



**NATIONAL TOXICOLOGY PROGRAM**  
**Technical Report Series**  
**No. 434**



# **TOXICOLOGY AND CARCINOGENESIS**

## **STUDIES OF 1,3-BUTADIENE**

**(CAS NO. 106-99-0)**

**IN B6C3F<sub>1</sub> MICE**

**(INHALATION STUDIES)**

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

## FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): the National Cancer Institute (NCI), National Institutes of Health; the National Institute of Environmental Health Sciences (NIEHS), National Institutes of Health; the National Center for Toxicological Research (NCTR), Food and Drug Administration; and the National Institute for Occupational Safety and Health (NIOSH), Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for NTP toxicology and carcinogenesis studies are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

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**NTP TECHNICAL REPORT**  
**ON THE**  
**TOXICOLOGY AND CARCINOGENESIS**  
**STUDIES OF 1,3-BUTADIENE**  
**(CAS NO. 106-99-0)**  
**IN B6C3F<sub>1</sub> MICE**  
**(INHALATION STUDIES)**

**NATIONAL TOXICOLOGY PROGRAM**  
**P.O. Box 12233**  
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## ABSTRACT



## 1,3-BUTADIENE

CAS No. 106-99-0

Chemical Formula:  $\text{C}_4\text{H}_6$       Molecular Weight: 54.09Synonyms:  $\alpha,\gamma$ -Butadiene; bivinyl; divinyl; erythrene; vinylethylene; biethylene; pyrrolylene

1,3-Butadiene is produced in large volumes for use in the manufacture of synthetic rubber and of thermoplastic resins. In previous inhalation studies conducted by the NTP (NTP, 1984) there was clear evidence of multiple organ carcinogenicity in male and female mice exposed to 625 or 1,250 ppm 1,3-butadiene for 60 or 61 weeks. To better characterize exposure-response relationships for neoplasms and nonneoplastic lesions, toxicology and carcinogenesis studies were conducted by exposing groups of male and female B6C3F<sub>1</sub> mice to air containing 1,3-butadiene (greater than 99% pure) for up to 2 years. An additional study in male B6C3F<sub>1</sub> mice, in which exposure to 1,3-butadiene was stopped after limited exposure periods (13, 26, 40, or 52 weeks), was performed to assess the effects of varying concentration and duration of exposure on the incidences of 1,3-butadiene-induced neoplasms. *In vitro* genetic toxicology studies were conducted in *Salmonella typhimurium* and mouse lymphoma cells. *In vivo* genetic effects were assayed in germ cells of male *Drosophila melanogaster* and in bone marrow and peripheral blood cells of B6C3F<sub>1</sub> mice.

**2-Year Studies:** Groups of 70 male and 70 female mice were exposed to air containing 0, 6.25, 20, 62.5, or 200 ppm 1,3-butadiene for 6 hours per day, 5 days per week for up to 2 years; groups of 90 male and 90 female mice were exposed to 625 ppm 1,3-butadiene on the same schedule. Up to 10 animals from

each group were examined after 9 and 15 months of exposure.

**Survival and Body Weight in the 2-Year Studies:** Two-year survival was decreased for males and females exposed to concentrations of 20 ppm or above, primarily due to the development of chemical-related malignant neoplasms. No female mice exposed to 200 or 625 ppm or males exposed to 625 ppm survived to the end of the studies (males: 35/50, 39/50, 24/50, 22/50, 4/50, 0/70; females: 37/50, 33/50, 24/50, 11/50, 0/50, 0/70). Mean body weights of exposed male and female mice were similar to those of the controls.

**Hematologic Effects in the 2-Year Studies:** Hematologic parameters were evaluated after 9 and 15 months of exposure. At 9 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume were observed in male mice exposed to 62.5 ppm or above and in female mice exposed to 200 or 625 ppm. Mean erythrocyte volume was increased in male mice exposed to 625 ppm and in females exposed to 200 or 625 ppm. At 15 months, decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume and increases in mean erythrocyte volume were observed in male and female mice exposed to 625 ppm.

*Neoplasms and Nonneoplastic Lesions in the 2-Year Studies:* Exposure of mice to 1,3-butadiene induced benign and malignant neoplasms at multiple sites. Statistically significant increases in the incidences of neoplasms at one or more sites were seen at concentrations of 20 ppm and higher in males and 6.25 ppm and higher in females. There was no exposure level in this study at which a significant carcinogenic response was not observed. Statistically significant increases occurred in the incidences of malignant lymphoma; histiocytic sarcoma; cardiac hemangiosarcoma; harderian gland adenoma; hepatocellular adenoma and carcinoma; alveolar/bronchiolar adenoma and carcinoma; mammary gland carcinoma, adenocanthoma, and malignant mixed tumor (females only); benign and malignant ovarian granulosa cell tumor; and forestomach squamous cell papilloma and carcinoma.

Low incidences of uncommon neoplasms also occurred in exposed male and female mice, including intestinal carcinomas in males, renal tubule adenomas in males and females, skin sarcomas (all types combined) in females, and Zymbal's gland adenomas and carcinomas in females.

Lymphocytic lymphomas appeared as early as week 23 and were the principal cause of death of male and female mice exposed to 625 ppm 1,3-butadiene. The early and extensive development of lethal lymphocytic lymphomas in mice exposed to 625 ppm resulted in a reduced number of mice at risk for neoplasms developing later at other sites. Exposure-response relationships for 1,3-butadiene-induced neoplasms were more clearly characterized at concentrations below 625 ppm and after adjustment for intercurrent mortality.

Increased incidences of nonneoplastic lesions in exposed mice included bone marrow atrophy; testicular atrophy; ovarian atrophy, angiectasis, germinal epithelial hyperplasia, and granulosa cell hyperplasia; uterine atrophy; cardiac endothelial hyperplasia and mineralization; alveolar epithelial hyperplasia; forestomach epithelial hyperplasia; and harderian gland hyperplasia.

*Stop-Exposure Study:* The stop-exposure study consisted of groups of 50 male mice exposed to 1,3-butadiene at concentrations of 200 ppm for 40 weeks, 625 ppm for 13 weeks, 312 ppm for 52 weeks, or 625 ppm for 26 weeks. After the

exposures were completed, these groups were placed in control chambers for the remainder of the 2-year study. The total exposure of 1,3-butadiene (concentration times duration of exposure) of the 13- and 40-week stop-exposure groups was approximately 8,000 ppm · weeks, while that of the 26- and 52-week stop-exposure groups was approximately 16,000 ppm · weeks.

The survival of all stop-exposure groups was markedly lower than that of the controls. The incidences of lymphocytic lymphoma, histiocytic sarcoma, cardiac hemangiosarcoma, alveolar/bronchiolar adenoma and carcinoma, forestomach squamous cell papilloma and carcinoma, hepatocellular adenoma, harderian gland adenoma and adenocarcinoma, and preputial gland carcinoma were significantly increased. Neoplasms were induced at most of these sites after only 13 weeks of exposure to 1,3-butadiene. Additionally, low numbers of malignant gliomas and neuroblastomas of the brain and Zymbal's gland carcinomas occurred in one or more stop-exposure groups.

At similar total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for an extended period (34% at 625 ppm for 13 weeks versus 12% at 200 ppm for 40 weeks; 60% at 625 ppm for 26 weeks versus 8% at 312 ppm for 52 weeks).

*Genetic Toxicology:* 1,3-Butadiene has been tested both *in vitro* and *in vivo* for mutagenic activity. *In vitro*, positive results were obtained in the *Salmonella typhimurium* gene mutation assay with strain TA1535; mutagenic activity was not observed in other *S. typhimurium* strains (TA100, TA97, and TA98). 1,3-Butadiene was negative in the mouse lymphoma assay for induction of trifluorothymidine resistance in L5178Y cells with and without S9.

*In vivo*, 1,3-butadiene did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster*; however, it did induce significant increases in chromosomal aberrations and sister chromatid exchanges in bone marrow cells of mice exposed for 2 weeks by inhalation. In addition, significant increases in micronucleated erythrocytes were observed in peripheral blood samples obtained

from male and female mice exposed to 1,3-butadiene for 2 or 13 weeks or 15 months by inhalation.

*Conclusions:* The previous inhalation studies of 1,3-butadiene in male and female B6C3F<sub>1</sub> mice provided *clear evidence of carcinogenicity\** at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies — 2-year exposures of 6.25, 20, 62.5, 200, or 625 ppm or shorter duration exposures of 200, 312, or 625 ppm — provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male

B6C3F<sub>1</sub> mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, preputial gland, brain, and kidney. There was *clear evidence of carcinogenicity* of 1,3-butadiene in female B6C3F<sub>1</sub> mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, ovary, and mammary gland.

Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also have been related to administration of 1,3-butadiene.

\* Explanation of Level of Evidence of Carcinogenic Activity is on page 11. A summary of peer review comments and the public discussion on this Technical Report appears on page 13.

**Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene**

	Male B6C3F <sub>1</sub> Mice		Female B6C3F <sub>1</sub> Mice
	(2-Year Study)	(Stop-Exposure Study)	
<b>Doses</b>	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks	200 ppm for 40 weeks, 312 ppm for 52 weeks, 625 ppm for 13 weeks, or 625 ppm for 26 weeks by inhalation for 6 hours daily, 5 days per week	0, 6.25, 20, 62.5, 200, or 625 ppm by inhalation for 6 hours daily, 5 days per week, for 103 weeks
<b>Body weights</b>	Exposed groups similar to controls	Exposed groups similar to controls	Exposed groups similar to controls
<b>2-Year survival rates</b>	35/50, 39/50, 24/50, 22/50, 4/50, 0/70	9/50, 1/50, 5/50, 0/50	37/50, 33/50, 24/50, 11/50, 0/50, 0/70
<b>Nonneoplastic effects</b>	<p>Bone marrow: atrophy (0/50, 0/50, 0/50, 0/48, 0/49, 23/73)</p> <p>Heart: endothelial hyperplasia (0/50, 1/49, 0/50, 2/48, 4/48, 5/73); mineralization (0/50, 0/49, 0/50, 1/48, 3/48, 20/73)</p> <p>Alveolar epithelium: hyperplasia (2/50, 9/50, 6/50, 13/49, 17/50, 12/73)</p> <p>Forestomach epithelium: hyperplasia (4/50, 3/50, 3/50, 6/48, 4/48, 40/72)</p> <p>Harderian gland: hyperplasia (1/50, 3/49, 4/50, 6/47, 8/47, 5/40)</p> <p>Testicle: atrophy (1/50, 3/50, 4/50, 2/48, 6/49, 53/72)</p>	<p>Heart: endothelial hyperplasia (6/50, 3/50, 7/50, 7/50); mineralization (0/50, 6/50, 9/50, 14/50)</p> <p>Alveolar epithelium: hyperplasia (18/50, 14/50, 10/50, 11/50)</p> <p>Forestomach epithelium: hyperplasia (10/48, 20/48, 8/50, 15/50)</p> <p>Harderian gland: hyperplasia (4/48, 6/48, 3/42, 7/36)</p> <p>Testicle: atrophy (5/50, 3/50, 3/50, 5/50)</p>	<p>Bone marrow: atrophy (0/50, 0/49, 0/48, 0/49, 0/50, 11/79)</p> <p>Heart: endothelial hyperplasia (0/50, 2/50, 1/50, 4/49, 5/50, 8/80); mineralization (0/50, 2/50, 0/50, 2/49, 2/50, 11/80)</p> <p>Alveolar epithelium: hyperplasia (5/50, 5/50, 3/50, 9/50, 11/50, 11/78)</p> <p>Forestomach epithelium: hyperplasia (4/50, 5/49, 4/47, 7/48, 14/50, 47/79)</p> <p>Liver: hepatocellular foci (8/49, 14/49, 19/50, 12/50, 5/50, 4/80)</p> <p>Harderian gland: hyperplasia (1/50, 5/49, 9/48, 4/49, 4/49, 7/66)</p> <p>Ovary: angiectasis (4/49, 6/49, 3/48, 13/50, 14/50, 17/79); granulosa cell hyperplasia (1/49, 0/49, 2/48, 3/50, 4/50, 2/79); germinal epithelial hyperplasia (2/49, 3/49, 8/48, 15/50, 14/50, 18/79); atrophy (4/49, 19/49, 32/48, 42/50, 43/50, 69/79)</p> <p>Uterus: atrophy (1/50, 0/49, 1/50, 1/49, 8/50, 41/78)</p>

(continued)

## Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)

	Male B6C3F <sub>1</sub> Mice		Female B6C3F <sub>1</sub> Mice
	(2-Year Study)	(Stop-Exposure Study)	
Neoplastic effects	Lymphoma (all lymphomas) (4/50, 2/50, 4/50, 6/50, 2/50, 51/73)	Lymphoma (all lymphomas) (8/50, 8/50, 22/50, 33/50)	Lymphoma (all lymphomas) (6/50, 12/50, 11/50, 7/50, 9/50, 32/80)
	Lymphocytic lymphoma (2/50, 0/50, 2/50, 4/50, 2/50, 49/73)	Lymphocytic lymphoma (6/50, 4/50, 17/50, 30/50)	Lymphocytic lymphoma (1/50, 3/50, 6/50, 3/50, 8/50, 31/80)
	Histiocytic sarcoma (0/50, 0/50, 4/50, 5/50, 7/50, 4/73)	Histiocytic sarcoma (5/50, 7/50, 2/50, 2/50)	Histiocytic sarcoma (3/50, 2/50, 7/50, 4/50, 7/50, 4/80)
	Heart: hemangiosarcoma (0/50, 0/49, 1/50, 5/48, 20/48, 4/73)	Heart: hemangiosarcoma (15/50, 33/50, 7/50, 13/50)	Heart: hemangiosarcoma (0/50, 0/50, 0/50, 1/49, 21/50, 23/80)
	Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (21/50, 23/50, 19/50, 31/49, 35/50, 3/73)	Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (36/50, 32/50, 28/50, 17/50)	Lung: alveolar/bronchiolar adenoma, adenocarcinoma, or carcinoma (4/50, 15/50, 19/50, 24/50, 25/50, 22/78)
	Forestomach: squamous cell papilloma or squamous cell carcinoma (1/50, 0/50, 0/50, 1/50, 8/50, 4/73)	Forestomach: squamous cell papilloma or squamous cell carcinoma (3/50, 9/50, 7/50, 10/50)	Forestomach: squamous cell papilloma or squamous cell carcinoma (0/50, 0/50, 3/50, 2/50, 4/50, 22/80)
	Liver: hepatocellular adenoma or carcinoma (21/50, 23/50, 30/50, 25/48, 33/48, 5/72)	Liver: hepatocellular adenoma (27/49, 19/50, 19/49, 11/50)	Liver: hepatocellular adenoma or carcinoma (15/49, 14/49, 15/50, 19/50, 16/50, 2/80)
	Harderian gland: adenoma or carcinoma (6/50, 7/50, 9/50, 20/50, 31/50, 6/73)	Harderian gland: adenoma or carcinoma (27/50, 30/50, 23/50, 13/50)	Harderian gland: adenoma or carcinoma (8/50, 10/50, 7/50, 15/50, 20/50, 9/80)
	Preputial gland: carcinoma (0/50, 0/50, 0/50, 0/50, 5/50, 0/73)	Preputial gland: carcinoma (1/50, 4/50, 4/50, 3/50)	Ovary: benign or malignant granulosa cell tumor (1/49, 0/49, 1/48, 9/50, 8/50, 6/79); adenoma or benign mixed tumor (2/49, 4/49, 1/48, 4/50, 6/50, 2/79)
	Kidney: renal tubule adenoma (0/50, 1/50, 0/50, 3/48, 1/49, 0/73)	Kidney: renal tubule adenoma (4/48, 3/49, 1/50, 1/50)	Mammary gland: adenocanthoma, carcinoma, or malignant mixed tumor (0/50, 2/50, 4/50, 12/50, 15/50, 16/80)
		Brain: malignant glioma (0/50, 0/50, 2/50, 1/50); neuroblastoma (0/50, 0/50, 2/50, 0/50)	

(continued)

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**Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of 1,3-Butadiene (continued)**


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	Male B6C3F <sub>1</sub> Mice		Female B6C3F <sub>1</sub> Mice
	(2-Year Study)	(Stop-Exposure Study)	
<b>Uncertain findings</b>	Small intestine: carcinoma (0/50, 1/50, 1/50, 1/50, 2/50, 0/73)	Zymbal's gland: carcinoma (1/50, 0/50, 2/50, 2/50)	Kidney: renal tubule adenoma (0/49, 0/49, 0/48, 0/50, 2/50, 0/80)  Skin, subcutaneous tissue: neurofibrosarcoma or sarcoma (1/50, 2/50, 3/50, 5/50, 3/50, 3/80)  Zymbal's gland: adenoma or carcinoma (0/50, 0/50, 0/50, 0/50, 0/50, 2/80)
<b>Level of evidence of carcinogenic activity</b>		Clear evidence	Clear evidence
<b>Genetic toxicology</b>			
<i>Salmonella typhimurium</i> gene mutation:		Positive in strain TA1535 Negative in strains TA100, TA97, and TA98 Negative with and without S9	
Mouse lymphoma gene mutation:			
Sex-linked recessive lethal mutations <i>Drosophila melanogaster</i> :		Negative by inhalation	
Chromosomal aberrations			
Mouse bone marrow <i>in vivo</i> :		Positive	
Sister chromatid exchanges			
Mouse bone marrow <i>in vivo</i> :		Positive	
Micronuclei			
Mouse peripheral blood erythrocytes <i>in vivo</i> :		Positive	

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## 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 1,3-butadiene on November 21, 1991, 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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## SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On November 21, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of 1,3-butadiene 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. R.L. Melnick, NIEHS, introduced the toxicology and carcinogenesis studies of 1,3-butadiene in B6C3F<sub>1</sub> mice by discussing the uses of the chemical and rationale for study, including the previous NTP study in mice; describing the experimental design for both standard and stop-exposure studies, which were intended to assess the relationship of exposure duration versus concentration on carcinogenicity; reporting on survival and toxicity, especially to the hematopoietic system and gonads in both sexes; and presenting data on neoplasms and nonneoplastic lesions caused by 1,3-butadiene at multiple sites in both sexes. Dr. Melnick reported on the expression of *K-ras* oncogenes in liver neoplasms and said that the *K-ras* is the most commonly detected oncogene in human cancers. Dr. C.C. Shackelford, NIEHS, provided a morphologic description of hemangiosarcomas of the heart induced by 1,3-butadiene. The proposed conclusions were that the present studies provided a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions, and confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male and female B6C3F<sub>1</sub> mice.

Dr. Goodman, a principal reviewer, agreed with the proposed overall conclusions in male and female mice but disagreed with the inclusion of brain and kidney neoplasms in males and liver neoplasms in females as support for the level of evidence. He said the last sentence should read "equivocal evidence of carcinogenicity" instead of "low incidence of" and the reference to Zymbal's gland carcinomas in males should be omitted. Dr. Melnick thought that for low numbers of rare neoplasms "low incidence" was meaningful; however, another wording would be considered. Dr. Goodman thought that the conclusions for the stop-exposure study should be presented separately from those for the 2-year studies. Dr. Melnick noted that the results are presented and

analyzed separately but, in evaluating the effect of 1,3-butadiene on an organ, the thinking was that all of the evidence should be brought to bear in drawing conclusions. Dr. Goodman asked that justification be given for the use of sex-linked recessive lethal mutations in *Drosophila melanogaster* and the micronucleus test. Dr. E. Zeiger, NIEHS, explained that the *D. melanogaster* assay is extremely predictive for carcinogenicity as there are very few false positives and that the micronucleus test is the only simple measure of somatic mutations *in vivo*.

Dr. Zeise, the second principal reviewer, agreed with the proposed conclusions. Because the study was designed to look at the issue of dose response, she thought a more extensive analysis of the dose-response data should be included, especially pertaining to the shape of the curve at lower doses. Dr. Melnick said some discussion could be given about the shape of the dose-response curve and the Poly-3 test used to provide neoplasm rates adjusted for intercurrent mortality. Dr. J.K. Haseman, NIEHS, expressed concern that mathematical modeling of the data might lead to extrapolation and risk assessment calculations, activities that are normally the purview of the regulatory agencies. Dr. Zeise noted that others are already using the NTP data for these purposes. She suggested that NTP not extrapolate, but evaluate the shape of the dose-response curve within the range of observations, because the study was designed to explore the dose response and NTP has the expertise to perform such statistical evaluations.

Dr. van Zwieten, the third principal reviewer, agreed with the proposed conclusions. He thought there should be a statement in the conclusions to the effect that a carcinogenic response was induced at all exposure levels. Also, a comment about duration of exposure necessary for a carcinogenic response in the stop-exposure study would be appropriate. Dr. Melnick said that statements would be brought forward to the Abstract.

Mr. Beliczky reported that the data from these studies had been recently used by NIOSH in conducting a risk assessment and the results have been provided to the Department of Labor for potential regulatory action by OSHA on allowable exposure

levels. Dr. Garman asked if separate classifications of lymphomas reflect the current recommendation of the NTP. Dr. S.L. Eustis, NIEHS, said accurate distinctions between types were difficult to make and of little value. Rather, identifying whether the lymphomas originated in the thymus or elsewhere was most useful.

Dr. Goodman moved that the Technical Report on 1,3-butadiene be accepted but with the conclusions for the 2-year studies separated from those for the stop-exposure study by inserting "chronic exposure to" in front of "1,3-butadiene" in the statements for male and female mice. "Brain" and "kidney" would be deleted from the listing for male mice and "liver" from the listing for female mice. Then a conclusion

for the stop-exposure study would be added: "There was *clear evidence of carcinogenicity* of 1,3-butadiene in the start/stop study in B6C3F<sub>1</sub> mice based on increased incidences of neoplasms in the hematopoietic system, lung, forestomach, and harderian gland." Finally in the last sentence, "low incidences" would be replaced with "marginal increases." The motion was tabled for lack of a second. Dr. Zeise moved that the Technical Report on 1,3-butadiene be accepted with the revisions discussed and with the conclusions as written for male and female B6C3F<sub>1</sub> mice, *clear evidence of carcinogenicity*. Mr. Beliczky seconded the motion, and it was accepted by eight yes votes to one no vote (Dr. Goodman) with one abstention (Dr. Bailey).

## INTRODUCTION



### 1,3-BUTADIENE

CAS No. 106-99-0

Chemical Formula:  $\text{C}_4\text{H}_6$       Molecular Weight: 54.09

**Synonyms:**  $\alpha,\gamma$ -Butadiene; bivinyll; divinyl; erythrene; vinylethylene; biethylene; pyrrolylene

#### PHYSICAL AND CHEMICAL PROPERTIES, PRODUCTION, USE, AND EXPOSURE

1,3-Butadiene is a colorless, noncorrosive gas with a boiling point of  $-4.4^\circ\text{C}$  and a vapor pressure of 1,900 mm Hg at  $20^\circ\text{C}$  (Kirshenbaum, 1978). The conversion factor for 1,3-butadiene at  $25^\circ\text{C}$  and 760 mm Hg is  $1\text{ ppm} = 2.21\text{ mg/m}^3$ . 1,3-Butadiene is a reactive material that can form the dimer 4-vinylcyclohexene and is flammable at atmospheric concentrations of 2% or higher. 1,3-Butadiene can form explosive peroxides in air, and therefore is shipped as a liquified gas under pressure with a peroxide inhibitor.

1,3-Butadiene is a coproduct in steam cracking of petroleum fractions for the manufacture of ethylene. The annual production volume of 1,3-butadiene is approximately 12 billion pounds worldwide and 3 billion pounds in the United States (Morrow, 1990; USITC, 1990). The major uses of 1,3-butadiene are in the manufacture of synthetic rubber (such as styrene-butadiene rubber or polybutadiene rubber) and of thermoplastic resins. Butadiene elastomers are used in the manufacture of rubber tires, footwear, sponges, hoses and piping, luggage, packaging, and a variety of other molded products.

According to a 1984 survey by the United States Environmental Protection Agency, atmospheric

emissions of 1,3-butadiene from facilities that produce or process 1,3-butadiene were approximately 10 million pounds per year; 70% of these emissions were attributed to equipment leaks and 30% to process venting (Mullins, 1990). 1,3-Butadiene has also been identified in automobile exhaust, cigarette smoke, and gasoline formulations; small amounts are released by the burning of plastics or rubber (Miller, 1978). Low levels of 1,3-butadiene (0.5 to 10 ppb) have been detected in ambient air in urban locations in the United States; however, levels of 1,3-butadiene in community air in Port Neches, Texas, a town with a butadiene production facility and two styrene-butadiene production plants, were measured by the Texas Air Control Board to be as high as 2 to 3 ppm (Durchin, 1990). Approximately 52,000 workers are potentially exposed to 1,3-butadiene annually, as estimated from data compiled from the National Occupational Exposure Survey (NIOSH, 1990). In-depth industrial hygiene surveys were conducted by the National Institute for Occupational Safety and Health at four monomer and five polymer manufacturing plants (Fajen *et al.*, 1990). Occupational exposures to 1,3-butadiene in most process areas were less than 10 ppm; however, maximum 8-hour time-weighted average exposures were frequently between 10 and 150 ppm, and in one case the average exposure was as high as 374 ppm. These exposures occurred in operations involving decontaminating and maintaining process equipment, sampling and

analyzing quality control samples, and loading or unloading tank trucks or rail cars. The odor threshold or recognition concentration for 1,3-butadiene in air is approximately 1 to 2 ppm (Amoore and Hautula, 1983).

The 8-hour, time-weighted, average workroom permissible exposure limit for 1,3-butadiene established by the United States Occupational Safety and Health Administration (OSHA) is 1,000 ppm (U.S. Department of Labor, 1981). Results of carcinogenicity studies of 1,3-butadiene in rats and mice prompted the American Conference of Governmental Industrial Hygienists to lower their recommended threshold limit value for 1,3-butadiene in the work environment from 1,000 ppm to 10 ppm (ACGIH, 1986). OSHA has proposed to lower the occupational exposure standard to a permissible exposure limit of 2 ppm with a 15-minute short-term exposure limit of 10 ppm (OSHA, 1990). A final decision on the proposed change is pending. An international symposium on the "Toxicology, Carcinogenesis, and Human Health Aspects of 1,3-Butadiene" was held at the National Institute of Environmental Health Sciences in 1988. The proceedings of that symposium were published in *Environmental Health Perspectives* (Melnick *et al.*, 1990a).

## TOXICITY IN ANIMALS

1,3-Butadiene has long been considered to have a low, noncumulative toxicity in animals and humans. For rats, the median lethal concentration ( $LC_{50}$ ) for a 4-hour exposure was 285 mg/L, equivalent to 129,000 ppm or 12.9%; for mice, the  $LC_{50}$  for a 2-hour exposure was 270 mg/L, equivalent to 123,000 ppm or 12.3% (Shugaev, 1969). Carpenter *et al.* (1944) exposed groups of 24 rats, 12 guinea pigs, 4 rabbits, and 1 dog to atmospheres containing 600, 2,300, or 6,700 ppm 1,3-butadiene for 7.5 hours a day, 6 days a week, for 8 months. The highest exposure concentration caused slight growth retardation and, in some animals, a mild reversible degeneration in the liver. This degeneration was reported as light cloudy swelling. There were no reported treatment-related effects in hematologic parameters or blood or urine chemistries, nor were there pathologic changes in the eye, adrenal gland, heart, kidney, skeletal muscle, pancreas, spleen, testis, or ovary. Exposure of rabbits to 250,000 ppm (25%) 1,3-butadiene for 2 minutes induced light anesthesia,

while exposure for 8 to 10 minutes induced deep anesthesia. Death due to respiratory paralysis occurred after a 25- to 35-minute exposure to this concentration of 1,3-butadiene (Carpenter *et al.*, 1944).

No treatment-related gross or microscopic changes or effects on growth, survival, hematologic or blood biochemical parameters, urinary measurements, or neuromuscular functions were observed in male or female Sprague-Dawley rats exposed to 1,000, 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6 hours a day, 5 days a week, for 13 weeks (Crouch *et al.*, 1979).

Nonneoplastic lesions associated with exposure of B6C3F<sub>1</sub> mice to 625 or 1,250 ppm 1,3-butadiene for up to 61 weeks included epithelial hyperplasia of the forestomach, endothelial hyperplasia of the heart, alveolar epithelial hyperplasia, hepatocellular necrosis, testicular atrophy, ovarian atrophy, and lesions in nasal tissues including chronic inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium (NTP, 1984; Melnick *et al.*, 1988). The proliferative lesions in the forestomach, heart, and lung may represent early preneoplastic changes in the development of neoplasms induced by 1,3-butadiene. The nasal lesions were seen only in male mice exposed to 1,250 ppm 1,3-butadiene.

Exposure of male B6C3F<sub>1</sub> mice or NIH Swiss mice to 1,250 ppm 1,3-butadiene for 6 weeks caused decreases in erythrocyte counts, hemoglobin concentrations, and hematocrit, and an increase in mean erythrocyte volume (Irons *et al.*, 1986a,b). Anemia due to exposure to 1,3-butadiene was not accompanied by increases in reticulocyte counts or in the frequency of nucleated erythrocytes in peripheral blood. These changes were considered to represent a macrocytic-megaloblastic anemia, because they were accompanied by mild megaloblastic changes in bone marrow cells. In related studies, Tice *et al.* (1987) reported that exposure of male B6C3F<sub>1</sub> mice to 1,3-butadiene for 10 days caused decreases in the number and rate of dividing cells in the bone marrow. These findings established the bone marrow as a site of toxicity for 1,3-butadiene in mice. Exposure of male B6C3F<sub>1</sub> mice to 1,250 ppm 1,3-butadiene for 6 hours a day, 5 days a week, for 6 or 12 weeks did not produce any persistent defects in humoral or cell-mediated immunity (Thurmond *et al.*, 1986).

## METABOLISM AND DISPOSITION

Malvoisin *et al.* (1979) identified 1,2-epoxy-3-butene as the first metabolite in 1,3-butadiene metabolism; this intermediate is formed by an inducible rat liver microsomal cytochrome P-450 monooxygenase (Figure 1; Bolt *et al.*, 1983). 1,2-Epoxy-3-butene was also detected in the expired air of Sprague-Dawley rats (Bolt *et al.*, 1983; Filser and Bolt, 1984) and of B6C3F<sub>1</sub> mice (Kreiling *et al.*, 1987) exposed to 1,3-butadiene, indicating that this epoxide intermediate is systemically available in exposed animals. Further metabolic transformation of 1,2-epoxy-3-butene involves conjugation with glutathione by glutathione-S-transferase, oxidation to 1,2:3,4-diepoxybutane, or hydrolysis by epoxide hydrolase and further oxidation to 3,4-epoxy-1,2-butanediol (Malvoisin and Roberfroid, 1982).

In studies by Laib *et al.* (1990), the metabolic elimination of 1,3-butadiene or 1,2-epoxy-3-butene was evaluated by measuring the decline in concentration of these chemicals in the gas phase of desiccator jars containing Sprague-Dawley rats or B6C3F<sub>1</sub> mice. Saturation of 1,3-butadiene metabolism in each species was reported at atmospheric concentrations between 1,000 and 2,000 ppm. At concentrations below 1,000 ppm, where first-order kinetics apply, the metabolic clearance was 1.6 times higher in mice (7,300 mL/kg per hour) than in rats (4,500 mL/kg per hour) (Bolt *et al.*, 1984; Kreiling *et al.*, 1986). The slightly higher metabolic elimination rate in mice is probably due to the higher respiratory frequency by this strain and species. This conclusion is based on the fact that the metabolic elimination rate constants of 7.6 hour<sup>-1</sup> for mice (Kreiling *et al.*, 1986) and 8.8 hour<sup>-1</sup> for rats (Bolt *et al.*, 1984) are nearly equivalent, and the exhalation rate constants are also similar for these species (Kreiling *et al.*, 1986), whereas the rate constant for the uptake of 1,3-butadiene (Kreiling *et al.*, 1986) and the minute air volume per body weight (Bond *et al.*, 1986) are about 2 to 2.5 times higher in mice than in rats.

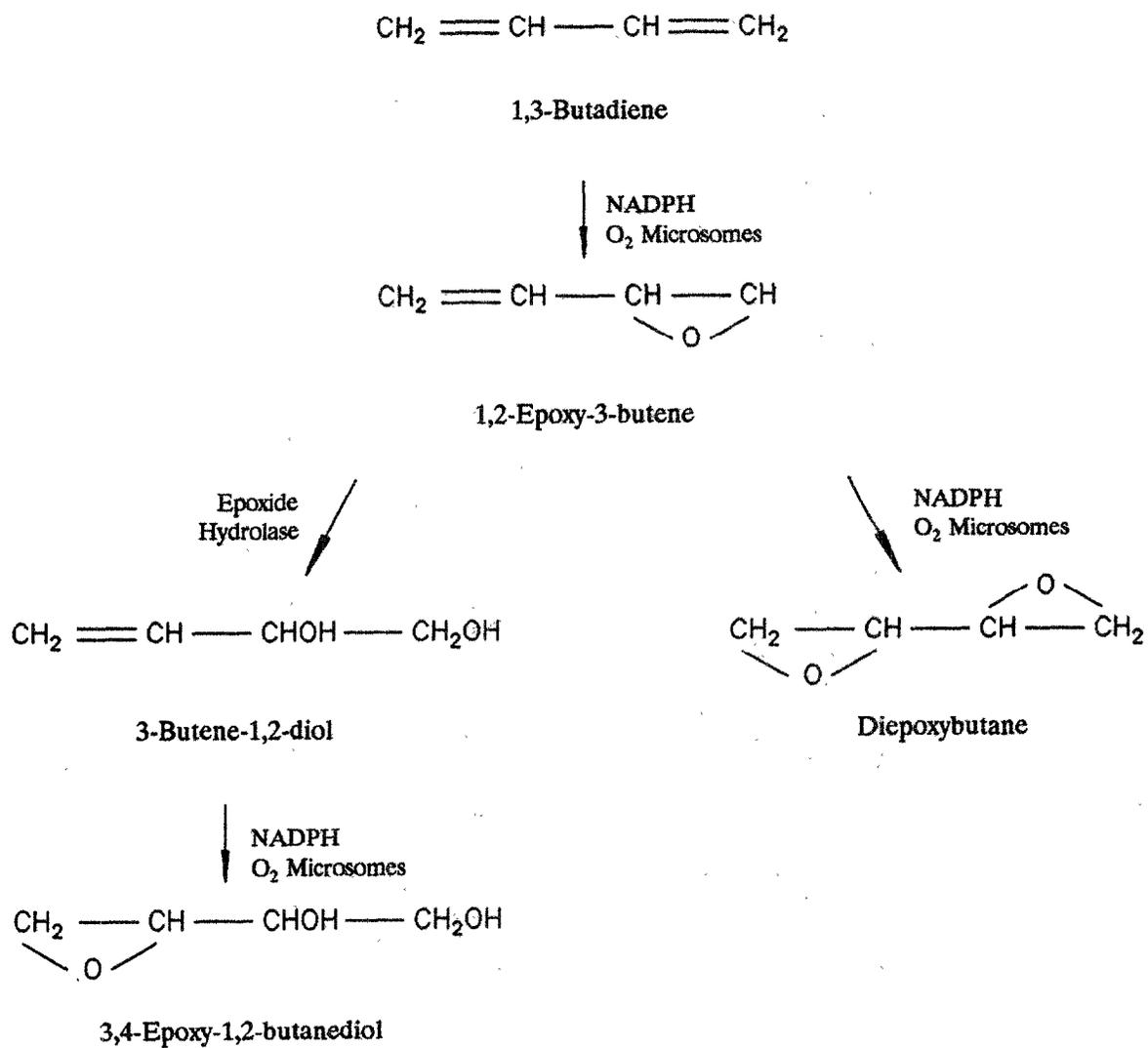
Bond *et al.* (1986) exposed Sprague-Dawley rats and B6C3F<sub>1</sub> mice to various airborne concentrations of 1-[<sup>14</sup>C]-1,3-butadiene and determined the uptake, distribution, and elimination of <sup>14</sup>C after specific periods of exposure. Respiratory measurements were also made to determine the uptake of inhaled 1,3-butadiene. However, because 1,3-butadiene and its metabolites eliminated during the exposure were not

collected, it was not possible to determine the actual percentage of 1,3-butadiene absorbed. Instead, only the percentage of inhaled [<sup>14</sup>C]-butadiene equivalents that was retained at the end of the exposure was reported. After 6 hours of exposure, the percentage of <sup>14</sup>C retained ranged from 1.5% to 17% in rats and 4% to 20% in mice; for each species, the percentage retained decreased as the exposure concentration of 1,3-butadiene increased, yet the total amount of 1,3-butadiene inhaled and retained was increased.

In a follow-up study (Dahl *et al.*, 1991), the respiratory data of Bond *et al.* (1986) were combined with the metabolic elimination rate data of Laib *et al.* (1990) to obtain values for the percentage of inhaled 1,3-butadiene that was eliminated as metabolites. The calculated values were 15% for rats and 12% for mice exposed to either 10 or 300 ppm 1,3-butadiene. The latter data reflect the constancy of uptake as the exposure concentration of 1,3-butadiene is increased in the range of first-order kinetics and also reflect the striking similarity between rats and mice after adjustment for species differences in breathing patterns.

In rats and mice exposed to [<sup>14</sup>C]-1,3-butadiene, measurements of tissue concentrations of <sup>14</sup>C did not reveal any apparent species differences (Bond *et al.*, 1987). <sup>14</sup>C was distributed to all tissues examined without any noticeably higher accumulations in the target organs for carcinogenicity of either species. These studies did not identify the metabolites in the tissues of exposed rats and mice.

Species differences in the metabolism of 1,3-butadiene have also been examined in *in vitro* studies. Rates of metabolism of 1,3-butadiene were slightly lower in microsomal fractions isolated from the liver or lung of Sprague-Dawley rats than from similar preparations obtained from B6C3F<sub>1</sub> mice (Bond *et al.*, 1988). Exposure of rats or mice to 1,3-butadiene for 6 hours a day for 5 days neither induced nor inhibited the microsomal metabolism of this chemical in either species. The rate of formation of 1,2-epoxy-3-butene from 1,3-butadiene was about seven times higher in lung postmitochondrial fractions obtained from mice than in similar fractions obtained from Sprague-Dawley rats (Schmidt and Loeser, 1985). No activity was detected in a human lung sample. In liver postmitochondrial fractions, the rate of formation of 1,2-epoxy-3-butene was only about 50% greater for mice than for rats or for a single human liver sample. Although this study



**FIGURE 1**  
**Metabolism of 1,3-Butadiene**

does not provide data on the variability of this activity in the human population, it does indicate that pathways for 1,3-butadiene metabolism in the liver may be qualitatively similar across species.

### CARCINOGENICITY IN ANIMALS

The carcinogenicity of 1,3-butadiene was studied by exposing groups of 100 Sprague-Dawley rats of each sex to 0, 1,000, or 8,000 ppm by inhalation for 6 hours a day, 5 days a week, for 2 years (IISRP, 1981a; Owen *et al.*, 1987). 1,3-Butadiene was carcinogenic at multiple organ sites in rats, as evidenced by increased incidences and dose-response trends for several organ-specific cancers: pancreatic exocrine neoplasms and Leydig cell tumors of the testis in males and uterine stromal sarcomas, Zymbal's gland carcinomas, mammary gland fibroadenomas and carcinomas, and thyroid follicular cell neoplasms in females. Further, the average number of mammary gland fibroadenomas per rat was increased in both exposure groups. The occurrence of nine glial cell neoplasms of the brain in exposed male rats (controls, 1/100; 1,000 ppm, 4/100; 8,000 ppm, 5/100) may also have been related to exposure to 1,3-butadiene because neuroglial neoplasms are uncommon in

laboratory rats, occurring at a rate of about 0.2% to 1.0% in untreated male Sprague-Dawley rats (Krinke *et al.*, 1985; Gopinath, 1986).

In long-term inhalation studies of 1,3-butadiene in B6C3F<sub>1</sub> mice (NTP, 1984; Huff *et al.*, 1985), groups of 50 male and 50 female mice were exposed for 6 hours a day, 5 days a week, to air containing 0, 625, or 1,250 ppm 1,3-butadiene. These studies, designed to last for 103 weeks, were terminated after 60 to 61 weeks because survival was decreased at both exposure concentrations due to malignant neoplasms occurring in multiple organs of males and females.

Malignant lymphomas, hemangiosarcomas of the heart, and lung neoplasms occurred with positive trends in male and female mice, and the incidences of these neoplasms at both exposure concentrations were higher than those of controls (Table 1). The high incidences of hemangiosarcomas of the heart were particularly unusual findings, because these endothelial cell neoplasms are uncommon in B6C3F<sub>1</sub> mice, occurring in none of 573 untreated males and 558 untreated females in recent NTP studies, and they have rarely been induced in long-term studies.

TABLE 1  
Incidences of Primary Neoplasms in Mice Exposed to 1,3-Butadiene for 61 Weeks<sup>a</sup>

Neoplasm	Males			Females		
	0 ppm	625 ppm	1,250 ppm	0 ppm	625 ppm	1,250 ppm
Malignant Lymphoma	0/50	23/50	29/50	1/50	10/49	10/49
Heart						
Hemangiosarcoma	0/50	16/49	7/49	0/50	11/48	18/49
Lung						
Alveolar/bronchiolar neoplasm	2/50	14/49	15/49	3/49	12/48	23/49
Forestomach						
Squamous cell neoplasm	0/49	7/40	1/44	0/49	5/42	10/49
Mammary Gland						
Acinar cell neoplasm	0/50	0/50	0/50	0/50	2/49	6/49
Ovary						
Granulosa cell tumor	—	—	—	0/49	6/45	12/48
Liver						
Hepatocellular neoplasm	8/50	6/49	2/49	0/50	2/47	5/49

<sup>a</sup> Incidences are expressed as number of neoplasm-bearing animals/number of animals examined microscopically.

Irons and coworkers confirmed by cytofluorometric analysis of cell surface markers that the type of lymphoma caused by 1,3-butadiene in B6C3F<sub>1</sub> mice is a T-cell lymphoma (Irons *et al.*, 1989; Irons, 1990). Early induction and increased incidences of forestomach neoplasms were also observed in females and low-dose males, and increased incidences of neoplasms of the mammary gland, ovary, and liver were observed in females. The rate of malignant lymphomas was lower in females than in males and the dose-responses for other neoplasms were better characterized in females. These studies demonstrated that 1,3-butadiene is a potent multiple-organ carcinogen in mice.

Irons and coworkers compared the induction of thymic lymphomas and the expression of murine leukemia retrovirus in B6C3F<sub>1</sub> mice and NIH Swiss mice exposed to 1,250 ppm 1,3-butadiene for 52 weeks (Irons *et al.*, 1987, 1989; Irons, 1990). The NIH Swiss mouse was used because it does not express the ecotropic murine leukemia viruses expressed in B6C3F<sub>1</sub> mice and it has a background rate of nearly zero for thymic lymphoma. The finding that exposure to 1,3-butadiene caused a 14% incidence of thymic lymphomas in NIH Swiss mice clearly shows that 1,3-butadiene induces this neoplasm independently of these activated retroviruses. Irons *et al.* (1989) suggested that ecotropic viruses were involved in the induction of lymphomas by 1,3-butadiene based on the higher incidence of thymic lymphoma in exposed B6C3F<sub>1</sub> mice than in exposed NIH Swiss mice.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Testicular and ovarian atrophy occurred in B6C3F<sub>1</sub> mice exposed to 625 or 1,250 ppm 1,3-butadiene for up to 61 weeks (NTP, 1984). Also, a concentration-related increase in sperm head abnormalities was observed in B6C3F<sub>1</sub> mice exposed to 200, 1,000, or 5,000 ppm 1,3-butadiene for 6 hours a day for 5 days (Morrissey *et al.*, 1990). Exposure of male Swiss CD-1 mice to 200, 1,000, or 5,000 ppm 1,3-butadiene for 6 hours a day for 5 days followed by cohabitation with untreated female mice for 1 week did not affect male fertility but did cause an increase in the percentage of female mice with two or more dead implantations. Because intrauterine deaths were not increased at 3 weeks or more after exposure, it was concluded that the more mature spermatozoa and

spermatids were adversely altered by exposure to 1,3-butadiene (Morrissey *et al.*, 1990).

Exposure of pregnant female Sprague-Dawley rats to 200, 1,000, or 8,000 ppm of 1,3-butadiene for 6 hours a day, from day 6 through day 15 of gestation, caused a dose-related increase in the incidence of major skeletal defects of pups (IISRP, 1981b). "Wavy" ribs was the most common major skeletal defect in the 1,000 ppm and 8,000 ppm exposure groups. Other major skeletal defects, particularly in the high-dose group, included abnormalities of the skull, spine, sternum, ribs, and ilium. An exposure-related retardation of maternal body weight gain was not accompanied by any observable effects on pregnancy incidence, implantation loss, or gravid uterine weight. Mean fetal weights and crown-to-rump lengths were lower in the high-dose group than in the controls; embryonic growth retardation may have been a consequence of reduced maternal weight gain at the 8,000 ppm exposure level.

In another teratogenicity study, pregnant Sprague-Dawley rats and Swiss CD-1 mice were exposed to 40, 200, or 1,000 ppm of 1,3-butadiene for 6 hours a day on gestation days 6 through 15 (Morrissey *et al.*, 1990). There was no evidence of developmental toxicity in rats, although maternal body weight gain was decreased in the 1,000 ppm group. In mice, maternal body weight gain was decreased at the 200 ppm and 1,000 ppm exposure levels, whereas body weights of male fetuses were reduced at concentrations of 40 ppm and above. Thus, the male fetus is more susceptible than the dam to inhaled 1,3-butadiene. Malformations were not increased in rats or mice.

## GENETIC TOXICITY

1,3-Butadiene, a potent *in vivo* clastogen, is mutagenic *in vitro* when tested in the presence of induced liver S9 activation systems. An overview of the mutagenicity of 1,3-butadiene and its oxidative intermediates is presented below.

1,3-Butadiene was mutagenic in *Salmonella typhimurium* TA1530 and TA1535, strains that are sensitive to base-pair substitutions, in the presence of induced liver S9 fractions (de Meester *et al.*, 1980; Arce *et al.*, 1990). Butadiene monoxide (1,2-epoxy-3-butene), *dl*-1,2:3,4-diepoxbutane, and 1,2:3,4-diepoxbutane, oxidative intermediates of

1,3-butadiene biotransformation, were also mutagenic to base-pair substitution strains of *S. typhimurium*, but without S9 (de Meester *et al.*, 1978; Simmon, 1979; Wade *et al.*, 1979; Dunkel *et al.*, 1984; Canter *et al.*, 1986; Zeiger and Pagano, 1989). 1,2:3,4-Diepoxybutane has been reported to induce sex-linked recessive lethal mutations and chromosomal translocations in *Drosophila melanogaster* (Watson, 1972; Shukla and Auerbach, 1980; Olsen and Green, 1982; Sankaranarayanan *et al.*, 1983).

In one study, 1,3-butadiene was negative for induction of sister chromatid exchanges in human lymphocyte cultures in the presence of rat, mouse, or noninduced human S9 (Arce *et al.*, 1990); however, in another study, 1,3-butadiene was positive for the induction of sister chromatid exchanges, with or without S9, in human lymphocytes (Sasiadek *et al.*, 1991). Increases in sister chromatid exchanges were reported in Chinese hamster ovary cells (Perry and Evans, 1975; Nishi *et al.*, 1984) and human lymphocytes and fibroblasts (Friedman *et al.*, 1982; Obe *et al.*, 1982; Porfirio *et al.*, 1983; Sasiadek *et al.*, 1991) treated with the metabolite 1,2:3,4-diepoxybutane. In addition, 1,2:3,4-diepoxybutane induced chromosomal aberrations in human lymphocytes and fibroblasts obtained from patients with chromosome breakage disorders (Auerbach and Wolman, 1979; Auerbach *et al.*, 1982; Marx *et al.*, 1983; Porfirio *et al.*, 1983).

1,3-Butadiene is a potent *in vivo* genotoxic agent to mouse bone marrow cells. Exposure of male B6C3F<sub>1</sub> mice to 6.25, 62.5, or 625 ppm 1,3-butadiene for 6 hours a day for 10 exposure days produced increases in chromosomal aberrations and sister chromatid exchanges in bone marrow cells and micronuclei in erythrocytes obtained from peripheral blood samples (Tice *et al.*, 1987). The lowest effective doses for each of these endpoints were 625 ppm for sister chromatid exchanges, 6.25 ppm for chromosomal aberrations, and 62.5 ppm for micronuclei. Exposure to 6.25 to 625 ppm 1,3-butadiene for 5 days a week for 13 weeks resulted in significant increases in micronucleated normochromatic erythrocytes isolated from peripheral blood of male and female B6C3F<sub>1</sub> mice (Shelby, 1990). The metabolite butadiene monoxide was also shown to be a strong inducer of sister chromatid exchanges and chromosomal aberrations in bone marrow cells of male C57Bl/6 mice given a single intraperitoneal injection of this chemical (Sharief *et al.*, 1986).

Comparative genotoxicity studies were performed with male B6C3F<sub>1</sub> mice and male Sprague-Dawley rats (Cunningham *et al.*, 1986). In these studies, bone marrow cells of mice exposed to 100 to 10,000 ppm 1,3-butadiene for 6 hours a day for 2 days showed significant increases in micronuclei and sister chromatid exchanges; no increase in either endpoint was seen in similarly exposed rats. No induction of unscheduled DNA synthesis was noted in hepatocytes isolated from mice and rats exposed to 10,000 ppm 1,3-butadiene for 2 days (Arce *et al.*, 1990).

In conclusion, 1,3-butadiene is mutagenic *in vitro*, inducing gene mutations in *S. typhimurium*, and *in vivo*, inducing sister chromatid exchanges, chromosomal aberrations, and micronuclei in mice.

## HUMAN EFFECTS

Early toxicology studies on 1,3-butadiene indicated that this chemical only caused irritation to mucous membranes, skin, and eyes or caused narcosis at high concentrations (Carpenter *et al.*, 1944). Human volunteers exposed to 2,000, 4,000, or 8,000 ppm 1,3-butadiene for 6 to 8 hours experienced minor irritation to the eyes and difficulty in visual focusing.

In tank farm workers at a styrene-butadiene synthetic rubber plant, erythrocyte counts, hemoglobin concentrations, and packed red cell volumes were slightly, but not significantly, lower, and mean erythrocyte volumes were slightly, but not significantly, higher than those of workers in other departments (Checkoway and Williams, 1982). These changes are similar to those observed in mice exposed to 1,3-butadiene.

Associations between occupational exposure to 1,3-butadiene and increased cancer risk have been evaluated in retrospective mortality studies of workers employed at facilities which produce 1,3-butadiene (Downs *et al.*, 1987; Divine, 1990) and at facilities which produce styrene-butadiene rubber (Meinhardt *et al.*, 1982; Matanoski and Schwartz, 1987; Matanoski *et al.*, 1990). Increased mortalities from lymphatic and hematopoietic cancers were detected among subgroups of occupationally exposed workers in each of these studies. Mortality rates for lymphosarcoma and reticulum cell sarcoma were increased by as much as 5.6-fold among workers in a 1,3-butadiene manufacturing plant (Downs *et al.*,

1987), while 5- to 6.6-fold increases in mortality from all lymphopietic cancers and leukemia were reported among black workers in production areas of styrene-butadiene-rubber plants (Matanoski *et al.*, 1990). In a nested-case control study comparing lymphopietic cancer cases to an internal population of workers who did not have cancer, Matanoski *et al.* (1989) found that the odds ratio was 9.4 for the association of the leukemia cases with 1,3-butadiene exposure.

### **STUDY RATIONALE**

Because 1,3-butadiene is an important chemical with a large production volume and a potential for

exposure, and because the first inhalation studies in mice had been terminated early, additional studies were performed to better characterize exposure-response relationships for neoplasms and nonneoplastic lesions induced by this chemical in mice. Five exposure levels ranging from 625 ppm, corresponding to the lowest concentration used in the previous inhalation studies, down to 6.25 ppm were included to extend the exposure range over two orders of magnitude. Additional studies in which exposure to 1,3-butadiene was stopped after limited periods of time were also included to assess the relationship between concentration and duration of exposure on the outcome of butadiene-induced carcinogenicity.

## MATERIALS AND METHODS

### PROCUREMENT AND CHARACTERIZATION

1,3-Butadiene was periodically shipped as a liquified gas directly to the study laboratory, Battelle Pacific Northwest (Richland, WA), from Phillips Chemical Company, Philtex Plant (Borger, TX). Although seven lots (J-014, J-025, J-038, J-050, J-149, J-217, and J-375) were used in the 2-year and stop-exposure studies, only one lot was used at any one time. Battelle Pacific Northwest analyzed each lot of 1,3-butadiene and confirmed the identity by infrared spectroscopy and established the purity as  $\geq 99\%$  by gas chromatography. The maximum allowable dimer (4-vinyl-1-cyclohexene) content in the headspace of any lot used in the studies was set at 500 ppm. The dimer content increased with time and was monitored daily. Any cylinder yielding a dimer value greater than 500 ppm was not used.

According to NTP practice, a complete chemical characterization is performed on the chemical lot that will be used in the toxicology study. The bulk chemical is then shipped to the study laboratory. Because the dimer content of 1,3-butadiene increased with time, it was impractical to follow the normal procedure. Therefore, to allow for the instability of the chemical and to determine the appropriate analytical tests, a representative lot (F-850) from the same supplier was subjected to a full characterization. The analyses were performed by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and are described in Appendix G. The study chemical, a clear, colorless gas, was identified as 1,3-butadiene by infrared and nuclear magnetic resonance spectroscopy. Lot F-850 was determined by gas chromatography to be greater than 99% pure, with no impurities with areas of 0.1% or greater relative to the major peak area. Approximation of the concentration of the inhibitor, *t*-butylcatechol, in the liquid phase indicated approximately 4 ppm. The level of 4-vinyl-1-cyclohexene was determined by gas chromatography to be  $35 \pm 1$  ppm for the liquid phase and less than 1 ppm for the headspace.

### GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

1,3-Butadiene was delivered from the headspace of the cylinders through stainless steel tubing to a distribution manifold and flow control system. Six metering valves and flow meters controlled the gas flow to each chamber (Figure G3). The gas entered the airstream near the top of the study chambers (Hazleton 2000, Lab Products, Inc.).

The concentration of 1,3-butadiene in the chambers and room air was monitored with an automated sampling system coupled to a gas chromatograph. The system automatically cycled through all ports once every 30 minutes. Calibration was performed by analyzing volumetrically prepared standards of 1,3-butadiene. At least 93% of all concentration measurements were within 10% of the target concentrations. Monthly mean exposure concentrations are presented in Figures G4 through G8. A summary of chamber concentrations is presented in Table G1.

### CHAMBER ATMOSPHERE CHARACTERIZATION

Uniformity of concentration of 1,3-butadiene in each exposure chamber with animals present was checked at approximately 3-month intervals from 12 chamber positions by the same system used for daily concentration monitoring. The chamber concentration was considered to be uniform if the variability was less than 5% relative standard deviation. During the studies, the variability did not exceed 4.8%.

The time ( $T_{90}$ ) following the start of generation for the concentration to build to 90% of the final stable concentration in the chamber and the times ( $T_{10}$  and  $T_1$ ) following cessation of generation for the concentration to decay to 10% and 1% of the stable concentration were determined with animals present. The  $T_{90}$ ,  $T_{10}$ , and  $T_1$  values were 15 minutes, 12 minutes, and 35 minutes, respectively.

The major 1,3-butadiene degradation product expected in the studies was the dimer, 4-vinyl-1-cyclohexene, formed from the condensation of two molecules of 1,3-butadiene. Other potential degradation products were the oxidation products 3,4-epoxy-1-butene and 1,3-butadiene diepoxide. As discussed previously, the dimer content increased with time. Once the headspace concentration in a cylinder reached 500 ppm, the cylinder was no longer used. To monitor for the oxidation products, a fraction of the chamber atmosphere was bubbled through dimethyl formamide; the dimethyl formamide was then analyzed by gas chromatography. No evidence of oxidative degradation products at the 1% level was observed.

## 2-YEAR STUDIES

### Study Design

Groups of 70 male and 70 female mice were administered 0 (chamber control), 6.25, 20, 62.5, or 200 ppm 1,3-butadiene by inhalation for 6 hours a day, 5 days a week, for up to 103 weeks; groups of 90 male and 90 female mice were administered 625 ppm 1,3-butadiene on the same schedule. After 9 months and again after 15 months of 1,3-butadiene administration, up to 10 male and 10 female mice were randomly selected from each group for interim evaluations.

## 2-YEAR STOP-EXPOSURE STUDY

### Study Design

Groups of 50 male mice were administered 1,3-butadiene by inhalation at concentrations of 200 ppm for 40 weeks, 312 ppm for 52 weeks, or 625 ppm for 13 or 26 weeks. After the exposures were stopped, animals were placed in control chambers and were evaluated at 103 weeks. Mice were exposed for 6 hours a day, 5 days a week.

The stop-exposure study was designed to test the hypothesis that carcinogenic responses due to exposure to 1,3-butadiene were directly related to the product of the exposure concentration times the duration of exposure. The exposure condition of 625 ppm for 26 weeks was selected because in the previous study, lymphomas were induced by that time in male B6C3F<sub>1</sub> mice exposed to this concentration of 1,3-butadiene (NTP, 1984). By reducing the exposure concentration by 50% (312 ppm) and doubling the

duration of exposure (52 weeks), the 1,3-butadiene concentration times exposure duration was kept constant. Similarly, the total exposure at 200 ppm for 40 weeks was nearly equivalent to the total exposure at 625 ppm for 13 weeks and equal to approximately half the total exposure given to the group exposed to 625 ppm for 26 weeks. Thus the total exposure to 1,3-butadiene was approximately 487,000 ppm-hr for the groups of mice exposed to 625 ppm for 26 weeks or to 312 ppm for 52 weeks, and approximately 242,000 ppm-hr for the groups of mice exposed to 625 ppm for 13 weeks or to 200 ppm for 40 weeks.

### Source and Specification of Animals

Male and female B6C3F<sub>1</sub> mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year chronic and stop-exposure studies. Mice were quarantined 13 or 15 days. Five male and five female mice were randomly selected and killed for parasite evaluation and gross observation of disease. Blood samples were collected for viral screens. Male mice were approximately 6 to 8 weeks old and females were approximately 7 to 8 weeks old when the studies began. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

### Animal Maintenance

Mice were housed individually during the studies. Water was available *ad libitum*; feed was available *ad libitum* except during exposure periods. Cages were rotated within the exposure chambers weekly during the studies. Further details of animal maintenance are given in Table 2. 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 the beginning of the studies, weekly for 13 weeks, and monthly thereafter.

Up to 10 mice from each group in the 2-year chronic studies were evaluated at 9 months and after 15 months of 1,3-butadiene administration. Blood was drawn from the supraorbital sinus for clinical pathology evaluations. Bone marrow was obtained from the right femur of animals evaluated at 15 months for determination of bone marrow

cellularity. The brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus of each animal selected for the 9- and 15-month interim evaluations were weighed at necropsy. Further details of the interim evaluations are presented in Table 2.

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. At the 9- and 15-month interim evaluations, complete histopathology was performed on all control mice, all mice in the highest exposure group with survival of at least 60%, and all mice in groups with higher exposure concentrations. All animals that died or were killed moribund, all 2-year core study mice, and all stop-exposure mice also received a complete histopathologic examination. Tissues examined are listed in Table 2.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management 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 heart, lung, forestomach, liver, ovary, uterus, harderian gland, Zymbal's gland, kidney, small intestine, and skin of male and female mice, the brain, preputial gland, and testis of male mice, and the mammary gland, ovary, and uterus of female 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 examples of lesions from the forestomach, liver, heart, harderian gland, and lung of male and female mice, kidney and brain of male mice, ovary and mammary gland of female mice, and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent

toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the 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 or were found to be missing; 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 incidence of neoplasms or nonneoplastic lesions is given as the number of animals bearing such lesions at a specific anatomic site and the number of animals in which that site was examined. In most instances, the denominators include only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin, intestine, mammary gland, or harderian gland neoplasms) prior to histologic sampling, or when lesions had multiple potential sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

### *Analysis of Neoplasm Incidence*

The large numbers of exposed mice that died or were killed moribund early in these studies were considered to be due primarily to lymphoma or to hemangiosarcoma of the heart. Moreover, carcinomas of the forestomach, lung, preputial gland, mammary gland, and Zymbal's gland and sarcomas of the skin were considered to be lethal neoplasms.

Consequently, for these particular neoplasms, primary emphasis in the analysis of neoplasm incidence was given to the life table test (Cox, 1972; Tarone, 1975), a survival-adjusted procedure appropriate for rapidly lethal neoplasms.

For incidental neoplasms (neoplasms discovered as a result of death from an unrelated cause), the primary statistical method used in these studies was logistic regression, which assumed that the diagnosed neoplasms were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, neoplasm prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The exposed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986). When neoplasms are incidental, this comparison of the time-specific neoplasm prevalences also provides a comparison of the time-specific neoplasm incidence (McKnight and Crowley, 1984).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of neoplasm-bearing animals.

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

Because the increased mortality in the 1,3-butadiene-exposed groups reduced the sensitivity of logistic regression analyses for detecting carcinogenic effects, supplemental analyses were performed by the survival-adjusted "Poly-3" quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989). This procedure, which is currently being evaluated by

the NTP, modifies the Cochran-Armitage/Fisher exact test by adjusting the denominators of the neoplasm rates to take into account survival differences. This adjustment was derived by Portier and Bailer (1989), based on their fitting a Weibull model to historical control neoplasm data for a variety of site-specific neoplasms. The use of the power of  $k=3$  in the poly- $k$  adjustment for intercurrent mortality in the 1,3-butadiene study is justified by the following: (1) For liver and lung neoplasms in historical control animals, the observed survival adjusted value of  $k$  that best fits the data is approximately 3 in both male and female mice (Portier *et al.*, 1986). For leukemia/lymphoma combined, a slightly higher value was observed (6 in females and 5 in males). In male mice, the value of  $k$  for all neoplasms combined was approximately 3 and for females it was approximately 4.5. (2) Simulations have shown that it is better (in terms of preserving false positive error rate) to underestimate the value of  $k$  than to overestimate it. Thus, for example, it is better to use  $k=3$  when in truth  $k=6$  than it is to use  $k=6$  when in truth  $k=3$ . Because none of the values for  $k$  estimated by Portier *et al.* (1986) were significantly less than 3, and few were significantly greater than 3, the choice of 3 seems reasonable. The corresponding survival-adjusted neoplasm rates are also reported.

#### *Determining Dose-Response Shape for 1,3-Butadiene*

For those neoplasms showing chemical-related effects, the shape of the dose-response curve was estimated by fitting the following modified Weibull model (Portier *et al.*, 1986) to the data:

$$P(\text{dose}) = 1 - e^{-(\text{intercept} + \text{scale} \cdot \text{dose}^{\text{shape}})}$$

where  $P(\text{dose})$  is the probability of a neoplasm prior to study termination for animals administered dose  $\text{dose}$  of 1,3-butadiene. The parameters *intercept*, *scale*, and *shape* are estimated via maximum likelihood estimation using the likelihood

$$L = \sum_{i=0}^5 x_i \log[P(d_i)] + (n_i - x_i) \log[1 - P(d_i)]$$

where  $x_i$  is the number of animals with neoplasm in dose group  $d_i$ , and  $n_i$  is the poly-3 adjusted number of animals at risk in dose group  $d_i$ ,  $i=0,1,2,\dots,5$ . A likelihood ratio test is used to test the hypothesis that the shape parameter equals 1. The test statistic

is given as  $-2$  times the differences in the log likelihoods. A one-sided test was used so that the critical values are 2.706 for  $P=0.05$  and 5.410 for  $P=0.01$  (these are the squares of the critical regions from standard normal distribution). The shape parameter was restricted to be less than or equal to 10.

If the estimated shape parameter is greater than 1, the resulting dose-response has more curvature than a linear model and exhibits "threshold-like" behavior. If the estimated shape parameter is less than 1, then the dose-response curve is very steep in the low-dose region.

### *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. Consequently, control neoplasm incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for neoplasms appearing to show compound-related effects.

### *Analysis of Continuous Variables*

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which have typically skewed distributions, were analyzed using the non-parametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance

of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test).

## QUALITY ASSURANCE METHODS

The 2-year studies were conducted in compliance with Food and Drug Administration 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 the 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 TOXICITY

The genetic toxicity of 1,3-butadiene was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, trifluorothymidine resistance in L5178Y mouse lymphoma cells, sister chromatid exchanges and chromosomal aberrations in mouse bone marrow cells, sex-linked recessive lethal mutations in *Drosophila melanogaster*, and micronuclei in peripheral blood erythrocytes of mice. The protocols for these studies and tabular presentations of their findings are in Appendix D.

**TABLE 2**  
**Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene**

2-Year Studies	Stop-Exposure Study
<b>Study Laboratory</b> Battelle Pacific Northwest Laboratories, Richland, WA	Battelle Pacific Northwest Laboratories, Richland, WA
<b>Strain and Species</b> B6C3F <sub>1</sub> mice	B6C3F <sub>1</sub> mice
<b>Animal Source</b> Frederick Cancer Research Facility, Frederick, MD	Frederick Cancer Research Facility, Frederick, MD
<b>Size of Study Groups</b> 70 males and 70 females. 90 males and 90 females in 625 ppm dose groups	50 males
<b>Doses</b> 0, 6.25, 20, 62.5, 200, or 625 ppm in air	200, 312, or 625 ppm in air
<b>Time Held Before Study</b> Males: 15 days Females: 13 days	15 days
<b>Average Age When Placed on Study</b> Males: 6 to 8 weeks Females: 7 to 8 weeks	6 to 8 weeks
<b>Date of First Exposure</b> 23 January 1986	23 January 1986
<b>Duration of Exposure</b> 6 hours daily, 5 days a week, for up to 103 weeks	6 hours daily, 5 days a week, for 13, 26, 40, or 52 weeks
<b>Date of Last Exposure</b> 9-month interims: 30 October 1986 15-month interims: 23 April 1987 2-year studies: 13 January 1988	13-week stop exposure: 23 April 1986 26-week stop exposure: 23 July 1986 40-week stop exposure: 29 October 1986 52-week stop exposure: 21 January 1987
<b>Average Age When Killed</b> 111 to 113 weeks	111 to 113 weeks
<b>Method of Sacrifice</b> 70% carbon dioxide	70% carbon dioxide
<b>Method of Animal Distribution</b> Animals randomly assigned to control and exposure groups with body weight as the blocking variable, using the XYBION PATH/TOX System.	Animals randomly assigned to control and exposure groups with body weight as the blocking variable, using the XYBION PATH/TOX System.
<b>Animals per Cage</b> 1	1

(continued)

**TABLE 2**  
**Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene (continued)**

2-Year Studies	Stop-Exposure Study
<b>Method of Animal Identification</b>	
Toe clip	Toe clip
<b>Diet</b>	
NIH-07 open-formula diet, pellets (Zeigler Bros., Inc., Gardners, PA), available <i>ad libitum</i> , except during exposure periods	NIH-07 open-formula diet, pellets (Zeigler Bros., Inc., Gardners, PA), available <i>ad libitum</i> , except during exposure periods
<b>Water</b>	
Tap water (City of Richland water supply) via automatic watering system (Systems Engineering, Napa, CA), available <i>ad libitum</i>	Tap water (City of Richland water supply) via automatic watering system (Systems Engineering, Napa, CA), available <i>ad libitum</i>
<b>Cages</b>	
Stainless steel wire-bottom cages (Hazleton Systems, Inc., Aberdeen, MD), changed weekly	Stainless steel wire-bottom cages (Hazleton Systems, Inc., Aberdeen, MD), changed weekly
<b>Chambers</b>	
Stainless steel chambers (Lab Products, Inc., Harford Division, Aberdeen, MD)	Stainless steel chambers (Lab Products, Inc., Harford Division, Aberdeen, MD)
<b>Animal Chamber Environment</b>	
Average temperature: 23.5° ± 1.5° C	Average temperature: 23.5° ± 1.5° C
Relative humidity: 55% ± 15%	Relative humidity: 55% ± 15%
Fluorescent light: 12 hours/day	Fluorescent light: 12 hours/day
Room air changes: 17-21/hour	Room air changes: 17-21/hour
<b>Type and Frequency of Observation</b>	
Observed twice daily; weighed initially, weekly for 13 weeks, monthly thereafter; clinical observations recorded monthly	Observed twice daily; weighed initially, weekly for 13 weeks, monthly thereafter; clinical observations recorded monthly
<b>Necropsy</b>	
Necropsy performed on all animals. The following organs were weighed at the 9- and 15-month interim evaluations: brain, heart, right kidney, liver, lungs, spleen, right testis, and thymus.	Necropsy performed on all animals.
<b>Clinical Pathology</b>	
Clinical pathology studies were performed at the 9- and 15-month interim evaluations.	None
<b>Hematology:</b> Packed red cell volume, hemoglobin, erythrocytes, Howell-Jolly bodies, mean erythrocyte volume, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration, platelets, reticulocytes, and leukocyte count and differential, total bone marrow cellularity (15 months only)	
<b>Clinical chemistry:</b> Creatine kinase and lactate dehydrogenase	

(continued)

**TABLE 2**  
**Experimental Design and Materials and Methods in the Inhalation Studies of 1,3-Butadiene (continued)**

2-Year Studies	Stop-Exposure Study
<p><b>Histopathology</b>            Complete histopathology was performed on all controls, 200, and 625 ppm mice at the 9-month interim evaluation; all controls, all exposed males, and 62.5, 200, and 625 ppm females at the 15-month interim evaluation; all animals dying early or killed moribund; and all 2-year core study mice. The following tissues were routinely examined microscopically: gross lesions and tissue masses with regional lymph nodes, adrenal gland, brain, epididymis, esophagus, gallbladder, harderian gland, heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph node (bronchial, mediastinal, mandibular, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, sternbrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, and uterus.</p>	<p>Complete histopathology was performed on all stop-exposure mice. The following tissues were routinely examined microscopically: gross lesions and tissue masses with regional lymph nodes, adrenal gland, brain, epididymis, esophagus, gallbladder, harderian gland, heart, kidney, large intestine (cecum, colon, rectum), larynx, liver, lung, lymph node (bronchial, mediastinal, mandibular, mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pharynx, pituitary gland, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spleen, sternbrae (including marrow), stomach (forestomach and glandular), testis, thymus, thyroid gland, and trachea.</p>

## RESULTS

### 2-YEAR STUDIES

#### Survival

Estimates of the probabilities of survival for male and female mice exposed to 1,3-butadiene and control mice are shown in Table 3 and in the Kaplan-Meier survival curves in Figure 2. Exposure-related mortalities first occurred during week 23, mainly in mice

exposed to 625 ppm, and were due primarily to the induction of fatal neoplasms and their associated lesions. No female mice exposed to 200 or 625 ppm or male mice exposed to 625 ppm survived to the end of the studies. Survival was decreased for males and females exposed to concentrations of 20 ppm or above. The decreases in survival were dose related.

TABLE 3  
Survival of Mice in the 2-Year Inhalation Studies of 1,3-Butadiene

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation <sup>a</sup>	10	10	10	10	10	10
15-Month interim evaluation <sup>a</sup>	10	10	10	10	10	7
Accidental deaths <sup>a</sup>	0	0	0	0	0	1
Moribund	9	6	15	15	23	33
Natural deaths	6	5	11	12	23	39
Missing <sup>a</sup>	0	0	0	1	0	0
Animals surviving until study termination	35	39	24	22	4 <sup>b</sup>	0
Percent probability of survival at end of study <sup>c</sup>	70	78	49	46	8	0
Mean survival (days) <sup>d</sup>	597	611	575	558	502	280
Survival analysis <sup>e</sup>	P<0.001	P=0.430N	P=0.044	P=0.021	P<0.001	P<0.001
<b>Female</b>						
Animals initially in study	70	70	70	70	70	90
9-Month interim evaluation <sup>a</sup>	10	10	10	10	10	8
15-Month interim evaluation <sup>a</sup>	10	10	10	10	10	2
Accidental deaths <sup>a</sup>	0	0	1	0	1	1
Moribund	10	10	14	31	37	46
Natural deaths	3	7	11	8	12	33
Animals surviving until study termination	37	33	24	11	0	0
Percent probability of survival at end of study	74	66	50	23	0	0
Mean survival (days)	608	597	573	548	441	320
Survival analysis	P<0.001	P=0.510	P=0.013	P<0.001	P<0.001	P<0.001

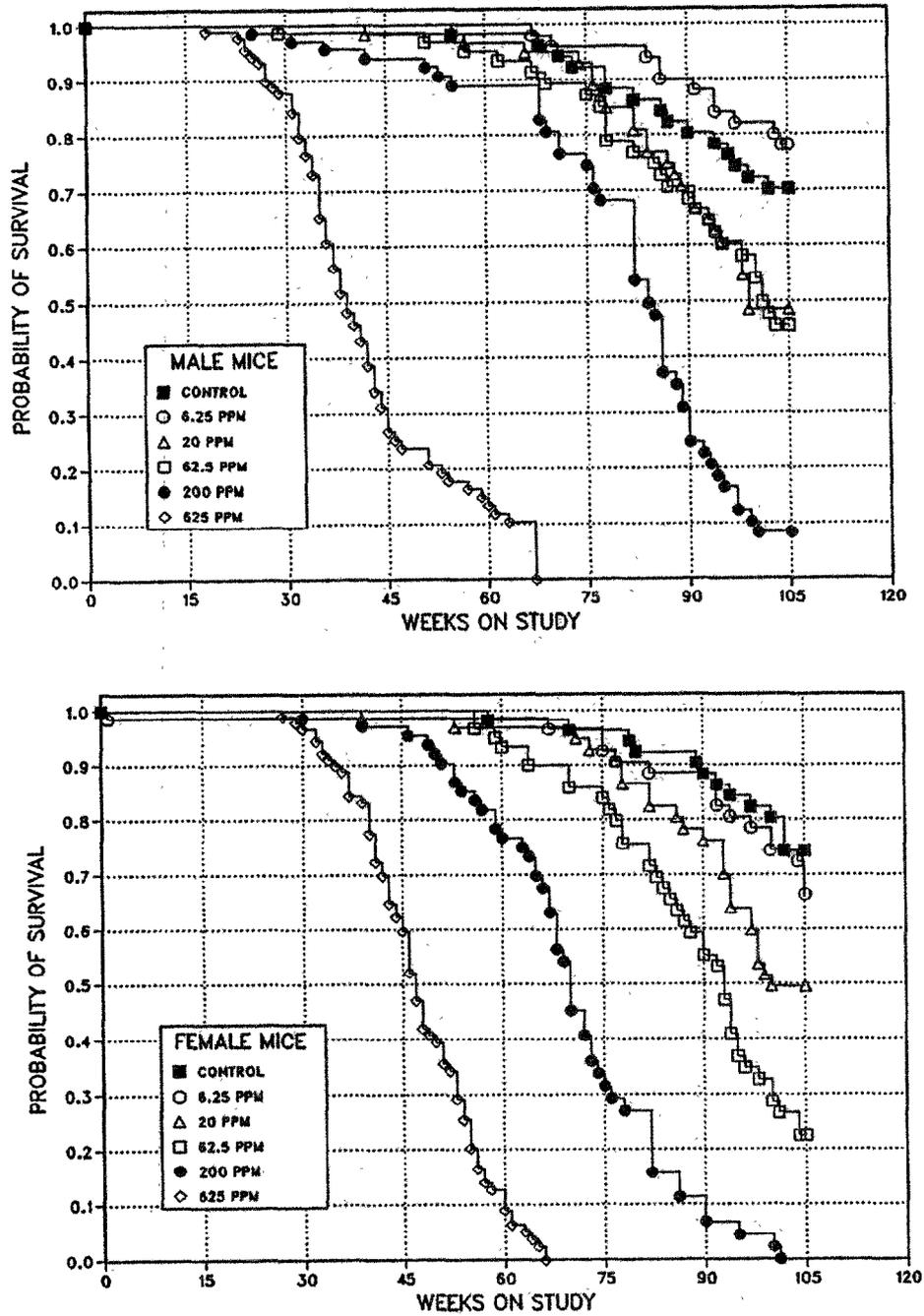
<sup>a</sup> Censored from survival analyses

<sup>b</sup> Includes one animal that died during the last week of the study

<sup>c</sup> Kaplan-Meier determinations. Survival rates adjusted for interim evaluations, accidental deaths, and missing animals.

<sup>d</sup> Mean of all deaths (uncensored, censored, terminal sacrifice)

<sup>e</sup> 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. A lower mortality in a dose group is indicated by N.



**FIGURE 2**  
**Kaplan-Meier Survival Curves for Mice Administered 1,3-Butadiene by Inhalation for 2 Years**

### ***Body Weights and Clinical Findings***

Mean body weights of exposed male and female mice were similar to those of controls throughout the study (Tables 4 and 5 and Figure 3). No clinical findings other than those associated with lesion development and moribundity were observed.

### ***Hematology and Clinical Chemistry***

At the 9-month interim evaluation, statistically significant, dose-related decreases in mean values for erythrocyte counts, hemoglobin concentration, and packed red cell volume occurred in male mice exposed to 62.5 ppm or above (Table F1). At the 625 ppm level, mean erythrocyte volume was significantly increased, although there was no evident reticulocytosis or excessive polychromasia compared to the control males. The percentage of erythrocytes with Howell-Jolly body inclusions was also significantly increased in males exposed to 625 ppm, and significant leukopenia and lymphopenia occurred in males exposed to 200 or 625 ppm. In females exposed to 200 or 625 ppm, erythroid parameters including erythrocyte counts, hemoglobin concentration, and packed red cell volume were significantly reduced. Macrocytosis and significant increases in the percentage of erythrocytes with Howell-Jolly bodies and mean erythrocyte hemoglobin, without a significant increase in reticulocytes or excessive polychromasia, occurred in females exposed to 200 or 625 ppm. Leukopenia and lymphopenia did not occur in females in the 200 and 625 ppm groups, although neutrophils and lymphocytes were lower in these groups than in the control females. These findings are consistent with a mild to moderate, poorly regenerative, macrocytic anemia. The mechanism for the anemia cannot be determined from the available data, but a mild megaloblastic anemia resulting from ineffective erythropoiesis in the bone marrow cannot be excluded. There were no significant differences or exposure-related trends in total serum enzyme activity of lactate dehydrogenase or creatine kinase of either sex at the 9-month interim evaluation.

At the 15-month interim evaluation, there was a statistically significant decrease in mean values for erythrocyte counts, hemoglobin concentrations, and packed red cell volumes, and a significant increase in the mean erythrocyte volumes in males and females exposed to 625 ppm (Table F2). Males and females in the 625 ppm groups had statistically significant

increases in the percentage of erythrocytes with Howell-Jolly body inclusions. Increases in numbers of Howell-Jolly bodies can occur in regenerative or megaloblastic anemias. Males exposed to 625 ppm 1,3-butadiene had a significant increase in mean platelet value, a finding that correlated with the occurrence of neoplasms. Bone marrow cell counts of the two surviving females in the 625 ppm group were greater than those of the controls. No statistical differences were noted in bone marrow cell counts between control and exposed males. Microscopic evaluations of the marrow smears revealed a slight left shift in the erythroid series of both sexes exposed to 200 or 625 ppm. There was also an increase in erythroblasts with some megaloblastic characteristics and an increase in necrobiotic erythroblasts and in erythroblasts with a multilobed nucleus. Two males in the 625 ppm group had many hypersegmented neutrophils present, and one contained a uniform population of immature mononuclear cells, which led to the diagnosis of malignant lymphoma. Total lactate dehydrogenase (LDH) values were increased in males and females exposed to 200 or 625 ppm (Table F2). The percentages of LDH-1 and LDH-2 were decreased and LDH-5 was increased in males and females exposed to 625 ppm. LDH-5 is the principal isoenzyme in skeletal muscle and liver, and the finding of a marked increase in hepatocellular necrosis may be the cause of the increased percent LDH-5; however, LDH is contained in all tissues in varying amounts. In no case was there any evidence of isoenzyme patterns suggestive of those observed in humans with myocardial infarction or muscle disease, lesions that are primarily associated with necrosis and not proliferation. There was no correlation between the total enzyme and isoenzyme values on an individual basis and the histopathologic lesion diagnosis.

### ***Pathology and Statistical Analyses of Results***

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the hematopoietic system, heart, lung, stomach, liver, ovary, uterus, harderian gland, mammary gland, preputial gland, kidney, skin, Zymbal's gland, small intestine, testis, and nose. Summaries of the incidences of neoplasms and nonneoplastic lesions, the individual animal tumor diagnoses, the statistical analyses of primary neoplasms that occurred with an incidence

**TABLE 4**  
**Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of 1,3-Butadiene**

Weeks on Study	0 ppm		6.25 ppm			20 ppm			62.5 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.0	70	23.1	100	70	22.9	100	70	22.9	100	70
2	24.4	70	24.2	99	70	24.1	99	70	24.1	99	70
3	25.8	70	25.6	99	70	25.3	98	70	24.9	97	69
4	27.1	70	26.6	98	70	26.5	98	70	26.3	97	69
5	27.8	70	27.4	99	70	27.7	100	70	27.5	99	69
6	28.7	70	28.5	99	70	28.6	100	70	28.3	99	69
7	29.2	70	28.9	99	70	29.2	100	70	29.1	100	69
8	30.1	70	29.5	98	70	30.0	100	70	29.7	99	69
9	30.7	70	29.9	97	70	30.6	100	70	30.1	98	69
10	31.1	70	30.7	99	70	31.2	100	70	30.9	99	69
11	32.0	70	31.4	98	70	32.3	101	70	32.0	100	69
12	32.7	70	31.7	97	70	32.3	99	70	32.1	98	69
13	33.0	70	31.9	97	70	32.7	99	70	32.2	98	69
14	33.7	70	32.4	96	70	33.4	99	70	32.9	98	69
18	35.2	70	34.7	99	70	36.0	102	70	35.6	101	69
22	36.6	70	36.8	101	70	37.9	104	70	36.3	99	69
26	38.9	70	37.6	97	70	39.5	102	70	37.5	96	69
30	40.8	70	40.0	98	70	42.0	103	70	40.7	100	68
34	41.7	70	41.0	98	70	42.9	103	70	41.9	101	68
38	43.2	70	42.5	98	70	44.5	103	70	42.2	98	68
42 <sup>a</sup>	43.3	60	42.8	99	60	44.2	102	60	43.8	101	58
46	44.1	60	43.6	99	60	45.1	102	59	45.0	102	58
49	45.5	60	44.3	97	60	45.9	101	59	45.1	99	58
54	46.0	60	44.8	97	60	46.6	101	59	45.7	99	57
58	45.7	59	45.1	99	60	46.1	101	58	46.6	102	56
62	46.1	59	45.9	100	60	46.9	102	58	46.5	101	56
66 <sup>a</sup>	45.7	49	45.4	99	50	46.1	101	48	46.5	102	45
70	45.8	48	46.4	101	48	46.6	102	47	47.0	103	43
74	45.3	46	46.0	102	48	46.5	103	47	46.8	103	43
78	45.1	46	45.4	101	48	46.5	103	43	46.7	104	41
82	44.8	44	44.9	100	48	45.5	102	42	46.5	104	38
86	44.3	43	45.5	103	47	44.9	101	38	46.4	105	36
90	44.0	41	45.8	104	45	44.8	102	35	45.2	103	34
94	43.1	39	44.5	103	44	43.7	101	32	43.9	102	31
96	42.9	39	44.6	104	42	43.0	100	30	43.6	102	29
97	43.0	37	44.6	104	41	41.6	97	30	41.6	97	29
99	42.3	37	44.0	104	41	41.9	99	25	41.4	98	28
101	41.6	36	43.4	104	41	42.0	101	24	40.2	97	25
103	41.5	35	43.7	105	40	41.6	100	24	40.3	97	22
Terminal sacrifice		35			39			24			22
Mean for weeks											
1-13	28.9		28.4	98		28.7	99		28.5	99	
14-52	40.3		39.6	98		41.1	102		40.1	100	
53-103	44.2		45.0	102		44.6	101		44.7	101	

(continued)

**TABLE 4**  
**Mean Body Weights and Survival of Male Mice in the 2-Year Inhalation Study of 1,3-Butadiene (continued)**

Weeks on Study	0 ppm		200 ppm			625 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.0	70	22.5	98	70	22.4	97	90
2	24.4	70	23.9	98	70	23.6	97	90
3	25.8	70	25.2	98	70	25.0	97	90
4	27.1	70	26.2	97	70	26.1	96	90
5	27.8	70	27.1	98	70	26.8	96	90
6	28.7	70	28.1	98	70	28.1	98	90
7	29.2	70	28.9	99	70	28.5	98	90
8	30.1	70	29.9	99	70	29.2	97	90
9	30.7	70	30.2	98	70	29.5	96	90
10	31.1	70	30.7	99	70	30.2	97	90
11	32.0	70	31.6	99	70	31.0	97	90
12	32.7	70	32.4	99	70	31.8	97	90
13	33.0	70	33.1	100	70	32.1	97	90
14	33.7	70	33.3	99	70	32.4	96	90
18	35.2	70	35.9	102	70	34.9	99	89
22	36.6	70	37.9	104	70	37.2	102	89
26	38.9	70	40.1	103	69	39.2	101	85
30	40.8	70	42.2	103	69	41.1	101	78
34	41.7	70	43.1	103	68	41.5	100	68
38	43.2	70	45.1	104	67	42.4	98	49
42 <sup>a</sup>	43.3	60	46.4	107	56	42.9	99	29
46	44.1	60	46.3	105	56	43.8	99	18
49	45.5	60	47.2	104	56	44.0	97	16
54	46.0	60	47.2	103	54	45.9	100	13
58	45.7	59	47.4	104	53	45.7	100	11
62	46.1	59	47.6	103	53	45.4	99	8
66 <sup>a</sup>	45.7	49	47.5	104	43			
70	45.8	48	47.0	103	39			
74	45.3	46	46.7	103	37			
78	45.1	46	47.2	105	33			
82	44.8	44	44.7	100	33			
86	44.3	43	43.7	99	23			
90	44.0	41	42.2	96	15			
94	43.1	39	41.5	96	10			
96	42.9	39	40.8	95	8			
97	43.0	37	40.0	93	6			
99	42.3	37	38.5	91	5			
101	41.6	36	39.5	95	4			
103	41.5	35	39.0	94	4			
Terminal sacrifice		35			4			0
Mean for weeks								
1-13	28.9		28.4	98		28.0	97	
14-52	40.3		41.8	104		39.9	99	
53-103	44.2		43.8	99				

<sup>a</sup> Interim evaluations occurred during weeks 41 and 66.

**TABLE 5**  
**Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of 1,3-Butadiene**

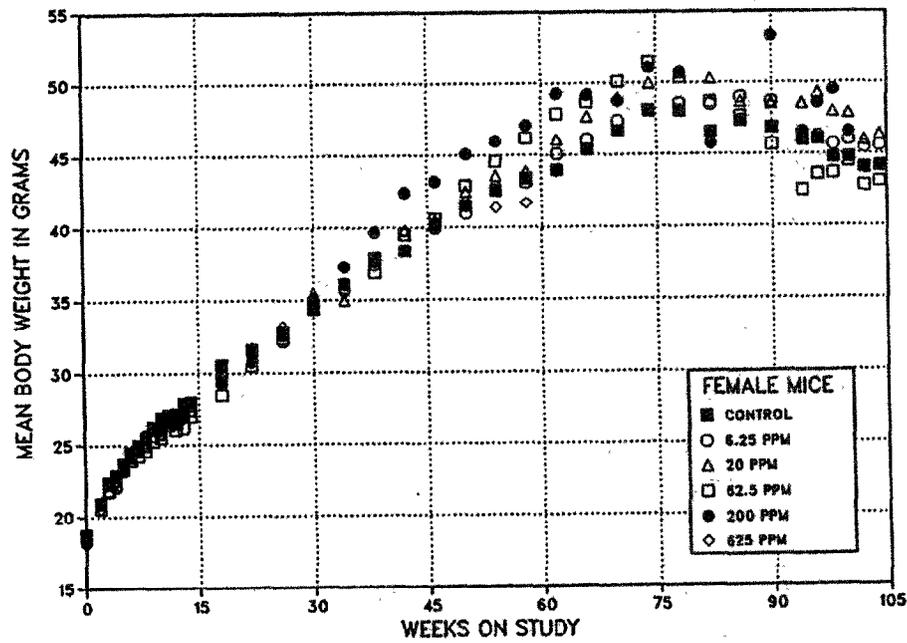
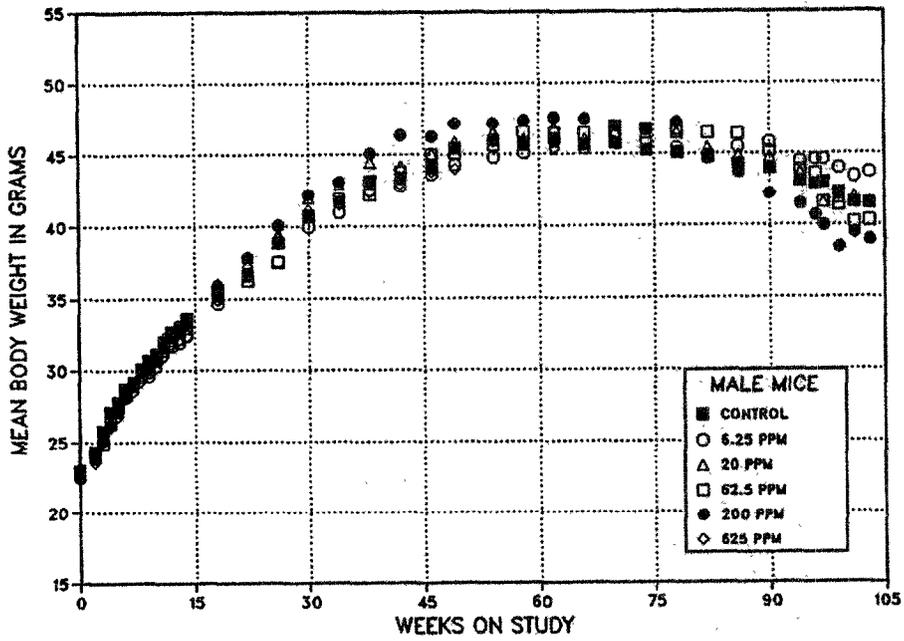
Weeks on Study	0 ppm		6.25 ppm			20 ppm			62.5 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	70	18.6	99	70	18.6	99	70	18.5	98	70
2	21.0	70	21.0	100	69	21.1	101	70	20.6	98	70
3	22.4	70	21.8	97	69	21.9	98	70	21.8	97	70
4	22.9	70	22.1	97	69	22.3	97	70	22.6	99	70
5	23.7	70	23.2	98	69	23.3	98	70	23.3	98	70
6	24.5	70	24.1	98	69	24.1	98	70	23.9	98	70
7	25.0	70	24.6	98	69	24.7	99	70	24.2	97	70
8	25.4	70	25.2	99	69	25.0	98	70	24.6	97	70
9	26.3	70	25.7	98	69	25.8	98	70	25.3	96	70
10	26.9	70	25.8	96	69	25.9	96	70	25.5	95	70
11	27.1	70	26.4	97	69	26.7	99	70	26.4	97	70
12	27.2	70	26.7	98	69	26.5	97	70	26.1	96	70
13	27.9	70	27.0	97	69	27.1	97	70	26.2	94	70
14	28.0	70	27.4	98	69	27.4	98	70	27.0	96	70
18	30.6	70	29.8	97	69	29.9	98	70	28.5	93	70
22	31.6	70	31.4	99	69	31.3	99	70	30.5	97	70
26	32.9	70	32.2	98	69	32.6	99	70	32.4	99	70
30	34.4	70	34.7	101	69	35.5	103	69	34.6	101	70
34	36.1	70	35.7	99	69	35.0	97	69	35.9	99	70
38	37.9	70	37.4	99	69	37.8	100	69	36.9	97	70
42 <sup>a</sup>	38.4	60	39.5	103	59	39.8	104	58	39.5	103	60
46	40.2	60	40.0	100	59	40.5	101	58	40.6	101	60
50	41.5	60	41.0	99	59	42.5	102	58	42.9	103	60
54	42.6	60	42.8	101	59	43.6	102	57	44.6	105	60
58	43.4	60	43.1	99	59	43.9	101	57	46.2	107	58
62	44.0	59	45.1	103	59	46.1	105	57	47.9	109	56
66 <sup>a</sup>	45.4	49	46.1	102	49	47.7	105	47	48.7	107	44
70	46.7	49	47.4	102	48	49.0	105	47	50.1	107	44
74	48.1	48	48.1	100	48	50.0	104	45	51.5	107	42
78	48.1	48	48.6	101	45	50.7	105	43	50.4	105	39
82	46.6	46	48.5	104	45	50.3	108	42	48.7	105	37
86	47.4	46	49.0	103	44	48.7	103	40	47.7	101	32
90	46.9	45	48.7	104	44	48.6	104	38	45.7	97	29
94	46.0	43	46.6	101	41	48.6	106	34	42.5	92	23
96	46.1	42	46.3	100	40	49.3	107	31	43.6	95	18
98	44.8	41	45.7	102	39	48.0	107	29	43.7	98	17
100	44.8	41	46.0	103	39	47.9	107	25	44.5	99	16
102	44.1	40	45.5	103	37	46.0	104	24	42.8	97	13
104	44.2	37	45.6	103	37	46.4	105	24	43.1	98	13
<b>Terminal sacrifice</b>		<b>37</b>			<b>33</b>			<b>24</b>			<b>11</b>
<b>Mean for weeks</b>											
1-13	24.5		24.0	98		24.1	98		23.8	97	
14-52	35.2		34.9	99		35.2	100		34.9	99	
53-104	45.6		46.4	102		47.8	105		46.4	102	

(continued)

**TABLE 5**  
**Mean Body Weights and Survival of Female Mice in the 2-Year Inhalation Study of 1,3-Butadiene**  
 (continued)

Weeks on Study	0 ppm		200 ppm			625 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.8	70	18.2	97	70	18.3	97	90
2	21.0	70	20.9	100	69	20.6	98	90
3	22.4	70	22.3	100	69	21.7	97	89
4	22.9	70	22.8	100	69	22.4	98	89
5	23.7	70	23.4	99	69	23.2	98	89
6	24.5	70	24.3	99	69	24.2	99	89
7	25.0	70	24.7	99	69	24.7	99	89
8	25.4	70	25.7	101	69	25.4	100	89
9	26.3	70	26.1	99	69	25.6	97	89
10	26.9	70	26.1	97	69	25.8	96	89
11	27.1	70	26.6	98	69	26.6	98	89
12	27.2	70	26.7	98	69	26.5	97	89
13	27.9	70	27.2	98	69	26.9	96	89
14	28.0	70	27.8	99	69	27.4	98	89
18	30.6	70	29.3	96	69	29.5	96	89
22	31.6	70	30.8	98	69	31.7	100	89
26	32.9	70	32.7	99	69	33.2	101	89
30	34.4	70	35.1	102	69	35.0	102	86
34	36.1	70	37.3	103	68	36.2	100	81
38	37.9	70	39.7	105	68	37.6	99	75
42 <sup>a</sup>	38.4	60	42.4	110	57	39.8	104	56
46	40.2	60	43.2	108	57	40.4	101	45
50	41.5	60	45.1	109	54	41.8	101	32
54	42.6	60	46.0	108	51	41.4	97	22
58	43.4	60	47.1	109	48	41.7	96	11
62	44.0	59	49.3	112	45	43.9	100	5
66 <sup>a</sup>	45.4	49	49.2	108	31			
70	46.7	49	48.8	105	23			
74	48.1	48	51.1	106	16			
78	48.1	48	50.8	106	13			
82	46.6	46	45.8	98	12			
86	47.4	46	47.4	100	7			
90	46.9	45	53.3	114	4			
94	46.0	43	46.6	101	3			
96	46.1	42	48.6	105	2			
98	44.8	41	49.5	111	2			
100	44.8	41	46.6	104	2			
102	44.1	40						
104	44.2	37						
Terminal sacrifice		37			0			0
Mean for weeks								
1-13	24.5		24.2	99		24.0	98	
14-52	35.2		36.3	103		35.3	100	
53-104	45.6		42.5	93				

<sup>a</sup> Interim evaluations occurred during weeks 41 and 66.



**FIGURE 3**  
**Growth Curves for Mice Administered 1,3-Butadiene by Inhalation for 2 Years**

of at least 5% in at least one animal group, and historical incidences for the biologically significant neoplasms mentioned in this section are presented in Appendix A for male mice and Appendix B for female mice.

**Hematopoietic System:** At the 9-month interim evaluation, the absolute and relative thymus weights of females receiving 625 ppm were lower than those of controls (Table E1). The relative thymus weight of 200 ppm females was also lower than that of controls. In addition, the relative spleen weight of females that received 62.5 ppm or more and the absolute spleen weight of females that received 200 ppm were lower than those of controls. At the 15-month interim evaluation, the absolute and relative spleen weights of males and females receiving 625 ppm were greater than those of controls (Table E2). The absolute spleen weight of the 200 ppm females was also greater than that of the controls.

Exposure of mice to 1,3-butadiene was associated with the development of malignant lymphoma, and to a lesser extent, histiocytic sarcoma (Table 6). The incidence of malignant lymphomas, particularly the lymphocytic type, was significantly greater in males exposed to 625 ppm and in females exposed to 200 or 625 ppm than in the controls. The lymphocytic lymphomas occurred as early as week 23 and peaked before the 15-month interim evaluations. Malignant lymphomas of the mixed and undifferentiated cell types occurred at incidences more typical of the spontaneous lesions seen in B6C3F<sub>1</sub> mice. The incidence of histiocytic sarcoma was significantly greater in males and females exposed to 200 ppm than in the controls. Moreover, the incidences in male mice exposed to 20, 62.5, or 625 ppm and in females exposed to 625 ppm were marginally higher than in the controls.

Although many organs, particularly the spleen, lymph nodes, liver, lung, and kidney, were affected in mice with lymphocytic lymphoma, the thymus was involved in most mice, and in some, the thymus was the predominant organ affected. The lymphocytic lymphomas consisted of uniform populations of small- to medium-sized lymphocytes, whereas the mixed and undifferentiated lymphomas generally consisted of more heterogeneous populations of lymphocytes with cellular pleomorphism and atypia. The histiocytic sarcomas have previously been referred to as

reticulum cell sarcoma, type A, or malignant lymphoma, histiocytic type, but currently are believed to be derived from nonlymphoid, mononuclear phagocytic cells. Histologically, the sarcoma cells were large and monomorphic, with dark basophilic nuclei and relatively abundant eosinophilic cytoplasm. Multinucleated giant cells were sometimes seen.

Bone marrow atrophy was observed in both sexes only at the highest exposure concentration, 625 ppm (Tables A5 and B5). Atrophy varied in severity from mild depletion of hematopoietic cells in mice evaluated at 9 months to marked depletion in mice that died or were killed before 15 months. Incidences of bone marrow hyperplasia in female mice exposed to 62.5, 200, or 625 ppm were greater than the incidences in the control group (Table B5). The hyperplasia was primarily an increase in granulocyte precursor cells, a response that correlated with the presence of large neoplasms in the skin, mammary gland, or other organs. Increased severity or incidence of hematopoiesis in the spleen, liver, and lung was also observed in females at the three highest exposure concentrations. The hematopoiesis in these organs paralleled the finding of bone marrow hyperplasia.

At the 9-month interim evaluations, absolute thymus weights were decreased in males and females exposed to 625 ppm (Table E1); the decrease in females was statistically significant. These decreased weights correlated with the presence of thymic necrosis (atrophy). The incidence of thymic necrosis was also increased in female mice exposed to 62.5, 200, or 625 ppm.

**Heart:** The absolute heart weights of females receiving 625 ppm were greater than those of the controls at both the 9- and 15-month interim evaluations (Tables E1 and E2). The absolute heart weight of 200 ppm females was also greater than that of controls at the 15-month interim evaluation.

The incidences of hemangiosarcoma of the heart in males exposed to 62.5 ppm or greater and in males and females exposed to 200 or 625 ppm were significantly higher than in the controls (Table 7). Endothelial hyperplasia was also observed in exposed mice. The cardiac hemangiosarcomas occurred in all ventricular locations, but were more frequent in the left ventricular wall. They were occasionally

**TABLE 6**  
**Malignant Lymphoma and Histiocytic Sarcoma in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>9-Month Interim Evaluation</b>						
Lymphocytic Malignant Lymphoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
<b>15-Month Interim Evaluation</b>						
Lymphocytic Malignant Lymphoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	2/7 (29%)
<b>2-Year Study</b>						
<b>Lymphocytic Malignant Lymphoma</b>						
Overall rate	2/50 (4%)	0/50 (0%)	2/50 (4%)	4/50 (8%)	2/50 (4%)	49/73 (67%)
Adjusted rate <sup>b</sup>	4.7%	0.0%	4.2%	12.0%	4.0%	95.1%
Terminal rate <sup>c</sup>	0/35 (0%)	0/39 (0%)	0/24 (0%)	0/22 (0%)	0/4 (0%)	0/0
First incidence (days)	511	- <sup>e</sup>	456	546	171	161
Life table test <sup>d</sup>	P<0.001	P=0.227N	P=0.671	P=0.253	P=0.529	P<0.001
<b>Malignant Lymphoma (Mixed or Undifferentiated Cell Type)</b>						
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	0/50 (0%)	0/50 (0%)	1/73 (1%)
<b>Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, NOS, or Undifferentiated Cell Type)<sup>f</sup></b>						
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	6/50 (12%)	2/50 (4%)	51/73 (70%)
Adjusted rate	9.8%	5.1%	12.2%	17.7%	4.0%	95.4%
Terminal rate	1/35 (3%)	2/39 (5%)	2/24 (8%)	1/22 (5%)	0/4 (0%)	0/0
First incidence (days)	511	733 (T)	456	200	171	161
Life table test	P<0.001	P=0.302N	P=0.528	P=0.238	P=0.627	P<0.001
<b>Histiocytic Sarcoma<sup>g</sup></b>						
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	5/50 (10%)	7/50 (14%)	4/73 (5%)
Adjusted rate	0.0%	0.0%	10.6%	14.3%	31.9%	10.8%
Terminal rate	0/35 (0%)	0/39 (0%)	0/24 (0%)	1/22 (5%)	0/4 (0%)	0/0
First incidence (days)	-	-	531	398	288	120
Life table test	P<0.001	-	P=0.051	P=0.021	P<0.001	P=0.043

(continued)

**TABLE 6**  
**Malignant Lymphoma and Histiocytic Sarcoma in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene**  
 (continued)

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Lymphocytic Malignant Lymphoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/8 (13%)
<b>15-Month Interim Evaluation</b>						
Lymphocytic Malignant Lymphoma	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/2 (0%)
<b>2-Year Study</b>						
<b>Lymphocytic Malignant Lymphoma</b>						
Overall rate	1/50 (2%)	3/50 (6%)	6/50 (12%)	3/50 (6%)	8/50 (16%)	31/80 (39%)
Adjusted rate	2.2%	8.5%	19.6%	13.5%	37.8%	70.5%
Terminal rate	0/37 (0%)	2/33 (6%)	3/24 (13%)	1/11 (9%)	0/0	0/0
First incidence (days)	623	695	533	407	205	203
Life table test	P<0.001	P=0.278	P=0.026	P=0.160	P<0.001	P<0.001
<b>Malignant Lymphoma (Mixed or Undifferentiated Cell Type)</b>						
Overall rate	5/50 (10%)	9/50 (18%)	5/50 (10%)	3/50 (6%)	1/50 (2%)	0/80 (0%)
<b>Malignant Lymphoma (Lymphocytic, Mixed, NOS, or Undifferentiated Cell Type)<sup>b</sup></b>						
Overall rate	6/50 (12%)	12/50 (24%)	11/50 (22%)	7/50 (14%)	9/50 (18%)	32/80 (40%)
Adjusted rate	14.6%	34.0%	38.7%	35.9%	39.7%	70.8%
Terminal rate	3/37 (8%)	10/33 (30%)	8/24 (33%)	3/11 (27%)	0/0	0/0
First incidence (days)	623	695	533	407	205	189
Life table test	P<0.001	P=0.068	P=0.029	P=0.055	P<0.001	P<0.001
<b>Histiocytic Sarcoma<sup>c</sup></b>						
Overall rate	3/50 (6%)	2/50 (4%)	7/50 (14%)	4/50 (8%)	7/50 (14%)	4/80 (5%)
Adjusted rate	6.9%	4.5%	20.0%	17.7%	28.1%	10.3%
Terminal rate	0/37 (0%)	0/33 (0%)	1/24 (4%)	0/11 (0%)	0/0	0/0
First incidence (days)	485	467	492	524	344	300
Life table test	P<0.001	P=0.518N	P=0.077	P=0.195	P=0.002	P=0.038

(1) Terminal sacrifice

<sup>a</sup> Incidences are given as number of neoplasm-bearing animals/number of animals necropsied.

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidences are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. A lower incidence in a dose group is indicated by N.

<sup>e</sup> Not applicable; no neoplasms in animal group

<sup>f</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean  $\pm$  standard deviation): 46/575 (8.0%  $\pm$  4.5%); range 2%-16%

<sup>g</sup> 2-Year historical incidence: 5/575 (0.9%  $\pm$  1.4%); range 0%-4%

<sup>h</sup> 2-Year historical incidence: 153/561 (27.3%  $\pm$  15.1%); range 8%-44%

<sup>i</sup> 2-Year historical incidence: 10/561 (1.8%  $\pm$  3.3%); range 0%-6%

TABLE 7  
Heart Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>9-Month Interim Evaluation</b>						
Endothelial Hyperplasia	0/10 (0%)	<sup>b</sup>	-	-	0/10 (0%)	1/10 (10%)
<b>15-Month Interim Evaluation</b>						
Endothelial Hyperplasia	0/10 (0%)	0/1 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	2/7 (29%)
Hemangiosarcoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	3/7 (43%)
<b>2-Year Study</b>						
<b>Endothelial Hyperplasia</b>						
Overall rate	0/50 (0%)	1/49 (2%)	0/50 (0%)	2/48 (4%)	4/48 (8%)	5/73 (7%)
Logistic regression test <sup>c</sup>	P=0.001	P=0.516	<sup>d</sup>	P=0.143	P=0.065	P=0.025
<b>Hemangiosarcoma<sup>e</sup></b>						
Overall rate	0/50 (0%)	0/49 (0%)	1/50 (2%)	5/48 (10%)	20/48 (42%)	4/73 (5%)
Adjusted rate <sup>f</sup>	0.0%	0.0%	3.4%	19.4%	93.3%	44.6%
Terminal rate <sup>g</sup>	0/35 (0%)	0/38 (0%)	0/24 (0%)	3/22 (14%)	3/4 (75%)	0/0
First incidence (days)	-	-	682	649	519	289
Life table test	P<0.001	-	P=0.451	P=0.011	P<0.001	P<0.001
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Endothelial Hyperplasia	0/10 (0%)	<sup>b</sup>	-	-	0/10 (0%)	1/8 (13%)
<b>15-Month Interim Evaluation</b>						
Endothelial Hyperplasia	0/10 (0%)	-	-	0/10 (0%)	4/10 (40%)*	0/2 (0%)
Hemangiosarcoma	0/10 (0%)	-	-	0/10 (0%)	1/10 (10%)	2/2 (100%)*
<b>2-Year Study</b>						
<b>Endothelial Hyperplasia</b>						
Overall rate	0/50 (0%)	2/50 (4%)	1/50 (2%)	4/49 (8%)	5/50 (10%)	8/80 (10%)
Logistic regression test	P=0.045	P=0.261	P=0.521	P=0.148	P=0.104	P=0.007
<b>Hemangiosarcoma<sup>h</sup></b>						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/49 (2%)	21/50 (42%)	23/80 (29%)
Adjusted rate	0.0%	0.0%	0.0%	4.0%	100.0%	100.0%
Terminal rate	0/37 (0%)	0/33 (0%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	-	-	-	649	343	307
Life table test	P<0.001	-	-	P=0.392	P<0.001	P<0.001

\* Significantly different (P<0.05) from the control group by the Fisher exact test

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals microscopically examined.

<sup>b</sup> Not examined

<sup>c</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.

<sup>d</sup> Not applicable; no neoplasms in animal group

<sup>e</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean ± standard deviation): 0/573 (0.0%); upper 95% confidence limit = 0.5%

<sup>f</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>g</sup> Observed incidence at terminal kill

<sup>h</sup> 2-Year historical incidence: 0/558 (0.0%); upper 95% confidence limit = 0.5%

multifocal and frequently coexisted with foci of endothelial hyperplasia distant and separate from the main neoplasm. A few hemangiosarcomas invaded through the epicardium and formed grossly visible, dark neoplastic growths. Typical hemangiosarcomas had solid foci of anaplastic, pleomorphic spindle cells at the center of the mass with a loose arrangement at the periphery (Plate 1). Mitotic figures were common. Variably sized clefts and spaces, frequently containing erythrocytes, lined by neoplastic cells with large vesicular nuclei were scattered throughout the masses (Plate 2). The endothelial relationship with the myofibers was most apparent at the periphery of the neoplasms, where the interfiber spaces were widened by capillary spaces lined by neoplastic cells. The foci of endothelial hyperplasia were characterized by variable separation of myofibers, presumably by distended capillaries, and increased cellularity of the capillary endothelium (Plates 3 and 4). Endothelial hyperplasia was considered a preneoplastic lesion by the Pathology Working Group, and some members considered them to represent incipient hemangiosarcomas.

When hemangiosarcomas occurred in multiple organs, the cardiac neoplasms were usually designated as primary, because the incidence of hemangiosarcomas was highest in the heart, the earliest lesions occurred in the heart, neoplastic emboli were seen in some animals, and liver and lung lesions were sometimes multifocal. It could not be determined definitively if the hemangiosarcomas in the other organs were metastases or primary neoplasms. The subcutaneous, splenic, and hepatic hemangiosarcomas that were present at low incidences in mice without cardiac hemangiosarcomas may reflect the spontaneous subcutaneous vascular neoplasms that occur in B6C3F<sub>1</sub> mice. The apparent low incidence (unadjusted for survival) of cardiac hemangiosarcomas in male mice exposed to 625 ppm may be explained by the high rate of development of lymphocytic lymphoma early in the study. Presumably, the lymphomas caused deaths before cardiac hemangiosarcomas developed.

Myocardial mineralization was also observed frequently in mice exposed to 625 ppm, but was not observed in the controls (Tables A5 and B5). The mineralization consisted of scattered, small basophilic

concretions replacing individual myofibers. The pathogenesis of this lesion was undetermined.

*Lung:* At the 15-month interim evaluation, the absolute and relative lung weights of males receiving 625 ppm and the absolute lung weight of females receiving 200 ppm were greater than those of the controls (Table E2).

Exposure of mice to 1,3-butadiene was associated with increased incidences of focal hyperplasia and pulmonary neoplasms (Table 8). Focal alveolar epithelial hyperplasia occurred with a significant positive trend, and the incidence in males exposed to 62.5, 200, or 625 ppm was significantly higher than in the controls by pairwise comparisons. Although the incidence of alveolar/bronchiolar adenoma in males was significantly increased only in the group exposed to 62.5 ppm, the combined incidences of alveolar/bronchiolar adenocarcinoma or carcinoma were significantly increased (by the logistic regression and life table analyses) in males exposed to 20, 62.5, or 200 ppm. In female mice, the incidence of focal hyperplasia was significantly greater than that of the control group in the 625 ppm group only, while the incidences of alveolar/bronchiolar adenoma and of adenocarcinoma or carcinoma in all exposure groups were significantly higher than in the control group.

Alveolar epithelial hyperplasia was considered the precursor of alveolar/bronchiolar adenoma and carcinoma. Hyperplasia consisted of a focal increase in cellularity of the alveolar epithelium with retention of the alveolar architecture. In contrast, the alveolar/bronchiolar adenomas exhibited distortion of alveolar structure due to the formation of complex, irregular papillary patterns lined by relatively uniform cuboidal or columnar cells. The alveolar/bronchiolar carcinomas were similar but consisted of heterogeneous cell populations with varying degrees of cellular pleomorphism and atypia (Plate 5). The adenocarcinomas were larger, highly anaplastic neoplasms, often containing areas of hemorrhage or necrosis.

*Stomach:* Focal hyperplasia of the forestomach epithelium occurred frequently in males exposed to 625 ppm, and the incidence of squamous cell papilloma in the 200 ppm group was significantly

**TABLE 8**  
**Lung Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>9-Month Interim Evaluation</b>						
Alveolar Epithelial Hyperplasia	0/10 (0%)	0/1 (0%)	0/2 (0%)	0/10 (0%)	3/10 (30%)	4/10 (40%)*
Alveolar/bronchiolar Adenoma	0/10 (0%)	1/1 (100%)	1/2 (50%)	0/10 (0%)	2/10 (20%)	2/10 (20%)
Alveolar/bronchiolar Carcinoma	1/10 (10%)	0/1 (0%)	0/2 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)
Alveolar/bronchiolar Adenoma or Carcinoma	1/10 (10%)	1/1 (100%)	1/2 (50%)	0/10 (0%)	2/10 (20%)	3/10 (30%)
<b>15-Month Interim Evaluation</b>						
Alveolar Epithelial Hyperplasia	0/10 (0%)	1/1 (100%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	6/7 (86%)**
Alveolar/bronchiolar Adenoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	5/7 (71%)**
Alveolar/bronchiolar Adenocarcinoma or Carcinoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	1/10 (10%)	2/10 (20%)	2/7 (29%)
Alveolar/bronchiolar Adenoma, Adenocarcinoma, or Carcinoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	2/10 (20%)	4/10 (40%)*	5/7 (71%)**
<b>2-Year Study</b>						
<b>Alveolar Epithelial Hyperplasia</b>						
Overall rate	2/50 (4%)	9/50 (18%)	6/50 (12%)	13/49 (27%)	17/50 (34%)	12/73 (16%)
Logistic regression test <sup>b</sup>	P<0.001	P=0.040	P=0.099	P<0.001	P<0.001	P=0.004
<b>Alveolar/bronchiolar Adenoma</b>						
Overall rate	18/50 (36%)	20/50 (40%)	10/50 (20%)	25/49 (51%)	21/50 (42%)	3/73 (4%)
Adjusted rate <sup>c</sup>	46.9%	47.3%	28.2%	74.2%	100.0%	59.4%
Terminal rate <sup>d</sup>	15/35 (43%)	17/39 (44%)	3/24 (13%)	14/22 (64%)	4/4 (100%)	0/0
First incidence (days)	572	587	517	434	351	251
Logistic regression test	P=0.200	P=0.517	P=0.080N	P=0.036	P=0.061	P=0.492
<b>Alveolar/bronchiolar Adenocarcinoma or Carcinoma</b>						
Overall rate	5/50 (10%)	6/50 (12%)	11/50 (22%)	12/49 (24%)	22/50 (44%)	3/73 (4%)
Adjusted rate	14.3%	15.4%	38.3%	42.9%	94.6%	59.4%
Terminal rate	5/35 (14%)	6/39 (15%)	7/24 (29%)	8/22 (36%)	3/4 (75%)	0/0
First incidence (days)	733 (T)	733 (T)	568	525	474	251
Life table test	P<0.001	P=0.577	P=0.017	P=0.006	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.577	P=0.034	P=0.027	P<0.001	P=0.207
<b>Alveolar/bronchiolar Adenoma, Adenocarcinoma, or Carcinoma<sup>e</sup></b>						
Overall rate	21/50 (42%)	23/50 (46%)	19/50 (38%)	31/49 (63%)	35/50 (70%)	3/73 (4%)
Adjusted rate	54.9%	54.5%	53.6%	87.9%	100.0%	59.4%
Terminal rate	18/35 (51%)	20/39 (51%)	9/24 (38%)	18/22 (82%)	4/4 (100%)	0/0
First incidence (days)	572	587	517	434	351	251
Life table test	P<0.001	P=0.552N	P=0.276	P<0.001	P<0.001	P<0.001
Logistic regression test	P=0.005	P=0.548	P=0.556N	P=0.005	P<0.001	P=0.422

(continued)

**TABLE 8**  
**Lung Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene (continued)**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Alveolar Epithelial Hyperplasia	0/10 (0%)	<sup>f</sup>	—	0/10 (0%)	1/10 (10%)	1/8 (13%)
Alveolar/bronchiolar Adenoma	0/10 (0%)	—	—	0/10 (0%)	2/10 (20%)	1/8 (13%)
<b>15-Month Interim Evaluation</b>						
Alveolar Epithelial Hyperplasia	0/10 (0%)	0/10 (0%)	1/10 (10%)	2/10 (20%)	5/10 (50%)*	1/2 (50%)
Alveolar/bronchiolar Adenoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	2/10 (20%)	1/2 (50%)
Alveolar/bronchiolar Carcinoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (10%)	1/10 (0%)	0/2 (0%)
Alveolar/bronchiolar Adenoma or Carcinoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	3/10 (30%)	1/2 (50%)
<b>2-Year Study</b>						
<b>Alveolar Epithelial Hyperplasia</b>						
Overall rate	5/50 (10%)	5/50 (10%)	3/50 (6%)	9/50 (18%)	11/50 (22%)	11/78 (14%)
Logistic regression test	P=0.092	P=0.558	P=0.510N	P=0.153	P=0.162	P=0.037
<b>Alveolar/bronchiolar Adenoma</b>						
Overall rate	4/50 (8%)	11/50 (22%)	12/50 (24%)	17/50 (34%)	14/50 (28%)	17/78 (22%)
Adjusted rate	10.5%	30.9%	40.7%	64.8%	100.0%	100.0%
Terminal rate	3/37 (8%)	9/33 (27%)	8/24 (33%)	4/11 (36%)	0/0	0/0
First incidence (days)	714	524	492	443	408	275
Logistic regression test	P=0.002	P=0.039	P=0.013	P<0.001	P=0.002	P=0.010
<b>Alveolar/bronchiolar Adenocarcinoma or Carcinoma</b>						
Overall rate	0/50 (0%)	5/50 (10%)	11/50 (22%)	9/50 (18%)	19/50 (38%)	8/78 (10%)
Adjusted rate	0.0%	13.3%	42.9%	40.8%	100.0%	100.0%
Terminal rate	0/37 (0%)	3/33 (9%)	10/24 (42%)	3/11 (27%)	0/0	0/0
First incidence (days)	<sup>g</sup>	519	507	532	373	307
Life table test	P<0.001	P=0.029	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test	P=0.001	P=0.034	P<0.001	P=0.002	P<0.001	P=0.012
<b>Alveolar/bronchiolar Adenoma, Adenocarcinoma, or Carcinoma<sup>h</sup></b>						
Overall rate	4/50 (8%)	15/50 (30%)	19/50 (38%)	24/50 (48%)	25/50 (50%)	22/78 (28%)
Adjusted rate	10.5%	39.5%	63.7%	78.5%	100.0%	100.0%
Terminal rate	3/37 (8%)	11/33 (33%)	14/24 (58%)	6/11 (55%)	0/0	0/0
First incidence (days)	714	519	492	443	373	275
Life table test	P<0.001	P=0.004	P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.005	P<0.001	P<0.001	P<0.001	P<0.001

\* Significantly different (P<0.05) from the control group by the Fisher exact test

\*\* P<0.01

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of lesion/bearing animals/number of animals microscopically examined.

<sup>b</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. A lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean ± standard deviation): 118/573 (20.6% ± 7.6%); range 10%-30%

<sup>f</sup> Not examined

<sup>g</sup> Not applicable; no neoplasms in animal group

<sup>h</sup> 2-Year historical incidence: 47/560 (8.4% ± 3.3%); range 0%-12%

higher than that of the control group (Table 9). Squamous cell carcinomas occurred in two males in the 625 ppm group and in one male in the 200 ppm group. In female mice, the incidences of hyperplasia, squamous cell papilloma, and squamous cell carcinoma were significantly higher in the 625 ppm group than in the control group. The lower rates of these forestomach lesions in males than in females in the 625 ppm group are likely related to the lower survival of the males. Squamous cell papillomas and carcinomas of the forestomach are relatively uncommon in B6C3F<sub>1</sub> mice, occurring with a combined incidence of 4 of 575 historical control males (0.7%) and 9 of 561 historical control females (1.6%) (Tables A4d and B4d).

Forestomach epithelial hyperplasia was typically a focal lesion consisting of thickened epithelium forming blunt rugose folds of varying length. Shallow ulcers were sometimes present in the center of the hyperplastic lesion with submucosal infiltrates of inflammatory cells. The papillomas were generally more complex, with a short stalk and branching papillae consisting of well-differentiated stratified squamous epithelium overlying a fibrovascular stroma (Plate 6). The squamous cell carcinomas exhibited invasion of the forestomach mucosa by cords and clusters of anaplastic cells (Plate 7).

*Liver:* The absolute liver weight of females receiving 62.5 ppm or more was greater than that of the controls at the 9-month interim evaluation (Table E1). At the 15-month interim evaluation, the absolute and relative liver weights of males exposed to 625 ppm and the absolute liver weight of females exposed to 200 ppm were greater than those of the controls (Table E2).

The incidence of hepatoproliferative lesions including hepatocellular adenomas and carcinomas was significantly increased in female mice exposed to 62.5 or 200 ppm 1,3-butadiene (Table 10). Hepatocellular foci including basophilic, clear cell, mixed cell, or eosinophilic foci occurred with increased incidences in female mice exposed to 20, 62.5, or 200 ppm. The incidences of hepatocellular neoplasms were significantly increased in male mice exposed to 200 ppm for 2 years. The low incidences of liver neoplasms in male and female mice exposed to 625 ppm probably reflect the occurrence of early deaths from malignant

lymphoma. Hepatocellular adenomas and carcinomas are common neoplasms in B6C3F<sub>1</sub> mice, occurring in 196 of 572 control males (34.3%) and 87 of 558 control females (15.6%) in the NTP historical database (Tables A4e and B4e).

Basophilic foci were characterized by cells with basophilic cytoplasm, while eosinophilic foci were composed of cells with cytoplasm that stained more intensely with eosin than did normal hepatocytes. Mixed cell foci consisted of mixtures of cells with eosinophilic cytoplasm and cells with clear cytoplasm. Hepatocellular adenomas were discrete, expansile masses that were larger than hepatic lobules and compressed the adjacent parenchyma. Hepatic plates within the adenomas were not organized in a normal lobular pattern and often intersected at near-right angles with plates in the adjacent normal liver. Hepatocellular carcinomas were larger than the adenomas and consisted of markedly disorganized hepatocytes that formed solid clusters, glandular structures, or broad trabeculae several cell layers thick (Plate 8). Neoplastic hepatocytes generally showed moderate to marked pleomorphism and atypia.

The incidence of liver necrosis was increased at the higher dose levels in male and female mice (Tables A5 and B5). This lesion consisted of patchy areas of necrosis that had no particular lobular distribution, and occurred frequently in animals with malignant lymphoma or hemangiosarcoma. Centrilobular hepatocellular necrosis also occurred with increased incidences in high-dose male and female mice (Tables A5 and B5), and occurred frequently in animals described as anemic or with atrial thrombi.

*Ovary:* A variety of ovarian neoplasms were observed, but only malignant and benign granulosa cell tumors were definitively related to exposure. Increased incidences of granulosa cell tumors were observed in females exposed to 62.5 or 200 ppm (Table 11). Granulosa cell tumors varied from small benign neoplasms with granulosa cells aligned on a scant stroma in a discretely packeted tubular pattern to large cystic neoplasms with thick trabeculae and spaces filled with blood or clear fluid (Plate 9). Malignant and benign granulosa cell tumors each have an NTP historical control incidence of 1 of 548 (0.2%) (Table B4f).

**TABLE 9**  
**Forestomach Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>9-Month Interim Evaluation</b>						
Epithelial Hyperplasia	0/10 (0%)	<sup>b</sup>	-	0/2 (0%)	0/10 (0%)	7/10 (70%)**
Squamous Cell Papilloma	0/10 (0%)	-	-	0/2 (0%)	0/10 (0%)	1/10 (10%)
<b>15-Month Interim Evaluation</b>						
Epithelial Hyperplasia	0/10 (0%)	-	0/10 (0%)	2/10 (20%)	2/10 (20%)	3/7 (43%)
Squamous Cell Papilloma	0/10 (0%)	-	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/7 (14%)
Squamous Cell Carcinoma	0/10 (0%)	-	0/10 (0%)	0/10 (0%)	1/10 (10%)	2/7 (29%)
Squamous Cell Papilloma or Squamous Cell Carcinoma	0/10 (0%)	-	0/10 (0%)	0/10 (0%)	1/10 (10%)	3/7 (43%)
<b>2-Year Study</b>						
<b>Epithelial Hyperplasia</b>						
Overall rate	4/50 (8%)	3/50 (6%)	3/50 (6%)	5/48 (10%)	4/48 (8%)	40/72 (56%)
Logistic regression test <sup>c</sup>	P<0.001	P=0.579N	P=0.414N	P=0.355	P=0.466N	P=0.089
<b>Squamous Cell Papilloma</b>						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	7/50 (14%)	2/73 (3%)
Adjusted rate <sup>d</sup>	2.5%	0.0%	0.0%	4.5%	51.7%	40.0%
Terminal rate <sup>e</sup>	0/35 (0%)	0/39 (0%)	0/24 (0%)	1/22 (5%)	1/4 (25%)	0/0
First incidence (days)	652	<sup>f</sup>	-	733 (T)	530	395
Logistic regression test	P<0.001	P=0.535N	P=0.486N	P=0.739	P=0.012	P=0.446
<b>Squamous Cell Carcinoma</b>						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	2/73 (3%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	5.9%	19.6%
Terminal rate	0/35 (0%)	0/39 (0%)	0/24 (0%)	0/22 (0%)	0/4 (0%)	0/0
First incidence (days)	-	-	-	-	620	302
Life table test	P<0.001	-	-	-	P=0.325	P=0.018
Logistic regression test	P=0.184	-	-	-	P=0.503	P=0.675
<b>Squamous Cell Papilloma or Squamous Cell Carcinoma<sup>g</sup></b>						
Overall rate	1/50 (2%)	0/50 (0%)	0/50 (0%)	1/50 (2%)	8/50 (16%)	4/73 (5%)
Adjusted rate	2.5%	0.0%	0.0%	4.5%	54.5%	51.8%
Terminal rate	0/35 (0%)	0/39 (0%)	0/24 (0%)	1/22 (5%)	1/4 (25%)	0/0
First incidence (days)	652	-	-	733 (T)	530	302
Life table test	P<0.001	P=0.481N	P=0.545N	P=0.679	P<0.001	P<0.001
Logistic regression test	P<0.001	P=0.535N	P=0.486N	P=0.739	P=0.006	P=0.199

(continued)

**TABLE 9**  
**Forestomach Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene (continued)**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Epithelial Hyperplasia	0/10 (0%)	<sup>b</sup>	—	0/10 (0%)	1/10 (10%)	8/8 (100%)**
<b>15-Month Interim Evaluation</b>						
Epithelial Hyperplasia	0/10 (0%)	0/1 (0%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	1/2 (50%)
Squamous Cell Papilloma	0/10 (0%)	0/1 (0%)	0/10 (0%)	1/10 (10%)	2/10 (20%)	0/2 (0%)
Squamous Cell Carcinoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/2 (50%)
Squamous Cell Papilloma or Squamous Cell Carcinoma	0/10 (0%)	0/1 (0%)	0/10 (0%)	1/10 (10%)	2/10 (20%)	1/2 (50%)
<b>2-Year Study</b>						
<b>Epithelial Hyperplasia</b>						
Overall rate	4/50 (8%)	5/49 (10%)	4/47 (9%)	7/48 (15%)	14/50 (28%)	47/79 (59%)
Logistic regression test	P<0.001	P=0.477	P=0.558	P=0.127	P=0.067	P=0.032
<b>Squamous Cell Papilloma</b>						
Overall rate	0/50 (0%)	0/50 (0%)	2/50 (4%)	1/50 (2%)	3/50 (6%)	16/80 (20%)
Adjusted rate	0.0%	0.0%	8.3%	9.1%	100.0%	100.0%
Terminal rate	0/37 (0%)	0/33 (0%)	2/24 (8%)	1/11 (9%)	0/0	0/0
First incidence (days)	<sup>f</sup>	—	733 (T)	733 (T)	470	246
Logistic regression test	P<0.001	—	P=0.149	P=0.260	P=0.078	P=0.002
<b>Squamous Cell Carcinoma</b>						
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)	1/50 (2%)	6/80 (8%)
Adjusted rate	0.0%	0.0%	4.2%	8.3%	3.8%	70.5%
Terminal rate	0/37 (0%)	0/33 (0%)	1/24 (4%)	0/11 (0%)	0/0	0/0
First incidence (days)	—	—	733 (T)	723	472	275
Life table test	P<0.001	—	P=0.414	P=0.277	P=0.374	P<0.001
Logistic regression tests	P=0.009	—	P=0.414	P=0.330	P=0.750	P=0.041
<b>Squamous Cell Papilloma or Squamous Cell Carcinoma<sup>h</sup></b>						
Overall rate	0/50 (0%)	0/50 (0%)	3/50 (6%)	2/50 (4%)	4/50 (8%)	22/80 (28%)
Adjusted rate	0.0%	0.0%	12.5%	16.7%	100.0%	100.0%
Terminal rate	0/37 (0%)	0/33 (0%)	3/24 (13%)	1/11 (9%)	0/0	0/0
First incidence (days)	—	—	733 (T)	723	470	246
Life table test	P<0.001	—	P=0.056	P=0.044	P=0.001	P<0.001
Logistic regression test	P<0.001	—	P=0.056	P=0.060	P=0.056	P<0.001

\*\* Significantly different (P<0.001) from the control group by the Fisher exact test

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals necropsied.

<sup>b</sup> Not examined

<sup>c</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidences are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions are nonfatal. A lower incidence in a dose group is indicated by N.

<sup>d</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>e</sup> Observed incidence at terminal kill

<sup>f</sup> Not applicable; no neoplasms in animal group

<sup>g</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean ± standard deviation): 4/575 (0.7% ± 1.4%); range 0%-4%

<sup>h</sup> 2-Year historical incidence: 9/561 (1.6% ± 2.1%); range 0%-6%

**TABLE 10**  
**Liver Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>9-Month Interim Evaluation</b>						
Mixed Cell Focus	0/10 (0%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
Hepatocellular Adenoma	4/10 (40%)	0/10 (0%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	1/10 (10%)
<b>15-Month Interim Evaluation</b>						
Foci (Basophilic, Eosinophilic, or Mixed Cell)	1/10 (10%)	0/10 (0%)	3/10 (30%)	0/10 (0%)	0/10 (0%)	2/7 (29%)
Hepatocellular Adenoma	2/10 (20%)	0/10 (0%)	4/10 (40%)	3/10 (30%)	4/10 (40%)	3/7 (43%)
Hepatocellular Carcinoma	0/10 (0%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	3/7 (43%)
Hepatocellular Adenoma or Carcinoma	2/10 (20%)	1/10 (10%)	4/10 (40%)	3/10 (30%)	4/10 (40%)	5/7 (71%)
<b>2-Year Study</b>						
<b>Foci (Basophilic, Clear Cell, Eosinophilic, or Mixed Cell)</b>						
Overall rate	9/50 (18%)	10/50 (20%)	6/50 (12%)	11/48 (23%)	6/48 (13%)	1/72 (1%)
Logistic regression test <sup>b</sup>	P=0.418	P=0.602N	P=0.414N	P=0.230	P=0.221	P=0.800
<b>Hepatocellular Adenoma</b>						
Overall rate	13/50 (26%)	13/50 (26%)	19/50 (38%)	16/48 (33%)	24/48 (50%)	5/72 (7%)
Adjusted rate <sup>c</sup>	32.1%	31.3%	52.1%	57.0%	92.2%	100.0%
Terminal rate <sup>d</sup>	9/35 (26%)	11/39 (28%)	8/24 (33%)	11/22 (50%)	3/4 (75%)	0/0
First incidence (days)	379	484	397	434	351	310
Logistic regression test	P=0.042	P=0.552	P=0.158	P=0.261	P=0.008	P=0.253
<b>Hepatocellular Carcinoma</b>						
Overall rate	11/50 (22%)	16/50 (32%)	16/50 (32%)	17/48 (35%)	26/48 (54%)	1/72 (1%)
Adjusted rate	26.0%	36.6%	44.8%	58.3%	100.0%	50.0%
Terminal rate	5/35 (14%)	12/39 (31%)	7/24 (29%)	11/22 (50%)	4/4 (100%)	0/0
First incidence (days)	540	484	517	434	351	422
Logistic regression test	P=0.036	P=0.142	P=0.389	P=0.088	P<0.001	P=0.347
<b>Hepatocellular Adenoma or Carcinoma<sup>e</sup></b>						
Overall rate	21/50 (42%)	23/50 (46%)	30/50 (60%)	25/48 (52%)	33/48 (69%)	5/72 (7%)
Adjusted rate	47.9%	53.0%	70.1%	79.2%	100.0%	100.0%
Terminal rate	13/35 (37%)	19/39 (49%)	12/24 (50%)	16/22 (73%)	4/4 (100%)	0/0
First incidence (days)	379	484	397	434	351	310
Logistic regression test	P=0.067	P=0.375	P=0.078	P=0.185	P=0.030	P=0.450
<b>Hepatocellular Foci, Adenoma, or Carcinoma</b>						
Overall rate	27/50 (54%)	29/50 (58%)	31/50 (62%)	30/48 (63%)	34/48 (71%)	6/72 (8%)
Adjusted rate	62.1%	67.1%	72.6%	87.5%	100.0%	100.0%
Terminal rate	19/35 (54%)	25/39 (64%)	13/24 (54%)	18/22 (82%)	4/4 (100%)	0/0
First incidence (days)	379	484	397	434	351	228
Logistic regression test	P=0.108	P=0.434	P=0.291	P=0.185	P=0.007	P=0.564

(continued)

**TABLE 10**  
**Liver Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene (continued)**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Basophilic Focus	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	0/8 (0%)
<b>15-Month Interim Evaluation</b>						
Foci (Basophilic, Clear Cell, Eosinophilic, or Mixed Cell)	0/10 (0%)	2/10 (20%)	1/10 (10%)	2/10 (20%)	2/10 (20%)	0/2 (0%)
Hepatocellular Adenoma	1/10 (10%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	1/2 (50%)
Hepatocellular Carcinoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/2 (0%)
Hepatocellular Adenoma or Carcinoma	1/10 (10%)	1/10 (10%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	1/2 (50%)
<b>2-Year Study</b>						
<b>Foci (Basophilic, Clear Cell, Eosinophilic, or Mixed Cell)</b>						
Overall rate	8/49 (16%)	14/49 (29%)	19/50 (38%)	12/50 (24%)	5/50 (10%)	4/80 (5%)
Logistic regression test	P=0.003	P=0.087	P=0.001	P=0.004	P=0.045	P=0.618
<b>Hepatocellular Adenoma</b>						
Overall rate	11/49 (22%)	10/49 (20%)	9/50 (18%)	14/50 (28%)	12/50 (24%)	1/80 (1%)
Adjusted rate	29.7%	27.8%	30.3%	65.8%	89.0%	100.0%
Terminal rate	11/37 (30%)	8/33 (24%)	6/24 (25%)	5/11 (45%)	0/0	0/0
First incidence (days)	733 (T)	519	365	485	370	450
Logistic regression test	P=0.599N	P=0.531N	P=0.519N	P=0.025	P=0.009	P=0.505
<b>Hepatocellular Carcinoma</b>						
Overall rate	4/49 (8%)	6/49 (12%)	8/50 (16%)	9/50 (18%)	8/50 (16%)	1/80 (1%)
Adjusted rate	10.3%	14.5%	25.0%	39.9%	82.7%	12.5%
Terminal rate	3/37 (8%)	2/33 (6%)	3/24 (13%)	2/11 (18%)	0/0	0/0
First incidence (days)	677	467	600	546	353	418
Logistic regression test	P=0.178	P=0.381	P=0.141	P=0.066	P=0.006	P=0.910
<b>Hepatocellular Adenoma or Carcinoma<sup>f</sup></b>						
Overall rate	15/49 (31%)	14/49 (29%)	15/50 (30%)	19/50 (38%)	16/50 (32%)	2/80 (3%)
Adjusted rate	39.3%	34.3%	45.5%	74.8%	91.7%	100.0%
Terminal rate	14/37 (38%)	8/33 (24%)	8/24 (33%)	6/11 (55%)	0/0	0/0
First incidence (days)	677	467	365	485	353	418
Logistic regression test	P=0.497	P=0.504N	P=0.441	P=0.027	P=0.008	P=0.302
<b>Hepatocellular Foci, Adenoma, or Carcinoma</b>						
Overall rate	18/49 (37%)	25/49 (51%)	27/50 (54%)	25/50 (50%)	18/50 (36%)	6/80 (8%)
Adjusted rate	47.2%	60.2%	80.5%	95.5%	100.0%	100.0%
Terminal rate	17/37 (46%)	17/33 (52%)	18/24 (75%)	10/11 (91%)	0/0	0/0
First incidence (days)	677	467	365	485	353	315
Logistic regression test	P=0.384	P=0.097	P=0.010	P<0.001	P=0.002	P=0.136

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals necropsied.

<sup>b</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean  $\pm$  standard deviation): 196/572 (34.3%  $\pm$  12.4%); range 12%-56%

<sup>f</sup> 2-Year historical incidence: 87/558 (15.6%  $\pm$  8.4%); range 3%-27%

**TABLE 11**  
**(Ovarian Lesions in Female Mice in the 2-Year Inhalation Study of 1,3-Butadiene<sup>a</sup>)**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>9-Month Interim Evaluation</b>						
Atrophy	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	9/10 (90%)**	8/8 (100%)**
Germinal Epithelium Hyperplasia	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/8 (13%)
Benign Granulosa Cell Tumor	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/8 (0%)
<b>15-Month Interim Evaluation</b>						
Atrophy	0/10 (0%)	0/10 (0%)	1/10 (10%)	9/10 (90%)**	7/10 (70%)**	2/2 (100%)*
Angiectasis	1/10 (10%)	1/10 (10%)	0/10 (0%)	0/10 (0%)	2/10 (20%)	0/2 (0%)
Germinal Epithelium Hyperplasia	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	3/10 (30%)	1/2 (50%)
Granulosa Cell Hyperplasia	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	1/2 (50%)
Benign Granulosa Cell Tumor	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	3/10 (30%)	1/2 (50%)
Malignant Granulosa Cell Tumor	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	0/2 (0%)
Benign or Malignant Granulosa Cell Tumor	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	4/10 (40%)*	1/2 (50%)
Adenoma or Benign Mixed Tumor	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/10 (10%)	1/10 (10%)	0/2 (0%)
<b>2-Year Study</b>						
<b>Atrophy</b>						
Overall rate	4/49 (8%)	19/49 (39%)	32/48 (67%)	42/50 (84%)	43/50 (86%)	69/79 (87%)
Logistic regression test <sup>b</sup>	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
<b>Angiectasis</b>						
Overall rate	4/49 (8%)	6/49 (12%)	3/48 (6%)	13/50 (26%)	14/50 (28%)	17/79 (22%)
Logistic regression test	P=0.259	P=0.366	P=0.606	P=0.017	P=0.021	P=0.425
<b>Germinal Epithelium Hyperplasia</b>						
Overall rate	2/49 (4%)	3/49 (6%)	8/48 (17%)	15/50 (30%)	15/50 (30%)	18/79 (23%)
Logistic regression test	P<0.001	P=0.460	P=0.017	P<0.001	P=0.010	P<0.001
<b>Granulosa Cell Hyperplasia</b>						
Overall rate	1/49 (2%)	0/49 (0%)	2/48 (4%)	3/50 (6%)	3/50 (6%)	2/79 (3%)
Logistic regression test	P=0.370N	P=0.517N	P=0.360	P=0.588	P=0.235	P=0.887
<b>Benign Granulosa Cell Tumor</b>						
Overall rate	1/49 (2%)	0/49 (0%)	1/48 (2%)	6/50 (12%)	6/50 (12%)	6/79 (8%)
Adjusted rate <sup>c</sup>	2.8%	0.0%	3.2%	28.5%	100.0%	27.1%
Terminal rate <sup>d</sup>	1/36 (3%)	0/33 (0%)	0/24 (0%)	1/11 (9%)	0/0	0/0
First incidence (days)	733 (T)	- <sup>e</sup>	677	608	393	311
Logistic regression test	P=0.030	P=0.517N	P=0.735	P=0.026	P=0.020	P=0.303
<b>Malignant Granulosa Cell Tumor</b>						
Overall rate	0/49 (0%)	0/49 (0%)	0/48 (0%)	3/50 (6%)	2/50 (4%)	0/79 (0%)
Adjusted rate	0.0%	0.0%	0.0%	19.3%	54.2%	0.0%
Terminal rate	0/36 (0%)	0/33 (0%)	0/24 (0%)	1/11 (9%)	0/0	0/0
First incidence (days)	-	-	-	645	569	-
Life table test	P<0.001	-	-	P=0.018	P=0.003	-
Logistic regression test	P=0.068	-	-	P=0.046	P=0.037	-

(continued)

TABLE 11  
Ovarian Lesions in Female Mice in the 2-Year Inhalation Study of 1,3-Butadiene (continued)

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>2-Year Study (continued)</b>						
<b>Benign or Malignant Granulosa Cell Tumor<sup>f</sup></b>						
Overall rate	1/49 (2%)	0/49 (0%)	1/48 (2%)	9/50 (18%)	8/50 (16%)	6/79 (8%)
Adjusted rate	2.8%	0.0%	3.2%	42.9%	100.0%	27.1%
Terminal rate	1/36 (3%)	0/33 (0%)	0/24 (0%)	2/11 (18%)	0/0	0/0
First incidence (days)	733 (T)	—	677	608	393	311
Life table test	P<0.001	P=0.517N	P=0.680	P<0.001	P<0.001	P<0.001
Logistic regression test	P=0.006	P=0.517N	P=0.735	P=0.001	P=0.001	P=0.303
<b>Adenoma or Benign Mixed Tumor</b>						
Overall rate	2/49 (4%)	4/49 (8%)	1/48 (2%)	4/50 (8%)	6/50 (12%)	2/79 (3%)
Adjusted rate	5.6%	12.1%	4.2%	26.4%	100.0%	4.1%
Terminal rate	2/36 (6%)	4/33 (12%)	1/24 (4%)	2/11 (18%)	0/0	0/0
First incidence (days)	733 (T)	733 (T)	733 (T)	664	460	257
Logistic regression test	P=0.244	P=0.296	P=0.640N	P=0.111	P=0.018	P=0.920N

\* Significantly different (P<0.05) from the control group by the Fisher exact test

\*\* P<0.01

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals microscopically examined.

<sup>b</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

<sup>f</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean ± standard deviation): 4/548 (0.7% ± 0.9%); range 0%-2%

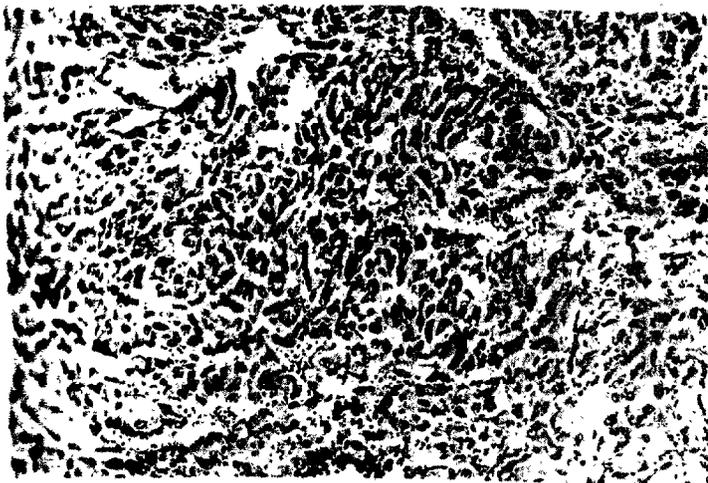
The incidence of germinal epithelial hyperplasia was markedly increased in the ovaries of mice exposed to 20 ppm or above. This lesion consisted of prominent downgrowth of the surface mesothelium into the parenchyma of the ovary, forming tubular and gland-like structures. Ovarian atrophy was an exposure-related effect and the incidence increased with dose. Characteristically, affected females had no evidence of oocytes, follicles, or corpora lutea.

**Uterus:** Uterine atrophy (and, therefore, less cystic hyperplasia) was a prominent exposure-related effect at 625 ppm and was also observed in the 200 ppm group (Table B5).

**Harderian Gland:** Male and female mice in the 62.5 and 200 ppm groups had increased incidences of harderian gland adenomas (Table 12; Plates 10 and 11). The occurrence of carcinomas in dosed mice,

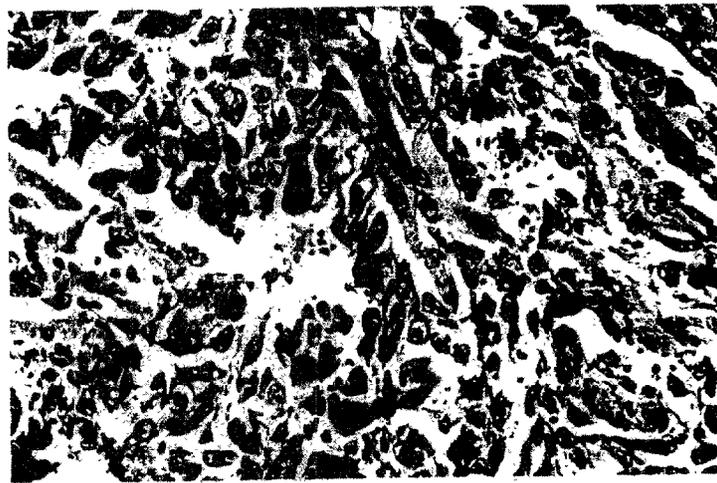
particularly males, is unusual. These malignant neoplasms are rare in NTP historical controls (males: 2/575 or 0.3%; females: 3/561 or 0.5%) (Tables A4f and B4h). The low incidence of harderian gland neoplasms in males and females that were exposed to 625 ppm probably reflects early deaths due to lymphocytic lymphoma which precluded the development of harderian gland neoplasms. The incidence of harderian gland hyperplasia was also increased in male mice exposed to 62.5 or 200 ppm 1,3-butadiene.

**Mammary Gland:** Increased incidences of mammary gland neoplasms in female mice exposed to 62.5, 200, or 625 ppm were related to 1,3-butadiene exposure. Most of the epithelial neoplasms were mammary carcinomas (Type B) (Plate 12); the rest were adenocarcinomas or malignant mixed tumors (Table 13). The adenocarcinomas are considered here to be variants of the carcinomas that have prominent



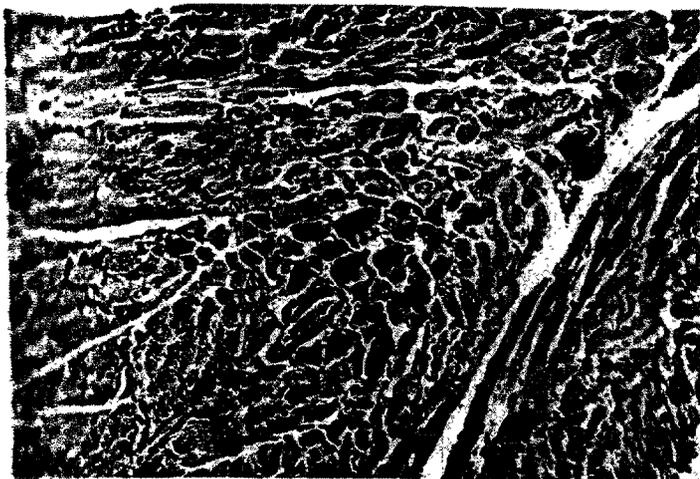
**PLATE 1**

Heart: Typical hemangiosarcoma with solid focus of anaplastic, pleomorphic spindle cells at the center of the mass with a loosened arrangement of anaplastic cells at the periphery. Female B6C3F<sub>1</sub> mouse exposed to 625 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×150



**PLATE 2**

Heart: Higher magnification of Plate 1 showing the variably sized clefts and spaces (frequently containing red blood cells) lined by plump cells with large vesicular nuclei. Female B6C3F<sub>1</sub> mouse exposed to 625 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×300



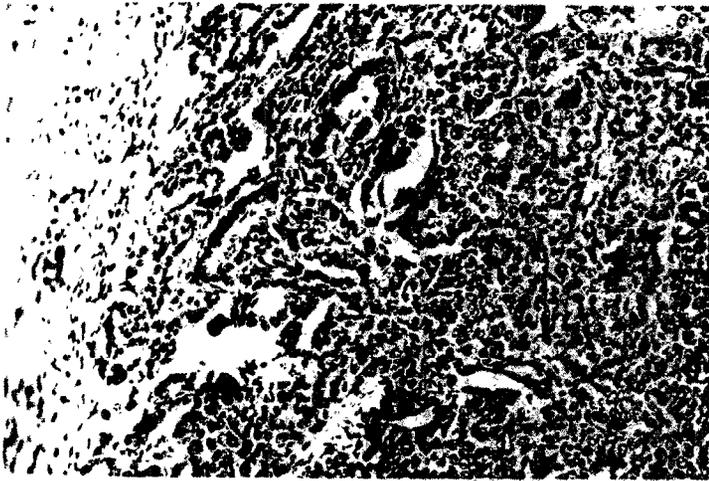
**PLATE 3**

Heart: Endothelial hyperplasia. Hyperplastic cells appear to originate in the pericyonium between myofibers. Male B6C3F<sub>1</sub> mouse exposed to 62.5 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×150

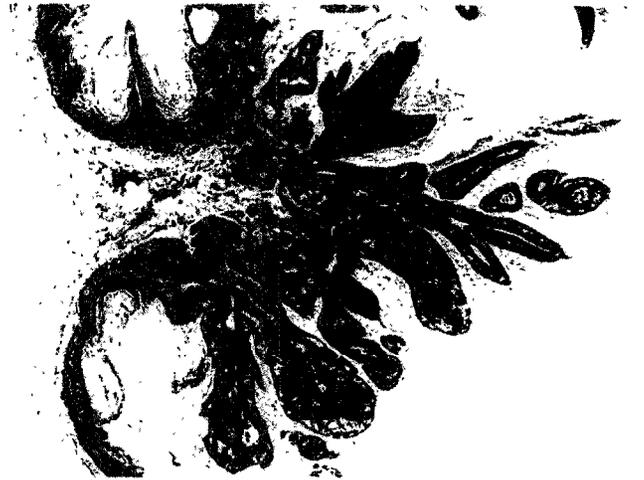


**PLATE 4**

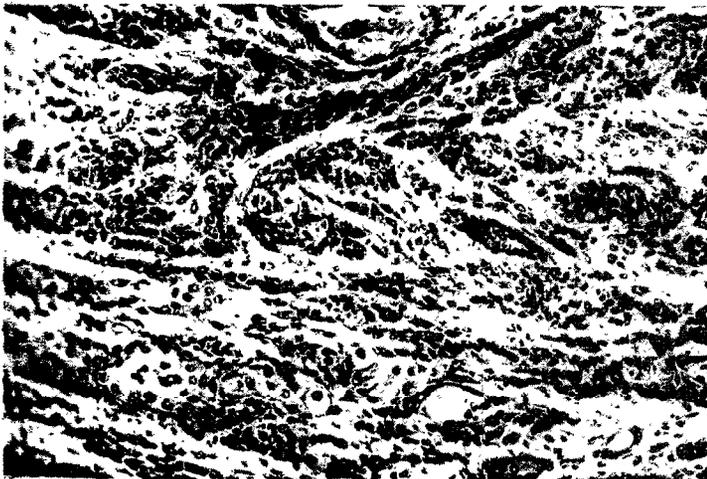
Heart: Higher magnification of Plate 3 showing the rounded or elongated hyperplastic cells in close apposition to the myofibers, and the space between adjacent myofibers is slightly increased. Male B6C3F<sub>1</sub> mouse exposed to 62.5 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×300



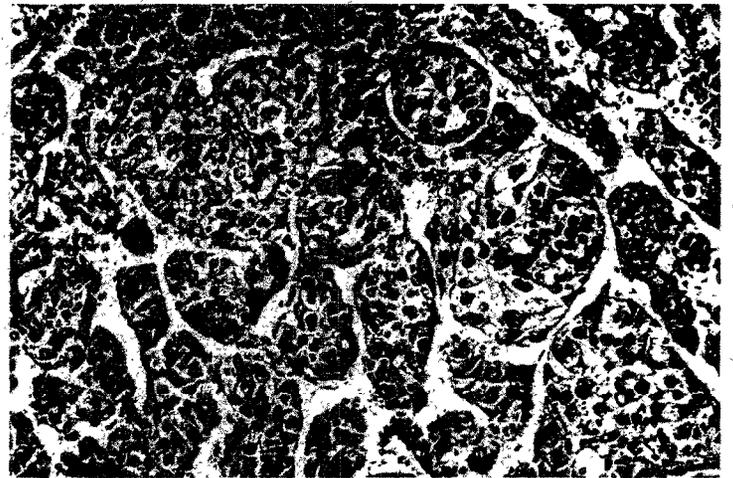
**PLATE 5**  
 Lung: Alveolar/bronchiolar carcinoma with cellular atypia and peripheral invasion into the remaining normal pulmonary parenchyma. Female B6C3F<sub>1</sub> mouse exposed to 200 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×150



**PLATE 6**  
 Forestomach: Squamous cell papilloma projects from the mucosal surface into the lumen of the forestomach. Note the thickened and folded keratinized epithelium. Female B6C3F<sub>1</sub> mouse exposed to 625 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×16



**PLATE 7**  
 Forestomach: Squamous cell carcinoma. Cords and clusters of neoplastic keratinized squamous epithelial cells with foci of keratinization have invaded the lamina propria. Female B6C3F<sub>1</sub> mouse exposed to 625 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×150



**PLATE 8**  
 Liver: Hepatocellular carcinoma with a trabecular pattern. Male B6C3F<sub>1</sub> mouse exposed to 200 ppm 1,3-butadiene in the 2-year inhalation study. H&E ×150

**TABLE 12**  
**Harderian Gland Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>15-Month Interim Evaluation</b>						
Hyperplasia	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	6/10 (60%)**	0/7 (0%)
Adenoma	0/10 (0%)	0/10 (0%)	2/10 (20%)	4/10 (40%)*	3/10 (30%)	3/7 (43%)
<b>2-Year Study</b>						
<b>Hyperplasia</b>						
Overall rate	1/50 (2%)	3/49 (6%)	4/50 (8%)	6/47 (13%)	8/47 (17%)	5/40 (13%)
Logistic regression test <sup>b</sup>	P=0.069	P=0.335	P=0.154	P=0.032	P=0.016	P=0.740
<b>Adenoma</b>						
Overall rate	6/50 (12%)	7/50 (14%)	8/50 (16%)	19/50 (38%)	30/50 (60%)	6/73 (8%)
Adjusted rate <sup>c</sup>	14.8%	17.3%	25.8%	63.4%	95.4%	100.0%
Terminal rate <sup>d</sup>	2/35 (6%)	6/39 (15%)	4/24 (17%)	12/22 (55%)	3/4 (75%)	0/0
First incidence (days)	543	652	536	464	382	289
Logistic regression test	P<0.001	P=0.497	P=0.395	P<0.001	P<0.001	P=0.264
<b>Carcinoma</b>						
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	3/50 (6%)	2/50 (4%)	0/73 (0%)
Adjusted rate	0.0%	2.6%	4.2%	11.7%	6.3%	0.0%
Terminal rate	0/35 (0%)	1/39 (3%)	1/24 (4%)	1/22 (5%)	0/4 (0%)	0/0
First incidence (days)	- <sup>e</sup>	733 (T)	733 (T)	680	495	-
Logistic regression test	P=0.720	P=0.522	P=0.425	P=0.086	P=0.352	-
<b>Adenoma or Carcinoma<sup>f</sup></b>						
Overall rate	6/50 (12%)	7/50 (14%)	9/50 (18%)	20/50 (40%)	31/50 (62%)	6/73 (8%)
Adjusted rate	14.8%	17.3%	29.5%	64.9%	95.5%	100.0%
Terminal rate	2/35 (6%)	6/39 (15%)	5/24 (21%)	12/22 (55%)	3/4 (75%)	0/0
First incidence (days)	543	652	536	464	382	289
Logistic regression test	P<0.001	P=0.497	P=0.217	P<0.001	P<0.001	P=0.002

(continued)

TABLE 12  
 Harderian Gland Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene (continued)

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Female</b>						
<b>9-Month Interim Evaluation</b>						
Hyperplasia	0/5 (0%)	<sup>g</sup>	-	-	1/1 (100%)	0/5 (0%)
Adenoma	0/5 (0%)	-	-	-	0/1 (0%)	1/5 (20%)
<b>15-Month Interim Evaluation</b>						
Adenoma	2/9 (22%)	1/1 (100%)	1/1 (100%)	1/10 (10%)	3/10 (30%)	0/2 (0%)
<b>2-Year Study</b>						
<b>Hyperplasia</b>						
Overall rate	1/50 (2%)	5/49 (10%)	9/48 (19%)	4/49 (8%)	4/49 (8%)	7/66 (11%)
Logistic regression test	P=0.213	P=0.097	P=0.012	P=0.138	P=0.271	P=0.507
<b>Adenoma</b>						
Overall rate	8/50 (16%)	10/50 (20%)	6/50 (12%)	15/50 (30%)	20/50 (40%)	9/80 (11%)
Adjusted rate	20.8%	29.2%	20.7%	61.0%	89.3%	45.2%
Terminal rate	7/37 (19%)	9/33 (27%)	4/24 (17%)	4/11 (36%)	0/0	0/0
First incidence (days)	658	722	569	532	370	307
Logistic regression test	P=0.046	P=0.356	P=0.511N	P=0.016	P=0.001	P=0.176
<b>Carcinoma</b>						
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	1/50 (2%)	0/80 (0%)
Adjusted rate	0.0%	2.7%	2.3%	0.0%	50.0%	0.0%
Terminal rate	0/37 (0%)	0/33 (0%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	- <sup>e</sup>	722	541	-	695	-
Logistic regression test	P=0.873N	P=0.493	P=0.631	-	P=0.085	-
<b>Adenoma or Carcinoma<sup>h</sup></b>						
Overall rate	8/50 (16%)	10/50 (20%)	7/50 (14%)	15/50 (30%)	20/50 (40%)	9/80 (11%)
Adjusted rate	20.8%	29.2%	22.5%	61.0%	89.3%	45.2%
Terminal rate	7/37 (19%)	9/33 (27%)	4/24 (17%)	4/11 (36%)	0/0	0/0
First incidence (days)	658	722	541	532	370	307
Logistic regression test	P=0.061	P=0.356	P=0.575N	P=0.016	P=0.001	P=0.176

(T) Terminal sacrifice

\* Significantly different ( $P < 0.05$ ) from the control group by the Fisher exact test

\*\*  $P < 0.01$

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals microscopically examined or number of animals necropsied.

<sup>b</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

<sup>f</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean  $\pm$  standard deviation): 25/575 (4.3%  $\pm$  3.8%); range 0%-12%

<sup>g</sup> Not examined

<sup>h</sup> 2-Year historical incidence: 13/561 (2.3%  $\pm$  2.5%); range 0%-8%

TABLE 13  
Mammary Gland Lesions in Female Mice in the 2-Year Inhalation Study of 1,3-Butadiene<sup>a</sup>

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>9-Month Interim Evaluation</b>						
Hyperplasia	0/10 (0%)	<sup>b</sup>	—	1/1 (100%)	1/1 (100%)	0/8 (0%)
<b>15-Month Interim Evaluation</b>						
Hyperplasia	0/10 (0%)	—	—	0/10 (0%)	1/10 (10%)	0/2 (0%)
Adenocarcinoma	0/10 (0%)	—	—	0/10 (0%)	2/10 (20%)	1/2 (50%)
<b>2-Year Study</b>						
<b>Hyperplasia</b>						
Overall rate	2/50 (4%)	0/50 (0%)	2/50 (4%)	4/50 (8%)	7/50 (14%)	2/80 (3%)
Logistic regression test <sup>c</sup>	P=0.078	P=0.285N	P=0.626	P=0.132	P=0.008	P=0.914
<b>Adenoacanthoma</b>						
Overall rate	0/50 (0%)	1/50 (2%)	2/50 (4%)	6/50 (12%)	4/50 (8%)	0/80 (0%)
Adjusted rate <sup>d</sup>	0.0%	2.9%	7.7%	32.5%	13.6%	0.0%
Terminal rate <sup>e</sup>	0/37 (0%)	0/33 (0%)	1/24 (4%)	2/11 (18%)	0/0	0/0
First incidence (days)	<sup>f</sup>	732	686	579	268	—
Logistic regression test	P=0.244N	P=0.492	P=0.193	P=0.005	P=0.331	—
<b>Carcinoma</b>						
Overall rate	0/50 (0%)	2/50 (4%)	2/50 (4%)	6/50 (12%)	11/50 (22%)	12/80 (15%)
Adjusted rate	0.0%	5.8%	5.7%	16.2%	39.1%	100.0%
Terminal rate	0/37 (0%)	1/33 (3%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	—	732	645	392	370	280
Logistic regression test	P=0.050	P=0.228	P=0.257	P=0.076	P=0.009	P=0.004
<b>Malignant Mixed Tumor</b>						
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	0/50 (0%)	4/80 (5%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	0.0%	29.4%
Terminal rate	0/37 (0%)	0/33 (0%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	—	—	—	—	—	335
Logistic regression test	P=0.041	—	—	—	—	P=0.276
<b>Adenoacanthoma, Carcinoma, or Malignant Mixed Tumor</b>						
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	12/50 (24%)	15/50 (30%)	16/80 (20%)
Adjusted rate	0.0%	5.8%	13.0%	43.4%	47.4%	100.0%
Terminal rate	0/37 (0%)	1/33 (3%)	1/24 (4%)	2/11 (18%)	0/0	0/0
First incidence (days)	—	732	645	392	268	280
Logistic regression test	P=0.026	P=0.228	P=0.056	P<0.001	P=0.004	P<0.001

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals necropsied.

<sup>b</sup> Not examined

<sup>c</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

<sup>d</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>e</sup> observed incidence at terminal kill

<sup>f</sup> Not applicable; no neoplasms in animal group

squamous differentiation. The malignant mixed tumors consisted of epithelial components arranged in glandlike structures and anaplastic spindle-cell components. Mammary gland carcinomas and adenocarcinomas are uncommon in female B6C3F<sub>1</sub> mice, with carcinomas occurring in 21 of 561 control females (3.7%) and adenocarcinomas occurring in 1 of 561 control females (0.2%) in the NTP historical database (Table B4i). Mammary gland hyperplasia occurred at a slightly increased incidence in females in the 62.5 and 200 ppm groups (Table 13).

*Preputial Gland:* In the 200 ppm group, five male mice had preputial carcinomas (Table A3). Preputial gland carcinomas are rare in B6C3F<sub>1</sub> mice; none were reported in one NTP survey of historical control data. Preputial gland carcinomas in the present study were considered to be related to exposure to 1,3-butadiene. Some preputial carcinomas were composed of large eosinophilic epithelial cells that were well differentiated toward squamous cells with keratin pearls, or toward sebaceous-like cells. More frequently, the carcinomas had necrotic cores and a thin layer of very anaplastic pleomorphic basophilic epithelial cells that aggressively invaded surrounding tissue and blood vessels.

*Kidney:* The absolute kidney weights of females receiving 625 ppm at the 9-month interim evaluation and females receiving 62.5 and 200 ppm at the 15-month interim evaluation were greater than those of the controls (Tables E1 and E2).

Renal tubule adenomas were not diagnosed in the control mice and are considered to be rare spontaneous neoplasms, with an incidence in control mice in NTP studies of 0.2% for males and 0.0% for females (Tables A4g and B4j). Two female mice exposed to 200 ppm had renal tubule adenomas (Table 14). Male mice had a higher incidence of renal tubule adenomas than females, with three in the 62.5 ppm group, one in the 200 ppm group, and one in the 625 ppm group. Histologically, the renal tubule adenomas contained multiple dilated tubules separated by thin connective tissue septa. Epithelial cells in these neoplasms caused a papillary appearance, and some cytoplasmic and nuclear pleomorphism was present. Renal tubule hyperplasia was characterized by one or two dilated renal tubules, usually in the outer cortex, lined by enlarged epithelial cells showing some degree of nuclear enlargement and piling up. Considering the rarity of these lesions,

they were probably related to exposure to 1,3-butadiene in males and were possibly related to exposure in females.

*Skin:* The incidences of neurofibrosarcoma or sarcoma of the subcutaneous tissue in female mice exposed to 62.5, 200, or 625 ppm were significantly increased by the life table test but not by the logistic regression test (Tables 15 and B3). Nevertheless, subcutaneous tissue sarcomas (all types) are uncommon spontaneous neoplasms and have occurred in only 2 of 561 NTP historical control females (Table B4k). Thus, these neoplasms may be exposure related.

*Zymbal's Gland:* Zymbal's gland neoplasms are rare spontaneous lesions in B6C3F<sub>1</sub> mice, occurring in no historical control animals. One adenoma was seen in a control male mouse (Table A1). In females, no Zymbal's gland neoplasms were seen in controls, and one adenoma and one carcinoma were diagnosed in the 625 ppm group (Table B1). These neoplasms may be related to chemical exposure.

*Small Intestine:* Carcinomas of the small intestine are uncommon in the B6C3F<sub>1</sub> mouse, with none occurring in historical control mice from recent NTP inhalation studies; therefore, the presence of carcinomas in two females exposed to 6.25 ppm and in one female exposed to 62.5 ppm is of interest. One adenoma was present in a female exposed to 6.25 ppm (Table B1). A carcinoma was noted in one male in each of the 6.25, 20, and 62.5 ppm groups; carcinomas were present in two animals exposed to 200 ppm (Tables A1 and A5). It is difficult to determine the relationship of these neoplasms to exposure to 1,3-butadiene; however, no proliferative intestinal lesions were noted in controls.

*Testis:* At the 9-month interim evaluation, the absolute testis weight of males receiving 62.5 ppm or more and the relative testis weight of males receiving 200 and 625 ppm were lower than those of the controls (Table E1). At the 15-month interim evaluation, the absolute and relative testis weights of males that received 200 and 625 ppm were lower than those of controls (Table E2). The decreases in testicular weights seen at the 9- and 15-month interim evaluations appeared to be dose related, and correlated well with the diagnosis of testicular atrophy.

TABLE 14  
Kidney Lesions in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>15-Month Interim Evaluation</b>						
Renal Tubule: Adenoma	0/10 (0%)	0/3 (0%)	0/4 (0%)	0/2 (0%)	0/10 (0%)	1/7 (14%)
<b>2-Year Study</b>						
<b>Renal Tubule: Hyperplasia</b>						
Overall rate	2/50 (4%)	0/50 (0%)	0/50 (0%)	3/48 (6%)	1/49 (2%)	1/73 (1%)
Logistic regression test <sup>b</sup>	P=0.353	P=0.215N	P=0.324N	P=0.397	P=0.673	P=0.924
<b>Renal Tubule: Adenoma<sup>c</sup></b>						
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/48 (6%)	1/49 (2%)	0/73 (0%)
Adjusted rate <sup>d</sup>	0.0%	2.6%	0.0%	13.6%	3.7%	0.0%
Terminal rate <sup>e</sup>	0/35 (0%)	1/39 (3%)	0/24 (0%)	3/22 (14%)	0/4 (0%)	0/0
First incidence (days)	- <sup>f</sup>	733 (T)	-	733 (T)	573	-
Logistic regression test	P=0.630	P=0.522	-	P=0.053	P=0.580	-
<b>Female</b>						
<b>2-Year Study</b>						
<b>Renal Tubule: Hyperplasia</b>						
Overall rate	0/49 (0%)	0/49 (0%)	0/48 (0%)	1/50 (2%)	0/50 (0%)	0/80 (0%)
<b>Renal Tubule: Adenoma<sup>g</sup></b>						
Overall rate	0/49 (0%)	0/49 (0%)	0/48 (0%)	0/50 (0%)	2/50 (4%)	0/80 (0%)
Adjusted rate	0.0%	0.0%	0.0%	0.0%	35.2%	0.0%
Terminal rate	0/37 (0%)	0/33 (0%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	-	-	-	-	418	-
Logistic regression test	P=0.816N	-	-	-	P=0.276	-

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of lesion-bearing animals/number of animals microscopically examined.

<sup>b</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression test regards neoplasms in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in a dose group is indicated by N.

<sup>c</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean  $\pm$  standard deviation): 1/571 (0.2%  $\pm$  0.3%); range 0%-1%

<sup>d</sup> Number of neoplasm-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this neoplasm type in any of the groups

<sup>e</sup> Observed incidence at terminal kill

<sup>f</sup> Not applicable; no neoplasms in animal group

<sup>g</sup> 2-Year historical incidence: 0/559 (0.0%); upper 95% confidence limit = 0.5%

**TABLE 15**  
**Subcutaneous Skin Neoplasms in Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm
<b>Male</b>						
<b>2-Year Study</b>						
<b>Hemangiosarcoma<sup>b</sup></b>						
Overall rate	0/50 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	3/50 (6%)	0/73 (0%)
Adjusted rate <sup>c</sup>	0.0%	2.6%	2.5%	0.0%	11.7%	0.0%
Terminal rate <sup>d</sup>	0/35 (0%)	1/39 (3%)	0/24 (0%)	0/22 (0%)	0/4 (0%)	0/0
First incidence (days)	- <sup>f</sup>	733 (T)	585	-	530	-
Life table test <sup>e</sup>	P=0.060	P=0.522	P=0.486	-	P=0.051	-
<b>Female</b>						
<b>15-Month Interim Evaluation</b>						
Neurofibrosarcoma	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	1/2 (50%)
<b>2-Year Study</b>						
<b>Hemangioma<sup>g</sup></b>						
Overall rate	0/50 (0%)	0/50 (0%)	1/50 (2%)	1/50 (2%)	0/50 (0%)	0/80 (0%)
<b>Hemangiosarcoma<sup>h</sup></b>						
Overall rate	1/50 (2%)	2/50 (4%)	2/50 (4%)	2/50 (4%)	2/50 (4%)	2/80 (3%)
Adjusted rate	2.7%	5.2%	8.3%	13.2%	21.4%	6.1%
Terminal rate	1/37 (3%)	1/33 (3%)	2/24 (8%)	0/11 (0%)	0/0	0/0
First incidence (days)	733 (T)	572	733 (T)	665	569	328
Life table test	P<0.001	P=0.471	P=0.350	P=0.173	P=0.014	P=0.153
<b>Neurofibrosarcoma or Sarcoma<sup>i</sup></b>						
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	5/50 (10%)	3/50 (6%)	3/80 (4%)
Adjusted rate	2.4%	5.3%	8.0%	20.3%	43.0%	19.3%
Terminal rate	0/37 (0%)	1/33 (3%)	0/24 (0%)	0/11 (0%)	0/0	0/0
First incidence (days)	677	642	569	597	524	316
Life table test	P<0.001	P=0.476	P=0.238	P=0.017	P=0.002	P=0.013

(T) Terminal sacrifice

<sup>a</sup> Incidences are given as number of neoplasm-bearing animals/number of animals necropsied.

<sup>b</sup> 2-Year historical incidence for untreated control groups in NTP inhalation studies (mean ± standard deviation): 0/575 (0.0% ± 0.0%); range 0%-0%

<sup>c</sup> Number of neoplasm-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this neoplasm type in any of the groups

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.

<sup>f</sup> Not applicable; no neoplasms in animal group

<sup>g</sup> 2-Year historical incidence: 0/561

<sup>h</sup> 2-Year historical incidence: 1/561 (0.2% ± 0.6%); range 0%-2%

<sup>i</sup> 2-Year historical incidence: 2/561 (0.4% ± 0.8%); range 0%-2%

Testicular atrophy was observed to be a prominent exposure-related response only in mice exposed to 625 ppm (Table A5). Testicular atrophy was characterized by a uniform minimal to mild decrease in cellularity of the seminiferous tubules.

*Nose:* Olfactory epithelial atrophy was observed in seven female mice exposed to 625 ppm and in one control (Table B5). Atrophy was associated with an inflamed tooth in one control mouse and was unilateral in the other control mouse. In female mice exposed to 625 ppm, the lesion was probably related to exposure to 1,3-butadiene; lesions were bilateral and were present in the middle and posterior nasal sections. Olfactory epithelial atrophy occurred in male mice exposed to 20 ppm or above; the incidence in males exposed to 625 ppm was lower than the incidence in females, but was possibly also related to exposure (Table A5). Atrophy was usually characterized by focal loss of olfactory sensory neurons,

with single layers of columnar, cuboidal, squamous, or respiratory epithelial cells covering the defect. The atrophy was usually minimal to mild in severity and usually affected the olfactory epithelium at the dorsal meatus of the posterior nasal section. These nasal lesions were similar to those seen in previous 1,3-butadiene studies (NTP, 1984), but no osseous or cartilaginous metaplasia was observed in the present studies. The low incidences of atrophy were not unexpected, because this lesion was not diagnosed in the 625 ppm groups in the previous 1,3-butadiene studies. In the previous studies, a strong predominance of olfactory epithelial lesions was observed in male mice exposed to 1,250 ppm as compared with the females, but this was not observed in animals exposed to 625 ppm in the present studies. Olfactory epithelial lesions observed in animals exposed to lower doses in the present studies were unilateral or not of the same character as those observed at the highest dose, but were still diagnosed as atrophy.

## 2-YEAR STOP-EXPOSURE STUDY

### Survival

Estimates of survival probabilities for male mice are shown in Table 16 and in the Kaplan-Meier curves in Figure 4. Survival of all groups of male mice exposed to 1,3-butadiene was significantly lower than that of the controls due to the development of malignant neoplasms, particularly malignant lymphomas and hemangiosarcomas of the heart. The reduced survival of male mice exposed to 625 ppm for 13 or 26 weeks became apparent between months 6 and 9 of the study, whereas that of male mice exposed to 312 ppm for 52 weeks and 200 ppm for 40 weeks became apparent between months 12 and 15 of the study.

Of the two groups receiving the total exposure equivalent of 8,000 ppm · weeks, the survival of males exposed to 625 ppm for 13 weeks was not significantly lower than that of males exposed to 200 ppm for 40 weeks. In contrast, of the two groups receiving the equivalent of 16,000 ppm · weeks, the survival of the group exposed to 625 ppm for 26 weeks was significantly lower than that of the group exposed to 312 ppm for 52 weeks.

### Body Weights

Mean body weights of exposed and control male mice were similar (Table 17 and Figure 5).

TABLE 16  
Survival in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene

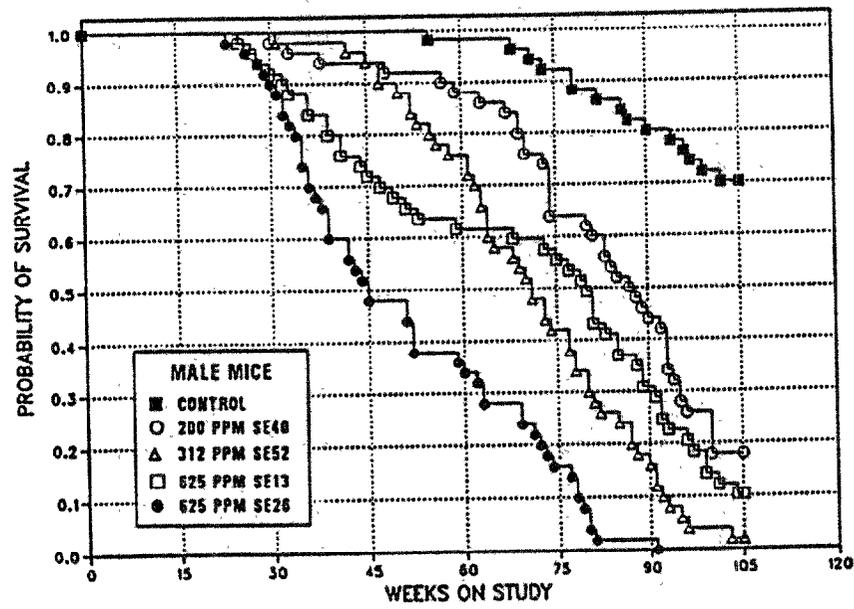
	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
Animals initially in study	70	50	50	50	50
9-Month interim evaluation <sup>a</sup>	10	0	0	0	0
15-Month interim evaluation <sup>a</sup>	10	0	0	0	0
Accidental deaths <sup>a</sup>	0	0	1	0	0
Moribund	9	29	25	25	22
Natural deaths	6	12	19	24	28
Animals surviving until study termination	35	9	5	1	0
Percent probability of survival at end of study <sup>b</sup>	70	18	10	2	0
Mean survival days <sup>c</sup>	685	579	487	496	357
Survival analysis <sup>d</sup>	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

<sup>a</sup> Censored from survival analyses

<sup>b</sup> Kaplan-Meier determinations. Survival rates adjusted for interim evaluations and accidental deaths.

<sup>c</sup> Mean of all deaths (uncensored, censored, terminal sacrifice)

<sup>d</sup> The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns.



**FIGURE 4**  
**Kaplan-Meier Survival Curves for Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

TABLE 17  
 Mean Body Weights and Survival of Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene

Week on Study <sup>a</sup>	0 ppm		200 ppm (40 weeks)			312 ppm (52 weeks)		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	23.0	70	22.6	98	50	22.7	99	50
2	24.4	70	25.6	105	50	25.1	103	50
3	25.8	70	26.7	103	50	26.4	102	50
4	27.1	70	27.9	103	50	27.1	100	50
5	27.8	70	28.8	104	50	27.9	100	50
6	28.7	70	29.4	102	50	28.2	98	50
7	29.2	70	30.3	104	50	29.0	99	50
8	30.1	70	31.2	104	50	29.6	98	50
9	30.7	70	32.0	104	50	30.1	98	50
10	31.1	70	32.5	105	50	30.9	99	50
11	32.0	70	33.6	105	50	31.2	98	50
12	32.7	70	33.7	103	50	31.0	95	50
13	33.0	70	34.0	103	50	31.5	96	50
17	35.2	70	36.7	104	50	34.5	98	50
21	36.6	70	39.7	109	50	36.2	99	50
25	38.9	70	41.7	107	50	38.0	98	50
29	40.8	70	43.6	107	50	40.5	99	50
33	41.7	70	43.8	105	49	41.4	99	49
37	43.2	70	45.1	104	48	43.1	100	49
41 <sup>b</sup>	43.3	60	46.3	107	47	44.1	102	49
45	44.1	60	46.3	105	47	45.5	103	47
49	45.5	60	47.4	104	46	46.1	101	45
53	46.0	60	47.2	103	46	45.8	100	41
57	45.7	59	47.4	104	46	46.8	102	39
61	46.1	59	48.1	104	44	47.1	102	36
65 <sup>b</sup>	45.7	49	46.7	102	43	47.5	104	29
69	45.8	48	46.0	100	40	47.9	105	27
73	45.3	46	45.0	99	37	47.0	104	22
77	45.1	46	44.6	99	32	44.9	100	21
81	44.8	44	43.9	98	31	46.7	104	14
85	44.3	43	43.9	99	27	46.9	106	12
89	44.0	41	42.1	96	24	42.7	97	9
93	43.1	39	39.2	91	20	37.6	87	4
95	42.9	39	40.0	93	15	35.8	83	3
97	43.0	37	40.4	94	13	38.3	89	2
99	42.3	37	38.6	91	13	36.1	85	2
101	41.6	36	37.8	91	9	34.6	83	2
103	41.5	35	37.1	89	9	31.8	77	1
Terminal sacrifice		35			9			1
Mean for weeks								
1-13	28.9		29.9	103		28.5	99	
14-52	41.0		43.4	106		41.0	100	
53-103	44.2		43.0	97		42.3	96	

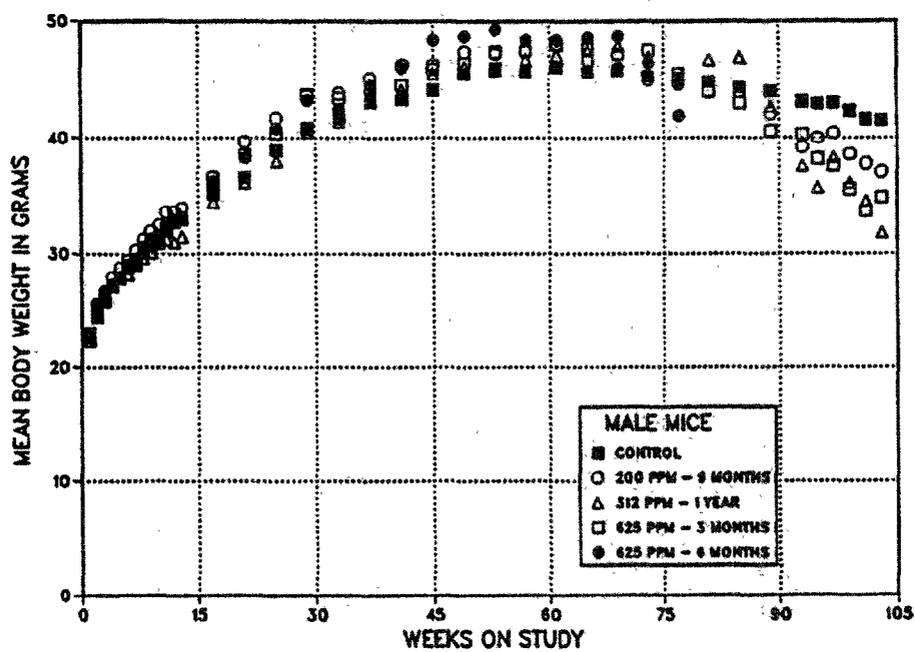
(continued)

**TABLE 17**  
**Mean Body Weights and Survival of Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**  
 (continued)

Week on Study	0 ppm		625 ppm (13 weeks)			625 ppm (26 weeks)		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	23.0	70	22.3	97	50	22.7	99	50
2	24.4	70	25.1	103	50	25.4	104	50
3	25.8	70	26.4	102	50	26.7	104	50
4	27.1	70	27.2	100	50	27.3	101	50
5	27.8	70	28.0	101	50	28.0	101	50
6	28.7	70	28.9	101	50	29.1	101	50
7	29.2	70	29.4	101	50	29.6	101	50
8	30.1	70	30.5	101	50	30.4	101	50
9	30.7	70	31.0	101	50	31.3	102	50
10	31.1	70	31.5	101	50	31.6	102	50
11	32.0	70	32.2	101	50	32.4	101	50
12	32.7	70	32.7	100	50	33.0	101	50
13	33.0	70	33.0	100	50	33.2	101	50
17	35.2	70	36.2	103	50	36.3	103	50
21	36.6	70	38.5	105	50	38.3	105	50
25	38.9	70	40.4	104	49	40.8	105	49
29	40.8	70	43.7	107	47	43.2	106	46
33	41.7	70	43.4	104	45	42.4	102	42
37	43.2	70	44.3	103	42	44.5	103	34
41 <sup>b</sup>	43.3	60	44.5	103	38	46.0	106	30
45	44.1	60	45.9	104	35	48.4	110	24
49	45.5	60	46.4	102	34	48.7	107	24
53	46.0	60	47.4	103	31	49.3	107	19
57	45.7	59	47.5	104	31	48.4	106	19
61	46.1	59	48.0	104	30	48.4	105	17
65 <sup>b</sup>	45.7	49	47.7	104	30	48.6	106	14
69	45.8	48	47.2	103	29	48.7	106	13
73	45.3	46	47.5	105	28	46.4	102	9
77	45.1	46	45.5	101	27	41.9	93	8
81	44.8	44	44.0	98	23			
85	44.3	43	43.0	97	20			
89	44.0	41	40.6	92	16			
93	43.1	39	40.3	94	11			
95	42.9	39	38.2	89	11			
97	43.0	37	37.6	87	9			
99	42.3	37	35.6	84	8			
101	41.6	36	33.7	81	7			
103	41.5	35	34.9	84	6			
Terminal sacrifice		35			5			0
Mean for weeks								
1-13	28.9		29.1	101		29.3	101	
14-52	41.0		42.6	104		43.2	105	
53-103	44.2		42.4	96		47.4	107	

<sup>a</sup> Week on study of stop-exposure groups. For weeks 17 through 95, control males were weighed one week after stop-exposure males.

<sup>b</sup> Interim evaluations in control males occurred during weeks 41 and 66.



**FIGURE 5**  
**Growth Curves for Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

### *Pathology and Statistical Analyses of Results*

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the hematopoietic system, heart, lung, liver, stomach, harderian gland, kidney, Zymbal's gland, brain, and preputial gland. Summaries of the incidences of neoplasms and nonneoplastic lesions, the individual animal tumor diagnoses, the statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one group, and the historical incidences for the biologically significant neoplasms mentioned in this section are presented in Appendix C.

Exposure of male mice to 200 ppm for 40 weeks, 625 ppm for 13 weeks, 312 ppm for 52 weeks, or 625 ppm for 26 weeks induced neoplasms at the same sites observed in the 2-year studies.

*Hematopoietic System:* The incidences of mice with malignant lymphoma in groups exposed to 625 ppm for 13 or 26 weeks were markedly greater than the incidence in the controls, while the incidences in groups exposed to 200 ppm for 40 weeks or 312 ppm for 52 weeks were only marginally increased (Table 18). The majority of the lymphomas were the lymphocytic type apparently arising from the thymus. During the first 9 months of the study, all early deaths except one were related to the development of malignant lymphomas; the cause of death of one male exposed to 200 ppm was not determined. The incidence of histiocytic sarcomas was also significantly increased in each of the stop-exposure groups, but they occurred more frequently in mice exposed to 200 or 312 ppm.

*Heart:* The incidences of endothelial hyperplasia and hemangiosarcoma in most stop-exposure groups were significantly greater than in the controls (Table 19). In contrast to the increased occurrence of malignant lymphomas in groups exposed to 625 ppm, hemangiosarcomas occurred more frequently in mice exposed to 200 or 312 ppm. Hemangiosarcomas were observed as early as 9 months in each of the 200, 312, and 625 ppm (26-week) stop-exposure groups. Myocardial mineralization, a lesion that occurred in

exposed male and female mice in the 2-year studies, was also seen in male mice exposed to 312 ppm for 52 weeks or 625 ppm for 13 or 26 weeks (Table C5).

*Lung:* The incidences of hyperplasia of the alveolar epithelium, alveolar/bronchiolar adenoma, and alveolar/bronchiolar adenocarcinoma or carcinoma were significantly greater in each of the stop-exposure groups than in the controls (Table 20).

*Liver:* The incidences of hepatocellular adenoma of the liver were significantly greater in the 200, 312, and 625 ppm (13-week) stop-exposure groups than in the controls, while the incidence of hepatocellular carcinomas was not increased in any of the stop-exposure groups (Table 21). Because the cause of death of mice with hepatocellular adenoma or carcinoma was generally attributed to other malignant neoplasms, the logistic regression test was considered the most appropriate analysis.

*Stomach:* The increased incidence of hyperplasia of the forestomach epithelium in each of the stop-exposure groups was not significantly greater than that in the control group (Table 22). Squamous cell papillomas occurred at low incidences in each of the groups, and the incidences were not significantly greater than the control incidence by the logistic regression test, the most appropriate analysis for these nonfatal lesions. Squamous cell carcinomas occurred in groups of mice exposed to 312 or 625 ppm (13 or 26 weeks), but not in mice exposed to 200 ppm nor in the control group. The incidences of carcinoma in the stop-exposure groups were significantly greater than the incidence in the control group by the life table test, the most appropriate analysis for these fatal neoplasms.

*Harderian Gland:* The incidences of adenoma of the harderian gland were significantly greater in each of the stop-exposure groups than in the controls (Table 23). Carcinomas occurred at low incidences in males exposed to 200 ppm for 40 weeks, 312 ppm for 52 weeks, or 625 ppm for 13 weeks; none were observed in the controls or in males exposed to 625 ppm for 26 weeks. Focal hyperplasia of the harderian gland occurred with low frequency in each of the stop-exposure groups.

**TABLE 18**  
**Malignant Lymphoma and Histiocytic Sarcoma in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Lymphocytic Malignant Lymphoma</b>					
Overall rate <sup>a</sup>	2/50 (4%)	6/50 (12%)	17/50 (34%)	4/50 (8%)	30/50 (60%)
Adjusted rate <sup>b</sup>	4.7%	26.7%	35.8%	100.0%	81.5%
Terminal rate <sup>c</sup>	0/35 (0%)	1/9 (11%)	0/5 (0%)	1/1 (100%)	0/0
First incidence (days)	511	208	169	289	159
Life table test <sup>d</sup>		P=0.033	P<0.001	P=0.034	P<0.001
<b>All Organs: Lymphoma (Mixed or NOS)</b>					
Overall rate	2/50 (4%)	2/50 (4%)	5/50 (10%)	4/50 (8%)	3/50 (6%)
Adjusted rate	5.3%	7.8%	34.8%	58.0%	43.3%
Terminal rate	1/35 (3%)	0/9 (0%)	1/5 (20%)	0/1 (0%)	0/0
First incidence (days)	666	514	251	217	251
Life table test		P=0.382	P=0.010	P=0.005	P=0.002
<b>Malignant Lymphoma (Lymphocytic, Mixed, or NOS)</b>					
Overall rate	4/50 (8%)	8/50 (16%)	22/50 (44%)	8/50 (16%)	33/50 (66%)
Adjusted rate	9.8%	32.4%	58.2%	100.0%	89.5%
Terminal rate	1/35 (3%)	1/9 (11%)	1/5 (20%)	1/1 (100%)	0/0
First incidence (days)	511	208	169	217	159
Life table test		P=0.023	P<0.001	P<0.001	P<0.001
<b>Histiocytic Sarcoma</b>					
Overall rate	0/50 (0%)	5/50 (10%)	2/50 (4%)	7/50 (14%)	2/50 (4%)
Adjusted rate	0.0%	21.3%	28.9%	43.0%	15.6%
Terminal rate	0/35 (0%)	0/9 (0%)	1/5 (20%)	0/1 (0%)	0/0
First incidence (days)	- <sup>e</sup>	576	692	314	364
Life table test		P=0.006	P=0.011	P<0.001	P=0.036

<sup>a</sup> Number of neoplasm-bearing animals/number of animals necropsied

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE 19**  
**Heart Lesions in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Endothelial Hyperplasia</b>					
Overall rate <sup>a</sup>	0/50 (0%)	6/50 (12%)	7/50 (14%)	3/50 (6%)	7/50 (14%)
Logistic regression test <sup>b</sup>		P=0.004	P=0.002	P=0.112	P=0.009
<b>Hemangiosarcoma</b>					
Overall rate	0/50 (0%)	15/50 (30%)	7/50 (14%)	33/50 (66%)	13/50 (26%)
Adjusted rate <sup>c</sup>	0.0%	76.2%	61.8%	100.0%	100.0%
Terminal rate <sup>d</sup>	0/35 (0%)	5/9 (56%)	2/5 (40%)	1/1 (100%)	0/0
First incidence (days)	- <sup>e</sup>	330	566	328	306
Life table test <sup>b</sup>		P<0.001	P<0.001	P<0.001	P<0.001

<sup>a</sup> Number of lesion-bearing animals/number of animals necropsied

<sup>b</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE 20**  
**Lung Lesions in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Alveolar Epithelial Hyperplasia</b>					
Overall rate <sup>a</sup>	2/50 (4%)	18/50 (36%)	10/50 (20%)	14/50 (28%)	11/50 (22%)
Logistic regression test <sup>b</sup>		P<0.001	P<0.001	P<0.001	P<0.001
<b>Alveolar/bronchiolar Adenoma</b>					
Overall rate	18/50 (36%)	24/50 (48%)	17/50 (34%)	26/50 (52%)	12/50 (24%)
Adjusted rate <sup>c</sup>	46.9%	94.3%	85.3%	100.0%	100.0%
Terminal rate <sup>d</sup>	15/35 (43%)	8/9 (89%)	3/5 (60%)	1/1 (100%)	0/0
First incidence (days)	572	399	327	344	358
Logistic regression test		P=0.015	P=0.044	P=0.001	P<0.001
<b>Alveolar/bronchiolar Adenocarcinoma or Carcinoma</b>					
Overall rate	5/50 (10%)	22/50 (44%)	18/50 (36%)	16/50 (32%)	11/50 (22%)
Adjusted rate	14.3%	89.5%	87.7%	100.0%	100.0%
Terminal rate	5/35 (14%)	7/9 (78%)	3/5 (60%)	1/1 (100%)	0/0
First incidence (days)	729 (T)	481	370	392	241
Life table test <sup>b</sup>		P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test		P<0.001	P<0.001	P<0.001	P<0.001
<b>Alveolar/bronchiolar Adenoma, Adenocarcinoma, or Carcinoma</b>					
Overall rate	21/50 (42%)	36/50 (72%)	28/50 (56%)	32/50 (64%)	17/50 (34%)
Adjusted rate	54.9%	100.0%	100.0%	100.0%	100.0%
Terminal rate	18/35 (51%)	9/9 (100%)	5/5 (100%)	1/1 (100%)	0/0
First incidence (days)	572	399	327	344	241
Life table test		P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test		P<0.001	P<0.001	P<0.001	P<0.001

(T) Terminal sacrifice

<sup>a</sup> Number of lesion-bearing animals/number of animals necropsied

<sup>b</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

**TABLE 21**  
**Liver Neoplasms in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Hepatocellular Adenoma</b>					
Overall rate <sup>a</sup>	13/50 (26%)	27/49 (55%)	19/49 (39%)	19/50 (38%)	11/50 (22%)
Adjusted rate <sup>b</sup>	32.1%	91.1%	91.0%	100.0%	100.0%
Terminal rate <sup>c</sup>	9/35 (26%)	7/9 (78%)	4/5 (80%)	1/1 (100%)	0/0
First incidence (days)	379	399	471	326	313
Logistic regression test <sup>d</sup>		P<0.001	P=0.042	P=0.045	P=0.284
<b>Hepatocellular Carcinoma</b>					
Overall rate	11/50 (22%)	14/49 (29%)	14/49 (29%)	10/50 (20%)	4/50 (8%)
Adjusted rate	26.0%	50.3%	90.9%	74.6%	50.5%
Terminal rate	5/35 (14%)	1/9 (11%)	4/5 (80%)	0/1 (0%)	0/0
First incidence (days)	540	407	520	382	483
Logistic regression test		P=0.530N	P=0.142	P=0.453N	P=0.393N
<b>Hepatocellular Adenoma or Carcinoma</b>					
Overall rate	21/50 (42%)	33/49 (67%)	24/49 (49%)	24/50 (48%)	13/50 (26%)
Adjusted rate	47.9%	93.4%	94.4%	100.0%	100.0%
Terminal rate	13/35 (37%)	7/9 (78%)	4/5 (80%)	1/1 (100%)	0/0
First incidence (days)	379	399	471	326	313
Logistic regression test		P=0.004	P=0.063	P=0.169	P=0.561
<b>Hepatoblastoma, Hepatocellular Adenoma, or Carcinoma</b>					
Overall rate	21/50 (42%)	33/49 (67%)	24/49 (49%)	25/50 (50%)	13/50 (26%)
Adjusted rate	47.9%	93.4%	94.4%	100.0%	100.0%
Terminal rate	13/35 (37%)	7/9 (78%)	4/5 (80%)	1/1 (100%)	0/0
First incidence (days)	379	399	471	326	313
Logistic regression test		P=0.004	P=0.063	P=0.103	P=0.561

<sup>a</sup> Number of neoplasm-bearing animals/number of animals microscopically examined

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard neoplasms in animals dying prior to terminal kill as nonfatal. A lower incidence in a dose group is indicated by N.

**TABLE 22**  
**Forestomach Lesions in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene**

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Epithelial Hyperplasia</b>					
Overall rate <sup>a</sup>	4/50 (8%)	10/48 (21%)	8/50 (16%)	20/48 (42%)	15/50 (30%)
Logistic regression test <sup>b</sup>		P=0.163	P=0.638N	P=0.095	P=0.566
<b>Squamous Cell Papilloma</b>					
Overall rate	1/50 (2%)	3/50 (6%)	4/50 (8%)	4/50 (8%)	4/50 (8%)
Adjusted rate <sup>c</sup>	2.5%	21.4%	28.3%	100.0%	20.1%
Terminal rate <sup>d</sup>	0/35 (0%)	1/9 (11%)	1/5 (20%)	1/1 (100%)	0/0
First incidence (days)	652	584	327	401	359
Logistic regression test		P=0.195	P=0.260	P=0.181	P=0.301
<b>Squamous Cell Carcinoma</b>					
Overall rate	0/50 (0%)	0/50 (0%)	4/50 (8%)	5/50 (10%)	6/50 (12%)
Adjusted rate	0.0%	0.0%	51.6%	33.1%	40.9%
Terminal rate	0/35 (0%)	0/9 (0%)	2/5 (40%)	0/1 (0%)	0/0
First incidence (days)	- <sup>e</sup>	-	370	422	288
Life table test <sup>b</sup>			P<0.001	P<0.001	P<0.001
Logistic regression test			P=0.013	P=0.017	P=0.061
<b>Squamous Cell Papilloma or Squamous Cell Carcinoma</b>					
Overall rate	1/50 (2%)	3/50 (6%)	7/50 (14%)	9/50 (18%)	10/50 (20%)
Adjusted rate	2.5%	21.4%	56.6%	100.0%	52.8%
Terminal rate	0/35 (0%)	1/9 (11%)	2/5 (40%)	1/1 (100%)	0/0
First incidence (days)	652	584	327	401	288
Life table test		P=0.065	P<0.001	P<0.001	P<0.001
Logistic regression test		P=0.195	P=0.025	P=0.004	P=0.313

<sup>a</sup> Number of lesion-bearing animals/microscopically examined or number of animals necropsied

<sup>b</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. A lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

TABLE 23  
 Harderian Gland Lesions in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Hyperplasia</b>					
Overall rate <sup>a</sup>	1/50 (2%)	4/48 (8%)	3/42 (7%)	6/48 (13%)	7/36 (19%)
Logistic regression test <sup>b</sup>		P=0.174	P=0.179	P=0.328	P=0.002
<b>Adenoma</b>					
Overall rate	6/50 (12%)	26/50 (52%)	20/50 (40%)	28/50 (56%)	13/50 (26%)
Adjusted rate <sup>c</sup>	14.8%	87.9%	94.3%	100.0%	100.0%
Terminal rate <sup>d</sup>	2/35 (6%)	6/9 (67%)	4/5 (80%)	1/1 (100%)	0/0
First incidence (days)	543	440	410	344	306
Logistic regression test		P<0.001	P<0.001	P<0.001	P=0.046
<b>Carcinoma</b>					
Overall rate	0/50 (0%)	2/50 (4%)	4/50 (8%)	2/50 (4%)	0/50 (0%)
Adjusted rate	0.0%	5.6%	38.8%	51.5%	0.0%
Terminal rate	0/35 (0%)	0/9 (0%)	1/5 (20%)	0/1 (0%)	0/0
First incidence (days)	- <sup>e</sup>	510	567	441	-
Life table test <sup>b</sup>		P=0.182	P<0.001	P=0.028	-
Logistic regression test		P=0.397	P=0.006	P=0.190	-
<b>Adenoma or Carcinoma</b>					
Overall rate	6/50 (12%)	27/50 (54%)	23/50 (46%)	30/50 (60%)	13/50 (26%)
Adjusted rate	14.8%	88.3%	100.0%	100.0%	100.0%
Terminal rate	2/35 (6%)	6/9 (67%)	5/5 (100%)	1/1 (100%)	0/0
First incidence (days)	543	440	410	344	306
Life table test		P<0.001	P<0.001	P<0.001	P<0.001
Logistic regression test		P<0.001	P<0.001	P<0.001	P=0.046

<sup>a</sup> Number of lesion-bearing animals/number of animals microscopically examined or number of animals necropsied

<sup>b</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

**Kidney:** Renal tubule epithelium focal hyperplasia or adenoma occurred at low incidences in each of the stop-exposure groups (Table 24). Although the incidences of adenoma in the exposed groups were not significantly greater than the incidence in the controls by the logistic regression analysis, renal tubule adenomas are rare spontaneous neoplasms in untreated male mice. The incidence of renal tubule neoplasms in NTP historical control male mice is 1/571 (Table C4). The small numbers of renal tubule adenomas in male mice exposed to 1,3-butadiene are considered to be related to chemical administration because of their rare occurrence in historical controls.

**Zymbal's Gland:** Carcinomas of the Zymbal's gland were seen in one male exposed to 200 ppm for 40 weeks, two males exposed to 625 ppm for 13 weeks, and two males exposed to 625 ppm for 26 weeks; an adenoma was seen in one control male (Table C1). The incidence of Zymbal's gland neoplasms (adenomas or carcinomas) in the group exposed to 625 ppm for 26 weeks was significantly

greater than the incidence in the controls by the life table test (Table C3a).

**Brain:** Malignant gliomas occurred in the brain of two male mice exposed to 625 ppm for 13 weeks and in one male mouse exposed to 625 ppm for 26 weeks (Table C1). In addition, malignant neuroblastomas were seen in two males exposed to 625 ppm for 13 weeks (Table C1). All of the neoplasms occurred in the anterior or olfactory lobe of the brain. Gliomas and neuroblastomas are rare spontaneous neoplasms; none have been observed in 574 NTP historical control male mice. For this reason, both the gliomas and the neuroblastomas are considered related to chemical administration.

**Preputial Gland:** Preputial gland carcinomas occurred at low incidences in each of the stop-exposure groups but none were seen in the controls (Table 25). An adenoma was seen in one male mouse exposed to 625 ppm for 13 weeks. The combined incidences of preputial gland adenoma or carcinoma were significantly increased in the groups exposed to 312 ppm for 52 weeks or 625 ppm for 13 or 26 weeks.

TABLE 24  
Kidney Lesions in Male Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene

	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>Renal Tubule: Hyperplasia</b>					
Overall rate <sup>a</sup>	2/50 (4%)	4/48 (8%)	1/50 (2%)	1/49 (2%)	2/50 (4%)
Logistic regression test <sup>b</sup>		P=0.163	P=0.678	P=0.013N	P=0.429N
<b>Renal Tubule: Adenoma</b>					
Overall rate	0/50 (0%)	4/48 (8%)	1/50 (2%)	3/49 (6%)	1/50 (2%)
Adjusted rate <sup>c</sup>	0.0%	17.4%	14.3%	27.8%	6.3%
Terminal rate <sup>d</sup>	0/35 (0%)	0/9 (0%)	0/5 (0%)	0/1 (0%)	0/0
First incidence (days)	- <sup>e</sup>	516	707	539	440
Logistic regression test		P=0.073	P=0.273	P=0.075	P=0.731

<sup>a</sup> Number of lesion-bearing animals/number of animals microscopically examined

<sup>b</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in a dose group is indicated by N.

<sup>c</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE 26**  
**Comparison of Selected Neoplasms in Male Mice Exposed to 1,3-Butadiene Concentrations**  
**of 200 ppm for 40 Weeks or 625 ppm for 13 Weeks**

	200 ppm (40 weeks)	625 ppm (13 weeks)
<b>All Organs: Lymphocytic Malignant Lymphoma</b>		
Overall rate <sup>a</sup>	6/50 (12%)	17/50 (34%)
Adjusted rate <sup>b</sup>	26.7%	35.8%
Terminal rate <sup>c</sup>	1/9 (11%)	0/5 (0%)
First incidence (days)	208	169
Life table test <sup>d</sup>		P=0.005
<b>All Organs: Malignant Lymphoma (Mixed or NOS)</b>		
Overall rate	2/50 (4%)	5/50 (10%)
Adjusted rate	7.8%	34.8%
Terminal rate	0/9 (0%)	1/5 (20%)
First incidence (days)	514	251
Life table test		P=0.117
<b>All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or NOS)</b>		
Overall rate	8/50 (16%)	22/50 (44%)
Adjusted rate	32.4%	58.2%
Terminal rate	1/9 (11%)	1/5 (20%)
First incidence (days)	208	169
Life table test		P=0.001
<b>Stomach (Forestomach): Squamous Cell Carcinoma</b>		
Overall rate	0/50 (0%)	4/50 (8%)
Adjusted rate	0.0%	51.6%
Terminal rate	0/9 (0%)	2/5 (40%)
First incidence (days)	- <sup>e</sup>	370
Life table test		P=0.019
Logistic regression test <sup>d</sup>		P=0.031
<b>Stomach (Forestomach): Squamous Cell Papilloma or Squamous Cell Carcinoma</b>		
Overall rate	3/50 (6%)	7/50 (14%)
Adjusted rate	21.4%	56.6%
Terminal rate	1/9 (11%)	2/5 (40%)
First incidence (days)	584	327
Life table test		P=0.045
Logistic regression test		P=0.099

<sup>a</sup> Number of neoplasm-bearing animals/number of animals necropsied

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal.

<sup>e</sup> Not applicable; no neoplasms in animal group

**TABLE 27**  
**Comparison of Selected Neoplasms in Male Mice Exposed to 1,3-Butadiene Concentrations of 312 ppm for 52 Weeks or 625 ppm for 26 Weeks**

	312 ppm (52 weeks)	625 ppm (26 weeks)
<b>All Organs: Lymphocytic Malignant Lymphoma</b>		
Overall rate <sup>a</sup>	4/50 (8%)	30/50 (60%)
Adjusted rate <sup>b</sup>	100.0%	81.5%
Terminal rate <sup>c</sup>	1/1 (100%)	0/0
First incidence (days)	289	159
Life table test <sup>d</sup>		P < 0.001
<b>All Organs: Malignant Lymphoma (Mixed or NOS)</b>		
Overall rate	4/50 (8%)	3/50 (6%)
Adjusted rate	58.0%	43.3%
Terminal rate	0/1 (0%)	0/0
First incidence (days)	217	251
Life table test		P = 0.244
<b>All Organs: Malignant Lymphoma (Lymphocytic, Mixed, or NOS)</b>		
Overall rate	8/50 (16%)	33/50 (66%)
Adjusted rate	100.0%	89.5%
Terminal rate	1/1 (100%)	0/0
First incidence (days)	217	159
Life table test		P < 0.001

<sup>a</sup> Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for lung and preputial gland; for other tissues, denominator is number of animals necropsied.

<sup>b</sup> Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality

<sup>c</sup> Observed incidence at terminal kill

<sup>d</sup> Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The life table analysis regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death.

by the "Poly-3" quantal response test (Bailer and Portier, 1988; Portier and Bailer, 1989). The survival-adjusted rates for those neoplasms showing chemical-related increases are summarized in Tables 28 and 29. The effect of this additional analysis was to detect as significant certain neoplastic responses that were not detected by the logistic regression analysis in mice exposed to 625 ppm. The results of the "Poly-3" quantal response test do not change the overall interpretation of these studies. Mortality-adjusted dose-response curves for neoplastic lesions induced by 1,3-butadiene are shown in Figures 6 (males) and 7 (females).

The results of fitting a modified Weibull model (Portier *et al.*, 1986) to the Poly-3 survival-adjusted

neoplasm rates are given as the shape parameter values in Table 28. For approximately half of the neoplasms evaluated, the dose-response trend is consistent with a linear model (i.e., shape parameter = 1). In most of the instances in which a departure from linearity was evident, the shape parameter was significantly less than a value of one (liver neoplasms in males, mammary gland neoplasms in females, and harderian gland neoplasms and lung neoplasms in males and females) implying that the dose-response curve is very steep in the low-dose region. Only for malignant lymphoma in male mice and hemangiosarcoma of the heart in female mice was there evidence of a "threshold-like" dose-response curve, i.e., a curve in which the shape parameter is significantly greater than one.

**TABLE 28**  
**Survival-Adjusted Neoplasm Rates for B6C3F<sub>1</sub> Mice in the 2-Year Inhalation Studies of 1,3-Butadiene<sup>a</sup>**

	0 ppm	6.25 ppm	20 ppm	62.5 ppm	200 ppm	625 ppm	Shape Parameter
<b>Male</b>							
Systemic neoplasms							
Malignant lymphoma	9.0	4.4	10.0	15.3	7.4	97.3**	10.000▲▲
Histiocytic sarcoma	0.0	0.0	10.0	12.9*	24.3**	50.7**	0.716
Heart							
Hemangiosarcoma	0.0	0.0	2.6	13.5*	64.1**	52.9**	1.033
Lung							
Adenoma or carcinoma	47.5	49.0	44.9	74.2*	87.8**	45.1	0.457□□
Harderian gland							
Adenoma or carcinoma	13.5	15.2	22.4	50.8**	80.6**	64.2** <sup>b</sup>	0.647□□
Forestomach							
Papilloma or carcinoma	2.3	0.0	0.0	2.7	28.7**	53.5** <sup>b</sup>	1.413
Liver							
Adenoma or carcinoma	44.6	48.2	65.2* <sup>b</sup>	61.6	85.9**	61.2	0.374□□
Preputial gland							
Carcinoma	0.0	0.0	0.0	0.0	18.9**	0.0	1.207
<b>Female</b>							
Systemic neoplasms							
Malignant lymphoma	13.1	27.2	27.5	20.2	40.1*	85.5**	1.690
Histiocytic sarcoma	6.5	4.4	17.2	11.8	34.0**	35.5*	0.583
Heart							
Hemangiosarcoma	0.0	0.0	0.0	3.1	71.9**	83.4**	1.293▲
Lung							
Adenoma or carcinoma	8.8	33.0*	46.5**	61.1**	81.5**	82.4**	0.374□□
Harderian gland							
Adenoma or carcinoma	17.5	22.7	17.4	41.2*	70.9**	58.0** <sup>b</sup>	0.572□
Forestomach							
Papilloma or carcinoma	0.0	0.0	7.8	6.1	22.5** <sup>b</sup>	82.6**	1.182
Liver							
Adenoma or carcinoma	33.3	30.3	36.4	51.4	64.9*	21.7	0.315
Ovary							
Granulosa cell tumor, benign or malignant	2.3	0.0	2.6	26.3**	41.1**	46.5** <sup>b</sup>	0.777
Mammary gland							
Carcinoma or adenocanthoma	0.0	4.5	10.2* <sup>b</sup>	32.6**	56.4**	66.8**	0.645□□

\* Significantly different ( $P < 0.05$ ) from the control group by the Poly-3 quantal response test (Portier and Bailer, 1989)

\*\*  $P < 0.01$

▲ Shape is significantly greater than 1,  $P < 0.05$  by likelihood ratio test

▲▲  $P < 0.01$

□ Shape is significantly less than 1,  $P < 0.05$  by likelihood ratio test

□□  $P < 0.01$

<sup>a</sup> Neoplasm rates determined by Poly-3 quantal response method

<sup>b</sup> Not significant by the logistic regression tests

**TABLE 29**  
**Survival-Adjusted Neoplasm Rates for B6C3F<sub>1</sub> Mice in the Stop-Exposure Inhalation Study of 1,3-Butadiene<sup>a</sup>**

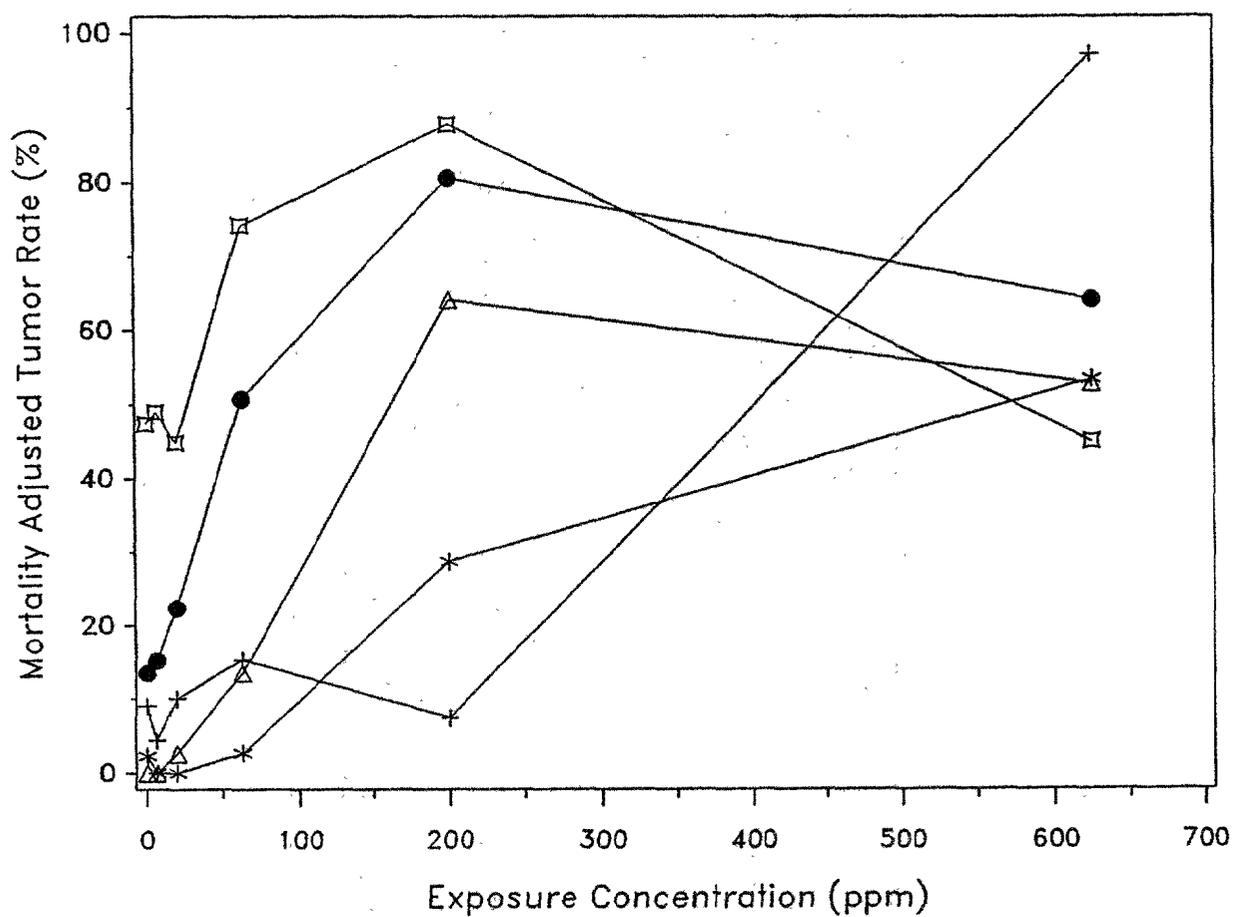
	0 ppm	200 ppm (40 weeks)	625 ppm (13 weeks)	312 ppm (52 weeks)	625 ppm (26 weeks)
Systemic neoplasms					
Malignant lymphoma	9.0	24.1	56.1**	35.0*	87.2**
Histiocytic sarcoma	0.0	16.3*	9.4	30.6**	20.4*
Heart					
Hemangiosarcoma	0.0	47.1**	30.9**	85.2**	74.5**
Lung					
Adenoma or carcinoma	47.5	88.6**	89.5**	88.0**	87.2**
Harderian gland					
Adenoma or carcinoma	13.5	72.1**	82.0**	88.6**	76.5**
Forestomach					
Papilloma or carcinoma	2.3	10.2	28.7**	39.2**	60.7** <sup>b</sup>
Preputial gland					
Adenoma or carcinoma	0.0	3.5	21.2**	21.2**	30.6**
Kidney					
Adenoma	0.0	13.3 <sup>ab</sup>	4.7	15.2 <sup>ab</sup>	11.0
Liver					
Adenoma or carcinoma	44.6	82.4**	80.3** <sup>b</sup>	75.9** <sup>b</sup>	77.3** <sup>b</sup>

\* Significantly different ( $P < 0.05$ ) from the control group by the Poly-3 quantal response test (Portier and Bailer, 1989)

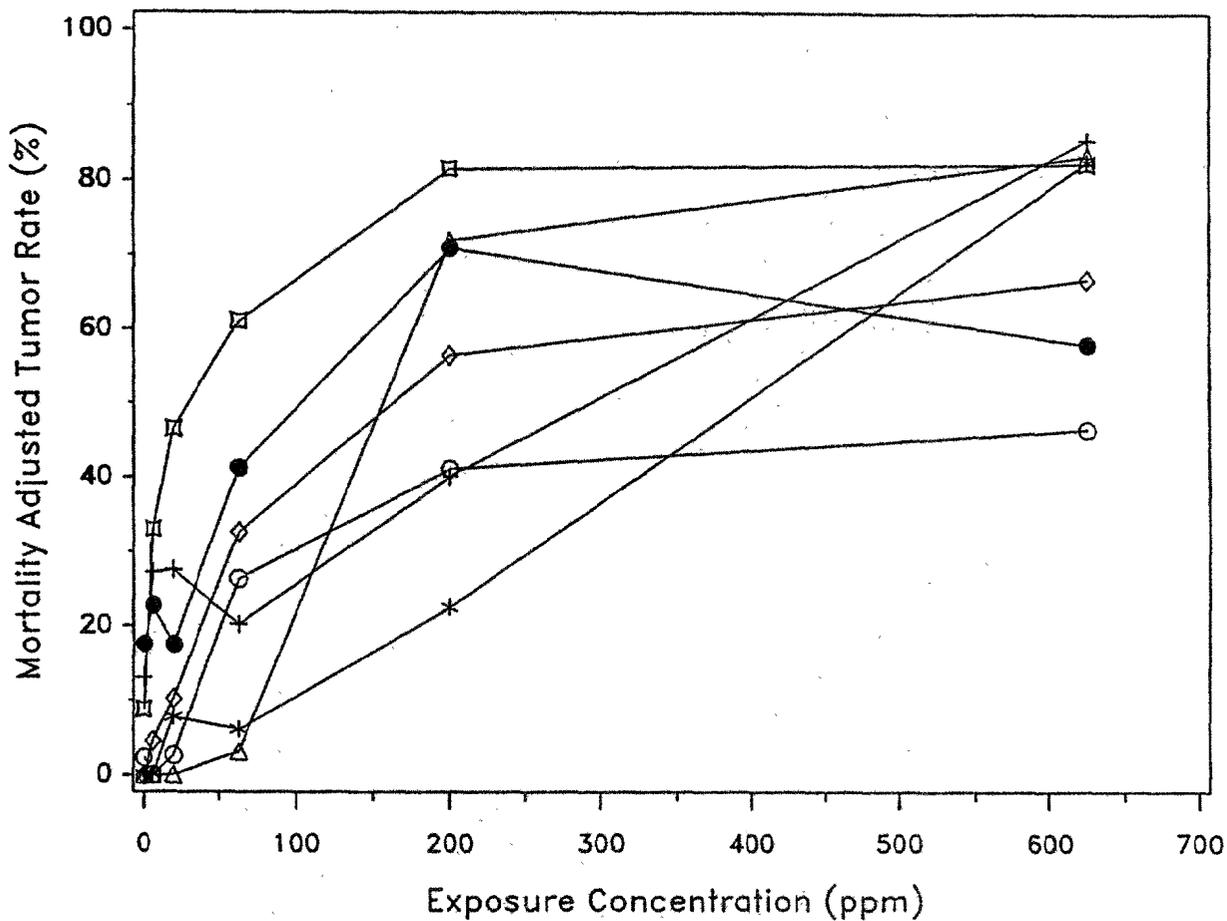
\*\*  $P < 0.01$

<sup>a</sup> Neoplasm rates determined by Poly-3 quantal response method

<sup>b</sup> Not significant by the logistic regression tests



**FIGURE 6**  
Dose-response curves for lymphomas (+), hemangiosarcomas of the heart (Δ), alveolar/bronchiolar neoplasms (◻), harderian gland neoplasms (●), and squamous cell neoplasms of the forestomach (\*) in male mice exposed to 1,3-butadiene.

**FIGURE 7**

Dose-response curves for lymphomas (+), hemangiosarcomas of the heart ( $\Delta$ ), alveolar/bronchiolar neoplasms ( $\boxplus$ ), harderian gland neoplasms ( $\bullet$ ), squamous cell neoplasms of the forestomach (\*), granulosa cell tumor of the ovary ( $\circ$ ), and mammary gland neoplasms ( $\diamond$ ) in female mice exposed to 1,3-butadiene.

## GENETIC TOXICOLOGY

1,3-Butadiene was mutagenic in *Salmonella typhimurium* strain TA1535 when tested as a gas in a sealed desiccator chamber, with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9 (Table D1). The positive response observed with TA1535 in the absence of exogenous metabolic activation was unexpected and may, in fact, be an artifact of the exposure protocol. To efficiently conduct these desiccator exposures, all plates to be exposed to a particular concentration of 1,3-butadiene, with or without S9, were housed together in a single desiccator and treated simultaneously. Previous investigations demonstrated that such an arrangement produced positive responses in cultures that did not contain S9 activation enzymes, whereas removing the S9-containing plates from the desiccator and treating only those cultures that did not contain S9 resulted in no increase in mutations (de Meester *et al.*, 1980). The induction of mutations in the cultures that did not contain S9 was believed to be caused by the formation of a volatile mutagenic intermediate in the S9-containing plates that migrated to the plates without S9. No mutagenic activity was detected for 1,3-butadiene in strain TA100, TA97, or TA98 under the same conditions.

No mutagenic activity was observed in the mouse lymphoma L5178Y cell assay, with or without Aroclor 1254-induced male Fischer rat liver S9 (Table D2; McGregor *et al.*, 1991). The maximum dose was 30% in air (v/v). One possible factor in the lack of mutagenic activity is the low solubility of 1,3-butadiene in the cell culture medium, which may have prevented adequate exposure. 1,3-Butadiene did not induce a significant increase in the number of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* exposed by inhalation to 360,000 ppm in air (Table D3). These negative results with 1,3-butadiene were somewhat surprising, given its demonstrated activity in mammalian cells *in vivo*.

Positive results were obtained with 1,3-butadiene in cytogenetic tests with mammalian cells *in vivo* (Tice *et al.*, 1987). Significant increases in the frequency of chromosomal aberrations (Table D4) and sister chromatid exchanges (Table D5) were observed in bone marrow cells of male mice exposed for 2 weeks to 1,3-butadiene (6.25 to 625 ppm in air). For both tests, the trend analyses were significant; both the mid- and high-dose animals showed increases in sister chromatid exchanges, while only the high-dose mice had elevated levels of chromosomal aberrations. In addition, cell cycle time was significantly lengthened as doses of 1,3-butadiene were increased, as indicated by the average generation time measurements (Table D5). Peripheral blood smears prepared from these same animals (exposed to 1,3-butadiene by inhalation for 2 weeks) revealed significant increases in micronucleated polychromatic erythrocytes and normochromatic erythrocytes (Table D6). Elevations in the frequency of micronucleated polychromatic erythrocytes (a measure of acute exposure) were observed in the mid- and high-dose mice, while only the high-dose mice showed a statistically significant increase in micronucleated normochromatic erythrocytes; for both types of cells, the trend analyses were significant. The rate of erythropoiesis was increased in exposed mice, particularly at the 625 ppm level, as indicated by the increase in the percentage of polychromatic erythrocytes in the total erythrocyte population in the peripheral blood (Table D6). This, along with the increase in average generation time (Table D5), indicates cellular (bone marrow) toxicity induced by 1,3-butadiene. The frequencies of micronucleated polychromatic erythrocytes and normochromatic erythrocytes were also scored in peripheral blood samples of male and female mice exposed for 13 weeks (Table D7) and 15 months (Table D8) to 6.25 to 625 ppm 1,3-butadiene; both exposure regimens produced positive results in both sexes. Also, the percentage of polychromatic erythrocytes in female mice exposed for 15 months to 1,3-butadiene was elevated at the two highest concentrations tested, which produced a positive trend (Table D8).

## DISCUSSION AND CONCLUSIONS

1,3-Butadiene is produced in large volumes for use mainly in the manufacture of synthetic rubber and thermoplastic resins. Previous long-term inhalation studies have shown that 1,3-butadiene is carcinogenic at multiple organ sites in Sprague-Dawley rats (IISRP, 1981a; Owen *et al.*, 1987) and B6C3F<sub>1</sub> mice (NTP, 1984; Huff *et al.*, 1985). The 2-year studies in rats, sponsored by the International Institute of Synthetic Rubber Producers (IISRP), were conducted at exposure concentrations of 1,000 and 8,000 ppm. The highest exposure level was limited by the safety requirement of being below 50% of the explosive limit of 1,3-butadiene in air, while the 1,000 ppm concentration was selected because it represented the occupational exposure standard for this chemical. The NTP usually conducts long-term studies in F344/N rats and B6C3F<sub>1</sub> mice; however, because the IISRP studies in rats were in progress at the time of chemical selection, the NTP studies were limited to long-term evaluations of 1,3-butadiene exposure in mice.

The exposure concentrations selected for the NTP studies, 625 and 1,250 ppm, were based on increased mortality and decreased body weight gains in mice exposed to concentrations of 2,500 ppm or higher for 14 weeks (NTP, 1984). The carcinogenicity studies in mice, designed to last for 103 weeks, were terminated after 61 weeks because of reduced survival due to malignant neoplasms involving multiple organs at both exposure concentrations. Malignant lymphomas, which appeared to originate in the thymus and were observed as early as week 20, were considered to be the major cause of early death, while hemangiosarcomas of the heart, an uncommon neoplasm in untreated B6C3F<sub>1</sub> mice (none occurred in 573 control males or 558 control females in recent NTP studies), were the second major cause of death. The incidences of primary neoplasms caused by exposure to 1,3-butadiene are shown in Table 1. Because these studies had been terminated early and dose-response relationships for various lesions were sometimes unclear (e.g., hemangiosarcomas of the heart in male mice), a second set of long-term inhalation studies of 1,3-butadiene in mice was performed to better characterize the carcinogenicity of this important industrial chemical. The latter studies, which are

presented in this Technical Report, were conducted at concentrations ranging from 6.25 to 625 ppm 1,3-butadiene. The exposure level of 625 ppm corresponds to the low-exposure level in the previous inhalation studies in mice, and 6.25 ppm is two orders of magnitude lower. A preliminary account of the results of these studies has been reported (Melnick *et al.*, 1990 b,c).

Exposure to 1,3-butadiene for up to 2 years had no apparent adverse effect on body weight gains for male or female mice; however, survival was reduced in all groups exposed to concentrations of 20 ppm or higher. As in the previous studies, lymphomas that occurred early, prior to 15 months, were the major cause of death for male and female mice exposed to 625 ppm 1,3-butadiene. T-cell lymphoma is caused by exposure to 1,3-butadiene (Irons *et al.*, 1989; Irons, 1990). In the present studies, most butadiene-induced lymphomas were well differentiated and lymphocytic, and appeared to originate in the thymus. After month 15, there was a marginal but statistically significant increase in histiocytic sarcomas. Additionally, other histological types of lymphoma (malignant mixed and malignant undifferentiated) commonly associated with the spontaneous lymphoma of aging B6C3F<sub>1</sub> mice were observed in all remaining groups. Lymphocytic lymphomas were analyzed separately from histiocytic sarcomas and all lymphomas, because they provide a clearer response of 1,3-butadiene-induced hematopoietic cancers.

The incidence of hemangiosarcomas of the heart was increased in male mice exposed to 62.5, 200, or 625 ppm and in female mice exposed to 200 or 625 ppm. In addition, one male exposed to 20 ppm and one female exposed to 62.5 ppm were observed to have this uncommon endothelial cell neoplasm; the occurrence of these rare sarcomas at the lower concentrations was also likely due to 1,3-butadiene exposure. Increased incidences of endothelial hyperplasia in the heart at all exposure concentrations probably represent preneoplastic changes caused by 1,3-butadiene.

The incidence of hemangiosarcomas of the heart was greater in male mice exposed to 200 ppm than in

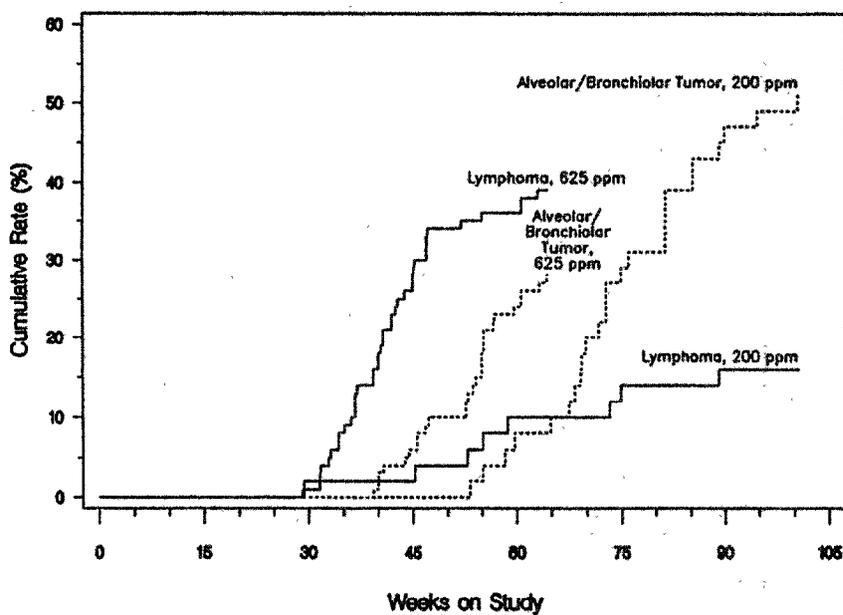
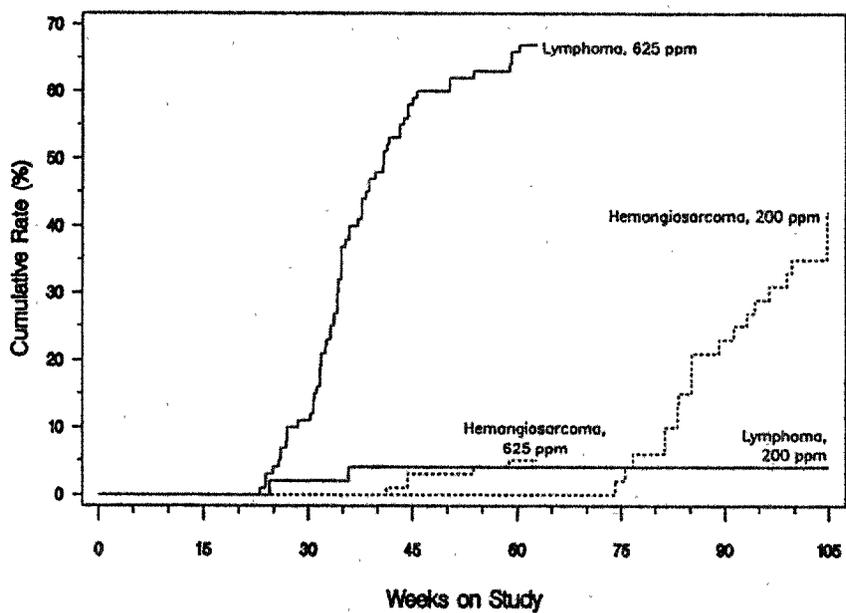
male mice exposed to 625 ppm. The lower incidence in males receiving 625 ppm was probably due to the early and extensive induction of lymphocytic lymphoma, which resulted in a significantly reduced number of mice at risk for the later-developing heart hemangiosarcomas. The median survival time was about 40 weeks for male mice exposed to 625 ppm and about 70 weeks for male mice exposed to 200 ppm. The effect of competing risks of early occurring lethal thymic lymphomas on the development of hemangiosarcomas of the heart is evident from the plots of the cumulative death-with-neoplasm rates of these neoplasms against the number of weeks on study for male mice exposed to 200 or 625 ppm 1,3-butadiene (Figure 8). In the 625 ppm group, the incidence of early lymphocytic lymphoma was very high (67%) and the incidence of hemangiosarcomas of the heart was low (5%); however, in the 200 ppm group, the incidence of early lymphocytic lymphoma was low (4%) and the incidence of hemangiosarcomas of the heart (42%) was much higher than that in the 625 ppm group. Furthermore, in male mice that died early after exposure to 200 or 625 ppm 1,3-butadiene, the incidences of hemangiosarcomas of the heart were nearly equivalent for the first 65 weeks of the study. After that time, a high incidence of hemangiosarcomas of the heart (approximately 50%) was observed in the 200 ppm group, whereas there were no surviving animals in the 625 ppm group. Thus, for male mice exposed to 1,3-butadiene concentrations below 625 ppm, the dose response for hemangiosarcomas of the heart is more clearly demonstrated. The impact of early mortality on the expression of later-developing neoplasms is largely accounted for in the mortality-adjusted neoplasm rates shown in Tables 30 and 31 for each neoplasm induced by exposure to 1,3-butadiene.

The incidence of alveolar/bronchiolar neoplasms in male mice was increased at concentrations of 62.5 and 200 ppm compared to that of the controls. In female mice, the incidence of alveolar/bronchiolar neoplasms was significantly increased in all exposure groups compared to that of the controls. Thus, even at a concentration of 6.25 ppm, 1,3-butadiene is carcinogenic to B6C3F<sub>1</sub> mice. Furthermore, in control female mice, all of the alveolar/bronchiolar neoplasms were adenomas, whereas in female mice exposed to 1,3-butadiene, including the 6.25 ppm exposure level, alveolar/bronchiolar carcinomas were observed. Because there was no exposure level at which a carcinogenic response was not induced, it is

likely that exposure concentrations below 6.25 ppm would also cause cancers in mice. The reduced incidence of lung neoplasms in mice exposed to 625 ppm compared with the incidence in mice exposed to 200 ppm is attributed to the high rate of early deaths due to competing risks of lymphocytic lymphoma in female mice exposed to 625 ppm (Figure 8). The time-to-neoplasm detection of alveolar/bronchiolar neoplasms was slightly shorter for animals exposed to 625 ppm than for animals exposed to 200 ppm; however, because all female mice exposed to 625 ppm 1,3-butadiene died by 65 weeks, the final incidence of this later developing and rarely lethal neoplasm was lower than that for female mice exposed to 200 ppm. Increased incidences of alveolar epithelial hyperplasia in exposed male and female mice probably represent preneoplastic changes caused by 1,3-butadiene in the lung.

Increased incidences of neoplasms of the forestomach (squamous cell papillomas or carcinomas), mammary gland (carcinomas, adenocarcinomas, and malignant mixed tumors), ovary (benign or malignant granulosa cell tumors), liver (hepatocellular adenomas or carcinomas), and other organ sites identified in the first studies were again observed in mice exposed to 1,3-butadiene. Additionally, the harderian gland and preputial gland were identified as sites of 1,3-butadiene-induced neoplasia. Increased incidences of proliferative nonneoplastic lesions in these organs, including epithelial hyperplasia of the forestomach, mammary gland hyperplasia, germinal epithelium and granulosa cell hyperplasia of the ovary, and hyperplasia of the harderian gland, probably represent preneoplastic changes at these sites. In control female mice, no neoplasms, or only benign neoplasms, were observed in the harderian gland, forestomach, and ovary; however, in female mice exposed to 1,3-butadiene, malignant neoplasms were observed at each of these sites. The greater tendency to malignant neoplasia in mice exposed to 1,3-butadiene further demonstrates the strong carcinogenic potency of this chemical.

The conclusion that the marginally increased incidences of hepatocellular neoplasms in male and female mice were related to chemical administration is strengthened by the detection of activated *K-ras* oncogenes with a specific codon 13 mutation in liver neoplasms obtained from mice exposed to 1,3-butadiene (Goodrow *et al.*, 1990). Activated



**FIGURE 8**  
**Cumulative Death-With-Neoplasm Rates of Selected Neoplasms**  
**in Mice Exposed to 1,3-Butadiene for 2 Years**

*K-ras* oncogenes have never been detected in liver neoplasms from untreated B6C3F<sub>1</sub> mice (Reynolds *et al.*, 1987). Activated *K-ras* genes with codon 13 mutations were also found in lung neoplasms and in some of the lymphomas induced by exposure to 1,3-butadiene.

In the stop-exposure study, groups of male mice were exposed to one of the following regimens: A) 200 ppm for 40 weeks; B) 625 ppm for 13 weeks; C) 312 ppm for 52 weeks; or D) 625 ppm for 26 weeks. After the exposures were terminated, these groups of animals were placed in control chambers for the remainder of the 2-year study. For the first two groups, the total exposure to 1,3-butadiene (concentration times duration of exposure) was approximately equivalent (8,000 ppm · weeks for regimens A and B) and was equal to about half the total exposure given to the latter two groups (16,000 ppm · weeks for regimens C and D).

Survival was markedly reduced in all of the stop-exposure groups due to the development of compound-related malignant neoplasms. The neoplasm incidence profiles in the stop-exposure groups show that the incidences of lymphocytic lymphomas, histiocytic sarcomas, hemangiosarcomas of the heart, alveolar/bronchiolar adenomas or carcinomas, squamous cell papillomas or carcinomas of the forestomach, hepatocellular adenomas or carcinomas, adenomas or adenocarcinomas of the harderian gland, and preputial gland carcinomas were increased even after only 13 weeks of exposure to 625 ppm 1,3-butadiene compared with the control males. It is likely that shorter exposure durations would also produce a positive multiple-organ carcinogenic response.

At similar total exposures, the incidence of lymphocytic lymphoma was greater with exposure to a higher concentration of 1,3-butadiene for a short time compared with exposure to a lower concentration for a longer time. This is evident by comparing the incidence of lymphocytic lymphoma in the 625 ppm 13-week stop-exposure group (34%) with that in the 200 ppm 40-week stop-exposure group (12%), or more notably by comparing the incidence in the 625 ppm 26-week stop-exposure group (60%) with that in the 312 ppm 52-week stop-exposure group (8%). Doubling the duration of exposure to 625 ppm from 13 weeks to 26 weeks resulted in less than a two-fold increase in the incidence of lymphocytic

lymphoma. Thus, for the induction of thymic lymphomas, the concentration of 1,3-butadiene is a much greater contributing factor than is the length of exposure.

Renal tubule cell adenomas were observed in 9 of the 200 male mice in the stop-exposure groups; the highest incidence was 4 in the 200 ppm 40-week stop-exposure group. The increased incidences of kidney neoplasms are particularly noteworthy, because these lesions rarely occur in untreated B6C3F<sub>1</sub> mice (historical incidence less than 0.2% in recent NTP studies). The detection of late-developing renal tubule cell adenomas in the stop-exposure groups is possibly due to the increased survival of these animals compared to those groups of male mice that were exposed to similar concentrations of 1,3-butadiene throughout their lifetimes or for up to 2 years.

Brain neoplasms including two neuroblastomas and three gliomas were observed in mice exposed to 625 ppm 1,3-butadiene for 13 or 26 weeks in the stop-exposure study. Because brain neoplasms are extremely rare in untreated B6C3F<sub>1</sub> mice, occurring in no historical control animals in the NTP database, their occurrence in this study was probably due to exposure to 1,3-butadiene. Furthermore, in the previous NTP inhalation studies of 1,3-butadiene in B6C3F<sub>1</sub> mice, gliomas were observed in one male mouse exposed to 1,250 ppm and two male mice exposed to 625 ppm (NTP, 1984). Although the incidence of brain neoplasms is low, the consistency between the previous studies and the present study is indicative of an exposure-related effect. It is also likely that the early and extensive development of lymphomas in mice exposed to 625 ppm 1,3-butadiene substantially reduced the number of mice at risk for later-developing brain neoplasms. Interestingly, glial cell neoplasms of the brain were also observed in male Sprague-Dawley rats exposed to 1,000 or 8,000 ppm 1,3-butadiene for up to 2 years (IISRP, 1981a).

In addition to the carcinogenic effects noted above, exposure to 1,3-butadiene caused a poorly regenerative anemia and gonadal toxicity in male and female mice (Melnick *et al.*, 1990b). Hematologic changes after 9 months of exposure to 1,3-butadiene included concentration-dependent decreases in erythrocyte counts, hemoglobin concentration, and packed red cell volume at exposure levels from 62.5 to 625 ppm

in males and at levels of 200 or 625 ppm in females. These changes were not accompanied by significant increases in reticulocyte counts or in the frequency of polychromatic erythrocytes in peripheral blood; however, there was a statistically significant increase in the percentage of erythrocytes with Howell-Jolly body inclusions. Other hematologic changes caused by exposure to 625 ppm 1,3-butadiene were an increase in mean erythrocyte volume and an increase in mean erythrocyte hemoglobin. Additionally, changes at other organ sites, bone marrow atrophy, and increases in splenic and hepatic extramedullary hematopoiesis were observed in mice exposed to 625 ppm 1,3-butadiene. These findings indicate partial or poorly regenerative, macrocytic anemia. The mechanism of the anemia cannot be determined from the data available from these studies; however, a mild megaloblastic anemia resulting from ineffective erythropoiesis in the bone marrow cannot be excluded. Tice *et al.* (1987) reported that exposure of male B6C3F<sub>1</sub> mice to 1,3-butadiene for 2 weeks caused a decrease in the number and rate of dividing cells in the bone marrow. Thus, in mice exposed to 1,3-butadiene, hematopoiesis in the bone marrow is suppressed, and younger, larger cells are probably released into the blood from extramedullary sites. Consistent with this explanation, Thurmond *et al.* (1986) observed extramedullary hematopoiesis in spleens of male B6C3F<sub>1</sub> mice exposed to 1,250 ppm 1,3-butadiene for approximately 6 months.

Testicular atrophy was induced in male B6C3F<sub>1</sub> mice exposed to 1,3-butadiene concentrations of 625 ppm or above in the current studies and in previous studies (NTP, 1984). In female mice exposed to 1,3-butadiene for 9 months, ovarian atrophy of moderate severity was observed in the 200 and 625 ppm groups; the ovaries of mice exposed to 62.5 ppm for 9 months appeared normal. The atrophic ovaries had no identifiable oocytes, follicles, or corpora lutea. After 15 months of exposure to 1,3-butadiene, ovarian atrophy was observed at exposure levels of 20 ppm and above. In female mice exposed to 1,3-butadiene for up to 2 years, the incidence of ovarian atrophy was increased at all exposure concentrations (6.25 to 625 ppm) compared with controls. Even though ovarian atrophy in the 6.25 ppm group was not observed until late in the study, when reproductive senescence was probably occurring, the dose-response data clearly establish the ovary as a target organ of 1,3-butadiene toxicity at

concentrations as low as 6.25 ppm, the lowest concentration studied.

The mechanism of butadiene-induced carcinogenicity is not known; however, oxidative intermediates of 1,3-butadiene biotransformation, 1,2-epoxy-3-butene, and diepoxybutane, or a combination of these (Malvoisin and Roberfroid, 1982), are likely involved. These metabolites are direct-acting mutagens in *Salmonella typhimurium* (de Meester *et al.*, 1978; Wade *et al.*, 1979), whereas the elicitation of *in vitro* mutagenicity of 1,3-butadiene appears to require metabolic activation (de Meester *et al.*, 1980). Furthermore, these epoxides have been shown to induce local (application site) neoplasms when applied to the skin of Swiss mice or when administered to Swiss mice or Sprague-Dawley rats by subcutaneous injection (Van Duuren *et al.*, 1963; 1966).

The carcinogenicity studies of 1,3-butadiene in Sprague-Dawley rats (IISRP, 1981a; Owen *et al.*, 1987) and B6C3F<sub>1</sub> mice (NTP, 1984; Huff *et al.*, 1985), including the current studies, demonstrate a species difference in the sites of neoplasm induction and the magnitude of the dose-dependent responses. In addition, in *in vivo* genotoxicity studies, 1,3-butadiene induced chromosomal aberrations, sister chromatid exchanges, and micronuclei in mice (Cunningham *et al.*, 1986; Tice *et al.*, 1987), but not in rats (Cunningham *et al.*, 1986). Biochemical and pharmacokinetic studies have been performed to determine the mechanism of neoplasm induction by 1,3-butadiene and to provide an explanation for the different toxic and carcinogenic responses between rats and mice. The possibility that the induction of thymic lymphomas in B6C3F<sub>1</sub> mice was a consequence of the expression of a murine leukemia retrovirus has been considered (Irons *et al.*, 1987, 1989; Irons, 1990). However, the finding that thymic lymphomas were induced by 1,3-butadiene in NIH Swiss mice, a strain that does not express the ecotropic murine leukemia viruses expressed in B6C3F<sub>1</sub> mice, demonstrates that these neoplasms were produced independently of these activated retroviruses.

*In vivo* alkylation of liver DNA was equivalent in B6C3F<sub>1</sub> mice and Wistar rats exposed to 1,3-butadiene (Kreiling *et al.*, 1986); however, expected reaction products between guanine and

1,2-epoxy-3-butene or diepoxybutane were detected in liver DNA from exposed mice, but not from exposed rats (Jelitto *et al.*, 1989). Further studies are needed on the dose-responses for DNA adduct formation in the major target organs of 1,3-butadiene-induced carcinogenicity in rats and mice and on the nature of the butadiene-derived material bound to rat liver DNA.

Differences in nonprotein sulfhydryl depletion in the liver, lung, and heart of rats and mice exposed to 1,3-butadiene were suggested as a basis for species differences in 1,3-butadiene-induced cytotoxicity and carcinogenicity (Kreiling *et al.*, 1988; Deutschmann and Laib, 1989). However, a causal relationship between decreases in nonprotein sulfhydryl levels in these organs and 1,3-butadiene-induced neoplasia after long-term exposure has not been established.

Studies on the pharmacokinetics of 1,3-butadiene in Sprague-Dawley rats and B6C3F<sub>1</sub> mice were designed to determine and compare the patterns of 1,3-butadiene metabolism in those species for which carcinogenicity data are available. At exposure concentrations below 1,000 ppm, where first-order kinetics apply, the metabolic elimination rate is nearly two times faster in B6C3F<sub>1</sub> mice than in Sprague-Dawley rats (Bolt *et al.*, 1984; Kreiling *et al.*, 1986). This difference has been attributed to the higher respiratory frequency of mice compared to rats (Kreiling *et al.*, 1986). The steady-state concentration of the epoxide metabolite, 1,2-epoxy-3-butene, is higher in mice than in rats exposed to similar atmospheric concentrations of 1,3-butadiene, largely because the metabolic elimination rate constant for this compound is five times higher in rats than in mice (Kreiling *et al.*, 1987; Laib *et al.*, 1990). Although quantitative differences in 1,3-butadiene metabolism have been observed among species, the differences are not of sufficient magnitude to account for the different dose-dependent toxic or carcinogenic responses seen for 1,3-butadiene in rats and mice. This is illustrated by a comparison of the carcinogenicity of 1,3-butadiene in mice exposed to 62.5 or 200 ppm 1,3-butadiene (as shown in Tables 8 through 17) to the carcinogenicity in rats exposed to 1,000 ppm (Owen *et al.*, 1987). Additional factors, such as steady-state levels of diepoxybutane, target organ levels and DNA reactivity of 1,3-butadiene intermediates, and differences in repair mechanisms, must be involved in distinguishing the site specificity

of 1,3-butadiene-induced carcinogenicity between species.

Dahl *et al.* (1991) reported that the blood concentrations of total 1,3-butadiene metabolites were lower in monkeys than in rats or mice exposed to equivalent concentrations of this gas. Based on these results, humans may be at lower risk for cancer than rodents following equivalent inhalation exposures to 1,3-butadiene. A number of important factors impact on this interpretation. First, a large part of the species differences observed by Dahl *et al.* (1991) is accounted for in risk assessment models that adjust for breathing rate differences between species. Second, because metabolic intermediates may vary greatly in their carcinogenic potential, measurement of total 1,3-butadiene metabolites in blood is not a good indication of cancer risk. In related studies, Bond *et al.* (1987) found that the accumulation of <sup>14</sup>C in rats and mice exposed to <sup>14</sup>C-labeled 1,3-butadiene was not greater in target organs of 1,3-butadiene carcinogenicity than in nontarget organs. A preliminary identification of 1,3-butadiene metabolites in the blood of rats, mice, and monkeys exposed to <sup>14</sup>C-labeled 1,3-butadiene was made by a vacuum line-cryogenic distillation procedure (Dahl *et al.*, 1991). In the one instance in which the material in the "1,2-epoxy-3-butene trap" was analyzed by high-performance liquid chromatography, it was found that only 5% to 15% of the trapped radioactivity was 1,2-epoxy-3-butene. Thus, there is uncertainty in the identification of specific metabolites by the procedures used. Third, only three monkeys of unmatched age were used in the studies of Dahl *et al.* (1991). Fourth, the results in monkeys are clouded because, unlike the rats or mice, the monkeys were anesthetized during their exposure to 1,3-butadiene. Alterations in respiratory rates and cardiac output caused by anesthesia likely influenced the inhalation pharmacokinetics of 1,3-butadiene. Finally, because metabolic differences are not of sufficient magnitude to account for the different target site carcinogenic responses of 1,3-butadiene in rats compared to mice, it is unreasonable to assume that the kinetic data obtained in monkeys are predictive of human risk.

The finding of increased mortalities from lymphatic and hematopoietic cancers among subgroups of occupationally exposed workers (Meinhardt *et al.*, 1982; Downs *et al.*, 1987; Matanoski and Schwartz, 1987; Divine, 1990; Matanoski *et al.*, 1990) raises

additional concern for the carcinogenicity of 1,3-butadiene to humans, particularly because these results correspond to increased incidences of lymphoma observed in mice exposed to 1,3-butadiene. The detection of *K-ras* oncogenes in neoplasms induced by 1,3-butadiene adds further relevance to the potential carcinogenicity of 1,3-butadiene in humans, because *K-ras* is the most commonly detected oncogene in human cancers (Bos, 1989).

## CONCLUSIONS

The previous inhalation studies of 1,3-butadiene in male and female B6C3F<sub>1</sub> mice provided *clear evidence of carcinogenicity\** at exposure concentrations of 625 or 1,250 ppm. The present inhalation studies — 2-year exposures of 6.25, 20, 62.5, 200, or 625 ppm or shorter duration exposures of 200, 312, or

625 ppm — provide a better characterization of the concentration-dependent responses for 1,3-butadiene-induced neoplasms and nonneoplastic lesions. The present studies confirmed the *clear evidence of carcinogenicity* of 1,3-butadiene in male B6C3F<sub>1</sub> mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, preputial gland, brain, and kidney. There was *clear evidence of carcinogenicity* of 1,3-butadiene in female B6C3F<sub>1</sub> mice based on increased incidences of neoplasms in the hematopoietic system, heart, lung, forestomach, liver, harderian gland, ovary, and mammary gland.

Low incidences of intestinal carcinomas in male mice, Zymbal's gland carcinomas in male and female mice, and renal tubule adenomas and skin sarcomas in female mice may also have been related to administration of 1,3-butadiene.

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



## REFERENCES

- American Conference of Governmental Industrial Hygienists (ACGIH) (1986). *Threshold Limit Values and Biological Exposure Indices for 1986-1987*, pp. 68-70. Cincinnati, OH.
- Amoore, J.E., and Hautula, E. (1983). Odor as an aid to chemical safety: Odor thresholds compared with threshold limit values and volatilities for 214 industrial chemicals in air and water dilution. *J. Appl. Toxicol.* 3, 272-290.
- Arce, G.T., Vincent, D.R., Cunningham, M.J., Choy, W.N., and Sarrif, A.M. (1990). *In vitro* and *in vivo* genotoxicity of 1,3-butadiene and metabolites. *Environ. Health Perspect.* 86, 75-78.
- Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.
- Auerbach, A.D., and Wolman, S.R. (1979). Carcinogen-induced chromosome breakage in chromosome instability syndromes. *Cancer Genet. Cytogenet.* 1, 21-28.
- Auerbach, A.D., Weiner, M.A., Warburton, D., Yeboa, K., Lu, L., and Broxmeyer, H.E. (1982). Acute myeloid leukemia as the first hematologic manifestation of Fanconi anemia. *Am. J. Hematol.* 12, 289-300.
- Bailer, A.J., and Portier, C.J. (1988). Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. *Biometrics* 44, 417-431.
- Bolt, H.M., Schmiedel, G., Filser, J.G., Rolzhäuser, H.P., Lieser, K., Wistuba, D., and Schurig, V. (1983). Biological activation of 1,3-butadiene to vinyl oxirane by rat liver microsomes and expiration of the reactive metabolite by exposed rats. *Cancer Res. Clin. Oncol.* 106, 112-116.
- Bolt, H.M., Filser, J.G., and Störmer, F. (1984). Inhalation pharmacokinetics based on gas uptake studies. V. Comparative pharmacokinetics of ethylene and 1,3-butadiene in rats. *Arch. Toxicol.* 55, 213-218.
- Bond, J.A., Dahl, A.R., Henderson, R.F., Dutcher, J.S., Mauderly, J.L., and Birnbaum, L.S. (1986). Species differences in the disposition of inhaled butadiene. *Toxicol. Appl. Pharmacol.* 84, 617-627.
- Bond, J.A., Dahl, A.R., Henderson, R.F., and Birnbaum, L.S. (1987). Species differences in the distribution of inhaled butadiene in tissues. *Am. Ind. Hyg. Assoc. J.* 48, 867-872.
- Bond, J.A., Martin, O.S., Birnbaum, L.S., Dahl, A.R., Melnick, R.L., and Henderson, R.F. (1988). Metabolism of 1,3-butadiene by lung and liver microsomes of rats and mice repeatedly exposed by inhalation to 1,3-butadiene. *Toxicol. Lett.* 44, 143-151.
- Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.
- Bos, J.L. (1989). *Ras* oncogenes in human cancer: A review. *Cancer Res.* 49, 4682-4689.
- Canter, D.A., Haworth, S., Mortelmans, K., Zeiger, E., Lawlor, T., and Speck, W. (1986). Comparative mutagenicity of aliphatic epoxides in *Salmonella*. *Mutat. Res.* 172, 105-138.
- Carpenter, C.P., Shaffer, C.B., Weil, C.S., and Smyth, H.F., Jr. (1944). Studies on the inhalation of 1,3-butadiene; with a comparison of its narcotic effect with benzol, toluol, and styrene, and a note on the elimination of styrene by the human. *J. Ind. Hyg. Toxicol.* 26, 69-78.

- Checkoway, H., and Williams T.M. (1982). A hematology survey of workers at a styrene-butadiene synthetic rubber manufacturing plant. *Am. Ind. Hyg. Assoc. J.* 43, 164-169.
- Choy, W.-N., Vlachos, D.A., Cunningham, M.J., Arce, G.T., and Sarrif, A.M. (1986). Genotoxicity of 1,3-butadiene. Induction of bone marrow micronuclei in B6C3F<sub>1</sub> mice and Sprague-Dawley rats *in vivo*. *Environ. Mutagen.* 8 (Suppl. 6), 18.
- Code of Federal Regulations (CFR) 21, Part 58.
- Cox, D.R. (1972). Regression models and life tables. *J. R. Stat. Soc.* B34, 187-220.
- Crouch, C.N., Pullinger, D.H., and Gaunt, I.F. (1979). Inhalation toxicity studies with 1,3-butadiene — 2. 3 Month toxicity studies in rats. *Am. Ind. Hyg. Assoc. J.* 40, 796-802.
- Cunningham, M.J., Choy, W.N., Arce, G.T., Rickard, L.B., Vlachos, D.A., Kinney, L.A., and Sarrif, A.M. (1986). *In vivo* sister chromatid exchange and micronucleus induction studies with 1,3-butadiene in B6C3F<sub>1</sub> mice and Sprague-Dawley rats. *Mutagenesis* 1, 449-452.
- Dahl, A.R., Sun, J.D., Birnbaum, L.S., Bond, J.A., Griffith, W.C., Jr., Mauderly, J.L., Muggenburg, B.A., Sabourin, P.J., and Henderson, R.F. (1991). Toxicokinetics of inhaled 1,3-butadiene in monkeys: Comparison to toxicokinetics in rats and mice. *Toxicol. Appl. Pharmacol.* 110, 9-19.
- de Meester, C., Poncelet, F., Roberfroid, M., and Mercier, M. (1978). Mutagenicity of butadiene and butadiene monoxide. *Biochem. Biophys. Res. Commun.* 80, 298-305.
- de Meester, C., Poncelet, F., Roberfroid, M., and Mercier, M. (1980). The mutagenicity of butadiene towards *Salmonella typhimurium*. *Toxicol. Lett.* 6, 125-130.
- Deutschmann, S., and Laib, R.J. (1989). Concentration-dependent depletion of non-protein sulfhydryl (NPSH) content in lung, heart and liver tissue of rats and mice after acute inhalation exposure to butadiene. *Toxicol. Lett.* 45, 175-183.
- Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* 6, 44-52.
- Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* 32, 236-248.
- Divine, B.J. (1990). An update on mortality among workers at a 1,3-butadiene facility — Preliminary results. *Environ. Health Perspect.* 86, 119-128.
- Downs, T.D., Crane, M.M., and Kim, K.W. (1987). Mortality among workers at a butadiene facility. *Am. J. Ind. Med.* 12, 311-329.
- Dunkel, V.C., Zeiger, E., Brusick, D., McCoy, E., McGregor, D., Mortelmans, K., Rosenkranz, H.S., and Simmon, V.F. (1984). Reproducibility of microbial mutagenicity assays. 1. Tests with *Salmonella typhimurium* and *Escherichia coli* using a standardized protocol. *Environ. Mutagen.* 6 (Suppl. 2), 1-251.
- Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* 6, 241-252.
- Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50, 1095-1121.
- Durchin, J. (1990). Sampling and analysis of the ambient air in the area of Port Neches, Jefferson County, Texas: Final Report. Sampling and Analysis Division, Monitoring Operations, Texas Air Control Board, Austin, TX.
- Fajen, J.M., Roberts, D.R., Ungers, L.J., and Krishnan, E.R. (1990). Occupational exposure of workers to 1,3-butadiene. *Environ. Health Perspect.* 86, 11-18.
- Filser, J.G., and Bolt, H.M. (1984). Inhalation pharmacokinetics based on gas uptake studies. VI. Comparative evaluation of ethylene oxide and butadiene monoxide as exhaled reactive metabolites of ethylene and 1,3-butadiene in rats. *Arch. Toxicol.* 55, 219-223.

- Friedman, E., Carnright, K., and Lipkin, M. (1982). Differential response of familial polyposis fibroblasts to two bifunctional alkylating agents. *Carcinogenesis* 3, 1481-1485.
- Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* 62, 957-974.
- Goodrow, T., Reynolds, S., Maronpot, R., and Anderson, M. (1990). Activation of K-ras by codon 13 mutations in C57BL/6 × C3H F<sub>1</sub> mouse tumors induced by exposure to 1,3-butadiene. *Cancer Res.* 50, 4818-4823.
- Gopinath, C. (1986). Spontaneous brain tumours in Sprague-Dawley rats. *Fd. Chem. Toxicol.* 24, 113-120.
- Goto, K., Akematsa, T., Shimazu, H., and Sugiyama, T. (1982). Simple differential Giemsa staining of sister chromatids after treatment with photosensitizing dyes and exposure to light and the mechanisms of staining. *Chromosoma* 53, 223-230.
- Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* 58, 385-392.
- Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* 12, 126-135.
- Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N × C3H/HeN)F<sub>1</sub> (B6C3F<sub>1</sub>) mice. *JNCI* 75, 975-984.
- Huff, J.E., Melnick, R.L., Solleveld, H.A., Haseman, J.K., Powers, M., and Miller, R.A. (1985). Multiple organ carcinogenicity of 1,3-butadiene in B6C3F<sub>1</sub> mice after 60 weeks of inhalation exposure. *Science* 227, 548-549.
- International Institute of Synthetic Rubber Producers (IISRP) (1981a). The toxicity and carcinogenicity of butadiene gas administered to rats by inhalation for approximately 24 months. Report No. 2653-522/2. Houston, TX.
- International Institute of Synthetic Rubber Producers (IISRP) (1981b). 1,3-Butadiene: Inhalation teratogenicity study in the rat. Report No. 2788-522/3. Houston, TX.
- Irons, R.D. (1990). Studies on the mechanism of 1,3-butadiene-induced leukemogenesis: The potential role of endogenous murine leukemia virus. *Environ. Health Perspect.* 86, 49-55.
- Irons, R.D., Smith, C.N., Stillman, W.S., Shah, R.S., Steinhagen, W.H., and Leiderman, L.J. (1986a). Macrocytic-megaloblastic anemia in male B6C3F<sub>1</sub> mice following chronic exposure to 1,3-butadiene. *Toxicol. Appl. Pharmacol.* 83, 95-100.
- Irons, R.D., Smith, C.N., Stillman, W.S., Shah, R.S., Steinhagen, W.H., and Leiderman, L.J. (1986b). Macrocytic-megaloblastic anemia in male NIH Swiss mice following repeated exposure to 1,3-butadiene. *Toxicol. Appl. Pharmacol.* 85, 450-455.
- Irons, R.D., Stillman, W.S., and Cloyd, M.W. (1987). Selective activation of endogenous ecotropic retrovirus in hematopoietic tissues of B6C3F<sub>1</sub> mice during the preleukemic phase of 1,3-butadiene exposure. *Virology* 161, 457-462.
- Irons, R.D., Cathro, H.P., Stillman, W.S., Steinhagen, W.H., and Shah, R.S. (1989). Susceptibility to 1,3-butadiene-induced leukemogenesis correlates with endogenous ecotropic retroviral background in the mouse. *Toxicol. Appl. Pharmacol.* 101, 170-176.
- Jelitto, B., Vangala, R.R., and Laib, R.J. (1989). Species differences in DNA damage by butadiene: Role of diepoxybutane. *Arch. Toxicol.* (Suppl. 13), 246-249.
- Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* 41, 133-145.
- Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53, 457-481.
- Kato, H. (1974). Spontaneous sister chromatid exchanges detected by a BudR-labeling method. *Nature* 251, 70-72.

- Kirshenbaum, I. (1978). Butadiene. In *Kirk-Othmer Encyclopedia of Chemical Technology*, Vol. 4, 3rd ed., pp. 313-337. John Wiley and Sons, New York.
- Kreiling, R., Laib, R.J., Filser, J.G., and Bolt, H.M. (1986). Species differences in butadiene metabolism between mice and rats evaluated by inhalation pharmacokinetics. *Arch. Toxicol.* **58**, 235-238.
- Kreiling, R., Laib, R.J., Filser, J.G., and Bolt, H.M. (1987). Inhalation pharmacokinetics of 1,2-epoxybutene-3 reveal species differences between rats and mice sensitive to butadiene-induced carcinogenesis. *Arch. Toxicol.* **61**, 7-11.
- Kreiling, R., Laib, R.J., and Bolt, H.M. (1988). Depletion of hepatic non-protein sulfhydryl content during exposure of rats and mice to butadiene. *Toxicol. Lett.* **41**, 209-214.
- Krinke, G., Naylor, D.C., Schmid, S., Fröhlich, E., and Schnider, K. (1985). The incidence of naturally-occurring primary brain tumours in the laboratory rat. *J. Comp. Pathol.* **95**, 175-192.
- Laib, R.J., Filser, J.G., Kreiling, R., Vangala, R.R., and Bolt, H.M. (1990). Inhalation pharmacokinetics of 1,3-butadiene and 1,2-epoxybutene-3 in rats and mice. *Environ. Health Perspect.* **86**, 57-63.
- MacGregor, J.T., Wehr, C.M., and Langlois, R.G. (1983). A simple fluorescent staining procedure for micronuclei and RNA in erythrocytes using Hoeschst 33258 and pyronin Y. *Mutat. Res.* **120**, 269-275.
- Malvoisin, E., and Roberfroid, M. (1982). Hepatic microsomal metabolism of 1,3-butadiene. *Xenobiotica* **12**, 137-144.
- Malvoisin, E., Lhoest, G., Poncelet, F., Roberfroid, M., and Mercier, M. (1979). Identification and quantitation of 1,2-epoxybutene-3 as the primary metabolite of 1,3-butadiene. *J. Chromatogr.* **178**, 419-425.
- Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.
- Margolin, B.H., Resnick, M.A., Rimo, J.V., Archer, P., Galloway, S.M., Bloom, A.D., and Zeiger, E. (1986). Statistical analysis for *in vitro* cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen.* **8**, 183-204.
- Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.
- Marx, M.P., Smith, S., Heyns, A. du P., and van Tonder, I.Z. (1983). Fanconi's anemia: A cytogenetic study on lymphocyte and bone marrow cultures utilizing 1,2:3,4-diepoxybutane. *Cancer Genet. Cytogenet.* **9**, 51-60.
- Matanoski, G.M., and Schwartz, L. (1987). Mortality of workers in styrene-butadiene polymer production. *J. Occup. Med.* **29**, 675-680.
- Matanoski, G.M., Santos-Burgoa, C., Zeger, S.L., and Schwartz, L. (1989). Epidemiologic data related to health effects of 1,3-butadiene. In *Assessment of Inhalation Hazards* (U. Mohr, D.V. Bates, D.L. Dungworth, P.N. Lee, R.O. McCellan, and F.J.C. Roe, Eds.), pp. 201-214. Springer-Verlag, New York.
- Matanoski, G.M., Santos-Burgoa, C., and Schwartz, L. (1990). Mortality of a cohort of workers in the styrene-butadiene polymer manufacturing industry (1943-1982). *Environ. Health Perspect.* **86**, 107-117.
- McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.
- McFee, A.F., Lowe, K.W., and San Sabastian, J.R. (1983). Improved sister-chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice. *Mutat. Res.* **119**, 83-88.

- McGregor, D.B., Brown, A., Cattanach, P., Edwards, I., McBride, D., Riach, C., and Caspary, W.J. (1988). Responses of the L5178Y tk+/tk- mouse lymphoma cell forward mutation assay. III. 72 coded chemicals. *Environ. Mol. Mutagen.* 12, 85-154.
- McGregor, D.B., Brown, A.G., Howgate, S., McBride, D., Riach, C., and Caspary, W.J. (1991). Responses of the L5178Y mouse lymphoma cell forward mutation assay. V. 27 coded chemicals. *Environ. Mol. Mutagen.* 17, 196-219.
- McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* 79, 639-648.
- Meinhardt, T.J., Lemen, R.A., Crandall, M.S., and Young, R.J. (1982). Environmental epidemiologic investigation of the styrene-butadiene rubber industry. *Scand. J. Work Environ. Health* 8, 250-259.
- Melnick, R.L., Huff, J.E., Haseman, J.K., and McConnell, E.E. (1988). Chronic toxicity results and ongoing studies of 1,3-butadiene by the National Toxicology Program. *Ann. N.Y. Acad. Sci.* 534, 648-662.
- Melnick, R.L., Huff, J.E., Bird, M.G., and Acquavella, J.F. (1990a). Symposium overview: Toxicology, carcinogenesis, and human health aspects of 1,3-butadiene. *Environ. Health Perspect.* 86, 3-5.
- Melnick, R.L., Huff, J.E., Roycroft, J.H., Chou, B.J., and Miller, R.A. (1990b). Inhalation toxicology and carcinogenicity of 1,3-butadiene in B6C3F<sub>1</sub> mice following 65 weeks of exposure. *Environ. Health Perspect.* 86, 27-36.
- Melnick, R.L., Huff, J., Chou, B.J., and Miller, R.A. (1990c). Carcinogenicity of 1,3-butadiene in C57BL/6 × C3H F<sub>1</sub> mice at low exposure concentrations. *Cancer Res.* 50, 6592-6599.
- Miller, L.M. (1978). Investigations of selected potential environmental contaminants: Butadiene and its oligomers. In: EPA-560/2-78-008. U.S. Environmental Protection Agency, Washington, DC.
- Morrissey, R.E., Schwetz, B.A., Hackett, P.L., Sikov, M.R., Hardin, B.D., McClanahan, B.J., Decker, J.R., and Mast, T.J. (1990). Overview of reproductive and developmental toxicity studies of 1,3-butadiene in rodents. *Environ. Health Perspect.* 86, 79-84.
- Morrow, N.L. (1990). The industrial production and use of 1,3-butadiene. *Environ. Health Perspect.* 86, 7-8.
- Mullins, J.A. (1990). Industrial emissions of 1,3-butadiene. *Environ. Health Perspect.* 86, 9-10.
- Myhr, B., Bowers, L., and Caspary, W.J. (1985). Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. *Prog. Mutat. Res.* 5, 555-568.
- National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH 11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.
- National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (NOES) (1981-1983), unpublished provisional data as of July 1, 1990.
- National Toxicology Program (NTP) (1984). Toxicology and Carcinogenesis Studies of 1,3-Butadiene (CAS No. 106-99-0) in B6C3F<sub>1</sub> Mice (Inhalation Studies). Technical Report Series No. 288, NIH Publication No. 84-2544. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.
- Nishi, Y., Hasegawa, M.M., Taketomi, M., Ohkawa, Y., and Inui, N. (1984). Comparison of 6-thioguanine-resistant mutation and sister chromatid exchanges in Chinese hamster V79 cells with forty chemical and physical agents. *Cancer Res.* 44, 3270-3279.

- Obe, G., Kalweit, S., Nowak, C., and Ali-Osman, F. (1982). Liquid holding experiments with human peripheral lymphocytes. 1. Effects of liquid holding on sister chromatid exchanges induced by trenimon, diepoxybutane, bleomycin, and x-rays. *Biol. Zbl.* **101**, 97-113.
- Occupational Safety and Health Administration (OSHA) (1990). Occupational exposure to 1,3-butadiene; proposed rule and notice of hearing. *Fed. Reg.* **55**, 32,736-32,826.
- Olsen, O.-A., and Green, M.M. (1982). The mutagenic effects of diepoxybutane in wild-type and mutagen-sensitive mutants of *Drosophila melanogaster*. *Mutat. Res.* **92**, 107-115.
- Owen, P.E., Glaister, J.R., Gaunt, I.F., and Pullinger, D.H. (1987). Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am. Ind. Hyg. Assoc. J.* **48**, 407-413.
- Perry, P., and Evans, H.J. (1975). Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. *Nature* **258**, 121-125.
- Porfirio, B., Dallapiccola, B., Mokini, V., Alimena, G., and Gandini, E. (1983). Failure of diepoxybutane to enhance sister chromatid exchange levels in Fanconi's anemia patients and heterozygotes. *Hum. Genet.* **63**, 117-120.
- Portier, C.J., and Bailer, A.J. (1989). Testing for increased carcinogenicity using a survival-adjusted quantal response test. *Fundam. Appl. Toxicol.* **12**, 731-737.
- Portier, C.J., Hedges, J.C., and Hoel, D.G. (1986). Age-specific models of mortality and tumor onset for historical control animals in the National Toxicology Program's carcinogenicity experiments. *Cancer Res.* **46**, 4372-4878.
- Reynolds, S.H., Stowers, S.J., Patterson, R.M., Maronpot, R.R., Aaronson, S.A., and Anderson, M.W. (1987). Activated oncogenes in B6C3F<sub>1</sub> mouse liver tumors: Implications for risk assessment. *Science* **237**, 1309-1316.
- Sadtler Standard Spectra. IR No. 893. Sadtler Research Laboratories, Philadelphia.
- Sankaranarayanan, K., Ferro, W., and Zijlstra, J.A. (1983). Studies on mutagen-sensitive strains of *Drosophila melanogaster*. III. A comparison of the mutagenic sensitivities of the *ebony* (UV and x-ray sensitive) and *Canton-S* (wild-type) strains to MMS, ENU, DEB, DEN and 2,4,6-Cl<sub>3</sub>-PDMT. *Mutat. Res.* **110**, 59-70.
- Sasiadek, M., Norppa, H., and Sorsa, M. (1991). 1,3-Butadiene and its epoxides induce sister-chromatid exchanges in human lymphocytes in vitro. *Mutat. Res.* **261**, 117-121.
- Schmidt, U., and Loeser, E. (1985). Species differences in the formation of butadiene monoxide from 1,3-butadiene. *Arch. Toxicol.* **57**, 222-225.
- Sharief, Y., Brown, A.M., Backer, L.C., Campbell, J.A., Westbrook-Collins, B., Stead, A.G., and Allen, J.W. (1986). Sister chromatid exchange and chromosome aberration analyses in mice after in vivo exposure to acrylonitrile, styrene, or butadiene monoxide. *Environ. Mutagen.* **8**, 439-448.
- Shelby, M.D. (1990). Results of NTP-sponsored mouse cytogenetic studies on 1,3-butadiene, isoprene, and chloroprene. *Environ. Health Perspect.* **86**, 71-73.
- Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.
- Shugaev, B.B. (1969). Concentrations of hydrocarbons in tissues as a measure of toxicity. *Arch. Environ. Health* **18**, 878-882.
- Shukla, P.T., and Auerbach, C. (1980). Genetic tests for the detection of chemically induced small deletions in *Drosophila* chromosomes. *Mutat. Res.* **72**, 231-243.
- Simmon, V.F. (1979). In vitro mutagenicity assays of chemical carcinogens and related compounds with *Salmonella typhimurium*. *J. Natl. Cancer Inst.* **62**, 893-899.
- Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* **62**, 679-682.

- Thurmond, L.M., Lauer, L.D., House, R.V., Stillman, W.S., Irons, R.D., Steinhagen, W.H., and Dean, J.H. (1986). Effect of short-term inhalation exposure to 1,3-butadiene on murine immune functions. *Toxicol. Appl. Pharmacol.* **86**, 170-179.
- Tice, R.R., Boucher, R., Luke, C.A., and Shelby, M.D. (1987). Comparative cytogenetic analysis of bone marrow damage induced in male B6C3F<sub>1</sub> mice by multiple exposures to gaseous 1,3-butadiene. *Environ. Mutagen.* **9**, 235-250.
- United States Department of Labor (1981). OSHA Safety and Health Standards. 29CFR 1910.1000. Table Z-1.
- United States International Trade Commission (USITC) (1990). Synthetic Organic Chemicals; United States Production and Sales, 1989. Publ. 2338. USITC, Washington, DC.
- Van Duuren, B.L., Nelson, N., Orris, L., Palmes, E.D., and Schmitt, F.L. (1963). Carcinogenicity of epoxides, lactones, and peroxy compounds. *J. Natl. Cancer Inst.* **31**, 41-55.
- Van Duuren, B.L., Langseth, L., Orris, L., Teebor, G., Nelson, N., and Kuschner, M. (1966). Carcinogenicity of epoxides, lactones, and peroxy compounds. IV. Tumor response in epithelial and connective tissue in mice and rats. *J. Natl. Cancer Inst.* **37**, 825-838.
- Wade, M.J., Moyer, J.W., and Hine, C.H. (1979). Mutagenic action of a series of epoxides. *Mutat. Res.* **66**, 367-371.
- Watson, W.A.F. (1972). Studies on a recombination-deficient mutant of *Drosophila* response to x-rays and alkylating agents. *Mutat. Res.* **14**, 299-307.
- Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.
- Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.
- Zeiger, E. (1990). Mutagenicity of 42 chemicals in *Salmonella*. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 32-54.
- Zeiger, E., and Pagano, D.A. (1989). Mutagenicity of the human carcinogen treosulphan in *Salmonella*. *Environ. Mol. Mutagen.* **13**, 343-346.
- Zeiger, E., Anderson, B., Haworth, S., Lawlor, T., and Mortelmans, K. (1992). *Salmonella* mutagenicity tests. V. Results from the testing of 311 chemicals. *Environ. Mol. Mutagen.* **19** (Suppl. 21), 2-141.
- Zimmering, S., Mason, J.M., Valencia, R., and Woodruff, R.C. (1985). Chemical mutagenesis testing in *Drosophila*. II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ. Mutagen.* **7**, 87-100.

