et al*., 1978).

TABLE 3. TUMOUR INCIDENCES IN FEMALE SWISS MICE AFTER DERMAL APPLICATION OF TRIS(2,3-DIBROMOPROPYL) PHOSPHATE

NUMBER OF ANIMALS TREATED	DOSE (mg/ ANIMALS)	NUMBER OF MICE WITH TUMOURS/NUMBER NECROPSIED[a]			
		FORESTOMACH	LUNG	SKIN	ORAL CAVITY
29	0	1/29	7/29	0/29	0/29
30	10	10/29	26/29	2/29	2/29
30	30	20/30	28/30	5/30	4/30

[a]Increases in incidences of tumours of forestomach, lung, skin and oral cavity in treated animals were statistically significant when compared with those in controls ($P < 0.05$).

3.2 Other relevant biological data

(a) Experimental data

Toxic effect

Tris(2,3-dibromopropyl) phosphate containing less than 1% volatile impurities has an oral LD_{50} of 5.24 g/kg bw in rats and a dermal LD_{50} of > 8 g/kg bw in rabbits.

In rabbits, administration of 0.22 g/animal to the eye or 1.1 g/animal to the skin caused no irritation (Daniher, 1976; Kerst, 1974). No evidence of skin sensitization was seen in guinea-pigs (Morrow *et al*., 1976).

Application of commercial, high-purity (99.76%) tris(2,3-dibromopropyl) phosphate weekly for 3 months at a dose of 2.27 g/kg bw to intact and abraded skin of the backs of female and male rabbits produced a 50% decrease in testicular weight in 7/8 males, with spermatogonia in the seminiferous tubules and occasional progression to secondary spermatocytes. In addition, chronic interstitial nephritis was seen in 6/8 males (Osterberg *et al*., 1977).

No data on the embryotoxicity or teratogenicity of tris(2,3-dibromopropyl) phosphate were available.

Absorption, distribution, excretion and metabolism

Tris(2,3-dibromopropyl) phosphate was absorbed from the digestive system of male weanling rats fed 100 and 1000 mg/kg of diet for 28 days. Dose-related bromine concentrations were detected by neutron activation analysis in muscle, liver and fat after 28 days' feeding; these concentrations were reduced to control levels 6 weeks after administration of the compound was discontinued (Kerst, 1974). The absorption of tris(2,3-dibromopropyl) phosphate was dose-dependent in rabbits that received daily skin applications of 500, 1000 and 2000 mg/kg of diet, as shown by increased blood bromide levels (Daniher, 1976).

After application of fabric treated with ^{14}C-tris(2,3-dibromopropyl) phosphate to the clipped skin of rabbits, up to 17% of the radiolabel in the cloth penetrated the skin over a 96-hr period of exposure. Most of the radiolabel appeared in the urine. Even higher absorption of radiolabel occurred when cloth moistened with human urine was applied to the skin (Ulsamer *et al*., 1978).

When a dose of 100 mg/animal was applied to the shaven skin of a male Lewis rat, a metabolic hydrolysis product, 2,3-dibromopropanol, was detected in free and conjugated form in the urine for several days (St John *et al.,* 1976).

Mutagenicity and other related short-term tests

Tris(2,3-dibromopropyl) phosphate is mutagenic *in Salmonella typhimurium* TA100 and TA1535, but not in TA1537 and TA1538, indicating that the mutations induced are of the base-pair substitution type (Blum & Ames, 1977; Brusick *et al.,* 1977; Prival *et al.,* 1977). In the study of Prival *et al.* (1977), although tris(2,3-dibromopropyl) phosphate behaved as a direct-acting mutagen at higher concentrations (> 1 μL/plate), much lower concentrations (0.01 μL/plate) had significant genetic activity only when microsomal preparations were present. On a quantitative basis, no significant difference in mutagenic activity was observed among 9 different commercial samples.

The urine of rats treated orally or dermally with tris(2,3-dibromopropyl) phosphate in doses of 500 and 5000 mg/kg bw also showed mutagenic activity in *Salmonella typhimurium* TA1535 (Brusick *et al.,* 1977).

Tris(2,3-dibromopropyl) phosphate was mutagenic in *Drosophila melanogaster,* inducing sex-linked recessive lethals in male germ-cell stages; the spermatids were the most sensitive (Valencia, 1978).

Results of a forward mutation assay with the thymidine kinase system in mouse lymphoma cells (L5178Y) were inconclusive, although a 2-3-fold increase in mutation frequency was obtained consistently with concentrations of 5 μg/mL (Brusick *et al.,* 1977).

Exposure to concentrations of 2 μL tris(2,3-dibromopropyl) phosphate per ml of growth medium for 4½ hrs induced reparable lesions (single-strand breaks) in the DNA of human cells (KB) in culture, as evidenced by a lowering of the sedimentation rate in alkaline sucrose gradients (Gutter & Rosenkranz, 1977).

2,3-Dibromo-l-propanol, a metabolite and also an impurity present in tris(2,3-dibromopropyl) phosphate, was mutagenic in *Salmonella typhimurium* TA100 and TA1535 but not in TA1538 (Carr & Rosenkranz, 1978).

A significant, dose-dependent increase in sister chromatid exchanges was observed in Chinese hamster V79 cells treated with tris(2,3-dibromopropyl) phosphate; chromosome aberrations were not significantly increased (Furukawa *et al.,* 1978).

(b) Humans

No skin irritation or sensitization was seen among 52 people who received 10 patch-test applications of the compound (Kerst, 1974). In another study with undiluted tris(2,3-dibromopropyl) phosphate, sensitization reactions occurred in 8/24 subjects; with a concentration of 20% in petroleum jelly, 2/25 subjects were sensitized. Seven of 8 treated fabrics elicited a response in the sensitized subjects (Morrow *et al.,* 1976).

Tris(2,3-dibromopropyl) phosphate is absorbed through the skin in humans (Blum & Ames, 1977). Approximately 180 μg/day (9 μg/kg bw) is absorbed through the skin of children wearing pyjamas treated with tris-(2,3-dibromopropyl) phosphate. Up to 29 ng/mL 2,3-dibromo-1-propanol, a mutagenic metabolite of tris(2,3-dibromopropyl) phosphate, has been found in the urine of children wearing such pyjamas (Blum *et al.,* 1978).

3.3 Case reports and epidemiological studies

No data were available to the Working Group.

4. Summary of Data Reported and Evaluation

4.1 Experimental data

Tris(2,3-dibromopropyl) phosphate was tested in one experiment in mice and in one in rats by oral administration and in one experiment in female mice by skin application. In mice, following oral administration, it produced tumours of the forestomach and lung in animals of both sexes, benign and malignant liver tumours in females and benign and malignant tumours of the kidney in males. In rats, it produced benign and malignant tumours of the kidney in males and benign kidney tumours in females.

After skin application to female mice, it produced tumours of the skin, lung, forestomach and oral cavity.

Tris(2,3-dibromopropyl) phosphate is mutagenic in *Salmonella typhimurium* and *Drosophila melanogaster*.

4.2 Human data

No case reports or epidemiological studies were available to the Working Group.

The extensive production and use of tris(2,3-dibromopropyl) phosphate over the past two decades, primarily as a flame retardant for textiles and plastics, indicate that widespread human exposure occurs. The Working Group knew of no published attempts to determine levels of this compound in the environment; however, estimates of the amounts released from industrial operations and textile leaching suggest that it is widely distributed. Its widespread use in childrens' sleepwear was noted.

4.3 Evaluation

There is *sufficient evidence* that tris(2,3-dibromopropyl) phosphate is carcinogenic in mice and rats. In the absence of adequate data in humans, it is reasonable, for practical purposes, to regard tris(2,3-dibromopropyl) phosphate as if it presented a carcinogenic risk to humans.

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