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TOXICOLOGY AND CARCINOGENESIS STUDIES OF 1,3-BUTADIENE (CAS NO. 106-99-0) IN B6C3F$_1$ MICE (INHALATION STUDIES)

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

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is made up of four charter DHHS agencies: 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, 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.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS STUDIES OF 1,3-BUTADIENE (CAS NO. 106-99-0) IN B6C3F$_1$ MICE (INHALATION STUDIES)

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

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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NOTE TO THE READER

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 testing in the NTP Carcinogenesis Program 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. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical has the potential for hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

Five categories of interpretative conclusions were adopted in June 1983 for use in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program. The study described in this Technical Report has been conducted under NTP health and safety requirements and/or guidelines for toxicity studies. Individual toxicology testing contractors are required to demonstrate corporate health and safety programs in compliance with NTP chemical health and safety requirements and to meet or exceed all applicable Federal, state, and local health and safety regulations.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

CONTENTS

TABLES

FIGURES

APPENDIXES

APPENDIXES (CONTINUED)

$CH_2=CH-CH=CH_2$

1,3-BUTADIENE

CAS NO. 106-99-0

C_4H_6 Mol. Wt. 54.09

Synonyms: butadiene, biethylene, bivinyl, divinyl, erythrene, vinylethylene, pyrrolylene

ABSTRACT

Male and female B6C3F$_1$ mice were exposed to air containing 1,3-butadiene (greater than 99% pure) at concentrations of 0-8,000 ppm in 15-day and 14-week inhalation studies. In the 15-day studies, survival was unaffected by dose, and no pathologic effects were observed; slight decreases in mean body weight occurred at the high concentrations. In the 14-week studies, mean body weight gain decreased with dose, and survival in the 5,000-ppm and 8,000-ppm groups of males was markedly reduced; no other compound-related effects were reported.

Inhalation carcinogenesis studies of 1,3-butadiene were conducted by exposing groups of 50 male and female B6C3F$_1$ mice 6 hours per day for 5 days per week to air containing the test chemical at concentrations of 0 (chamber controls), 625, or 1,250 ppm. These studies were planned for 103-week exposures but were terminated at week 60 for male mice and week 61 for female mice because of the rapidly declining survival, primarily due to neoplasia. Body weights were not affected by 1,3-butadiene.

Significantly increased incidences of neoplasms at multiple sites were observed in mice exposed to 1,3-butadiene. Hemangiosarcomas of the heart occurred at increased incidences in exposed males and females (male: control, 0/50; low dose, 16/49; high dose, 7/49; female: 0/50; 11/48; 18/49). Hemangiosarcomas were also observed in the peritoneal cavity (one high dose male), subcutaneous tissue (two low dose females), and liver (one high dose female).

Malignant lymphomas, diagnosed as early as week 20, were observed at increased incidences in exposed male and female mice (male: 0/50; 23/50; 29/50; female: 1/50; 10/49; 10/49).

Alveolar/bronchiolar adenomas and alveolar/bronchiolar carcinomas (both separately and combined) occurred at increased incidences in exposed male and female mice (combined incidences--male: 2/50; 14/49; 15/49; female: 3/49; 12/48; 23/49).

Epithelial hyperplasia of the forestomach occurred at increased incidences in dosed mice (male: 0/49; 5/40; 7/44; female: 0/49; 5/42; 9/49). Papillomas of the forestomach occurred in low dose male and in low dose and high dose female mice (male: 0/49; 5/40; 0/44; female: 0/49; 4/42; 10/49). Squamous cell carcinomas of the forestomach were observed in dosed mice (male: 0/49, 2/40, 1/44; female: 0/49, 1/42, 1/49).

Acinar cell carcinomas of the mammary gland were observed at an increased incidence in high dose female mice (0/50; 2/49; 6/49); adenosquamous carcinomas were found in four low dose females. The

incidences of granulosa cell tumors of the ovary were increased in dosed females (0/49; 6/45; 12/48). A granulosa cell carcinoma was observed in another high dose female. Gliomas were observed in two 68- to 69-week-old low dose and one high dose male mice; brain tumors are uncommon even in 2-year-old mice.

Liver necrosis occurred at increased incidences in dosed male and low dose female mice (male: 1/50, 8/49, 8/49; female: 6/50, 15/47, 6/49). Hepatocellular adenomas or carcinomas (combined) were observed at an increased incidence in high dose female mice (0/50, 2/47, 5/49).

No neoplastic lesions of the nasal cavity were observed at any dose level. The following nonneoplastic lesions of the nasal cavity occurred in mice exposed at 1,250 ppm: chronic inflammation (male, 35/50; female, 2/49); fibrosis (male, 35/50; female, 2/49); cartilaginous metaplasia (male, 16/50; female, 1/49); osseous metaplasia (male, 11/50; female, 2/49); and atrophy of the sensory epithelium (male, 32/50). No nonneoplastic lesions of the nasal cavity were found in the controls. The incidence of testicular atrophy (0/50, 19/49, 11/48) or ovarian atrophy (2/49, 40/45, 40/48) was increased in exposed male or female mice.

An audit of the experimental data from these studies on 1,3-butadiene was conducted by the National Toxicology Program. No data discrepancies were found that influenced the final interpretation of these experiments.

Under the conditions of these studies, there was *clear evidence of carcinogenicity** for 1,3-butadiene in male and female B6C3F$_1$ mice, as shown by increased incidences and early induction of hemangiosarcomas of the heart, malignant lymphomas, alveolar/bronchiolar adenomas and carcinomas, and papillomas of the stomach in males and females; and of acinar cell carcinomas of the mammary gland, granulosa cell tumors of the ovary, and hepatocellular adenomas and adenomas or carcinomas (combined) in females. 1,3-Butadiene was associated with nonneoplastic lesions in the respiratory epithelium, liver necrosis, and testicular or ovarian atrophy.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of 1,3-Butadiene is based on the 14-week studies that began in May 1977 and ended in September 1977 at Industrial Biotest Laboratories and on the 61-week studies that began in April 1981 and ended in June 1982 at Battelle Pacific Northwest Laboratories.

National Toxicology Program
P.O. Box 12233
Research Triangle Park, NC 27709
(Evaluated Experiment, Interpreted Results, and Reported Findings)

Marcelina Powers, D.V.M.,Chemical Manager

Gary A. Boorman, D.V.M., Ph.D.
Rajendra S. Chhabra, Ph.D.
David M. DeMarini, Ph.D.
Joseph K. Haseman, Ph.D.
James Huff, Ph.D.
C.W. Jameson, Ph.D.
E.E. McConnell, D.V.M.
John A. Moore, D.V.M.
G. Rao, D.V.M., Ph.D.
B. A. Schwetz, D.V.M., Ph.D.
Raymond W. Tennant, Ph.D.

NTP Pathology Working Group
(Evaluated Slides and Prepared Pathology Report on 4/4/83)

Henk Solleveld, D.V.M., Ph.D., NTP (Chairman)

Gary A. Boorman, D.V.M., Ph.D.
NTP
Scot L. Eustis, D.V.M., Ph.D.
NTP
Marilyn Wolfe, D.V.M., Ph.D.
NTP
Kenneth Ayres, D.V.M.
Burroughs Wellcome
Kevin Morgan, Ph.D.
Chemical Industry Institute of Toxicology

Principal Contributors at Battelle Pacific Northwest Laboratories
(Conducted Studies and Evaluated Tissues)

William J. Clarke, D.V.M., Ph.D.
Principal Investigator
Rodney Miller, D.V.M., Ph.D.
Pathologist
R.B. Westerberg, Ph.D.
Chemist

Principal Contributors at Experimental Pathology Laboratory
(Provided Pathology Quality Assurance)

Deborah A. Banas, D.V.M.

Principal Contributors at Carltech Associates, Inc.
(Contractor for Technical Report Preparation)

William D. Theriault, Ph.D.
Project Manager
Abigail C. Jacobs, Ph.D.
Senior Scientist
John Warner, M.S.
Chemist/Statistician

PEER REVIEW PANEL

The members of the Peer Review Panel who evaluated this Technical Report 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: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

National Toxicology Program Board of Scientific Counselors Technical Reports Review Subcommittee

Jerry B. Hook, Ph.D. (Chairperson)
Vice President, Preclinical Research and Development
Smith Kline & French Laboratories
Philadelphia, Pennsylvania

Curtis Harper, Ph.D. (Principal Reviewer)
Associate Professor of Pharmacology
School of Medicine
University of North Carolina
Chapel Hill, North Carolina

James Swenberg, Ph.D., D.V.M.
Chief of Pathology
Chemical Industry Institute of Toxicology
Research Triangle Park, North Carolina

Ad Hoc Subcommittee Panel of Experts

Louis S. Beliczky, M.S., M.P.H. (Principal Reviewer)
Director, Department of Industrial Hygiene
United Rubber Workers International Union
Akron, Ohio

Devra L. Davis, Ph.D.
Science Policy Director
Environmental Law Institute
Washington, D.C.

Robert M. Elashoff, Ph.D
University of California at Los Angeles
Jonsson Comprehensive Cancer Center
Los Angeles, California

Seymour L. Friess, Ph.D. (Principal Reviewer)
Arlington
Virginia

J. Michael Holland, Ph.D., D.V.M.
Chevron Environmental Health Center
Richmond, California

Robert A. Scala, Ph.D.
Exxon Corporation
East Millstone, New Jersey

Tom Slaga, Ph.D.*
University of Texas System Cancer Center
Science Park, Research Division
Smithville, Texas

John R. Van Ryzin, Ph.D. (Principal Reviewer)
Division of Biostatistics
School of Public Health
Columbia University
New York, New York

Stan D. Vesselinovitch, Ph.D.*
Professor, Departments of Radiology and Pathology
University of Chicago
Chicago, Illinois

Mary Vore, Ph.D.*
Assistant Professor
Pharmacology Department
College of Medicine
University of Kentucky
Lexington, Kentucky

* Unable to attend October 28, 1983, meeting

SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF 1,3-BUTADIENE

On October 28, 1983, the technical report on 1,3-butadiene received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9:00 a.m. in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. J. Van Ryzin, a principal reviewer for the technical report on the carcinogenesis studies of 1,3-butadiene, agreed with the conclusions given in the technical report. He said a statement in the abstract should emphasize that this inhalation study was designed for 103-104 weeks' exposure but was terminated at 60 and 61 weeks because of low survival concomitant with neoplasms.

As a second principal reviewer, Mr. L. Beliczky agreed with the conclusions. He suggested that an immunotoxicology profile on butadiene might be worthwhile, given the potential carcinogenicity of the chemical in the Zymbal gland. Also, in view of limited epidemiologic evidence associating employment in certain industries with occurrence of brain tumors, he suggested that more studies to evaluate the significance of the gliomas in male mice would be useful. He also noted that the reported hemangiosarcomas of the heart are an unusual neoplastic response. Dr. J. Swenberg supported the need for highlighting the occurrence of gliomas in view of their rarity in mice. Mr. Beliczky recommended comparative pharmacokinetic studies in Sprague-Dawley rats and $B6C3F_1$ mice because of apparent differences in sensitivity to tumor induction by 1,3-butadiene in the two species. Dr. M. Powers, NTP, responded that such pharmacokinetic studies were being designed.

As a third principal reviewer, Dr. S. Friess agreed with the major conclusions as to "clear evidence of carcinogenicity." However, he suggested that the strength of evidence for papillomas and carcinomas of the forestomach in male and female mice better fits in the category of "some evidence of carcinogenicity," based on lack of dose response: in both sexes the incidences in the low dose groups were greater than those in the high dose groups. Dr. Friess also suggested adding liver adenomas and adenomas and carcinomas (combined) in females to the conclusions under the category of "clear evidence" because the dose trends and enhanced incidence rates at the high dose were clear and significant. Dr. J. Huff, NTP, indicated that a single category of evidence was generally selected for each sex and species and reflected the highest degree of evidence. In the discussion that followed, several Panel members concurred with this concept. [A statement has been added to the conclusions concerning the increased incidence of liver lesions in mice.] Dr. Friess also noted the increased incidence of nasal lesions in males, with no increase in neoplasia and the almost complete lack of such lesions in females.

As a fourth principal reviewer, Dr. C. Harper also agreed with the conclusions. He asked for clarification regarding the major cause of early deaths; in one section malignant lymphomas were stated as causative and elsewhere a number of deaths were attributed to hemangiosarcomas. Dr. G. Boorman, NTP, replied that the lymphomas occurred principally in the thymus and likely caused the animals to suffocate, while in some cases the heart lesions were contributory.

In other discussion, Dr. R. Scala noted that 1,3-butadiene is highly explosive and the concentrations used in the 13-week studies seemed near the explosive level. He emphasized the importance of listing the safety procedures used at the contract laboratory [see p. 96] and expressed concern that other inhalation studies with potent chemicals such as ethylene oxide and 1,2-epoxybutane were conducted in the same chamber room. Dr. E. McConnell, NTP, agreed and said that conducting several inhalation studies in the same chamber room would not be done routinely. He mentioned that the chambers

used for each chemical were essentially closed systems and cross-contamination was unlikely. Dr. Scala stated that there was apparent inadequate randomization of the animals by weight. Dr. J. Haseman, NIEHS, agreed and said analysis showed that the initial weights in both sexes were significantly lower in the control groups than in the dosed groups [see p. 32]. Dr. Swenberg commented that although results were given for the 14-week studies, no pathology information was given; he requested that pathology findings or lack thereof be included in the report. [None was reported; see p. 31.]

In response to questions about the long-term inhalation study in Sprague-Dawley rats performed at Hazleton Laboratories, Europe, under the sponsorship of the International Institute of Synthetic Rubber Producers, Dr. Scala noted that the overall report had not been published but the findings of the 2-year carcinogenicity study would be submitted to a toxicology journal. Dr. B. Schwetz, NTP, stated that these data had been made available to the NTP and that an ongoing correspondence had been initiated.

Dr. Van Ryzin moved that the technical report on the carcinogenesis studies of 1,3-butadiene be accepted with the modifications discussed. To the conclusions would be added "hepatocellular adenomas and adenomas or carcinomas (combined)" in female mice. Dr. J. Holland seconded the motion and the technical report was approved by seven affirmative votes with two abstentions (Dr. Holland and Dr. Scala).

I. INTRODUCTION

ANIMAL TOXICITY STUDIES

METABOLISM

MUTAGENICITY

TERATOGENICITY AND REPRODUCTIVE EFFECTS

CARCINOGENICITY IN ANIMALS

EFFECTS ON HUMANS

REASON FOR TESTING

I. INTRODUCTION

$CH_2=CH-CH=CH_2$

1,3-BUTADIENE

CAS NO. 106-99-0

C_4H_6 **Mol. Wt. 54.09**

Synonyms: butadiene, biethylene, bivinyl, divinyl, erythrene, vinylethylene, pyrrolylene

1,3-Butadiene is a colorless gas produced commercially as an ethylene coproduct (about 60% of U.S. production), by oxidative dehydrogenation of *n*-butenes (about 25% of U.S. production), and by dehydrogenation of *n*-butane (about 15% of U.S. production) (SRI, 1980). Between 2.1 and 7.3 billion pounds of 1,3-butadiene were produced or imported in 1977 (USEPA, 1981). 1,3-Butadiene ranked 36th in U.S. production (2.31 billion pounds) in 1983 (Chem. & Eng. News, 1984). During the previous year, 1.38 billion pounds of 1,3-butadiene-derived thermoplastic resins and 1.33 million metric tons of synthetic rubber were produced (Chem. &. Eng. News, 1983).

1,3-Butadiene is used as an intermediate in the production of elastomers, polymers, and other chemicals. Of the 1,3-butadiene used in 1978, 44% was used to manufacture styrene-butadiene rubber (a substitute for natural rubber, produced by copolymerization of 1,3-butadiene with styrene), and 19% was used to produce polybutane elastomer (a substance that increases resistance of tire products to wear, heat degradation, and blowouts). Chloroprene monomer, derived from 1,3-butadiene, is used exclusively to manufacture neoprene elastomers for non-tire and latex applications. Commercial nitrile rubber, used largely in rubber hoses, seals, and gaskets for automobiles, is a copolymer of 1,3-butadiene and acrylonitrile. Acrylonitrile-butadiene-styrene resins, usually containing 20%-30% 1,3-butadiene by weight, are used to make parts for automobiles and appliances. Other polymer uses include specialty polybutadiene polymers, thermoplastic elastomers, nitrile barrier resins, and K resins®. 1,3-Butadiene is used as an intermediate in the production of a variety of industrial chemicals, including two fungicides, captan and captofol (USEPA, 1981). It is approved by the U.S. Food and Drug Administration for use in the production of adhesives used in articles for packaging, transporting, or holding food; in components of paper and paperboard that are in contact with dry food; and as a modifier in the production of semirigid and rigid vinyl chloride plastic food-contact articles (USCFR, 1978, rev. 1983). No information was located on the levels of monomer or on its elution rate from any of the commercially available polymers. It is not known if unreacted 1,3-butadiene migrates from packaging materials.

1,3-Butadiene has been detected in drinking water in the United States (Kraybill, 1980; USEPA, 1978); however, it is primarily an air contaminant. 1,3-Butadiene reacts photochemically in the atmosphere to generate smog; acrolein and formaldehyde are apparent by-products (Parsons and Wilkins, 1976). It has been detected in cigarette smoke (Osborne et al., 1956), gasoline vapor (Stephens and Burleson, 1967), incineration products of fossil fuels (Natusch, 1978), and automobile exhaust (Neligan, 1962). 1,3-Butadiene has been detected in urban atmospheres in the United States at concentrations that generally range from 1 to 5 ppb (Natusch, 1978). Concentrations of 0-19 ppb have been measured at industrial sites near Houston, TX (Siddiqi and Worley, 1977); a level of 30 ppb was detected at another industrial area (Stephens, 1973). Concentrations of 40-45 ppm in 1960 and 2-10 ppm in 1970 were reported in air samples and factory emissions at USSR petrochemical plants

(Batkina, 1976; D'Yachkov, 1972; Faustov, 1972; Cyashenko and Sidenko, 1976).

Occupational exposure to 1,3-butadiene occurs mainly through inhalation and, to a lesser extent, by dermal contact. Approximately 62,000 U.S. workers are exposed annually (NIOSH, 1980). Most exposures occur in plants manufacturing the chemical or using it to produce polymers or elastomers. The international occupational exposure limits, expressed as time-weighted averages (TWA's), are: 1,000 ppm in the United States, United Kingdom, Finland, West Germany, the Netherlands, Australia, Belgium, and Switzerland; 682 ppm in Rumania; 454 ppm in Italy; 223 ppm in East Germany, Yugoslavia, and Czechoslovakia; and 45 ppm in the Union of Soviet Socialist Republics, Bulgaria, and Poland (International Labor Office, 1977; ACGIH, 1981).

ANIMAL TOXICITY STUDIES

The median LC_{50} value of 1,3-butadiene in short-term inhalation studies was reported to be 117,700 ppm for mice during an unspecified exposure period (Shugaev, 1969) and 122,700 ppm for a 2-hour exposure period (Zlobina and Dueva, 1974). In rats, the LC_{50} value for a 4-hour exposure was 129,500 ppm (Shugaev, 1969). Short-term oral LD_{50} values of 5.48 g/kg for rats and 3.21 g/kg for mice have been reported (Ripp, 1968). Rats exposed to 1,3-butadiene for 8 months at concentrations ranging from 600 to 6,700 ppm (7.5 hours/day, 6 days/week) showed no adverse effects except for slight growth retardation at the highest concentration (Carpenter et al., 1944). Rabbits and rats exposed at a concentration of 4.5 ppm for 4 hours per day for 4 months showed no effects; changes in the nervous system function occurred at 45 ppm and 1,000 ppm. Changes in liver, kidney, and spleen morphology, the central nervous system, and immunologic status have been observed in rats exposed for 81 days at 0.45 ppm; morphologic changes in the nasopharynx occurred at 1.35 ppm; and hemodynamic changes, increased permeability of the vessels, and alteration of the structure of the kidney and heart were seen at 13.5 ppm (Nikiforova et al., 1969; Crouch et al., 1979). Guinea pigs exposed briefly at 4.5-6.8 ppm had allergic reactions (Zlobina and Dueva, 1974).

Rats exposed to 1,3-butadiene at concentrations of 1,000-8,000 ppm for 6 hours per day, 5 days per week for 3 months showed moderately increased salivation at the higher concentrations; no effects on survival, growth rate, food consumption, hematologic and biochemical parameters, or gross or microscopic pathologic findings were observed (Crouch et al., 1979).

METABOLISM

The metabolism of 1,3-butadiene has not been studied extensively. Nine minutes after rabbits were exposed to 1,3-butadiene at concentrations of 250,000 ppm, the test chemical was found in the femoral artery at a concentration of 0.26 mg/ml and in the femoral vein at 0.18 mg/ml (Carpenter et al., 1944). Concentrations of 1,3-butadiene were determined in major organs of cats, rats, and mice exposed at or near the LC_{50} values for 1-4 hours (Shugaev, 1969). Rats (strain and sex unspecified) exposed to 1,3-butadiene for 2 hours at concentrations of 130,000 ppm had the test chemical at a high concentration in the perirenal fat (152 mg/100 g) and lower concentrations in the liver (51 mg/100 g), brain (50 mg/100 g), spleen (45 mg/100 g), and kidney (36 mg/100 g). After rats were exposed at 130,000 ppm for up to 1 hour, 1,3-butadiene concentrations in the brain and liver were similar (34.6 mg/100 g and 33.6 mg/100 g, respectively) immediately after exposure, 2.9 mg/100 g and 3.3 mg/100 g 60 minutes after exposure, and 0 and trace amounts 90 minutes after exposure (Shugaev, 1969).

In vitro studies have shown that 1,3-butadiene is converted to 1,2-epoxybutene by cytochrome P-450 dependent mixed-function oxidases in rat liver microsomes (Malvoisin et al., 1979). Pretreatment with phenobarbital increased the 1,3-butadiene epoxydase activity. Incubation of the epoxide with rat liver microsomes and a NADPH-generating system produced four products: an unidentified metabolite, two metabolites resembling DL-1,2:3,4-diepoxybutane, and 3,4-epoxy-1,2-butane diol (Malvoisin and Roberfroid, 1982; Malvoisin et al., 1980). Pretreatment of rats with phenobarbital apparently enhanced the formation of the diol up to fifteenfold. The authors suggested that biotransformation of 3,4-epoxy-1-butene to

3,4-epoxy-1,2-butane diol is mediated by a microsomal epoxide hydrase and by mixed function oxidases. These pathways have not been confirmed by in vivo studies.

MUTAGENICITY

All reported studies on the mutagenicity of 1,3-butadiene used *Salmonella typhimurium* TA1530, a base-pair substitution strain. 1,3-Butadiene was not found to be mutagenic when tested by the usual plate-incorporation protocol or by the preincubation protocol in the presence or absence of S9 prepared from the livers of Aroclor-treated male Wistar rats (Poncelet et al., 1980). When bacterial cells and S9 were incorporated into an agar overlay and exposed to gaseous 1,3-butadiene in a desiccator, the compound was mutagenic. Using the same protocol for gaseous 1,3-butadiene, de Meester et al. (1980) confirmed the findings of Poncelet et al. (1980) and showed that 1,3-butadiene was not mutagenic at atmospheric concentrations of 2%-35% (v/v) in the absence of S9. When Petri plates containing only cells (no S9) were placed in the same desiccator with plates containing Aroclor-induced S9 only (no cells) and exposed to 1,3-butadiene gas, the cells were mutated. These experiments demonstrated that 1,3-butadiene is not a direct-acting mutagen as previously reported by de Meester et al. (1978) and that the mutagenic form of 1,3-butadiene is an oxidative metabolite of 1,3-butadiene, a volatile compound, and a base-pair substitution mutagen. The investigators suggested that epoxides are probably the ultimate mutagenic forms of 1,3-butadiene.

TERATOGENICITY AND REPRODUCTIVE EFFECTS

The fertility of rats was not severely impaired when they were exposed to 1,3-butadiene at concentrations of 600-6,700 ppm for 7.5 hours per day, 6 days per week, for 8 months; however, the decreased fecundity observed may have been related to exposure. No evidence of degenerative testicular changes in males was seen, and all embryos appeared normal at necropsy (Carpenter et al., 1944). When female rats were exposed to 1,3-butadiene for 4 months at 45 ppm, increased embryonic mortality and teratogenesis were reported (Serebrennikov and Ogleznev, 1978). Pregnant female Sprague-Dawley rats exposed to 1,3-butadiene at concentrations of 0, 200, 1,000, or 8,000 ppm for 6 hours per day during days 6-15 of gestation showed embryonic growth retardation and slight embryo mortality at all concentrations (Hazleton Labs Europe, 1981). At the highest exposure concentration, evidence of teratogenicity (major fetal defects such as cardiovascular, sternebral, and thoracic abnormalities) was seen.

CARCINOGENICTY IN ANIMALS

Groups of 100 Sprague-Dawley rats of each sex were exposed to 1,3-butadiene at concentrations of 0, 1,000, or 8,000 ppm for 6 hours per day, 5 days per week for 105-111 weeks (Hazleton Labs Europe, 1981). Significantly increased incidences of mammary gland tumors, Zymbal gland carcinomas, follicular cell tumors of the thyroid gland, and uterine stromal carcinomas in fmales and increased incidences of Leydig cell tumors and pancreatic exocrine tumors in males were observed. Survival was 20% for males at week 105 and 25% for females at week 111.

EFFECTS ON HUMANS

Workers exposed to 1,3-butadiene at concentrations of 8,000 ppm for 8 hours complained of eye irritation, blurred vision, coughing, and drowsiness (Carpenter et al, 1944). Routine examination of workers exposed to 1,3-butadiene for a number of years at concentrations of less than 500 ppm revealed no overt adverse effects (Wilson and McCormick, 1954). Effects on the skin and on nervous, gastrointestinal, circulatory, and respiratory systems have been reported in workers in the synthetic rubber industry (Abdullaeva, 1973; Alekperov et al., 1970; Revnova, 1973) and workers exposed to 1,3-butadiene and other chemicals have reported headaches, irritability, cardiac pain, and general weakness (Mukhametova et al., 1976); details of exposure concentrations and duration were not provided.

Epidemiologic evaluations of the potential hazards associated with the production of synthetic rubber polymers showed marginal increases in the incidences of diseases of the lymphatic and hematopoietic systems in workers in one of three plants surveyed, but other suspect or known carcinogens were present (Meinhardt et al., 1978; McMichael et

al., 1976). A NIOSH survey of two plants showed an overall mortality that was lower (80% and 68%) than that expected for the U.S. population; however, excessive but not significant mortality rates for specific categories of neoplasms of the lymphatic and hematopoietic systems were observed in workers in both plants (Meinhardt et al., 1982). In a study of the styrene-butadiene rubber polymer industry in the United States and Canada, male workers were found to have a low overall mortality compared with the general population; mortality rates from Hodgkin's disease and from cancers of the gastrointestinal tract, larynx, kidney, and testis were increased but not significantly (Matanoski et al., 1982).

The International Agency for Research on Cancer has evaluated the available evidence linking cancer in humans to occupational exposures in the rubber industry (IARC, 1982). The evidence for carcinogenicity to humans was considered sufficient for certain exposures and was summarized as: "A large number of retrospective follow-up studies of cohorts of rubber workers and case-control studies of individuals with cancer have been conducted in the US, the UK, Switzerland, Canada, Sweden and Finland. These studies indicate that an excess incidence of bladder tumours occurred in the UK, which was probably associated with exposure to aromatic amines of workers employed before 1950. The evidence is less strong for US workers. US workers, however, showed increased rates of lymphatic leukaemia, probably due to exposure to organic solvents. Stomach and lung cancer rates were elevated in both the US and UK studies. There is limited evidence that other cancers (skin, colon, prostate, lymphoma) are associated with work in the industry. Cancers of the brain, thyroid, pancreas and oesophagus have also been reported."

REASON FOR TESTING

1,3-Butadiene was tested because of its large production, the widespread exposure of the population, and the lack of long-term toxicity and carcinogenicity information. The chemical was tested only in mice because a 2-year inhalation study in rats (sponsored by the International Institute of Synthetic Rubber Producers, Inc.) was already in progress (now completed) at the time of chemical selection.

II. MATERIALS AND METHODS

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF 1,3-BUTADIENE

1,3-Butadiene (rubber grade, containing 0.02% t-butyl catechol) was obtained from Phillips Petroleum Co. The 1,3-butadiene was packaged in low-pressure steel cylinders as a liquefied gas under its own vapor pressure and stored at or slightly below room temperature in the testing laboratory throughout the studies. Lot no. Y-634 was used for the 15-day studies and lot nos. Y-634 and Y-679 were used for the 14-week studies, both at Industrial Biotest Laboratories. In the 61-week studies at Battelle Pacific Northwest Laboratories, several 5-gallon cylinders of 1,3-butadiene were used as an emergency resource (Table 1). Because some cylinders contained nitrogen as an inert blanket for the cylinder headspace, cylinders were briefly bled into a hood before being used.

A special acceptance and handling protocol for 1,3-butadiene cylinders was employed during the 61-week studies. 1,3-Butadiene was shipped at approximately 5-week intervals. Each cylinder was analyzed upon receipt and was considered acceptable for use in the study only if the 4-ethenylcyclohexene (4-vinyl-1-cyclohexene, referred to in this report as dimer) content was less than 100 ppm. The concentration of dimer, often found in commercial 1,3-butadiene, increases with time under normal storage conditions. Generally cylinders were used for no longer than 6 weeks to minimize the amount of dimer delivered to the exposure chambers (Appendix D). Three cylinders that had dimer contents of slightly more than 100 ppm were used because replacement cylinders were not readily available.

Each lot of 1,3-butadiene was analyzed by infrared spectrometry and gas chromatography (Appendix D). Based on the peak area in the gas chromatogram, the percent purity of the various lots of 1,3-butadiene varied from 98.94% to 100%. One of the impurities that was commonly observed had the same retention time as methane. A second impurity eluting right after 1,3-butadiene was also commonly observed. This impurity was not identified; the highest concentration observed was 0.24% (lot no. F-193). The amount of dimer was determined by a second gas chromatographic system.

GENERATION OF CHAMBER CONCENTRATIONS FOR THE SIXTY-ONE-WEEK STUDIES

During the 60- and 61-week studies, 1,3-butadiene gas was metered to the exposure chambers and diluted in the fresh air chamber inlets. The uniformity of the vapor concentration in the exposure chambers was measured periodically throughout the studies. The generation system is illustrated and described in Appendix E.

1,3-Butadiene concentrations in the chamber atmospheres were monitored 7-12 times during each exposure day with a photoionization detector (PID) for the first 150 days or with a gas chromatograph for the remainder of the studies. Weekly and monthly concentrations are presented in Appendix E. The exposure concentrations for the 61-week studies are summarized as follows:

Target Concentration (ppm)	Average Chamber Concentration ± Standard Deviation (ppm)	No. of Samples
625	627 ± 25	2,406
1,250	1,236 ± 41	2,415

Throughout the studies, samples taken from the chambers several times each exposure day indicated that mean daily concentrations were within 3%-4% of the target concentrations. The distribution of mean daily concentrations are as follows:

Range of Target Concentration (percent of target concentration)	No. of Days Mean Within Range	
	625 ppm	1,250 ppm
> 110	0	0
100-110	157	84
90-100	128	199
80-90	1	4
70-80	1	0
< 70	0	0

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDIES OF 1,3-BUTADIENE (a)

	Fifteen-Day Studies	Fourteen-Week Studies	Sixty-One-Week Studies
EXPERIMENTAL DESIGN			
Testing Laboratory	Industrial Biotest Laboratories	Same as 15-d studies	Battelle Pacific Northwest Laboratories
Size of Test Groups	5 male and 5 female mice	10 male and 10 female mice	50 male and 50 female mice
Concentrations	0, 625, 1,250, 2,500, 5,000, or 8,000 ppm 1,3-butadiene via inhalation	0, 625, 1,250, 2,500, 5,000, or 8,000 ppm 1,3-butadiene via inhalation; restart--0 or 8,000 ppm	0, 625, or 1,250 ppm 1,3-butadiene via inhalation
Date of First Exposure	3/4/77	5/27/77; restart, 6/22/77	4/15/81
Date of Last Exposure	3/17/77	8/28/77; restart, 9/21/77	6/7/82 (males); 6/15/82 (females)
Duration of Exposures	6 h/d, 5 d/wk for 2 wk	6 h/d, 5 d /wk, for 64 or 63 exposures (restart)	6 h/d, 5 d/wk for 60 wk (males) or 61 wk (females)
Type and Frequency of Observation	Observed 1 × d for signs of moribundity and mortality; weighed on d 0, 5, 10, and 15	Observed 1 × d for signs of moribundity and mortality; weighed on d 0, then 1 × wk	Observed 2 × d for signs of moribundity and mortality; weighed 1 × wk for 12 wk, then 1 × mo
Necropsy and Histologic Examination	The following tissues were examined during necropsy of all animals: gross lesions, skin, mandibular lymph node, mammary gland, salivary gland, thigh muscle, sciatic nerve, sternebrae, vertebrae or femur, including marrow, costochondral junction (rib), thymus, larynx and pharynx, trachea, lungs and bronchi, heart, thyroid glands, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, cecum, rectum, mesenteric lymph node, liver, gallbladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, seminal vesicles/prostate/testes or ovaries/uterus, nasal cavity and nasal turbinates, brain, pituitary, spinal cord, eyes	Necropsies performed on all animals; tissues examined: same as in 14-d study; histopath exam performed on all controls, high dose, and early deaths	Complete necropsy and histopath exam performed on all animals; tissues examined: gross lesions, mandibular lymph node, mammary gland, sternebrae including marrow, thymus, trachea, lungs and bronchi, heart, thyroid glands, parathyroids, esophagus, stomach, colon, small intestine (2 sections), liver, gallbladder, pancreas, spleen, kidneys, adrenal glands, urinary bladder, prostate/testes or ovaries/uterus, nasal cavity and nasal turbinates (3 sections), brain (3 sections), pituitary, pharynx, and (if abnormal) eyes

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDIES OF 1,3-BUTADIENE (Continued)

	Fifteen-Day Studies	Fourteen-Week Studies	Sixty-One-Week Studies
ANIMALS AND ANIMAL MAINTENANCE			
Species	$B6C3F_1$ mice	$B6C3F_1$ mice	$B6C3F_1$ mice
Animal Source	Frederick Cancer Research Center (Frederick, MD)	Frederick Cancer Research Center (Frederick, MD)	Charles River Breeding Labs (Portage, MI)
Time Held Before Start of Test	14 d	8 d; restart, 13 d	21 d
Age When Placed on Study	Approximately 5 wk	Approximately 4-5 wk	8-9 wk
Age When Killed	Approximately 7 wk	Approximately 18-19 wk	68-69 wk (males); 69-70 wk (females)
Necropsy Dates	3/18/77	8/29/77; restart, 9/22/77	6/8/82 (males); 6/16/82 (females)
Method of Distribution	According to a table of random numbers	Same as 15-d studies	Distributed to weight blocks, then to groups according to table of random numbers
Feed	Wayne Lab-Blox® (Allied Mills, Inc., Chicago, IL); freely available except during inhalation exposure	Same as 15-d studies	NIH 07 diet (Ziegler Bros, Gardners, PA); freely available except during inhalation exposure; Wayne Lab-Blox® for 2 wk
Water	Provided ad libitum	Provided ad libitum	Tap water freely available through automatic watering system (Edstrom Industries, Waterford, WI)
Cages	Stainless steel mesh (Unifab Corp., Kalamazoo, MI)	Same as 15-d studies	Stainless steel wire (Lab Products, Rochelle Pk, NJ)
Cage Rotation	None	None	Rotated last 5 mo of studies
Animals per Cage	1	1	1

TABLE 1. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE INHALATION STUDIES OF 1,3-BUTADIENE (Continued)

	Fifteen-Day Studies	Fourteen-Week Studies	Sixty-One-Week Studies
Animal Room Environment	Fluorescent light 12 h/d; information on temperature, air changes, and humidity not available	Fluorescent light 12 h/d; information on temperature, air changes, and humidity not available	Av temp--21° C during exposure, 24° C during nonexposure; rel humidity--54%-57%; fluorescent light 12 h/d; 20 room air changes/h; chamber environment: temp--23° ± 1° C; rel humidity--56% ± 7%
Other Chemicals on Test in Same Room	Propylene	Propylene	Ethylene Oxide 1,2-Epoxybutane
CHEMISTRY			
Lot No. Used	Y-634	Y-634, Y-679	B-915, B-899B, B-962, B-996, F-037, F-047, F-089A, F-089B, F-089C, F-120, F-159, F-193, F-207
Date of Initial Use of Subsequent Lots	N/A	6/24/77	4/15/81, 5/12/81, 6/15/81, 7/13/81, 8/25/81, 9/14/81, 11/10/81, 12/14/81, 12/15/81, 1/18/82, 2/22/82, 4/2/82, 4/23/82
Supplier	Phillips Petroleum Co. (Phillips, TX)	Same as 15-d studies	Same as 15-d studies
CHEMICAL/VEHICLE			
Preparation	Test material was metered into the chamber air supply so that it was well mixed with incoming air by turbulence	Same as 15-d studies	Same as 15-d studies

(a) No single-exposure studies were conducted.

II. MATERIALS AND METHODS

FIFTEEN-DAY STUDIES

Male and female B6C3F$_1$ mice were obtained from Frederick Cancer Research Center and observed for 14 days before being placed on study at Industrial Biotest Laboratories. Groups of five mice of each sex were exposed to air containing 1,3-butadiene at target concentrations of 0, 625, 1,250, 2,500, 5,000, or 8,000 ppm for 6 hours per day, 5 days per week for 2 weeks. Mice were observed daily for signs of moribundity and mortality and were weighed on days 0, 5, 10, and 15. Necropsies were performed on all animals. Details of animal maintenance are presented in Table 1.

FOURTEEN-WEEK STUDIES

Fourteen-week studies were conducted at Industrial Biotest Laboratories to evaluate the cumulative effects of 1,3-butadiene and to determine the concentrations to be used in the intended 2-year studies. Three- to four-week-old male and female mice were obtained from Frederick Cancer Research Center, observed for 8 days or 13 days (a supplemental study was started because most of the males in the highest dose group died) and then assigned to groups according to a table of random numbers. Feed and water were freely available, except during exposure periods, when water only was available.

Groups of 10 mice of each sex were exposed to air containing 1,3-butadiene at concentrations of 0, 625, 1,250, 2,500, 5,000, or 8,000 ppm, 6 hours per day, 5 days per week for 14 weeks (64 exposures). Because four male mice in the highest dose group were dead by day 4, another two groups of 10 male mice each were started: a group of controls and an 8,000-ppm exposure group. Further experimental details are summarized in Table 1.

Animals were checked once per day for signs of moribundity and mortality; moribund animals were killed. Body weights were recorded weekly. At the end of the 95-day or 93-day (restart) studies, survivors were killed. Necropsies were performed on all animals, except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 1.

SIXTY-ONE-WEEK STUDIES

Study Design

Two long-term studies were initiated at Battelle Pacific Northwest Laboratories. Groups of 50 mice of each sex were exposed 6 hours per day, 5 days per week to air containing 1,3-butadiene at target concentrations of 0, 625, or 1,250 ppm. The first study was terminated after 13 weeks because the low dose group had been exposed inadvertently at the same concentration as the high dose group. No clinical signs or mortality were observed in these animals. After study termination, all animals were examined grossly and 10 animals from each sex and dose group were selected randomly for histopathologic examination. No compound-related lesions were observed. The second study was intended to last 103 weeks but was terminated after 60 weeks (males) or 61 weeks (females) because of low survival. Results of this second study are given and interpreted in this Technical Report.

Source and Specifications of Test Animals

The male and female B6C3F$_1$ (C57BL/6N $\times$ C3H/HeN MTV$^-$) mice used in this study were produced under strict barrier conditions at Charles River Breeding Laboratories under a contract to the Carcinogenesis Program. Breeding starts for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals shipped for testing were progeny of defined microflora-associated parents that were transferred from isolators to barrier maintained rooms. Animals were shipped to the testing laboratory at 5-6 weeks of age. The animals were quarantined at the testing facility for 3 weeks. Thereafter, a complete pathologic examination was performed on a selected number of animals to assess their health. The rodents were placed on study at 8-9 weeks of age. The health of the animals was monitored during the course of the study. Results of serologic analyses for murine viruses in the control animals at the end of the studies are given in Appendix F.

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid B6C3F$_1$ test animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoretograms that demonstrate phenotype expressions of known genetic loci.

The C57BL/6 mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6 colony were used as parents for the hybrid B6C3F$_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because matched concurrent controls were included in each study.

Animal Maintenance

Mice were housed individually in stainless steel cages within the exposure chambers. Feed (Appendix G) and water were freely available except during exposure periods, when only water was available. Details of animal maintenance are summarized in Table 1.

Clinical Examinations and Pathology

All animals were observed twice daily for signs of moribundity or mortality. Clinical signs were recorded once per week. Individual body weights were recorded once per week for the first 12 weeks of the study and once per month thereafter. Mean body weights were determined for each group. Examination of mice for palpable masses began 6 months after the study started and continued monthly thereafter. Moribund animals were killed, as were animals that survived to the end of the study. Necropsies were performed on all animals, including those found dead, unless they were excessively autolyzed or cannibalized. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Three separate sections of nasal turbinate were examined. Section one was at the level just caudal to the incisor teeth, section two was midway between the incisors and first molar, and section three was at the middle of the second molar. Tissues examined microscopically are listed in Table 1.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Chairperson of the NTP Pathology Working Group (PWG) for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (In Press). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group.

Nonneoplastic lesions are not specifically examined routinely by the quality assurance pathologist or the PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and the PWG if they are considered part of the toxic response to a chemical or if they are deemed of special interest.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

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) extensions of Cox's method for testing for a dose-related trend. All reported P values for the survival analysis are two-sided.

Calculation of Incidence: The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site to the number of animals in which that site was examined. In most instances, the denominators included only those animals for which the site was examined histologically. However, when macroscopic examination was required to detect lesions (e.g., skin or mammary tumors) prior to histologic sampling, or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the number of animals on which necropsies were performed.

Analysis of Tumor Incidence: Three statistical methods are generally used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with controls and tests for overall dose-response trends.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals in each group examined during the time period. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975).

Incidental Tumor Analyses--This method, which is appropriate for nonfatal tumors (Peto et al., 1980), was not employed for these data, since the marked survival differences between dosed and control groups reduced the sensitivity of this procedure for detecting carcinogenic effects.

Unadjusted Analyses--In addition to the life table analyses, the results of the Fisher's exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are presented. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences. All reported P values for tumor analyses are one-sided.

III. RESULTS

FIFTEEN-DAY STUDIES

FOURTEEN-WEEK STUDIES

SIXTY-ONE-WEEK STUDIES

Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

FIFTEEN-DAY STUDIES

All mice survived to the end of the exposure period. Male mice that were exposed to 1,3-butadiene at 1,250 ppm or more and female mice that were exposed at 5,000 or 8,000 ppm lost weight between days 10 and 15. The mean body weights of males and females that were exposed at 5,000 or 8,000 ppm were lower on day 15 than on day 0 (Table 2). No compound-related effects were observed at necropsy.

TABLE 2. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FIFTEEN-DAY INHALATION STUDIES OF 1,3-BUTADIENE

Concentration (ppm)	Survival(a)	Mean Body Weight (grams) Initial	Final	Change (b)	Final Body Weight Relative to Controls (c) (percent)
MALE					
0	5/5	22.8 ± 1.0	25.8 ± 0.6	+ 3.0 ± 1.0	--
625	5/5	23.4 ± 0.4	26.0 ± 0.0	+ 2.6 ± 0.4	+ 0.8
1,250	5/5	23.4 ± 0.5	25.4 ± 0.2	+ 2.0 ± 0.6	- 1.6
2,500	5/5	23.4 ± 0.5	24.6 ± 0.7	+ 1.2 ± 0.8	- 4.7
5,000	5/5	22.6 ± 1.4	21.6 ± 1.5	- 1.0 ± 0.6	- 16.3
8,000	5/5	23.2 ± 0.7	23.2 ± 0.4	0.0 ± 0.6	- 10.1
FEMALE					
0	5/5	19.8 ± 0.6	23.6 ± 0.8	+ 3.8 ± 1.2	--
625	5/5	20.0 ± 0.5	22.6 ± 0.4	+ 2.6 ± 0.2	- 4.2
1,250	5/5	20.0 ± 0.7	21.8 ± 0.7	+ 1.8 ± 0.7	- 7.6
2,500	5/5	20.0 ± 0.7	22.2 ± 0.4	+ 2.2 ± 1.0	- 5.9
5,000	5/5	20.2 ± 0.7	19.4 ± 1.1	- 0.8 ± 0.7	- 17.8
8,000	5/5	20.0 ± 0.7	19.6 ± 0.2	- 0.4 ± 0.5	- 16.9

(a) Number surviving/number initially in the group
(b) Mean weight change of the group ± standard error of the mean
(c) Final weight of the dosed survivors relative to the survivors of the controls =

$$\frac{\text{Final Body Weight (Dosed Group)} - \text{Final Body Weight (Control Group)}}{\text{Final Body Weight (Control Group)}} \times 100$$

FOURTEEN-WEEK STUDIES

Six of 10 males and 1/10 females that were exposed at 8,000 ppm, 6/10 males and 1/10 females exposed at 5,000 ppm, and 1/10 males exposed at 1,250 or 2,500 ppm died or were killed in a moribund condition (Table 3). In addition, one male and one female exposed at 625 ppm died as a result of accidents. Body weight gains were decreased in males at the three highest concentrations and in females at the two highest concentrations. In the supplemental study, 6/10 males exposed at 8,000 ppm died; the survivors lost weight. No compound-related histopathologic effects were observed. Because of the mortality and the depressions in weight gain observed in mice exposed at 2,500 ppm, concentrations of 625 and 1,250 ppm 1,3-butadiene were selected for mice in the 2-year studies.

TABLE 3. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

Concentration (ppm)	Survival(a)	Mean Body Weight (grams) Initial (b)	Final	Change (c)	Final Body Weight Relative to Controls (d) (percent)
MALE					
0	10/10	17.0 ± 0.5	30.6 ± 0.4	+13.6 ± 0.5	--
625	(e) 9/10	17.6 ± 0.5	29.9 ± 0.4	+12.1 ± 0.6	- 2.3
1,250	9/10	16.6 ± 0.5	30.9 ± 0.6	+14.1 ± 0.5	+ 1.0
2,500	9/10	17.0 ± 0.5	26.7 ± 0.7	+ 9.5 ± 0.4	-12.7
5,000	4/10	17.2 ± 0.4	23.0 ± 0.7	+ 6.5 ± 1.0	-24.8
8,000	4/10	17.5 ± 0.6	19.5 ± 1.7	+ 3.2 ± 1.0	-36.3
(f) 0	10/10	20.8 ± 0.3	30.9 ± 0.8	+10.1 ± 0.7	--
(f) 8,000	4/10	21.1 ± 0.3	19.0 ± 0.8	- 2.0 ± 0.4	-38.5
FEMALE					
0	10/10	15.0 ± 0.3	27.3 ± 0.4	+12.3 ± 0.5	
625	(e) 9/10	14.9 ± 0.3	25.9 ± 1.1	+10.9 ± 1.3	- 5.1
1,250	10/10	14.6 ± 0.4	27.3 ± 0.3	+12.7 ± 0.3	0.0
2,500	10/10	14.8 ± 0.4	25.8 ± 0.4	+11.0 ± 0.3	- 5.5
5,000	9/10	15.0 ± 0.4	24.1 ± 0.7	+ 9.2 ± 0.7	-11.7
8,000	9/10	15.7 ± 0.3	22.1 ± 0.5	+ 6.4 ± 0.5	-19.0

(a) Number surviving/number initially in the group
(b) Initial mean body weight of all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean weight change of the survivors of the group ± standard error of the mean
(d) Final weight of the dosed survivors relative to the survivors of the controls =

$$\frac{\text{Final Body Weight (Dosed Group)} - \text{Final Body Weight (Control Group)}}{\text{Final Body Weight (Control Group)}} \times 100$$

(e) Accidental death
(f) Restart mice

III. RESULTS

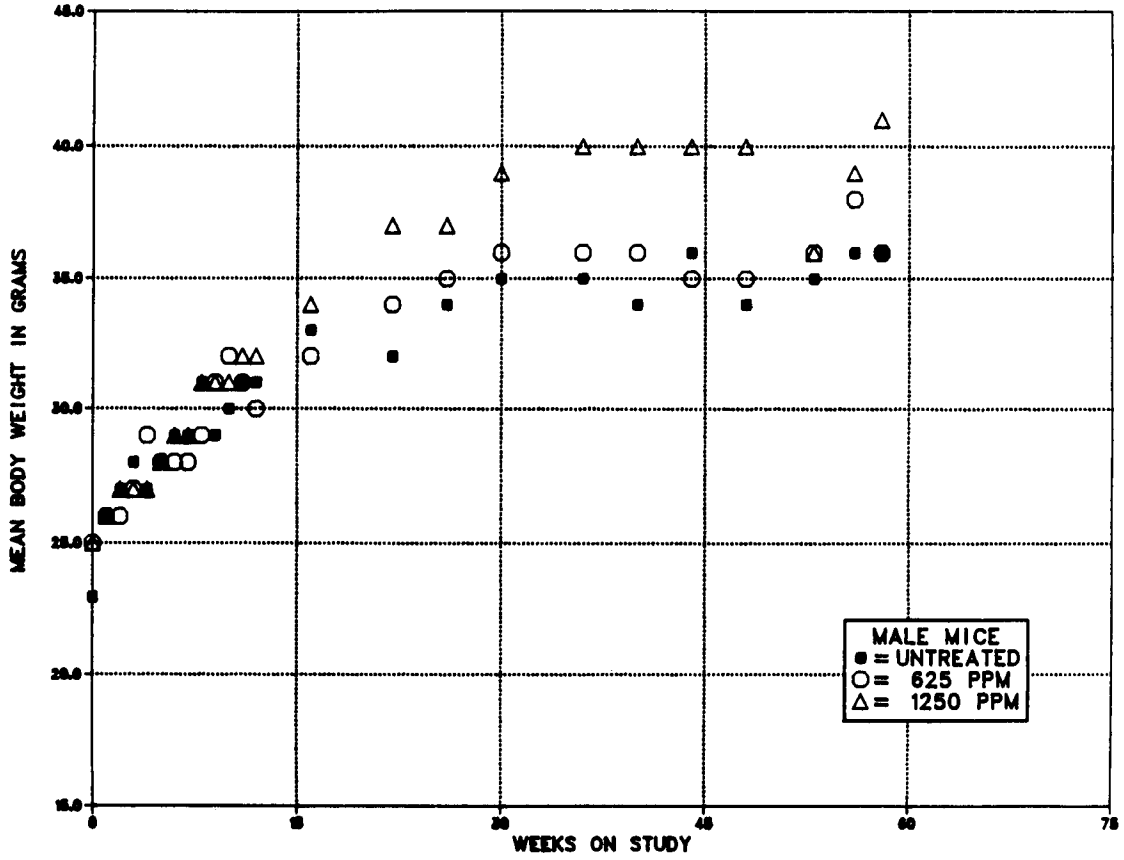
SIXTY-ONE-WEEK STUDIES

Body Weights and Clinical Signs

Body weights were not affected by inhalation exposure to 1,3-butadiene. Due to an apparent inadequate randomization, initial weights in dosed male and female mice were 9%-11% higher than those of the controls ($P<0.01$ by Mann-Whitney U test), and these approximate relationships were seen throughout much of the studies (Table 4 and Figure 1). There were no characteristic clinical signs observed other than those associated with tumor development and moribundity.

TABLE 4. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

Weeks on Study	Control Av. Wt. (grams)	Control No. of Survivors	625 ppm Av. Wt. (grams)	625 ppm Wt. (percent of controls)	625 ppm No. of Survivors	1,250 ppm Av. Wt. (grams)	1,250 ppm Wt. (percent of controls)	1,250 ppm No. of Survivors
MALE								
0	23	50	25	108.7	50	25	108.7	50
1	26	50	26	100.0	50	26	100.0	50
2	27	50	26	96.3	50	27	100.0	50
3	28	50	27	96.4	50	27	96.4	50
4	27	50	29	107.4	50	27	100.0	50
5	28	50	28	100.0	50	28	100.0	50
6	29	50	28	96.6	50	29	100.0	50
7	29	50	28	96.6	50	29	100.0	50
8	31	50	29	93.5	50	31	100.0	50
9	29	50	31	106.9	50	31	106.9	50
10	30	50	32	106.7	49	31	103.3	50
11	31	50	31	100.0	49	32	103.2	50
12	31	50	30	96.8	49	32	103.2	49
16	33	50	32	97.0	49	34	103.0	49
22	32	50	34	106.3	49	37	115.6	44
26	34	50	35	102.9	46	37	108.8	41
30	35	50	36	102.9	44	39	111.4	38
36	35	50	36	102.9	39	40	114.3	37
40	34	50	36	105.9	35	40	117.6	32
44	36	50	35	97.2	32	40	111.1	27
48	34	50	35	102.9	26	40	117.6	22
53	35	50	36	102.9	24	36	102.9	14
56	36	49	38	105.6	18	39	108.3	9
58	36	49	36	100.0	13	41	113.9	7
FEMALE								
0	18	50	20	111.1	50	20	111.1	50
1	21	50	22	104.8	50	21	100.0	50
2	21	50	23	109.5	48	20	95.2	50
3	23	49	23	100.0	48	23	100.0	50
4	23	49	24	104.3	48	24	104.3	50
5	24	49	25	104.2	48	23	95.8	50
6	24	49	24	100.0	48	23	95.8	50
7	24	48	24	100.0	48	25	104.2	50
8	26	48	26	100.0	48	26	100.0	50
9	26	48	26	100.0	48	25	96.2	50
10	27	48	26	96.3	48	25	92.6	50
11	27	48	26	96.3	48	27	100.0	50
12	26	48	27	103.8	48	25	96.2	50
16	28	48	27	96.4	48	26	92.9	50
22	28	48	29	103.6	48	28	100.0	48
26	29	48	30	103.4	48	29	100.0	48
30	30	47	29	96.7	46	28	93.3	48
36	27	47	30	111.1	44	29	107.4	48
40	29	47	31	106.9	40	30	103.4	47
44	31	47	31	100.0	39	32	103.2	45
48	29	46	32	110.3	35	33	113.8	43
53	31	46	33	106.5	32	32	103.2	42
56	31	46	33	106.5	23	33	106.5	36
58	30	46	33	110.0	19	33	110.0	35
60	31	46	34	109.7	16	34	109.7	31



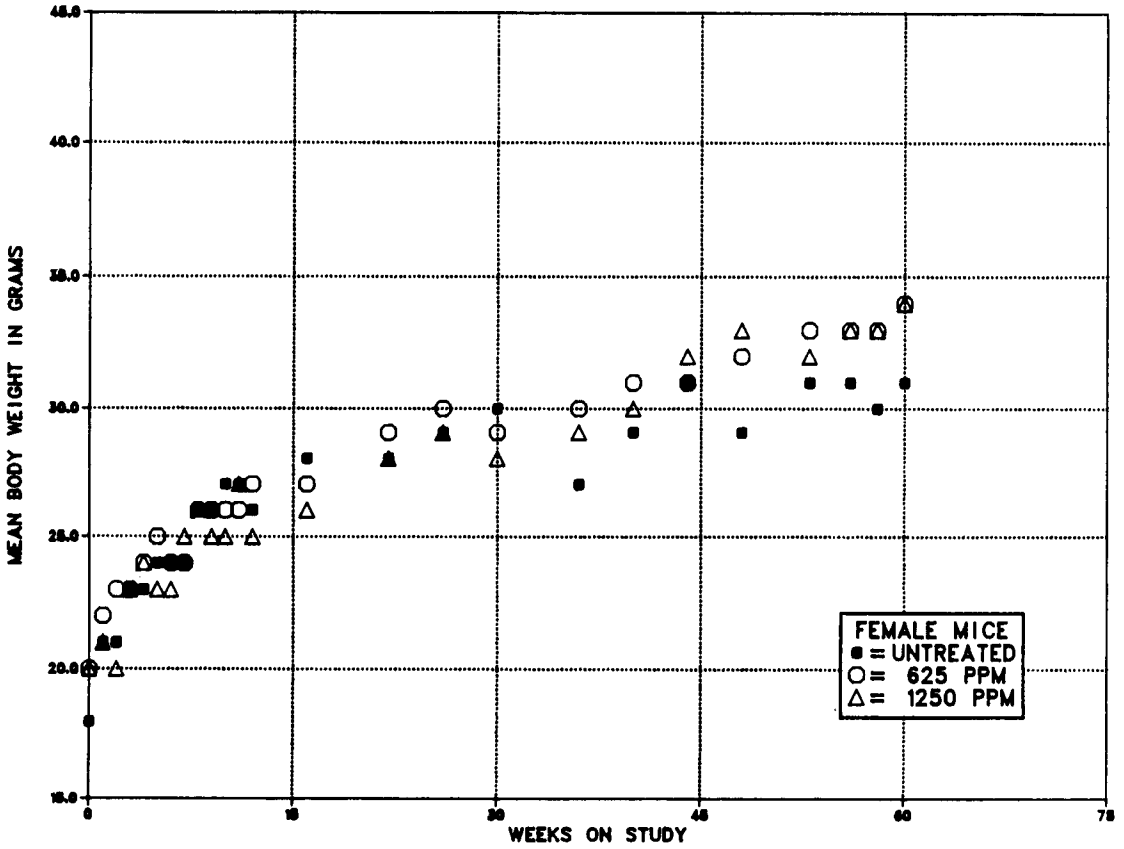


FIGURE 1. GROWTH CURVES FOR MICE EXPOSED TO 1,3-BUTADIENE BY INHALATION FOR SIXTY-ONE WEEKS

Survival

Estimates of the probabilities of survival of male and female mice exposed to 1,3-butadiene at the concentrations used in these studies and those of the controls are shown in the Kaplan and Meier curves in Figure 2. The survival of both dosed groups of mice of each sex was significantly less than that of the corresponding controls (Table 5). The 2-year studies were later abbreviated to 60 weeks for males and 61 weeks for females because of poor survival.

TABLE 5. SURVIVAL OF MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
MALE (a)			
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	1	39	39
Accidentally Killed	0	0	(c) 4
Killed at Termination	49	11	7
Survival P Values (d)	<0.001	<0.001	<0.001
FEMALE (a)			
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	4	32	18
Accidentally Killed	0	3	1
Animals Missing	0	0	1
Killed at Termination	46	14	30
Died During Termination Period	0	1	0
Survival P Values (d)	<0.001	<0.001	0.002

(a) Terminal kill period: male--week 60; female--week 61
(b) Includes moribund animals that were killed
(c) Cause of death was food deprivation.
(d) Results of the life table trend test are in the control column, and those of the life table pairwise comparisons with the controls are in the dosed columns.

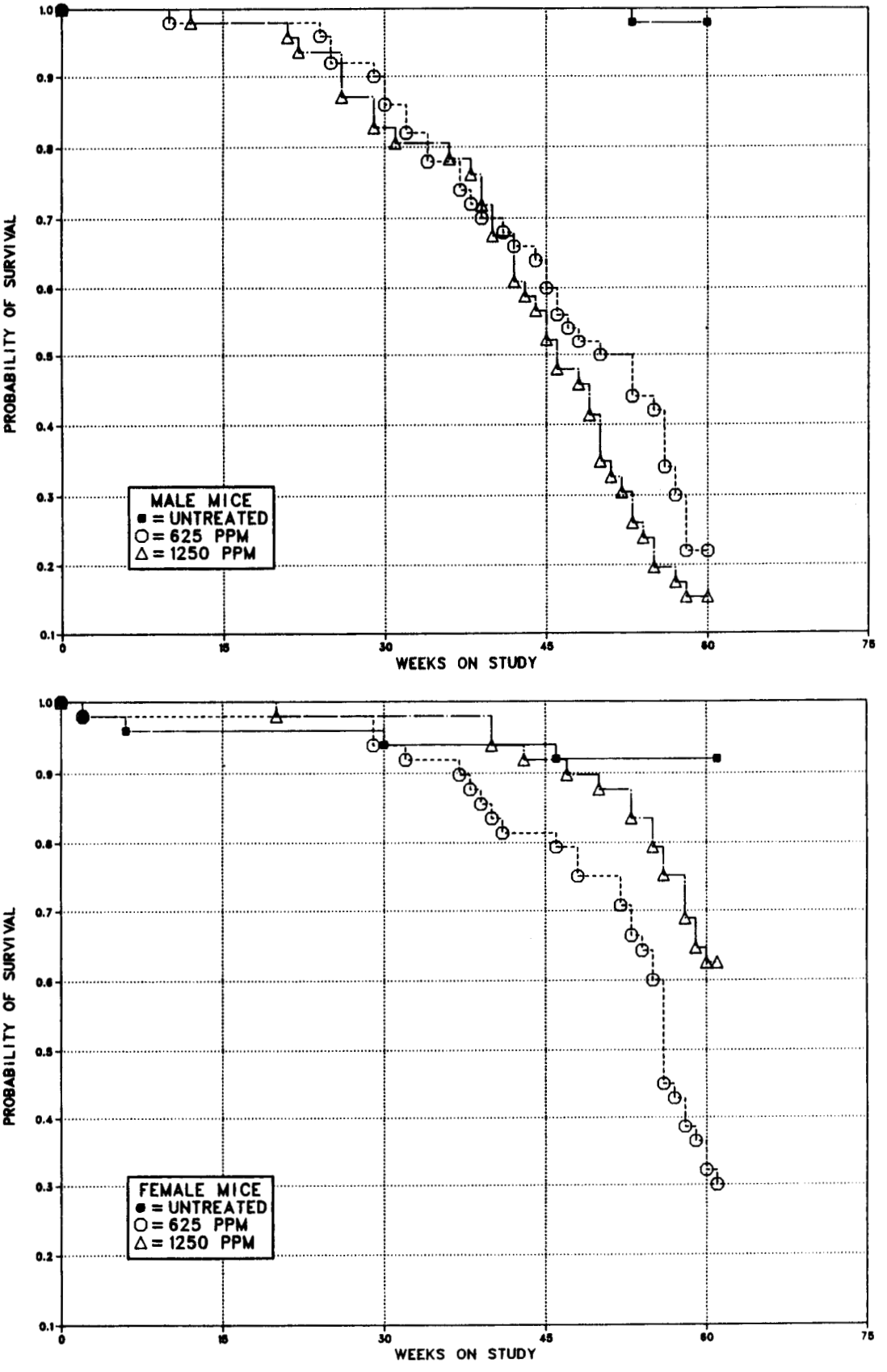


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR MICE EXPOSED TO 1,3-BUTADIENE FOR SIXTY-ONE WEEKS

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidence of animals with neoplastic or nonneoplastic lesions. Histopathologic findings on neoplasms in mice are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for individual male and female mice.

Findings on nonneoplastic lesions are summarized in Appendix B, Tables B1 and B2. Appendix C, Tables C1 and C2, contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix C (footnotes).

Lung: Alveolar epithelial hyperplasia was observed at increased incidences in dosed male and female mice (Table 6). Alveolar/bronchiolar adenomas, carcinomas, and adenomas or carcinomas (combined) in males and females occurred with significant positive trends; and the incidences of adenomas in dosed males and high dose females, carcinomas in high dose males and dosed females, and adenomas or carcinomas (combined) in dosed males and females were significantly higher than those in the controls. Grossly, the neoplasms protruded from the surface of the lung and were nodular in appearance.

TABLE 6. ANALYSIS OF LUNG LESIONS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE (a)

	Control	625 ppm	1,250 ppm
MALE			
Alveolar Epithelial Hyperplasia			
Overall Rates	1/50 (2%)	5/49 (10%)	2/49 (4%)
Alveolar/Bronchiolar Adenoma			
Overall Rates	2/50 (4%)	12/49 (24%)	11/49 (22%)
Adjusted Rates	4.1%	72.3%	75.0%
Terminal Rates	2/49 (4%)	7/11 (64%)	4/7 (57%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.010		
Fisher Exact Tests		P=0.003	P=0.007
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/50 (0%)	2/49 (4%)	5/49 (10%)
Adjusted Rates	0.0%	18.2%	47.6%
Terminal Rates	0/49 (0%)	2/11 (18%)	3/7 (43%)
Life Table Tests	P<0.001	P=0.018	P<0.001
Cochran-Armitage Trend Test	P=0.016		
Fisher Exact Tests		P=0.242	P=0.027
Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates	2/50 (4%)	14/49 (29%)	15/49 (31%)
Adjusted Rates	4.1%	86.2%	92.4%
Terminal Rates	2/49 (4%)	9/11 (82%)	6/7 (86%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
FEMALE			
Alveolar Epithelial Hyperplasia			
Overall Rates	0/49 (0%)	8/48 (17%)	7/49 (14%)
Alveolar/Bronchiolar Adenoma			
Overall Rates	3/49 (6%)	9/48 (19%)	20/49 (41%)
Adjusted Rates	6.5%	48.1%	56.7%
Terminal Rates	3/46 (7%)	6/15 (40%)	15/30 (50%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.056	P<0.001
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/49 (0%)	6/48 (13%)	8/49 (16%)
Adjusted Rates	0.0%	36.7%	24.7%
Terminal Rates	0/46 (0%)	5/15 (33%)	6/30 (20%)
Life Table Tests	P=0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.005		
Fisher Exact Tests		P=0.012	P=0.003
Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates	3/49 (6%)	12/48 (25%)	23/49 (47%)
Adjusted Rates	6.5%	61.7%	63.6%
Terminal Rates	3/46 (7%)	8/15 (53%)	17/30 (57%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.010	P<0.001

(a) The statistical analyses used are described in Chapter II (Statistical Methods) and Appendix C (footnotes).

Hematopoietic System: Malignant lymphomas in mice of each sex occurred with significant positive trends, and the incidences in the dosed groups were significantly higher than those in the controls (Table 7). The lymphoma appeared to orginate in the thymus in most animals, although the precise origin and pathogenesis of this neoplasm is difficult to trace because of the advanced degree of development observed at the time of necropsy. In one mouse, the lymphoma was limited to the thymus, and no other tissue was involved. In one high dose animal, a lobe of the thymus which was involved by the lymphoma was completely surrounded by a mediastinal mass of lymphoma cells; the lymphoma completely obscured the architecture of the thymus, but the capsule was intact. Grossly, the tumors appeared as large, pale, firm masses (up to 2.5 × 2.0 × 1.5 cm) in the mediastinum often accompanied by hydrothorax. Lymphomas also involved the spleen, lymph nodes, liver, lung, kidney, heart, pancreas, and stomach. The lymphomas varied from undifferentiated to moderately well differentiated; most were well-differentiated lymphocytic lymphomas composed of medium-sized lymphoid cells that formed monotonous sheets which focally or diffusely invaded multiple organs in a manner characteristic of lymphomas in mice. The malignant lymphomas were considered to be the major cause of early deaths in these studies.

TABLE 7. ANALYSIS OF HEMATOPOIETIC SYSTEM TUMORS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE (a)

	Control	625 ppm	1,250 ppm
MALE			
Lymphoma, All Malignant			
Overall Rates	0/50 (0%)	23/50 (46%)	29/50 (58%)
Adjusted Rates	0.0%	59.4%	75.5%
Terminal Rates	0/49 (0%)	2/11 (18%)	1/7 (14%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
FEMALE			
Lymphoma, All Malignant			
Overall Rates	1/50 (2%)	10/49 (20%)	10/49 (20%)
Adjusted Rates	2.2%	32.0%	22.9%
Terminal Rates	1/46 (2%)	3/15 (20%)	1/30 (3%)
Life Table Tests	P=0.006	P<0.001	P=0.003
Cochran-Armitage Trend Test	P=0.006		
Fisher Exact Tests		P=0.003	P=0.003

Heart: Early ("preneoplastic") lesions were diagnosed as atypical endothelial hyperplasia. Atypical hyperplasia was observed at increased incidences in dosed mice of each sex (Table 8). Hemangiosarcomas in mice of each sex occurred with significant positive trends, and the incidences in the dosed groups were significantly higher than those in the controls. Grossly, the hemangiosarcomas appeared as white areas at the apex of the heart or as dark sac-like protrusions. The growth pattern as well as the close contact between proliferating endothelial cells and erythrocytes led to the diagnosis of hemangiosarcoma. The hemangiosarcomas were not confined to the heart but were also found in the liver, lung, and kidney. The lesions in these organs were regarded as metastatic because early lesions were observed only in the heart, the incidence was highest in the heart, and, with one exception, a heart hemangiosarcoma was also found in each animal having a hemangiosarcoma in the liver, lung, or kidney. The hemangiosarcomas may have caused the death of a number of animals.

Forestomach: Epithelial hyperplasia was observed at increased incidences in dosed mice of each sex (Table 9). The incidences of papillomas, of squamous cell papillomas or carcinomas (combined), and of papillomas or carcinomas (combined) in low dose male mice were significantly higher than those in the controls. The incidence of papillomas and of papillomas or carcinomas (combined) in female mice occurred with significant positive trends. Significant positive trends occurred in the incidences of all types of papillomas and of all types of papillomas or carcinomas (combined) in female mice.

Grossly, the papillomas appeared as small white nodules. The carcinomas were solid pale masses, cells of which infiltrated the walls of the stomach. In some animals, the carcinomas extended into the peritoneal cavity and invaded adjacent structures.

TABLE 8. ANALYSIS OF HEART LESIONS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
MALE			
Atypical Hyperplasia			
Overall Rates	0/50 (0%)	5/49 (10%)	2/49 (4%)
Hemangiosarcoma			
Overall Rates	0/50 (0%)	16/49 (33%)	(a) 7/49 (14%)
Adjusted Rates	0.0%	57.5%	57.3%
Terminal Rates	0/49 (0%)	2/11 (18%)	3/7 (43%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P=0.032		
Fisher Exact Tests		P<0.001	P=0.006
FEMALE			
Atypical Hyperplasia			
Overall Rates	0/49 (0%)	5/48 (10%)	8/49 (16%)
Hemangiosarcoma			
Overall Rates	0/50 (0%)	(b) 11/48 (23%)	(c) 18/49 (37%)
Adjusted Rates	0.0%	40.6%	46.3%
Terminal Rates	0/46 (0%)	3/15 (20%)	10/30 (33%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001

(a) An hemangiosarcoma of the peritoneal cavity was also observed.
(b) Two hemangiosarcomas of the subcutaneous tissue were also observed.
(c) An hemangiosarcoma of the liver was also observed.

TABLE 9. ANALYSIS OF FORESTOMACH LESIONS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
MALE			
Epithelial Hyperplasia			
Overall Rates	0/49 (0%)	5/40 (13%)	7/44 (16%)
All Papilloma			
Overall Rates	0/49 (0%)	5/40 (13%)	0/44 (0%)
Adjusted Rates	0.0%	45.5%	0.0%
Terminal Rates	0/48 (0%)	5/11 (45%)	0/7 (0%)
Life Table Tests	P=0.036	P<0.001	(a)
Cochran-Armitage Trend Test	P=0.568		
Fisher Exact Tests		P=0.016	(a)
Squamous Cell Carcinoma			
Overall Rates	0/49 (0%)	2/40 (5%)	1/44 (2%)
Squamous Cell Papilloma or Carcinoma			
Overall Rates	0/49 (0%)	4/40 (10%)	1/44 (2%)
Adjusted Rates	0.0%	26.2%	7.1%
Terminal Rates	0/48 (0%)	2/11 (18%)	0/7 (0%)
Life Table Tests	P=0.032	P=0.001	P=0.248
Cochran-Armitage Trend Test	P=0.354		
Fisher Exact Tests		P=0.037	P=0.473
All Papilloma or Carcinoma			
Overall Rates	0/49 (0%)	7/40 (18%)	1/44 (2%)
Adjusted Rates	0.0%	50.8%	7.1%
Terminal Rates	0/48 (0%)	5/11 (45%)	0/7 (0%)
Life Table Tests	P=0.006	P<0.001	P=0.248
Cochran-Armitage Trend Test	P=0.363		
Fisher Exact Tests		P=0.003	P=0.473
FEMALE			
Epithelial Hyperplasia			
Overall Rates	0/49 (0%)	5/42 (12%)	9/49 (18%)
Squamous Cell Papilloma			
Overall Rates	0/49 (0%)	3/42 (7%)	1/49 (2%)
Adjusted Rates	0.0%	20.0%	3.3%
Terminal Rates	0/46 (0%)	3/15 (20%)	1/30 (3%)
Life Table Tests	P=0.248	P=0.008	P=0.415
Cochran-Armitage Trend Test	P=0.381		
Fisher Exact Tests		P=0.094	P=0.500
All Papilloma			
Overall Rates	0/49 (0%)	4/42 (10%)	10/49 (20%)
Adjusted Rates	0.0%	26.7%	31.8%
Terminal Rates	0/46 (0%)	4/15 (27%)	9/30 (30%)
Life Table Tests	P<0.001	P=0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.042	P<0.001
Squamous Cell Carcinoma			
Overall Rates	0/49 (0%)	1/42 (2%)	1/49 (2%)
Squamous Cell Papilloma or Carcinoma			
Overall Rates	0/49 (0%)	4/42 (10%)	1/49 (2%)
Adjusted Rates	0.0%	22.9%	3.4%
Terminal Rates	0/46 (0%)	3/15 (20%)	1/29 (3%)
Life Table Tests	P=0.249	P=0.003	P=0.408
Cochran-Armitage Trend Test	P=0.393		
Fisher Exact Tests		P=0.042	P=0.500
All Papilloma or Carcinoma			
Overall Rates	0/49 (0%)	5/42 (12%)	10/49 (20%)
Adjusted Rates	0.0%	29.3%	31.8%
Terminal Rates	0/46 (0%)	4/15 (27%)	9/30 (30%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.018	P<0.001

(a) No P value is presented because no tumors were observed in the 1,250-ppm and control groups.

Liver: Necrosis was observed at increased incidences in dosed male and low dose female mice (male: control, 1/50, 2%; low dose, 8/49, 16%; high dose, 8/49, 16%; female: 6/50, 12%; 15/47, 32%; 6/49, 12%). Hepatocellular adenomas and hepatocellular adenomas or carcinomas (combined) in female mice occurred with significant positive trends (Table 10). The incidence of hepatocellular adenomas or carcinomas (combined) in high dose female mice was significantly higher than that in the controls. The carcinomas were found in one low dose and one high dose female. In male mice, hepatocellular adenomas or carcinomas (combined) were observed in 8/50 (16%) of the controls, 6/49 (12%) of the low dose group, and 2/49 (4%) of the high dose group.

TABLE 10. ANALYSIS OF LIVER TUMORS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
Hepatocellular Adenoma			
Overall Rates	0/50 (0%)	1/47 (2%)	4/49 (8%)
Adjusted Rates	0.0%	6.7%	12.1%
Terminal Rates	0/46 (0%)	1/15 (7%)	3/30 (10%)
Life Table Tests	P=0.015	P=0.278	P=0.030
Cochran-Armitage Trend Test	P=0.025		
Fisher Exact Tests		P=0.485	P=0.056
Hepatocellular Carcinoma			
Overall Rates	0/50 (0%)	1/47 (2%)	1/49 (2%)
Hepatocellular Adenoma or Carcinoma			
Overall Rates	0/50 (0%)	2/47 (4%)	5/49 (10%)
Adjusted Rates	0.0%	13.3%	14.3%
Terminal Rates	0/46 (0%)	2/15 (13%)	3/30 (10%)
Life Table Tests	P=0.009	P=0.048	P=0.015
Cochran-Armitage Trend Test	P=0.016		
Fisher Exact Tests		P=0.232	P=0.027

Mammary Gland: Acinar cell carcinomas in female mice occurred with a significant positive trend, and the incidences in the dosed groups were significantly higher than that in the controls (Table 11). The acinar cell carcinomas metastasized to the lung in one low dose and one high dose female. Four adenosquamous carcinomas were found in low dose female mice. Adenosquamous carcinomas metastasized to the lung in three low dose females.

TABLE 11. ANALYSIS OF MAMMARY GLAND LESIONS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
Hyperplasia, NOS			
Overall Rates	5/50 (10%)	2/49 (4%)	0/49 (0%)
Acinar Cell Carcinoma			
Overall Rates	0/50 (0%)	2/49 (4%)	6/49 (12%)
Adjusted Rates	0.0%	13.3%	16.7%
Terminal Rates	0/46 (0%)	2/15 (13%)	3/30 (10%)
Life Table Tests	P=0.004	P=0.048	P=0.007
Cochran-Armitage Trend Test	P=0.007		
Fisher Exact Tests		P=0.242	P=0.012
Adenosquamous Carcinoma			
Overall Rates	0/50 (0%)	4/49 (8%)	0/49 (0%)
Adjusted Rates	0.0%	11.9%	0.0%
Terminal Rates	0/46 (0%)	0/15 (0%)	0/30 (0%)
Life Table Tests	P=0.575	P=0.030	(a)
Cochran-Armitage Trend Test	P=0.615		
Fisher Exact Tests		P=0.056	(a)

(a) No P value is presented because no tumors were observed in the 1,250-ppm and control groups.

Ovary: The incidences of ovarian lesions in female mice are presented in Table 12. Epithelial hyperplasia refers to a proliferation of tubular structures and is usually the early stage of a tubular adenoma. Lesions that showed a tubular cell component as well as a granulosa cell component were diagnosed as "mixed tumor, benign." Granulosa cell tumors occurred with significant positive trends, and the incidences in the dosed groups were significantly higher than that in the controls (Table 13).

Uterus: Involution of the uterus, characterized by fewer and less prominent endometrial glands, was observed at increased incidence in dosed female mice (control, 0/49; low dose, 7/46, 15%; high dose, 14/49, 29%).

TABLE 12. NUMBERS OF FEMALE MICE WITH NEOPLASTIC AND NONNEOPLASTIC LESIONS IN THE OVARY IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
No. of mice examined	49	45	48
Lesion			
Granulosa cell tumor	0	6	12
Granulosa cell carcinoma	0	0	1
Granulosa cell tumor or carcinoma	0	6	13
Tubular adenoma	0	2	0
Benign mixed tumor	0	0	2
Cystadenoma	0	1	0
Granulosa cell hyperplasia	0	2	0
Epithelial hyperplasia	0	3	0
Atrophy	2	40	40

TABLE 13. ANALYSIS OF OVARIAN TUMORS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
Granulosa Cell Tumor			
Overall Rates	0/49 (0%)	6/45 (13%)	12/48 (25%)
Adjusted Rates	0.0%	33.4%	36.6%
Terminal Rates	0/46 (0%)	4/15 (27%)	9/29 (31%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test	P<0.001		
Fisher Exact Tests		P=0.010	P<0.001

Nasal Cavity: Inflammation, fibrosis, osseous and cartilaginous metaplasia, and atrophy of the olfactory epithelium were observed in the region of the ethmoturbinates at increased incidences in high dose male mice (Table 14). No neoplastic lesions were observed at any concentration.

Preputial Gland: Carcinomas were observed at an increased incidence in dosed male mice (control, 0/50; low dose, 3/50, 6%; high dose, 2/50, 4%).

Brain: Gliomas were found in the brains of one high dose and two low dose male mice. An ependymoma was found in the brain of one low dose male mouse.

Zymbal Gland: Carcinomas were observed in two high dose male mice and one high dose female mouse.

Testis: Testicular atrophy was observed at an increased incidence in dosed male mice (control, 0/50; low dose, 19/47, 40%; high dose, 11/48, 23%).

TABLE 14. NUMBERS OF MICE WITH NONNEOPLASTIC LESIONS OF THE NASAL CAVITY IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
MALE			
No. of mice examined	50	50	50
Lesion			
Chronic inflammation	0	0	35
Fibrosis	0	0	35
Cartilaginous metaplasia	0	0	16
Osseous metaplasia	0	0	11
Atrophy of olfactory sensory epithelium	0	0	32
FEMALE			
No. of mice examined	50	49	49
Lesion			
Chronic inflammation	0	0	2
Fibrosis	0	0	2
Cartilaginous metaplasia	0	0	1
Osseous metaplasia	0	0	2
Atrophy of olfactory sensory epithelium	0	0	0

IV. DISCUSSION AND CONCLUSIONS

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Male and female B6C3F$_1$ mice in 15-day and 14-week studies were exposed to air containing 1,3-butadiene at concentrations of 0-8,000 ppm. In the 15-day studies, survival was unaffected by dose and no pathologic effects were observed; mean body weight gain decreased slightly at the high concentrations. In the 14-week studies, mean body weight gain decreased with dose, and survival in the 5,000-ppm and 8,000-ppm groups of males was markedly reduced; no other compound-related effects were observed. Concentrations for the long-term studies were set at 0, 625, or 1,250 ppm. The doses used in the mouse studies conducted for the NTP were about one-sixth (high dose) and five-eighths (low dose) those used in the rat studies (0, 1,000, or 8,000 ppm) done by the Hazleton Labs Europe (1981); the duration of the mouse studies was approximately one-half that of the rat studies.

In the long-term studies of mice, increased mortality was apparent in groups of dosed males at week 22 and females at week 30. These studies were terminated at week 60 for males and week 61 for females because survival in dosed groups was reduced (male: control, 49/50; low dose, 11/50; high dose, 7/50; female: 46/50; 15/50; 30/50). Mean body weights of exposed mice were not significantly different from those of the controls. In spite of the shortened survival (mice lived to be 68-70 weeks old instead of 108-110 weeks of age in a 2-year study), significantly increased incidences of mesenchymal and epithelial neoplasms were observed at multiple sites. Neoplasms occurred in 1,3-butadiene-exposed mice at increased incidences in the lung, hematopoietic system, heart, and stomach in males and females and in the ovary, mammary gland (carcinomas), and liver in females. In the rat studies, compound-related neoplasms occurred in the pancreas and testes in males and in the uterus and mammary gland (fibroadenomas) in females (Hazleton Labs Europe, 1981). Neoplasms of the Zymbal gland and brain (glioma) were observed in both studies (Table 15). Brain neoplasms have not been found in 2,343 untreated male B6C3F$_1$ mice in 2-year studies in the NTP Program. Zymbal gland neoplasms have been diagnosed in 1/2,343 untreated male B6C3F$_1$ mice and in 0/2,486 untreated female B6C3F$_1$ mice in the NTP Program.

The decreased survival of mice exposed to 1,3-butadiene in these studies differed markedly from the survival of Sprague-Dawley rats exposed to the chemical at higher concentrations for 105-111 weeks (Hazleton Labs Europe, 1981). Twenty percent of the rats exposed at 1,000 or 8,000 ppm survived until week 111. In the studies in mice, the early deaths were considered to be due primarily to malignant tumors, including lymphomas and hemangiosarcomas involving multiple organs. Overall tumor incidences in these 61-week studies were 20% and 12% in concurrent controls as compared with a range of 80%-94% in exposed mice (male: 10/50; 44/50; 40/50; female: 6/50; 40/49; 46/49).

Toxic and proliferative lesions of the nasal cavity occurred at increased incidences in the high dose groups: chronic inflammation, fibrosis, cartilaginous metaplasia, and osseous metaplasia were found in males and females, and atrophy of the olfactory sensory epithelium was seen in male but not female mice. In Sprague-Dawley rats exposed to 1,3-butadiene at 8,000 ppm for 105-111 weeks, focal alveolar epithelialization in the respiratory tract was observed; investigators in that study did not consider the effect to be compound related (Hazleton Labs Europe, 1981). In humans, mucosal changes in the upper respiratory tract (which altered sensory perception), laryngotracheitis, and bronchitis were reported in workers in the synthetic rubber industry in Eastern Europe (Gunter and Lucas, 1973; Ripp, 1968; Volkova and Bagdinov, 1969; Faustov, 1972).

Alveolar/bronchiolar adenomas or carcinomas (combined) occurred at significantly increased incidences in dosed mice of each sex (male: 2/50; 14/49; 15/49; female: 3/49; 12/48; 23/49). Compound-related neoplasms were not found in the lungs of the Sprague-Dawley rats exposed at a higher concentration for a longer period of time.

Malignant lymphomas occurred at various sites in male and female mice at increased incidences and were considered to be the primary cause of early deaths. These lesions were found in 21/39 low dose and 28/43 high dose males and in 7/34 low dose and 9/19 high dose females that died or were killed before week 60. Lymphomas were

TABLE 15. SIGNIFICANT EFFECTS OF EXPOSURE TO 1,3-BUTADIENE ON SPRAGUE-DAWLEY RATS AND B6C3F$_1$ MICE IN INHALATION STUDIES

	RATS (a) (Hazleton Labs Europe, 1981)	
	1,000 ppm	8,000 ppm
NEOPLASMS		
Male		
	Leydig cell adenoma (b)	Leydig cell adenoma (b) Pancreas: exocrine tumors (b) Brain: glioma
Female		
	Mammary gland: fibroadenoma/carcinoma (b) Thyroid: follicular cell adenoma (b) Uterus: stromal sarcoma (b)	Mammary gland: fibroadenoma/carcinoma (b) Thyroid: follicular cell adenoma (b) Uterus: stromal sarcoma (b) Zymbal gland: carcinoma (b)
NONNEOPLASTIC LESIONS		
Male		
		Increased focal alveolar epithelialization Nephropathy

	MICE (c) (NTP, this report)	
	625 ppm	1,250 ppm
NEOPLASMS		
Male		
	Heart: hemangiosarcoma (b) Malignant lymphoma (b) Lung: alveolar/bronchiolar adenoma and carcinoma (b) Forestomach: papilloma (b) Preputial gland: squamous cell carcinoma (d) Brain: glioma (d)	Heart: hemangiosarcoma (b) Malignant lymphoma (b) Lung: alveolar/bronchiolar adenoma and carcinoma (b) Preputial gland: squamous cell carcinoma (d) Zymbal gland: carcinoma (d) Brain: glioma (d)
Female		
	Heart: hemangiosarcoma (b) Malignant lymphoma (b) Lung: alveolar/bronchiolar adenoma and carcinoma (b) Forestomach: papilloma (b) Ovary: granulosa cell tumor (b)	Heart: hemangiosarcoma (b) Malignant lymphoma (b) Lung: alveolar/bronchiolar adenoma and carcinoma (b) Forestomach: papilloma (b) Mammary gland: acinar cell carcinoma (b) Ovary: granulosa cell tumor (b) Liver: hepatocellular adenoma or carcinoma (combined) (b)
NONNEOPLASTIC LESIONS		
Male		
	Forestomach: epithelial hyperplasia (b) Liver necrosis (b) Testicular atrophy (b)	Forestomach: epithelial hyperplasia (b) Liver necrosis (b) Nasal cavity lesions (chronic inflammation, fibrosis, cartilaginous metaplasia, osseous metaplasia, atrophy of sensory epithelium (b) Testicular atrophy (b)
Female		
	Liver necrosis (b) Forestomach: epithelial hyperplasia (b) Ovary: atrophy (b) Uterus: involution (b)	Forestomach: epithelial hyperplasia (b) Ovary: atrophy (b) Uterus: involution (b)

(a) Groups of 100 male and female Sprague-Dawley rats were exposed to air containing 0, 1,000, or 8,000 ppm 1,3-butadiene 6 h/d, 5 d/wk for 105 wk (female), or 111 wk (male); survival in dosed groups decreased.
(b) Statistically significant ($P < 0.05$)
(c) Groups of 50 male and female B6C3F$_1$ mice were exposed to air containing 0, 625, or 1,250 ppm 1,3-butadiene 6 h/d, 5 d/wk for 60 wk (male), or 61 wk (female); survival in dosed groups decreased and was the reason for early termination.
(d) Considered uncommon at 60 wk

observed as early as week 20 in a high dose female; most of the deaths attributed to this tumor occurred between weeks 40 and 45. Increased incidences of lymphomas were not observed in the Hazleton rat study.

The incidences of hemangiosarcoma of the heart were increased in dosed male and female mice after the animals had been on test for only 60-61 weeks. The heart lesions were striking in that a spectrum of changes was observed: changes varied from the presence of more prominent endothelial cells (diagnosed as atypical hyperplasia) to frank tumor masses. Hemangiomas and hemangiosarcomas of the heart are rare tumors; only 1/2,372 and 1/2,372 have been observed in untreated male and 0/2,443 and 1/2,443 in untreated female B6C3F$_1$ mice in 2-year studies in the NTP Carcinogenesis Program. These heart lesions were not observed in the Sprague-Dawley rats.

Although increased incidences of necrosis of the liver were associated with exposure to 1,3-butadiene in male and female mice (male: 1/50, 8/49, 8/49; female: 6/50, 15/47, 6/49), a decreased incidence of hepatocellular neoplasms was observed in dosed male mice. The increased incidence of hepatocellular adenomas or carcinomas (combined) (0/50, 2/47, 5/49) in dosed female mice, however, was considered to be due to exposure to 1,3-butadiene, largely because of the short latency period. Increased incidences of hepatocellular neoplasms were not observed in rats in the Hazleton study (Hazleton Labs Europe, 1981).

Epithelial hyperplasia of the forestomach in male and female mice and papillomas or carcinomas of the forestomach in males occurred at significantly increased incidences. Although unlikely, these lesions could have resulted from ingesting the test chemical when the mice licked their fur or from swallowing the chemical during exposure. These lesions were not increased in Sprague-Dawley rats.

Testicular and ovarian atrophy and uterine involution observed in mice were associated with exposure to 1,3-butadiene. Testicular or Leydig cell tumors were reported in the long-term inhalation studies in Sprague-Dawley rats (Hazleton Labs Europe, 1981), but they were not seen in the 60- 61-week studies in mice. The testicular atrophy (0/50, 19/47, 11/48), ovarian atrophy (2/49, 40/45, 40/48), and uterine involution (0/49, 7/46, 14/49) observed in exposed mice were not seen in the rat studies. The morphology of the uterine epithelium changed from hyperplastic endometrial epithelium in the controls to a less active involuted appearance in some of the low dose and high dose animals. The involuted uteri were characterized by fewer and less prominent endometrial glands. The observed uterine involution is considered to be secondary to ovarian atrophy. In dosed female mice, granulosa cell tumors of the ovary and acinar cell carcinomas of the mammary gland were seen at increased incidences. Tumors of the ovary were not seen at increased incidences in the Hazleton inhalation study conducted with Sprague-Dawley rats; however, those rats had increased incidences of acinar cell tumors of the mammary gland (females) and Leydig cell tumors of the testes. Follicular cell adenomas of the thyroid gland were seen in only one control male and one low dose female mouse, although the thyroid was one of the primary sites affected in Sprague-Dawley rats (Hazleton Labs Europe, 1981).

Several types of neoplasms occurred in dosed mice at incidences that were marginally increased relative to those of the controls: squamous cell carcinomas of the preputial gland in low dose males, gliomas of the brain in two low dose males and one high dose male, carcinomas of the Zymbal gland in two high dose males and one high dose female, and adenosquamous carcinomas of the mammary gland in four low dose females (three metastasized to the lungs). Brain and Zymbal gland neoplasms were also noted in the study of Sprague-Dawley rats (Hazleton Labs Europe, 1981).

The results of these two carcinogenesis studies in rodents strongly suggest that B6C3F$_1$ mice are more susceptible to the carcinogenic effects of 1,3-butadiene than are Sprague-Dawley rats. Comparative tissue-disposition studies in Sprague-Dawley rats and B6C3F$_1$ mice are being conducted by NTP. Additional metabolism studies (F344/N vs Sprague-Dawley rats, males vs females) are scheduled by NTP.

In vitro tests indicate that the mutagenic form of 1,3-butadiene is one or more volatile oxidative metabolites that are base-pair substitution

mutagens. Although in vivo tests have not determined the chemical's metabolic pathways, the observed carcinogenicity of 1,3-butadiene may be due to the formation of such metabolites. The epoxy derivatives diepoxybutane, 1,2-epoxybutane, 2,3-epoxybutane, and 3,4-epoxybutene have been shown to be mutagenic in a variety of test systems.

Diepoxybutane has been shown to be mutagenic in bacteria (McCann et al., 1975; Voogd et al., 1981), yeast (Olszewska and Kilbey, 1975), Neurospora (Ong and de Serres, 1975), Drosophila (Sankaranarayanan, 1983), and mammalian cells (NTP, unpublished results). Diepoxybutane also has been shown to cause sister-chromatid exchanges in the mouse in vivo (Conner et al., 1983) and transformation of mammalian cells in vitro (Pienta, 1980). 1,2-Epoxybutane has been shown to be mutagenic in bacteria (McCann et al., 1975; Voogd et al., 1981), yeast (Migliore et al., 1982), Neurospora (Kolmark and Giles, 1955), Drosophila (Knaap et al., 1982), and mammalian cells (Amacher et al., 1980). It also has been shown to transform mammalian cells in vitro (Pienta, 1980). 2,3-Epoxybutane has been shown to be mutagenic in bacteria (Voogd et al., 1981), yeast (Migliore et al., 1982), and Neurospora (Kolmark and Giles, 1955). 3,4-Epoxybutene has been shown to be mutagenic in bacteria (Voogd et al., 1981). The mutagenic potency of the four epoxides was compared in only one study (Voogd et al., 1981), and the results in bacteria showed that the diepoxide was more potent than the monoepoxides.

Several of the epoxides of 1,3-butadiene were tested for carcinogenicity by subcutaneous injection in mice (Van Duuren, 1969). Although the duration, number of animals and controls used, and overall description of the studies are inadequate for a full carcinogenesis evaluation, the results indicated that 3,4-epoxybutene and both isomers of diepoxybutane were carcinogenic under the conditions of the study. In addition, the diepoxides appeared to be more potent than the monoepoxides, a finding in agreement with the mutagenic potencies of these epoxides in bacteria. Although the diepoxides are the most potent mutagenic/carcinogenic forms of the parent compound, further studies (e.g., metabolism, tissue distribution, kinetics) would assist in identifying which metabolite or combination of metabolites may be associated with the observed carcinogenic activity of 1,3-butadiene. 4-Vinyl-1-cyclohexene, the dimer of 1,2-butadiene, is currently on test in the NTP Carcinogenesis Program.

Conclusions: Under the conditions of these studies, there was *clear evidence of carcinogenicity** for 1,3-butadiene in male and female $B6C3F_1$ mice, as shown by increased incidences and early induction of hemangiosarcomas of the heart, malignant lymphomas, alveolar/bronchiolar adenomas and carcinomas, and papillomas of the stomach in males and females; and of acinar cell carcinomas of the mammary gland, granulosa cell tumors of the ovary, and hepatocellular adenomas and adenomas or carcinomas (combined) in females. 1,3-Butadiene was associated with nonneoplastic lesions in the respiratory epithelium, liver necrosis, and testicular or ovarian atrophy.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

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V. REFERENCES

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
GLIOMA, INVASIVE		1 (2%)	1 (2%)
#LUNG	(50)	(49)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	12 (24%)	11 (22%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	5 (10%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		22 (44%)	28 (56%)
#THYMUS	(43)	(20)	(13)
MALIGNANT LYMPHOMA, NOS		1 (5%)	1 (8%)
CIRCULATORY SYSTEM			
*MEDIASTINUM	(50)	(50)	(50)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
*PERITONEAL CAVITY	(50)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
#LUNG	(50)	(49)	(49)
HEMANGIOSARCOMA, METASTATIC		4 (8%)	
#HEART	(50)	(49)	(49)
HEMANGIOSARCOMA		16 (33%)	7 (14%)
#LIVER	(50)	(49)	(49)
HEMANGIOSARCOMA, METASTATIC		11 (22%)	5 (10%)
#PANCREAS	(50)	(46)	(46)
HEMANGIOSARCOMA, INVASIVE			1 (2%)
#KIDNEY	(50)	(48)	(48)
HEMANGIOSARCOMA, INVASIVE			1 (2%)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
HEPATOCELLULAR ADENOMA	5 (10%)	4 (8%)	1 (2%)
HEPATOCELLULAR CARCINOMA	3 (6%)	2 (4%)	1 (2%)
#FORESTOMACH	(49)	(40)	(44)
PAPILLOMA, NOS		3 (8%)	
SQUAMOUS CELL PAPILLOMA		2 (5%)	
SQUAMOUS CELL CARCINOMA		2 (5%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(48)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(50)	(43)	(47)
FOLLICULAR-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		3 (6%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(49)
GLIOMA, NOS		2 (4%)	1 (2%)
EPENDYMOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS			2 (4%)
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF THORAX	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	1	30	31
MORIBUND SACRIFICE		9	8
SCHEDULED SACRIFICE	49	11	7
TERMINAL SACRIFICE			
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			4
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	10	44	40
TOTAL PRIMARY TUMORS	11	73	61
TOTAL ANIMALS WITH BENIGN TUMORS	8	16	11
TOTAL BENIGN TUMORS	8	21	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	40	40
TOTAL MALIGNANT TUMORS	3	52	49
TOTAL ANIMALS WITH SECONDARY TUMORS##		14	9
TOTAL SECONDARY TUMORS		17	13
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(49)
SARCOMA, NOS		2 (4%)	2 (4%)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
OSTEOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	9 (19%)	20 (41%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		6 (12%)	8 (16%)
ACINAR-CELL CARCINOMA, METASTATIC		1 (2%)	1 (2%)
ADENOSQUAMOUS CARCINOMA, METASTATIC		3 (6%)	
GRANULOSA-CELL CARCINOMA, METASTATIC			1 (2%)
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)	8 (16%)	10 (20%)
#LYMPH NODE	(46)	(41)	(44)
ALVEOLAR/BRONCHIOLAR CA, METASTATIC			1 (2%)
GRANULOSA-CELL CARCINOMA, METASTATIC			1 (2%)
#LIVER	(50)	(47)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#THYMUS	(47)	(21)	(33)
MALIGNANT LYMPHOMA, NOS		1 (5%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(49)
HEMANGIOSARCOMA		2 (4%)	
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
#LYMPH NODE	(46)	(41)	(44)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
#LUNG	(49)	(48)	(49)
HEMANGIOSARCOMA, METASTATIC		4 (8%)	6 (12%)
#HEART	(49)	(48)	(49)
HEMANGIOSARCOMA		11 (23%)	18 (37%)
NEUROFIBROSARCOMA			1 (2%)
#LIVER	(50)	(47)	(49)
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC		7 (15%)	9 (18%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(46)	(45)	(46)
CARCINOSARCOMA			1 (2%)
#LIVER	(50)	(47)	(49)
HEPATOCELLULAR ADENOMA		1 (2%)	4 (8%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#PANCREAS	(49)	(45)	(48)
ADENOCARCINOMA, NOS			1 (2%)
#FORESTOMACH	(49)	(42)	(49)
PAPILLOMA, NOS		1 (2%)	9 (18%)
SQUAMOUS CELL PAPILLOMA		3 (7%)	1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(49)
TUBULAR-CELL ADENOMA		1 (2%)	
NEUROFIBROSARCOMA, METASTATIC			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(48)	(46)	(49)
FOLLICULAR-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
ACINAR-CELL CARCINOMA		2 (4%)	6 (12%)
ADENOSQUAMOUS CARCINOMA		4 (8%)	
#UTERUS	(49)	(46)	(49)
ENDOMETRIAL STROMAL POLYP	1 (2%)		
#OVARY	(49)	(45)	(48)
CYSTADENOMA, NOS		1 (2%)	
GRANULOSA-CELL TUMOR		6 (13%)	12 (25%)
GRANULOSA-CELL CARCINOMA			1 (2%)
TUBULAR ADENOMA		2 (4%)	
MIXED TUMOR, BENIGN			2 (4%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*ZYMBAL GLAND	(50)	(49)	(49)
CARCINOMA,NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*BONE	(50)	(49)	(49)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	4	26	12
MORIBUND SACRIFICE		7	6
SCHEDULED SACRIFICE	46	14	30
TERMINAL SACRIFICE			
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			1
ACCIDENTALLY KILLED, NOS		3	
ANIMAL MISSING			1
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	6	40	46
TOTAL PRIMARY TUMORS	6	66	100
TOTAL ANIMALS WITH BENIGN TUMORS	4	15	28
TOTAL BENIGN TUMORS	4	19	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	35	36
TOTAL MALIGNANT TUMORS	2	41	52
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	18	17
TOTAL SECONDARY TUMORS	1	22	24
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		6	12
TOTAL UNCERTAIN TUMORS		6	12
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

** PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: CHAMBER CONTROL

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060	060
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+
THYMUS	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA							X												X		X	X			
HEPATOCELLULAR CARCINOMA										X															
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA					X																				
PARATHYROID	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
S: ANIMAL MIS-SEXED
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: CHAMBER CONTROL (Continued)

ANIMAL NUMBER	5 2 6	5 2 7	5 2 8	5 2 9	5 3 0	5 3 1	5 3 2	5 3 3	5 3 4	5 3 5	5 3 6	5 3 7	5 3 8	5 3 9	5 4 0	5 4 1	5 4 2	5 4 3	5 4 4	5 4 5	5 4 6	5 4 7	5 4 8	5 4 9	5 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 5 3	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	0 6 0	
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA											X				X											2
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	-	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	41
THYMUS	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	43
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA						•																				5
HEPATOCELLULAR CARCINOMA											X						X						X			3
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	N	+	N	50N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	47
LARGE INTESTINE	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	46
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	-	+	+	+	+	+	+	+	+	41
ADRENAL	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL ADENOMA																										1
PARATHYROID	-	+	+	-	+	+	-	+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	39
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	45
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

N ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: LOW DOSE

ANIMAL NUMBER	50 1	50 2	50 3	50 4	50 5	50 6	50 7	50 8	50 9	50 10	51 1	51 2	51 3	51 4	51 5	51 6	51 7	51 8	51 9	51 10	52 1	52 2	52 3	52 4	52 5
WEEKS ON STUDY	60 5 6	60 2 5	60 2 6	60 4 8	60 4 6	60 4 8	60 3 4	60 3 7	60 4 6	60 5 0	60 3 6	60 3 0	60 3 8	60 4 3	60 4 2	60 4 4	60 4 6	60 4 4	60 4 4	60 5 1	60 4 6	60 5 3	60 4 0	60 5 3	60 5 2
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA				X	X	X			X						X					X				X	
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
HEMANGIOSARCOMA, METASTATIC								X																X	
TRACHEA	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
NASAL CAVITY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, INVASIVE																		X							
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
THYMUS	-	-	+	+	+	+	+	-	-	+	+	-	-	+	-	-	+	-	-	-	-	-	+	-	+
MALIGNANT LYMPHOMA, NOS																									
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA	X							X		X				X	X						X			X	
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA																					X			X	
HEPATOCELLULAR CARCINOMA																									
HEMANGIOSARCOMA, METASTATIC	X													X										X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	M	M	+	+	+	M	M	M	+	+	M	M	M	M	M	+	+	+	M	M	M	M	+	M	M
PANCREAS	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	-	+	+	-	-	+	+	-	+	+	+	+	+	+	+	+	+	+
PAPILLOMA, NOS						X																			
SQUAMOUS CELL PAPILLOMA																									
SQUAMOUS CELL CARCINOMA					X																				
SMALL INTESTINE	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	-	+	+	-	+	-	-	+	+	-
LARGE INTESTINE	-	+	+	-	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+
PARATHYROID	+	-	-	+	+	+	-	+	-	-	-	+	-	-	+	-	-	+	+	+	+	+	-	+	-
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
PROSTATE	+	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	+	-	+	+	+	+	+	-	+
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
SQUAMOUS CELL CARCINOMA																							X		
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, NOS																		X							
EPENDYMOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
SQUAMOUS CELL CARCINOMA, METASTAT					X																				
FIBROUS HISTIOCYTOMA, MALIGNANT																									
MALIGNANT LYMPHOMA, NOS		X	X				X			X	X	X			X	X		X	X				X	X

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE : LOW DOSE (Continued)

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL TISSUES / TUMORS
RESPIRATORY SYSTEM	
LUNGS AND BRONCHI	49
ALVEOLAR/BRONCHIOLAR ADENOMA	12
ALVEOLAR/BRONCHIOLAR CARCINOMA	2
HEMANGIOSARCOMA, METASTATIC	4
TRACHEA	45
NASAL CAVITY	50M
GLIOMA, INVASIVE	1
HEMATOPOIETIC SYSTEM	
BONE MARROW	48
SPLEEN	46
LYMPH NODES	42
THYMUS	28
MALIGNANT LYMPHOMA, NOS	1
CIRCULATORY SYSTEM	
HEART	49
HEMANGIOSARCOMA	16
DIGESTIVE SYSTEM	
SALIVARY GLAND	47
LIVER	49
HEPATOCELLULAR ADENOMA	4
HEPATOCELLULAR CARCINOMA	2
HEMANGIOSARCOMA, METASTATIC	11
BILE DUCT	49
GALLBLADDER & COMMON BILE DUCT	50M
PANCREAS	48
ESOPHAGUS	47
STOMACH	48
PAPILLOMA, NOS	1
SQUAMOUS CELL PAPILLOMA	2
SQUAMOUS CELL CARCINOMA	2
SMALL INTESTINE	26
LARGE INTESTINE	37
URINARY SYSTEM	
KIDNEY	48
URINARY BLADDER	46
ENDOCRINE SYSTEM	
PITUITARY	41
ADRENAL	48
THYROID	43
PARATHYROID	26
REPRODUCTIVE SYSTEM	
MAMMARY GLAND	50M
TESTIS	47
PROSTATE	37
PREPUTIAL/CLITORAL GLAND	50M
SQUAMOUS CELL CARCINOMA	3
NERVOUS SYSTEM	
BRAIN	48
GLIOMA, NOS	2
EPENDYMOMA	1
ALL OTHER SYSTEMS	
MULTIPLE ORGANS NOS	50M
SQUAMOUS CELL CARCINOMA, METASTAT	1
FIBROUS HISTIOCYTOMA, MALIGNANT	1
MALIGNANT LYMPHOMA, NOS	22

* ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: HIGH DOSE

ANIMAL NUMBER	5 6 1 1	5 6 0 2	5 6 0 3	5 6 0 4	5 6 0 5	5 6 0 6	5 6 0 7	5 6 0 8	5 6 0 9	5 6 1 0	5 6 1 1	5 6 1 2	5 6 1 3	5 6 1 4	5 6 1 5	5 6 1 6	5 6 1 7	5 6 1 8	5 6 1 9	5 6 2 0	5 6 2 1	5 6 2 2	5 6 2 3	5 6 2 4	5 6 2 5
WEEKS ON STUDY	0 6 8	0 6 0	0 6 0	0 6 4	0 6 5	0 6 5	0 6 5	0 7 4	0 7 6	0 7 8	0 7 8	0 8 1	0 8 1	0 8 1	0 8 3	0 8 5	0 8 9	0 8 9	0 8 9	0 8 9	0 9 3	0 9 5	0 9 7	0 9 7	0 9 7
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA	X	X								X										X					
ALVEOLAR/BRONCHIOLAR CARCINOMA	X																								
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+
NASAL CAVITY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, INVASIVE																									
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+
SPLEEN	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+
LYMPH NODES	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+
THYMUS	-	+	-	-	+	-	+	-	+	+	+	+	+	+	-	-	A	-	-	-	-	-	-	-	+
MALIGNANT LYMPHOMA, NOS		X																							
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA		X	X																	X				X	
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA																				X					
HEPATOCELLULAR CARCINOMA		X	X																	X					
HEMANGIOSARCOMA, METASTATIC																									
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	N	N	N	N	N	N	+	N	N	N	N	+	N	N	N	+	+	+	N	+	N	+
PANCREAS	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA, INVASIVE																									
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	-	+	+	+	-	+	+	-	+
SQUAMOUS CELL CARCINOMA						X																			
SMALL INTESTINE	+	+	-	+	-	+	-	-	-	+	-	+	+	-	-	A	-	-	+	+	-	-	+	-	-
LARGE INTESTINE	+	+	+	-	+	+	-	-	+	+	+	+	+	+	-	A	-	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR CA, METASTAT																									
HEMANGIOSARCOMA, INVASIVE																									
HEMANGIOSARCOMA, METASTATIC			X																						
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	A	-	+	+	+	+	+	+	-	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	-	+	+	+
ADRENAL	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+
PARATHYROID	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	A	-	-	+	+	-	+	-	-	-
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
PROSTATE	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	A	-	+	+	+	-	+	+	-	+
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA, NOS		X																							
SQUAMOUS CELL CARCINOMA	X																								
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, NOS																									
SPECIAL SENSE ORGANS																									
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA, NOS										X															
MUSCULOSKELETAL SYSTEM																									
MUSCLE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																									
BODY CAVITIES																									
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
HEMANGIOSARCOMA, METASTATIC																									
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
HEMANGIOSARCOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SQUAMOUS CELL CARCINOMA, METASTAT						X																			
MALIGNANT LYMPHOMA, NOS				X	X			X	X				X	X			X	X		X	X		X	X	

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	3 2 6	3 2 7	3 2 8	3 2 9	3 3 0	3 3 1	3 3 2	3 3 3	3 3 4	3 3 5	3 3 6	3 3 7	3 3 8	3 3 9	3 4 0	3 4 1	3 4 2	3 4 3	3 4 4	3 4 5	3 4 6	3 4 7	3 4 8	3 4 9	3 5 0	TOTAL
WEEKS ON STUDY	0 3 8	0 5 1	0 6 1	0 8 1	0 3 1	0 4 2	0 6 0	0 4 3	0 4 8	0 5 7	0 4 0	0 4 1	0 4 2	0 5 1	0 4 5	0 2 2	0 4 2	0 4 9	0 4 2	0 4 9	0 4 2	0 1 1	0 2 1	0 5 1	0 4 4	TISSUES TUMORS
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA	X	X	X	X				X		X								X			X					11
ALVEOLAR/BRONCHIOLAR CARCINOMA							X						X	X												5
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+	+	+	+	+	46
NASAL CAVITY	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
GLIOMA, INVASIVE			X																							1
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	-	+	+	+	+	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	45
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	-	+	+	+	+	+	+	+	+	45
LYMPH NODES	-	+	+	+	+	+	+	+	-	-	+	A	+	+	+	+	+	+	+	+	-	-	+	-	+	40
THYMUS	-	-	-	-	-	+	-	+	-	-	-	A	-	-	-	-	-	-	-	-	+	-	-	-	+	13
MALIGNANT LYMPHOMA, NOS																										1
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMANGIOSARCOMA				X						X			X													7
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA																										1
HEPATOCELLULAR CARCINOMA		X																								1
HEMANGIOSARCOMA, METASTATIC				X						X																5
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	+	N	N	+	+	N	+	N	+	N	N	+	+	+	N	+	N	+	+	N	N	+	+	38M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	46
HEMANGIOSARCOMA, INVASIVE	X																									1
ESOPHAGUS	+	+	+	+	+	+	+	+	+	-	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	47
STOMACH	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	-	+	+	+	+	+	+	+	+	44
SQUAMOUS CELL CARCINOMA																										1
SMALL INTESTINE	+	+	+	-	-	-	+	-	+	+	+	A	-	+	+	-	-	+	-	+	+	+	+	+	+	26
LARGE INTESTINE	-	+	+	-	+	+	+	+	-	+	+	A	-	+	+	+	-	-	+	+	+	+	+	+	+	37
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ALVEOLAR/BRONCHIOLAR CA, METASTAT													X													1
HEMANGIOSARCOMA, INVASIVE	X																									1
HEMANGIOSARCOMA, METASTATIC																										1
URINARY BLADDER	-	+	+	+	+	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+	+	+	-	+	42
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	-	+	-	+	A	+	+	+	-	+	+	+	+	+	+	-	+		41
ADRENAL	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	46
THYROID	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	47
PARATHYROID	+	+	-	-	-	+	-	+	+	-	-	A	+	-	-	-	+	-	-	+	-	-	+	+		25
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
TESTIS	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	48
PROSTATE	+	-	+	+	+	-	+	+	-	+	+	A	-	+	+	+	+	-	+	-	-	+	-	+	+	33
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
CARCINOMA, NOS																										1
SQUAMOUS CELL CARCINOMA																										1
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GLIOMA, NOS			X																							1
SPECIAL SENSE ORGANS																										
ZYMBAL'S GLAND	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
CARCINOMA, NOS			X																							2
MUSCULOSKELETAL SYSTEM																										
MUSCLE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE													X													1
BODY CAVITIES																										
MEDIASTINUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
HEMANGIOSARCOMA, METASTATIC	X																									1
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	N	N	50M
HEMANGIOSARCOMA	X																									1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
SQUAMOUS CELL CARCINOMA, METASTAT																										1
MALIGNANT LYMPHOMA, NOS		X			X	X		X	X		X			X	X	X	X	X	X	X			X	X	X	28

M ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: CHAMBER CONTROL

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	061	061	061	061	061	061	061	061	002	061	061	061	061	061	061	061	061	061	061	030	061	061	061	061	061
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
OSTEOSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA													X												
OSTEOSARCOMA, METASTATIC																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	-	+	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-
LARGE INTESTINE	+	+	+	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PARATHYROID	-	-	+	+	+	-	-	+	-	+	+	+	-	+	-	-	-	+	+	+	+	+	+	-	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	N	+	+	+	+	+	+	N	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOMETRIAL STROMAL POLYP		X																							
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIGNANT LYMPHOMA, NOS							X																		

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
S: ANIMAL MIS-SEXED
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: CHAMBER CONTROL (Continued)

ANIMAL NUMBER	52061	52071	52081	52091	53011	53021	53031	53041	53051	53061	53071	53081	53091	53101	54011	54021	54031	54041	54051	54061	54071	54081	54091	54101	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	0 6 4	0 6 1	0 6 1	0 6 1	0 6 1	0 6 1	
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	50M
OSTEOSARCOMA								X																	1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA										X					X				X						3
OSTEOSARCOMA, METASTATIC								X																		1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
LYMPH NODES	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	44
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	47
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
DIGESTIVE SYSTEM																									
SALIVARY GLAND	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	44
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	+	M	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
ESOPHAGUS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	47
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
SMALL INTESTINE	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	41
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	-	45
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
URINARY BLADDER	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	45
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	45
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	48
PARATHYROID	+	-	+	-	+	+	+	+	-	+	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	30
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	50M
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
ENDOMETRIAL STROMAL POLYP																									1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	49
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
MALIGNANT LYMPHOMA, NOS																									1

M ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: LOW DOSE

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	058	046	002	040	061	059	055	056	056	056	061	052	033	061	061	061	061	061	052	061	032	061	057	047	039
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	N	+	+	+	+	+	N	+	+	+	+	N	+	N	+	+	+	+	+	+	+	+	B	+
SARCOMA, NOS						X																			
FIBROUS HISTIOCYTOMA, MALIGNANT																									
HEMANGIOSARCOMA																						X			
HEMANGIOSARCOMA, METASTATIC																							X		
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B	+
HEPATOCELLULAR CARCINOMA, METASTA														X											
ALVEOLAR/BRONCHIOLAR ADENOMA									X					X						X			X		
ALVEOLAR/BRONCHIOLAR CARCINOMA															X			X							
ACINAR-CELL CARCINOMA, METASTATIC																				X					
ADENOSQUAMOUS CARCINOMA, METASTAT												X													
HEMANGIOSARCOMA, METASTATIC															X			X	X						
TRACHEA	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B	+
SPLEEN	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
LYMPH NODES	+	+	-	+	+	-	A	-	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	B	+
THYMUS	-	-	+	-	+	-	A	+	+	-	+	-	-	+	+	+	-	-	-	+	-	+	+	B	-
MALIGNANT LYMPHOMA, NOS																									
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B	+
HEMANGIOSARCOMA	X								X	X					X			X	X				X		
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	-	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
LIVER	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
HEPATOCELLULAR ADENOMA																									
HEPATOCELLULAR CARCINOMA														X											
HEMANGIOSARCOMA, METASTATIC	X									X					X				X				X		
MALIGNANT LYMPHOMA, NOS																									
BILE DUCT	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
GALLBLADDER & COMMON BILE DUCT	+	+	N	N	+	N	N	N	+	N	+	N	N	+	+	+	+	+	N	+	N	+	N	B	+
PANCREAS	+	+	+	+	+	+	A	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	B	-
STOMACH	+	+	+	+	+	+	A	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	B	+
PAPILLOMA, NOS											X														
SQUAMOUS CELL PAPILLOMA					X									X			X								
SQUAMOUS CELL CARCINOMA																									
SMALL INTESTINE	+	+	+	-	+	+	A	-	+	+	+	-	-	+	+	+	+	+	-	+	-	-	+	B	-
LARGE INTESTINE	+	+	+	+	+	+	A	+	+	+	+	-	-	+	+	-	+	+	-	+	-	+	+	B	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
TUBULAR-CELL ADENOMA																						X			
URINARY BLADDER	+	+	+	+	+	+	A	+	+	+	+	-	+	+	+	+	+	+	-	+	-	+	+	B	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	A	-	-	+	+	-	-	+	+	+	+	+	+	+	+	+	+	B	+
ADRENAL	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
THYROID	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	B	+
FOLLICULAR-CELL ADENOMA																									
PARATHYROID	+	-	-	+	-	+	A	-	-	+	-	-	+	+	-	+	+	+	+	+	-	+	-	B	-
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	N	+	N	+	+	N	+	+	+	N	+	N	N	+	+	+	+	N	+	+	+	+	B	+
ACINAR-CELL CARCINOMA																		X		X					
ADENOSQUAMOUS CARCINOMA												X													
UTERUS	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	B	+
OVARY	+	+	+	+	+	+	A	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	B	+
CYSTADENOMA, NOS																	X								
GRANULOSA-CELL TUMOR					X				X		X									X					
TUBULAR ADENOMA											X							X							
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	B	+
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	B	N
OSTEOSARCOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	B	N
SQUAMOUS CELL CARCINOMA, METASTAT																									
SARCOMA, NOS, METASTATIC						X																			
FIBROUS HISTIOCYTOMA, METASTATIC																									
HEMANGIOSARCOMA, METASTATIC									X																
MALIGNANT LYMPHOMA, NOS		X		X										X							X				X

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE : LOW DOSE (Continued)

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL TISSUES / TUMORS
INTEGUMENTARY SYSTEM	
SUBCUTANEOUS TISSUE	49M
SARCOMA, NOS	2
FIBROUS HISTIOCYTOMA, MALIGNANT	1
HEMANGIOSARCOMA	2
HEMANGIOSARCOMA, METASTATIC	1
RESPIRATORY SYSTEM	
LUNGS AND BRONCHI	48
HEPATOCELLULAR CARCINOMA, METASTA	1
ALVEOLAR/BRONCHIOLAR ADENOMA	9
ALVEOLAR/BRONCHIOLAR CARCINOMA	6
ACINAR-CELL CARCINOMA, METASTATIC	1
ADENOSQUAMOUS CARCINOMA, METASTAT	3
HEMANGIOSARCOMA, METASTATIC	1
TRACHEA	44
HEMATOPOIETIC SYSTEM	
BONE MARROW	48
SPLEEN	46
LYMPH NODES	41
THYMUS	21
MALIGNANT LYMPHOMA, NOS	1
CIRCULATORY SYSTEM	
HEART	48
HEMANGIOSARCOMA	11
DIGESTIVE SYSTEM	
SALIVARY GLAND	48
LIVER	47
HEPATOCELLULAR ADENOMA	1
HEPATOCELLULAR CARCINOMA	1
HEMANGIOSARCOMA, METASTATIC	7
MALIGNANT LYMPHOMA, NOS	1
BILE DUCT	47
GALLBLADDER & COMMON BILE DUCT	49M
PANCREAS	45
ESOPHAGUS	45
STOMACH	42
PAPILLOMA, NOS	1
SQUAMOUS CELL PAPILLOMA	3
SQUAMOUS CELL CARCINOMA	1
SMALL INTESTINE	28
LARGE INTESTINE	36
URINARY SYSTEM	
KIDNEY	47
TUBULAR-CELL ADENOMA	1
URINARY BLADDER	44
ENDOCRINE SYSTEM	
PITUITARY	48
ADRENAL	47
THYROID	46
FOLLICULAR-CELL ADENOMA	1
PARATHYROID	23
REPRODUCTIVE SYSTEM	
MAMMARY GLAND	49M
ACINAR-CELL CARCINOMA	2
ADENOSQUAMOUS CARCINOMA	4
UTERUS	46
OVARY	45
CYSTADENOMA, NOS	1
GRANULOSA-CELL TUMOR	6
TUBULAR ADENOMA	2
NERVOUS SYSTEM	
BRAIN	48
MUSCULOSKELETAL SYSTEM	
BONE	49M
OSTEOSARCOMA	1
ALL OTHER SYSTEMS	
MULTIPLE ORGANS NOS	49M
SQUAMOUS CELL CARCINOMA, METASTAT	1
SARCOMA, NOS, METASTATIC	2
FIBROUS HISTIOCYTOMA, METASTATIC	1
HEMANGIOSARCOMA, METASTATIC	1
MALIGNANT LYMPHOMA, NOS	8

M ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE: HIGH DOSE

ANIMAL NUMBER	501	502	503	504	505	506	507	508	509	510	511	512	513	514	515	516	517	518	519	520	521	522	523	524	525
WEEKS ON STUDY	061	043	060	061	022	061	061	061	061	058	061	061	061	061	056	048	061	061	061	028	061	061	048	061	058
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS																					X				
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA			X					X		X		X	X				X	X	X		X			X	X
ALVEOLAR/BRONCHIOLAR CARCINOMA			X															X						X	
ACINAR-CELL CARCINOMA, METASTATIC																									
GRANULOSA-CELL CARCINOMA, METASTA																									
HEMANGIOSARCOMA, METASTATIC										X					X										
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+
SPLEEN	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	-	+	+
ALVEOLAR/BRONCHIOLAR CA, METASTAT			X																						
GRANULOSA-CELL CARCINOMA, METASTA																									
HEMANGIOSARCOMA, METASTATIC																									
THYMUS	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	-	-	+	+	-	+	+	-	+	-
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA		X	X	X		X				X	X				X		X		X					X	
NEUROFIBROSARCOMA			X																						
DIGESTIVE SYSTEM																									
SALIVARY GLAND	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
CARCINOSARCOMA																	X								
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA											X														X
HEPATOCELLULAR CARCINOMA																									
HEMANGIOSARCOMA													X												
HEMANGIOSARCOMA, METASTATIC		X		X						X					X									X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	N	+	+	N	+	+	+	+	N	+	+	+	+	N	N	N	+	+	+	N	+	N	+	N
PANCREAS	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS																			X						
ESOPHAGUS	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PAPILLOMA, NOS	X			X				X																	
SQUAMOUS CELL PAPILLOMA																									
SQUAMOUS CELL CARCINOMA																									
SMALL INTESTINE	+	+	-	-	-	+	+	+	+	-	+	+	+	+	-	-	+	+	+	+	+	+	+	+	-
LARGE INTESTINE	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEUROFIBROSARCOMA, METASTATIC			X																						
URINARY BLADDER	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PARATHYROID	+	+	-	+	+	+	+	-	+	-	+	+	+	+	-	-	+	+	+	-	+	-	-	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	N	N	+	N	+	+	+	+	+	+	+	+	+	+	N	N	+	N	N	+	+	+	+	+
ACINAR-CELL CARCINOMA													X												
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GRANULOSA-CELL TUMOR									X				X	X							X				
GRANULOSA-CELL CARCINOMA																									
MIXED TUMOR, BENIGN																		X				X			
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
ZYMBAL'S GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA,NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SQUAMOUS CELL CARCINOMA, METASTAT																									
SARCOMA, NOS, METASTATIC																					X				
CARCINOSARCOMA, METASTATIC																	X								
MALIGNANT LYMPHOMA, NOS																X				X			X		X

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL TISSUES / TUMORS
INTEGUMENTARY SYSTEM	
SUBCUTANEOUS TISSUE	49M
SARCOMA, NOS	2
RESPIRATORY SYSTEM	
LUNGS AND BRONCHI	49
ALVEOLAR/BRONCHIOLAR ADENOMA	28
ALVEOLAR/BRONCHIOLAR CARCINOMA	8
ACINAR-CELL CARCINOMA, METASTATIC	1
GRANULOSA-CELL CARCINOMA, METASTA	1
HEMANGIOSARCOMA, METASTATIC	4
TRACHEA	48
HEMATOPOIETIC SYSTEM	
BONE MARROW	47
SPLEEN	48
LYMPH NODES	44
ALVEOLAR/BRONCHIOLAR CA, METASTAT	1
GRANULOSA-CELL CARCINOMA, METASTA	1
HEMANGIOSARCOMA, METASTATIC	1
THYMUS	33
CIRCULATORY SYSTEM	
HEART	49
HEMANGIOSARCOMA	18
NEUROFIBROSARCOMA	1
DIGESTIVE SYSTEM	
SALIVARY GLAND	46
CARCINOSARCOMA	1
LIVER	49
HEPATOCELLULAR ADENOMA	4
HEPATOCELLULAR CARCINOMA	1
HEMANGIOSARCOMA	1
HEMANGIOSARCOMA, METASTATIC	9
BILE DUCT	49
GALLBLADDER & COMMON BILE DUCT	49M
PANCREAS	48
ADENOCARCINOMA, NOS	1
ESOPHAGUS	46
STOMACH	49
PAPILLOMA, NOS	9
SQUAMOUS CELL PAPILLOMA	1
SQUAMOUS CELL CARCINOMA	1
SMALL INTESTINE	38
LARGE INTESTINE	43
URINARY SYSTEM	
KIDNEY	49
NEUROFIBROSARCOMA, METASTATIC	1
URINARY BLADDER	46
ENDOCRINE SYSTEM	
PITUITARY	43
ADRENAL	48
THYROID	49
PARATHYROID	38
REPRODUCTIVE SYSTEM	
MAMMARY GLAND	49M
ACINAR-CELL CARCINOMA	6
UTERUS	49
OVARY	48
GRANULOSA-CELL TUMOR	12
GRANULOSA-CELL CARCINOMA	1
MIXED TUMOR, BENIGN	2
NERVOUS SYSTEM	
BRAIN	49
SPECIAL SENSE ORGANS	
ZYMBAL'S GLAND	49M
CARCINOMA,NOS	1
ALL OTHER SYSTEMS	
MULTIPLE ORGANS NOS	49M
SQUAMOUS CELL CARCINOMA, METASTAT	1
SARCOMA, NOS, METASTATIC	1
CARCINOSARCOMA, METASTATIC	1
MALIGNANT LYMPHOMA, NOS	17

M ANIMALS NECROPSIED

APPENDIX B

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
FOREIGN BODY, NOS	1 (2%)		
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SEROUS			1 (2%)
INFLAMMATION, SUPPURATIVE	3 (6%)	1 (2%)	5 (10%)
INFLAMMATION, CHRONIC			35 (70%)
FIBROSIS			35 (70%)
METAPLASIA, CARTILAGINOUS			16 (32%)
METAPLASIA, SQUAMOUS			1 (2%)
METAPLASIA, OSSEOUS			11 (22%)
#TRACHEA	(49)	(45)	(46)
INFLAMMATION, SUPPURATIVE			1 (2%)
METAPLASIA, SQUAMOUS		3 (7%)	3 (7%)
#LUNG	(50)	(49)	(49)
MINERALIZATION			1 (2%)
CONGESTION, NOS	2 (4%)	5 (10%)	13 (27%)
HEMORRHAGE	6 (12%)	6 (12%)	7 (14%)
INFLAMMATION, FOCAL			1 (2%)
PERIVASCULAR CUFFING	3 (6%)		
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)	5 (10%)	2 (4%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(48)	(45)
NECROSIS, NOS		1 (2%)	
ATROPHY, NOS		6 (13%)	6 (13%)
HYPERPLASIA, GRANULOCYTIC		3 (6%)	3 (7%)
#SPLEEN	(50)	(46)	(45)
HYPERPLASIA, GRANULOCYTIC		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS		3 (7%)	2 (4%)
#LYMPH NODE	(41)	(42)	(40)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)
NECROSIS, NOS			1 (3%)
HYPERPLASIA, NOS	8 (20%)	1 (2%)	
#LUNG	(50)	(49)	(49)
HEMATOPOIESIS	1 (2%)		
#LIVER	(50)	(49)	(49)
HEMATOPOIESIS			1 (2%)
#THYMUS	(43)	(20)	(13)
CYST, NOS	2 (5%)		
ATROPHY, NOS		3 (15%)	4 (31%)
CIRCULATORY SYSTEM			
#LUNG	(50)	(49)	(49)
THROMBOSIS, NOS			1 (2%)
#HEART	(50)	(49)	(49)
MINERALIZATION		2 (4%)	5 (10%)
THROMBOSIS, NOS			2 (4%)
HEMORRHAGE		1 (2%)	1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM (Continued)			
FIBROSIS	1 (2%)		
DEGENERATION, NOS			2 (4%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
HYPERPLASIA, ATYPICAL		5 (10%)	2 (4%)
DIGESTIVE SYSTEM			
*TOOTH	(50)	(50)	(50)
NECROSIS, NOS	2 (4%)		
#SALIVARY GLAND	(50)	(47)	(49)
INFLAMMATION, NOS	15 (30%)	4 (9%)	1 (2%)
#LIVER	(50)	(49)	(49)
HEMORRHAGE		7 (14%)	4 (8%)
HEMATOMA, NOS		1 (2%)	
DEGENERATION, NOS		1 (2%)	2 (4%)
NECROSIS, NOS	1 (2%)	5 (10%)	5 (10%)
NECROSIS, FOCAL		2 (4%)	3 (6%)
NECROSIS, CENTRAL		1 (2%)	
EOSINOPHILIC CYTO CHANGE	1 (2%)		
CLEAR-CELL CHANGE			1 (2%)
#LIVER/PERIPORTAL	(50)	(49)	(49)
FIBROSIS		1 (2%)	
#PANCREAS	(50)	(46)	(46)
INFLAMMATION, NOS	1 (2%)		
ATROPHY, NOS			2 (4%)
#ESOPHAGUS	(50)	(47)	(47)
DILATATION, NOS	1 (2%)		
#GLANDULAR STOMACH	(49)	(40)	(44)
ULCER, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#FORESTOMACH	(49)	(40)	(44)
ULCER, NOS			1 (2%)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL		5 (13%)	7 (16%)
HYPERKERATOSIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(48)
HEMORRHAGE	1 (2%)		
PYELONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, NOS	42 (84%)	8 (17%)	2 (4%)
PYELONEPHRITIS, ACUTE			2 (4%)
GLOMERULONEPHRITIS, CHRONIC	1 (2%)		
INFARCT, NOS		1 (2%)	1 (2%)
#KIDNEY/GLOMERULUS	(50)	(48)	(48)
MINERALIZATION			1 (2%)
#KIDNEY/PELVIS	(50)	(48)	(48)
INFLAMMATION, SUPPURATIVE			1 (2%)
#URINARY BLADDER	(49)	(40)	(42)
INFLAMMATION, SUPPURATIVE		1 (3%)	1 (2%)
HYPERPLASIA, EPITHELIAL			3 (7%)
ENDOCRINE SYSTEM			
#ADRENAL/CAPSULE	(49)	(48)	(46)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#ADRENAL CORTEX	(49)	(48)	(46)
HYPERTROPHY, NOS			1 (2%)
HYPERPLASIA, NOS	1 (2%)	4 (8%)	
#THYROID	(50)	(43)	(47)
ECTOPIA	1 (2%)		
CYST, NOS	1 (2%)		1 (2%)
INFLAMMATION, NOS	1 (2%)		
#PARATHYROID	(39)	(26)	(25)
CYST, NOS	1 (3%)	1 (4%)	

TABLE B1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
CYST, NOS			1 (2%)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
ABSCESS, NOS	1 (2%)	3 (6%)	
*SEMINAL VESICLE	(50)	(50)	(50)
DILATATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE			2 (4%)
#TESTIS	(50)	(47)	(48)
INFLAMMATION, NOS			1 (2%)
ATROPHY, NOS		19 (40%)	11 (23%)
NERVOUS SYSTEM			
#BRAIN	(50)	(48)	(49)
HEMORRHAGE		1 (2%)	1 (2%)
CORPORA AMYLACEA	19 (38%)	8 (17%)	2 (4%)
*OLFACTORY SENSORY EPITHELIUM	(50)	(50)	(50)
ATROPHY, NOS			25 (50%)
ATROPHY, FOCAL			7 (14%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
MICROPHTHALMIA			1 (2%)
*LACRIMAL APPARATUS	(50)	(50)	(50)
HYPERPLASIA, EPITHELIAL			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
HEMATOMA, NOS		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MINERALIZATION			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		
AUTO/NECROPSY/HISTO PERF		1	1
AUTO/NECROPSY/NO HISTO			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, BASAL CELL			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE	3 (6%)	2 (4%)	1 (2%)
INFLAMMATION, CHRONIC			2 (4%)
FIBROSIS			2 (4%)
METAPLASIA, CARTILAGINOUS			1 (2%)
METAPLASIA, OSSEOUS			2 (4%)
*LARYNX	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
METAPLASIA, SQUAMOUS	1 (2%)		
#TRACHEA	(49)	(44)	(48)
INFLAMMATION, SUPPURATIVE	2 (4%)		
METAPLASIA, SQUAMOUS	1 (2%)		
#LUNG	(49)	(48)	(49)
CONGESTION, NOS	5 (10%)	3 (6%)	1 (2%)
EDEMA, NOS	1 (2%)		
HEMORRHAGE	3 (6%)	5 (10%)	13 (27%)
PERIVASCULAR CUFFING	36 (73%)	4 (8%)	6 (12%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		8 (17%)	7 (14%)
HISTIOCYTOSIS		4 (8%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)		
*PERITONEAL CAVITY	(50)	(49)	(49)
HEMATOPOIESIS			1 (2%)
#BONE MARROW	(49)	(48)	(47)
FIBROSIS		1 (2%)	
ATROPHY, NOS		1 (2%)	2 (4%)
HYPERPLASIA, GRANULOCYTIC	1 (2%)	3 (6%)	4 (9%)
#SPLEEN	(49)	(46)	(48)
NECROSIS, NOS		1 (2%)	
HISTIOCYTOSIS		2 (4%)	
HYPERPLASIA, LYMPHOID	3 (6%)		
HEMATOPOIESIS		8 (17%)	5 (10%)
#LYMPH NODE	(46)	(41)	(44)
HYPERPLASIA, NOS	28 (61%)	3 (7%)	2 (5%)
HISTIOCYTOSIS		1 (2%)	
ERYTHROPHAGOCYTOSIS		1 (2%)	
#LUNG	(49)	(48)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
ERYTHROPHAGOCYTOSIS		1 (2%)	
#LIVER	(50)	(47)	(49)
HEMATOPOIESIS		1 (2%)	2 (4%)
#ADRENAL	(49)	(47)	(48)
HEMATOPOIESIS		1 (2%)	2 (4%)
#THYMUS	(47)	(21)	(33)
NECROSIS, NOS	1 (2%)	2 (10%)	
ATROPHY, NOS			2 (6%)

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
THROMBOSIS, NOS			1 (2%)
#LYMPH NODE	(46)	(41)	(44)
THROMBOSIS, NOS		1 (2%)	
#LUNG	(49)	(48)	(49)
THROMBOSIS, NOS		2 (4%)	1 (2%)
EMBOLUS, SEPTIC		1 (2%)	
#HEART	(49)	(48)	(49)
THROMBOSIS, NOS		2 (4%)	
HYPERPLASIA, ATYPICAL		5 (10%)	8 (16%)
#OVARY	(49)	(45)	(48)
THROMBOSIS, NOS		1 (2%)	1 (2%)
#ADRENAL CORTEX	(49)	(47)	(48)
THROMBOSIS, NOS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(46)	(45)	(46)
INFLAMMATION, NOS	29 (63%)	6 (13%)	13 (28%)
ATROPHY, FOCAL	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#LIVER	(50)	(47)	(49)
HEMORRHAGE		4 (9%)	3 (6%)
INFLAMMATION, NOS	2 (4%)	2 (4%)	2 (4%)
DEGENERATION, NOS	2 (4%)	1 (2%)	
NECROSIS, NOS	1 (2%)	12 (26%)	5 (10%)
NECROSIS, FOCAL	5 (10%)	3 (6%)	1 (2%)
EOSINOPHILIC CYTO CHANGE		1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	
#BILE DUCT	(50)	(47)	(49)
DILATATION, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#PANCREAS	(49)	(45)	(48)
INFLAMMATION, NOS	15 (31%)	4 (9%)	2 (4%)
NECROSIS, NOS			1 (2%)
ATROPHY, NOS		1 (2%)	2 (4%)
#FORESTOMACH	(49)	(42)	(49)
HYPERPLASIA, EPITHELIAL		5 (12%)	9 (18%)
HYPERKERATOSIS		1 (2%)	
*RECTUM	(50)	(49)	(49)
HEMORRHAGE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(47)	(49)
INFLAMMATION, NOS	41 (84%)	9 (19%)	13 (27%)
PERIVASCULAR CUFFING	1 (2%)		
INFARCT, NOS			1 (2%)
AMYLOIDOSIS	1 (2%)	1 (2%)	
#KIDNEY/TUBULE	(49)	(47)	(49)
PIGMENTATION, NOS		2 (4%)	
#URINARY BLADDER	(45)	(44)	(46)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(40)	(43)
CYST, NOS		1 (3%)	
DEGENERATION, CYSTIC		1 (3%)	
#ADRENAL	(49)	(47)	(48)
CYST, NOS		2 (4%)	1 (2%)
DEGENERATION, NOS			1 (2%)
NECROSIS, CORTICAL		1 (2%)	1 (2%)
#ADRENAL/CAPSULE	(49)	(47)	(48)
HYPERPLASIA, NOS	45 (92%)	25 (53%)	35 (73%)

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#ADRENAL CORTEX	(49)	(47)	(48)
HYPERPLASIA, NOS			3 (6%)
#THYROID	(48)	(46)	(49)
CYST, NOS	2 (4%)		
DEGENERATION, CYSTIC	3 (6%)	4 (9%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(49)	(49)
HYPERPLASIA, NOS	5 (10%)	2 (4%)	
#UTERUS	(49)	(46)	(49)
DILATATION, NOS			1 (2%)
CYST, NOS		1 (2%)	1 (2%)
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
INVOLUTION, NOS		7 (15%)	14 (29%)
#UTERUS/ENDOMETRIUM	(49)	(46)	(49)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	12 (24%)		2 (4%)
HYPERPLASIA, NOS	40 (82%)	10 (22%)	11 (22%)
#FALLOPIAN TUBE	(49)	(46)	(49)
INFLAMMATION, NOS			1 (2%)
#OVARY	(49)	(45)	(48)
CYST, NOS	4 (8%)	3 (7%)	5 (10%)
HEMORRHAGE	2 (4%)	4 (9%)	10 (21%)
INFLAMMATION, NOS	3 (6%)	2 (4%)	1 (2%)
ATROPHY, NOS	2 (4%)	40 (89%)	40 (83%)
HYPERPLASIA, GRANULOSA-CELL		2 (4%)	
HYPERPLASIA, EPITHELIAL		3 (7%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(48)	(49)
HEMORRHAGE		1 (2%)	1 (2%)
PERIVASCULAR CUFFING	1 (2%)		
CORPORA AMYLACEA	13 (27%)	8 (17%)	11 (22%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(49)	(49)
MINERALIZATION			1 (2%)
INFLAMMATION, NOS	1 (2%)		
DEGENERATION, NOS		1 (2%)	1 (2%)
*LACRIMAL APPARATUS	(50)	(49)	(49)
HYPERPLASIA, EPITHELIAL		1 (2%)	
*NASOLACRIMAL DUCT	(50)	(49)	(49)
INFLAMMATION, SUPPURATIVE	7 (14%)	3 (6%)	
MUSCULOSKELETAL SYSTEM			
*STERNUM	(50)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
FIBROUS OSTEODYSTROPHY	5 (10%)	2 (4%)	3 (6%)
BODY CAVITIES			
*MEDIASTINUM	(50)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
*PERITONEAL CAVITY	(50)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, MESOTHELIAL		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

TABLE B2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	CONTROL (CHAM)	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	
ANIMAL MISSING/NO NECROPSY			1
ACCIDENTAL DEATH		1	
AUTO/NECROPSY/HISTO PERF		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX C

ANALYSES OF PRIMARY TUMORS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

TABLE C1. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	2/50 (4%)	12/49 (24%)	11/49 (22%)
Adjusted Rates (b)	4.1%	72.3%	75.0%
Terminal Rates (c)	2/49 (4%)	7/11 (64%)	4/7 (57%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P=0.010		
Fisher Exact Tests		P=0.003	P=0.007
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	0/50 (0%)	2/49 (4%)	5/49 (10%)
Adjusted Rates (b)	0.0%	18.2%	47.6%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	3/7 (43%)
Life Table Tests (d)	P<0.001	P=0.018	P<0.001
Cochran-Armitage Trend Test (d)	P=0.016		
Fisher Exact Tests		P=0.242	P=0.027
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	14/49 (29%)	15/49 (31%)
Adjusted Rates (b)	4.1%	86.2%	92.4%
Terminal Rates (c)	2/49 (4%)	9/11 (82%)	6/7 (86%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	0/50 (0%)	23/50 (46%)	29/50 (58%)
Adjusted Rates (b)	0.0%	59.4%	75.5%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	1/7 (14%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Heart: Hemangiosarcoma			
Overall Rates (a)	0/50 (0%)	16/49 (33%)	7/49 (14%)
Adjusted Rates (b)	0.0%	57.5%	57.3%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	3/7 (43%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P=0.032		
Fisher Exact Tests		P<0.001	P=0.006
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	0/50 (0%)	16/50 (32%)	8/50 (16%)
Adjusted Rates (b)	0.0%	57.5%	62.6%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	3/7 (43%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P=0.020		
Fisher Exact Tests		P<0.001	P=0.003
Liver: Hepatocellular Adenoma			
Overall Rates (a)	5/50 (10%)	4/49 (8%)	1/49 (2%)
Adjusted Rates (b)	10.2%	26.5%	14.3%
Terminal Rates (c)	5/49 (10%)	2/11 (18%)	1/7 (14%)
Life Table Tests (d)	P=0.230	P=0.078	P=0.627
Cochran-Armitage Trend Test (d)	P=0.085N		
Fisher Exact Tests		P=0.513N	P=0.107N

TABLE C1. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	Control	625 ppm	1,250 ppm
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	3/50 (6%)	2/49 (4%)	1/49 (2%)
Adjusted Rates (b)	6.1%	15.2%	9.1%
Terminal Rates (c)	3/49 (6%)	1/11 (9%)	0/7 (0%)
Life Table Tests (d)	P=0.262	P=0.269	P=0.534
Cochran-Armitage Trend Test (d)	P=0.229N		
Fisher Exact Tests		P=0.510N	P=0.316N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	8/50 (16%)	6/49 (12%)	2/49 (4%)
Adjusted Rates (b)	16.3%	39.0%	22.1%
Terminal Rates (c)	8/49 (16%)	3/11 (27%)	1/7 (14%)
Life Table Tests (d)	P=0.114	P=0.027	P=0.424
Cochran-Armitage Trend Test (d)	P=0.040N		
Fisher Exact Tests		P=0.403N	P=0.049N
Forestomach: All Papilloma			
Overall Rates (a)	0/49 (0%)	5/40 (13%)	0/44 (0%)
Adjusted Rates (b)	0.0%	45.5%	0.0%
Terminal Rates (c)	0/48 (0%)	5/11 (45%)	0/7 (0%)
Life Table Tests (d)	P=0.036	P<0.001	(e)
Cochran-Armitage Trend Test (d)	P=0.568		
Fisher Exact Tests		P=0.016	(e)
Forestomach: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	0/49 (0%)	4/40 (10%)	1/44 (2%)
Adjusted Rates (b)	0.0%	26.2%	7.1%
Terminal Rates (c)	0/48 (0%)	2/11 (18%)	0/7 (0%)
Life Table Tests (d)	P=0.032	P=0.001	P=0.248
Cochran-Armitage Trend Test (d)	P=0.354		
Fisher Exact Tests		P=0.037	P=0.473
Forestomach: All Papilloma or Carcinoma			
Overall Rates (a)	0/49 (0%)	7/40 (18%)	1/44 (2%)
Adjusted Rates (b)	0.0%	50.8%	7.1%
Terminal Rates (c)	0/48 (0%)	5/11 (45%)	0/7 (0%)
Life Table Tests (d)	P=0.006	P<0.001	P=0.248
Cochran-Armitage Trend Test (d)	P=0.363		
Fisher Exact Tests		P=0.003	P=0.473
Preputial Gland: Squamous Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	1/50 (2%)
Adjusted Rates (b)	0.0%	21.9%	14.3%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	1/7 (14%)
Life Table Tests (d)	P=0.017	P=0.005	P=0.128
Cochran-Armitage Trend Test (d)	P=0.378		
Fisher Exact Tests		P=0.121	P=0.500
Preputial Gland: All Carcinoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	0.0%	21.9%	28.6%
Terminal Rates (c)	0/49 (0%)	2/11 (18%)	2/7 (29%)
Life Table Tests (d)	P=0.001	P=0.005	P=0.003
Cochran-Armitage Trend Test (d)	P=0.202		
Fisher Exact Tests		P=0.121	P=0.247

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) 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 that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is presented because no tumors were observed in the 1,250-ppm and control groups.

TABLE C2. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE

	Control	625 ppm	1,250 ppm
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/49 (6%)	9/48 (19%)	20/49 (41%)
Adjusted Rates (b)	6.5%	48.1%	56.7%
Terminal Rates (c)	3/46 (7%)	6/15 (40%)	15/30 (50%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.056	P<0.001
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	0/49 (0%)	6/48 (13%)	8/49 (16%)
Adjusted Rates (b)	0.0%	36.7%	24.7%
Terminal Rates (c)	0/46 (0%)	5/15 (33%)	6/30 (20%)
Life Table Tests (d)	P=0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P=0.004		
Fisher Exact Tests		P=0.012	P=0.003
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	3/49 (6%)	12/48 (25%)	23/49 (47%)
Adjusted Rates (b)	6.5%	61.7%	63.6%
Terminal Rates (c)	3/46 (7%)	8/15 (53%)	17/30 (57%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.010	P<0.001
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	1/50 (2%)	10/49 (20%)	10/49 (20%)
Adjusted Rates (b)	2.2%	32.0%	22.9%
Terminal Rates (c)	1/46 (2%)	3/15 (20%)	1/30 (3%)
Life Table Tests (d)	P=0.006	P<0.001	P=0.003
Cochran-Armitage Trend Test (d)	P=0.006		
Fisher Exact Tests		P=0.003	P=0.003
Heart: Hemangiosarcoma			
Overall Rates (a)	0/50 (0%)	11/48 (23%)	18/49 (37%)
Adjusted Rates (b)	0.0%	40.6%	46.3%
Terminal Rates (c)	0/46 (0%)	3/15 (20%)	10/30 (33%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Circulatory System: Hemangiosarcoma			
Overall Rates (a)	0/50 (0%)	13/49 (27%)	19/49 (39%)
Adjusted Rates (b)	0.0%	50.5%	49.0%
Terminal Rates (c)	0/46 (0%)	5/15 (33%)	11/30 (37%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Liver: Hepatocellular Adenoma			
Overall Rates (a)	0/50 (0%)	1/47 (2%)	4/49 (8%)
Adjusted Rates (b)	0.0%	6.7%	12.1%
Terminal Rates (c)	0/46 (0%)	1/15 (7%)	3/30 (10%)
Life Table Tests (d)	P=0.015	P=0.278	P=0.030
Cochran-Armitage Trend Test (d)	P=0.025		
Fisher Exact Tests		P=0.485	P=0.056

TABLE C2. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	Control	625 ppm	1,250 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	0/50 (0%)	2/47 (4%)	5/49 (10%)
Adjusted Rates (b)	0.0%	13.3%	14.3%
Terminal Rates (c)	0/46 (0%)	2/15 (13%)	3/30 (10%)
Life Table Tests (d)	P=0.009	P=0.048	P=0.015
Cochran-Armitage Trend Test (d)	P=0.016		
Fisher Exact Tests		P=0.232	P=0.027
Forestomach: Squamous Cell Papilloma			
Overall Rates (a)	0/49 (0%)	3/42 (7%)	1/49 (2%)
Adjusted Rates (b)	0.0%	20.0%	3.3%
Terminal Rates (c)	0/46 (0%)	3/15 (20%)	1/30 (3%)
Life Table Tests (d)	P=0.248	P=0.008	P=0.415
Cochran-Armitage Trend Test (d)	P=0.381		
Fisher Exact Tests		P=0.094	P=0.500
Forestomach: All Papilloma			
Overall Rates (a)	0/49 (0%)	4/42 (10%)	10/49 (20%)
Adjusted Rates (b)	0.0%	26.7%	31.8%
Terminal Rates (c)	0/46 (0%)	4/15 (27%)	9/30 (30%)
Life Table Tests (d)	P<0.001	P=0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.042	P<0.001
Forestomach: Squamous Cell Papilloma or Carcinoma			
Overall Rates (a)	0/49 (0%)	4/42 (10%)	1/49 (2%)
Adjusted Rates (b)	0.0%	22.9%	3.4%
Terminal Rates (c)	0/46 (0%)	3/15 (20%)	1/29 (3%)
Life Table Tests (d)	P=0.249	P=0.003	P=0.408
Cochran-Armitage Trend Test (d)	P=0.393		
Fisher Exact Tests		P=0.042	P=0.500
Forestomach: All Papilloma or Carcinoma			
Overall Rates (a)	0/49 (0%)	5/42 (12%)	10/49 (20%)
Adjusted Rates (b)	0.0%	29.3%	31.8%
Terminal Rates (c)	0/46 (0%)	4/15 (27%)	9/30 (30%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.018	P<0.001
Mammary Gland: Acinar Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	2/49 (4%)	6/49 (12%)
Adjusted Rates (b)	0.0%	13.3%	16.7%
Terminal Rates (c)	0/46 (0%)	2/15 (13%)	3/30 (10%)
Life Table Tests (d)	P=0.004	P=0.048	P=0.007
Cochran-Armitage Trend Test (d)	P=0.007		
Fisher Exact Tests		P=0.242	P=0.012
Mammary Gland: Adenosquamous Carcinoma			
Overall Rates (a)	0/50 (0%)	4/49 (8%)	0/49 (0%)
Adjusted Rates (b)	0.0%	11.9%	0.0%
Terminal Rates (c)	0/46 (0%)	0/15 (0%)	0/30 (0%)
Life Table Tests (d)	P=0.575	P=0.030	(e)
Cochran-Armitage Trend Test (d)	P=0.615		
Fisher Exact Tests		P=0.056	(e)

TABLE C2. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE SIXTY-ONE-WEEK INHALATION STUDY OF 1,3-BUTADIENE (Continued)

	Control	625 ppm	1,250 ppm
Ovary: Granulosa Cell Tumor			
Overall Rates (a)	0/49 (0%)	6/45 (13%)	12/48 (25%)
Adjusted Rates (b)	0.0%	33.4%	36.6%
Terminal Rates (c)	0/46 (0%)	4/15 (27%)	9/29 (31%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.010	P<0.001
Ovary: Granulosa Cell Tumor or Carcinoma			
Overall Rates (a)	0/49 (0%)	6/45 (13%)	13/48 (27%)
Adjusted Rates (b)	0.0%	33.4%	39.8%
Terminal Rates (c)	0/46 (0%)	4/15 (27%)	10/29 (34%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.010	P<0.001

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) 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 that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The Cochran-Armitage and Fisher's exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is presented because no tumors were observed in the 1,250-ppm and control groups.

APPENDIX D

CHEMICAL CHARACTERIZATION OF 1,3-BUTADIENE

APPENDIX D. CHEMICAL CHARACTERIZATION

1,3-Butadiene was analyzed by infrared spectroscopy and gas chromatography by the testing laboratory. More than 15 lots were used. The analysis of Lot No. B-915, the first lot used in the 60- to 61-week studies, follows. Results for analysis of all lots for purity and dimer content are presented in Table D1.

I. Infrared Spectroscopy

A. Method: Approximately 6 ml of gas was placed in an evacuated 10-cm path gas cell with sodium chloride windows. A Beckman Acculab 6 spectrophotometer was used.

B. Results: The spectrum was consistent with the previous spectra and those from Midwest Research Institute (Figure 3).

II. Gas Chromatography

A. Method:

Sample size: 0.5 ml
Column: 2.35 m × 2 mm ID, Porapak QS
Detector: Flame ionization
Detector temperature: 250° C
Oven temperature: 100° C isothermal for 20 min
Injection temperature: 200° C
Flow rate: 20 ml/min

B. Results: A main peak and three trace impurities were detected:

Injection	Purity (percent (of total area)
1	98.890
2	99.118
3	98.818
Mean	98.94

III. Gas Chromatography, Dimer Analysis

A. Method:

Sample size: 0.3 ml
Column: 1 m × 2 mm ID, Porapak PS 80/100
Detector: Flame ionization
Detector temperature: 275° C
Oven temperature: 150° C isothermal for 8 min
Injection temperature: 200° C
Flow rate: 30 ml/min

B. Results: The largest impurity had a retention time of 0.65 minutes and a mean area of 1.05%. One peak greater than 0.5% by area percent was noted but no dimer was detected.

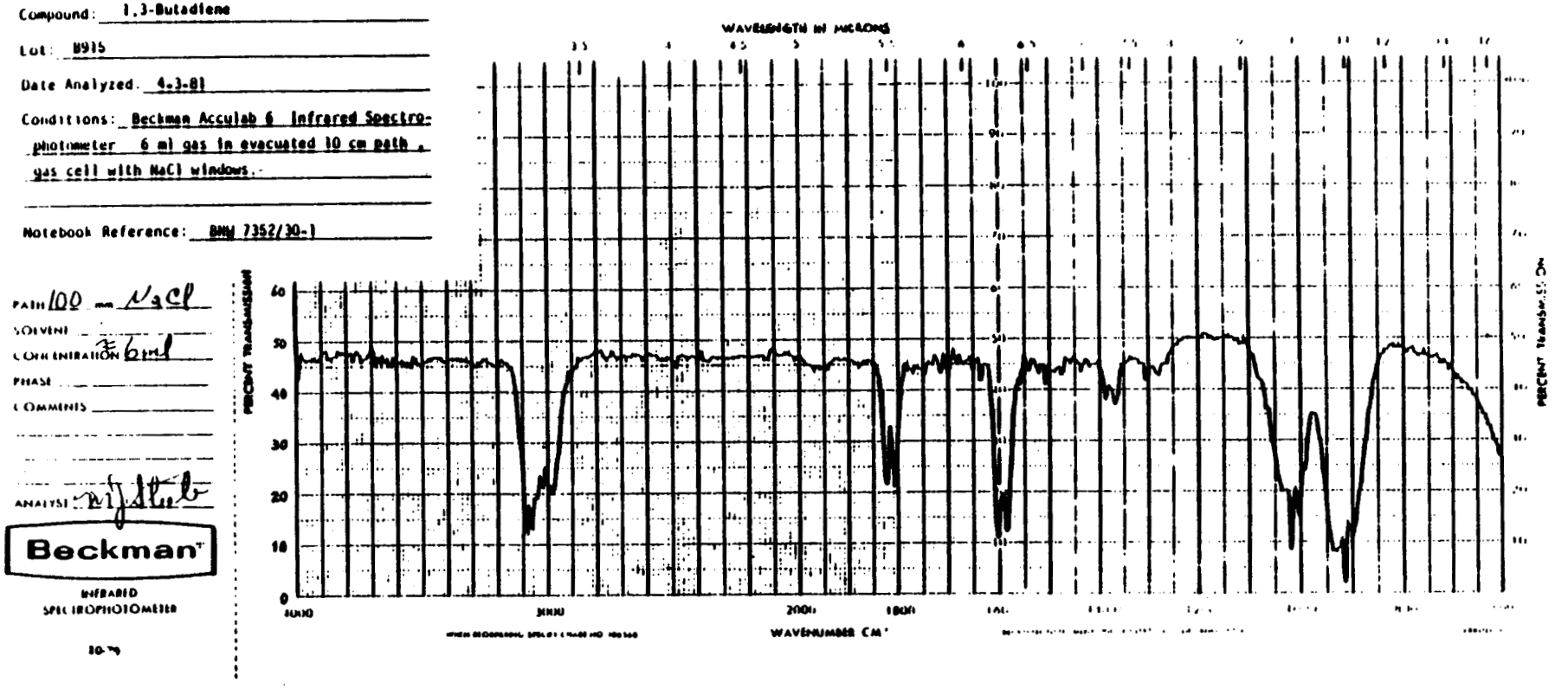

FIGURE 3. INFRARED ABSORPTION SPECTRUM OF 1,3-BUTADIENE (LOT NO. B915)

IV. Determination of Rapidly Eluting Impurity

A. Method: Gas chromatography

Sample size: 0.05 ml vapor
Column: 1% SP1000 on Carbopack B 60/80, 4 feet × 2 mm ID, glass
Carrier: Helium
Flow rate: 27 ml/min
Detector: Flame ionization
Detector temperature: 250° C
Oven temperature: 70° C, isothermal
Injection temperature: 200° C

B. Results: This chromatographic analysis verified the presence of the rapidly eluting impurity in the butadiene sample. The average concentration was 0.67% by area. Analysis of propane and methane standards indicated that the impurity was probably methane. The impurity shows a retention time of 0.50-0.43 minutes. Methane exhibits a 0.44-minute retention time.

V. Conclusions: The infrared spectrum is consistent with previous data and with the Midwest Research Institute spectra. The purity is 98.94% as determined by gas chromatography. A rapidly migrating impurity of greater than 0.5% by area, but no dimer, was detected. Analysis of standards indicated that the rapidly eluting impurity was probably methane.

TABLE D1. SUMMARY OF PURITY OF 1,3-BUTADIENE IN THE SIXTY-ONE-WEEK INHALATION STUDIES

Lot Number	Date Received	Dates Analyzed	Date on Test	Purity by Area Percent	Dimer Content (ppm) (a)	Dimer Content (mole percent) (b)	Cylinder Capacity (gallons)
B-915	03/31/81	04/03/81	04/15/81	98.94	0	0.05	16
B-899B	05/06/81	05/08/81	05/12/81	99.72	0	0.10	28
B-962	05/29/81	06/03/81	06/15/81	99.34	102	0.20	28
B-996	06/30/81	07/09/81	07/13/81	99.73	18	0.45	28
F-037	08/20/81	08/21/81	08/25/81	99.87	104	0.40	28
F-047	09/09/81	09/10/81	09/14/81	100.0	74	0.35	28
F-089A(c)	10/29/81	10/30/81	11/10/81	99.85	43	0.10	5
F-105	12/10/81	12/11/81	Rejected	99.98	328	0.07	28
F-089B(d)	10/29/81	12/11/81	12/14/81	(e)	140		5
F-089C(f)	10/29/81	12/14/81	12/15/81	100.0	44		5
F-120	01/14/82	01/15/82	01/18/82	99.99	37		28
F-159	02/09/82	02/12/82	02/22/82	99.99	51	0.05	28
F-193	03/31/82	03/31/82	04/02/82	99.67	38		28
F-207	04/21/82	04/22/82	04/23/82	99.86	27		28
F-238	05/28/82	06/07/82	Rejected	99.80	119	0.17	28
F-207	04/21/82	06/08/82	04/23/82	(e)	111		28
F-207(g)	04/21/82	06/29/82 06/30/82	04/23/82	99.85	116		28

(a) Dimer content in headspace, determined at testing laboratory
(b) Dimer content in the liquid, determined by the manufacturer. Dimer content in liquid and headspace are not directly comparable.
(c) Tank one of three of lot F-089
(d) Tank two of three of lot F-089
(e) Only dimer was determined.
(f) Tank three of three of lot F-089; assayed for dimer only on 01/07/82 (316 ppm) and 01/18/82 (1,540 ppm)
(g) Purity assay performed after end of 61-week studies

APPENDIX E

GENERATION AND MONITORING OF CHAMBER CONCENTRATIONS

I. Atmospheric Generation System: The generation system used to deliver butadiene gas to each exposure chamber is depicted in Figure 4. Butadiene was supplied in 5- or 28-gallon gas cylinders located in the animal exposure room. The natural bottle pressure (about 147 psi at room temperature) was reduced to an operating pressure of 54 psi by a Union Carbide single-stage regulator. A nitrogen purge tee and check valve preceded this regulator to allow clearing of the entire gas distribution system for system maintenance or exchange of gas bottles.

The butadiene was piped to a polyethylene vapor hood containing safety devices, comprised of two emergency shutoff valves and a pressure gauge. These safety devices were incorporated in the hood (vented to the room exhaust) to minimize the hazard to animals and personnel in the event of a leak. The gas was then piped to a second hood containing two double-pattern metering valves. Since the upstream pressure to these valves was well regulated, these valves provided stable control of the gas flow rate and ultimately the concentration in the chambers. To provide the proper chamber concentration, the valves were set and periodically checked, by matching the calculated with the actual flow measured by a bubble meter. From the double-pattern metering valves, the gas was piped to each exposure chamber. A shutoff valve at the entrance to the chamber permitted easy, rapid termination of gas flow. All materials in the gas distribution system were stainless steel, Teflon®, Viton®, or brass.

To help prevent the possibility of concentration excursions in the exposure chambers, several safety systems were incorporated. One of each of the two series valves, incorporated in the double-pattern metering valves, was used as a flow-limiting valve, the other as a flow-control valve. The limiting valve was set to limit the maximum flow to 20% greater than the nominal flow calculated to provide the target chamber concentration. Two explosion-proof solenoid shutoff valves connected in series were operated by the general alarm systems. These valves would automatically shut off the flow of gas to the chambers whenever an alarm situation occurred.

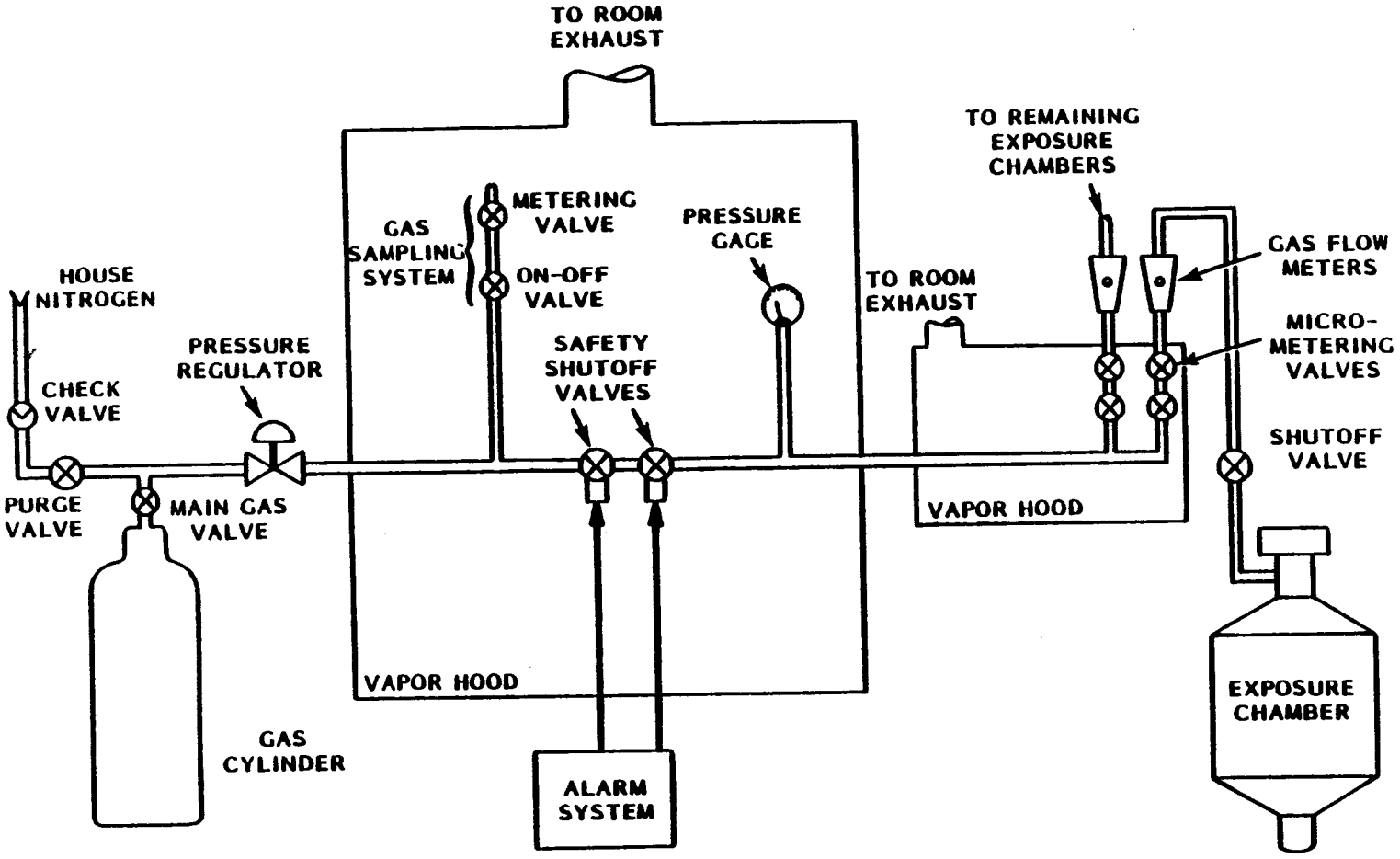

FIGURE 4. SCHEMATIC DIAGRAM OF THE 1,3-BUTADIENE GAS DISTRIBUTION SYSTEM

II. Vapor Concentration Uniformity in the Chamber: The uniformity of vapor concentrations in the exposure chambers was measured periodically throughout the study with a portable photoionization detector at 12 positions (two positions, one in front [F] and one in back [B], for each of the six animal cage units per chamber). The sample point was just above and about 10 cm in from the front or back center of each cage unit (Figure 5). The data, normalized at all 12 sample positions for each chamber, are presented in Table E1.

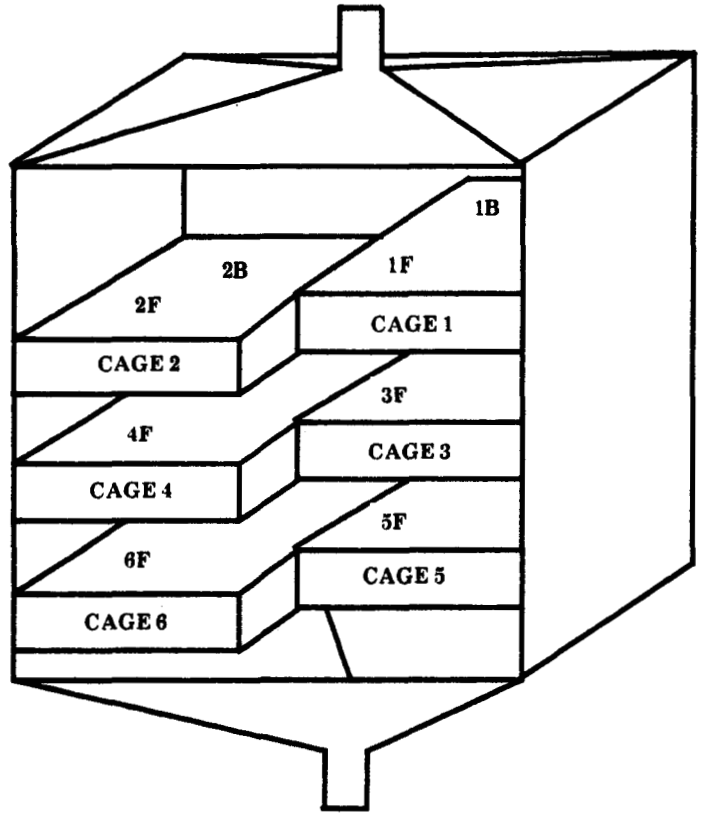

FIGURE 5. SCHEMATIC FRONT VIEW OF CHAMBER SHOWING APPROXIMATE SAMPLE SITES

TABLE E1. 1,3-BUTADIENE VAPOR CONCENTRATION UNIFORMITY TEST

Sample Date Sample Location	04/01/81 Chamber A(a) (percent)	04/01/81 Chamber B (b) (percent)	03/09/82 Chamber A (percent)	03/09/82 Chamber B (percent)	05/20/82 Chamber A (percent)	05/20/82 Chamber B (percent)
1F	101	103	100	99	100	102
1B	100	100	101	100	102	100
2F	100	98	100	101	102	101
2B	101	100	100	101	102	103
3F	100	100	99	100	98	99
3B	99	98	100	98	99	100
4F	99	101	99	101	102	100
4B	100	98	100	98	99	99
5F	100	101	99	102	98	99
5B	100	98	100	98	99	99
6F	101	103	101	103	102	97
6B	100	100	100	100	99	103
Mean ± SD	100 ± 1	100 ± 1	100 ± 1	100 ± 2	100 ± 2	100 ± 2

(a) Chamber A housed animals exposed at 625 ppm 1,3-butadiene.
(b) Chamber B housed animals exposed at 1,250 ppm 1,3-butadiene.

III. Chamber Concentration Monitoring System: Butadiene concentrations in the exposure chambers, control chamber, and exposure room were automatically monitored 7-12 times during each exposure day with a photoionization detector (PID) for the first 150 days or a Hewlett Packard 5840A gas chromatograph (GC) equipped with a flame ionization detector from day 151 to termination. The calibration of the PID was checked every 10 days with a "bag" standard prepared by the testing facility and daily with a propylene on-line standard. The GC was calibrated at least monthly with the "bag" standard.

During exposures, samples from each sampling location were continuously drawn by vacuum through stainless steel sample lines to the input of an automatic, multiplexed, 8-port sample valve. The constant flow assured fresh samples at the 8-port valve.

Weekly concentrations are graphically represented in Figures 6 and 7.

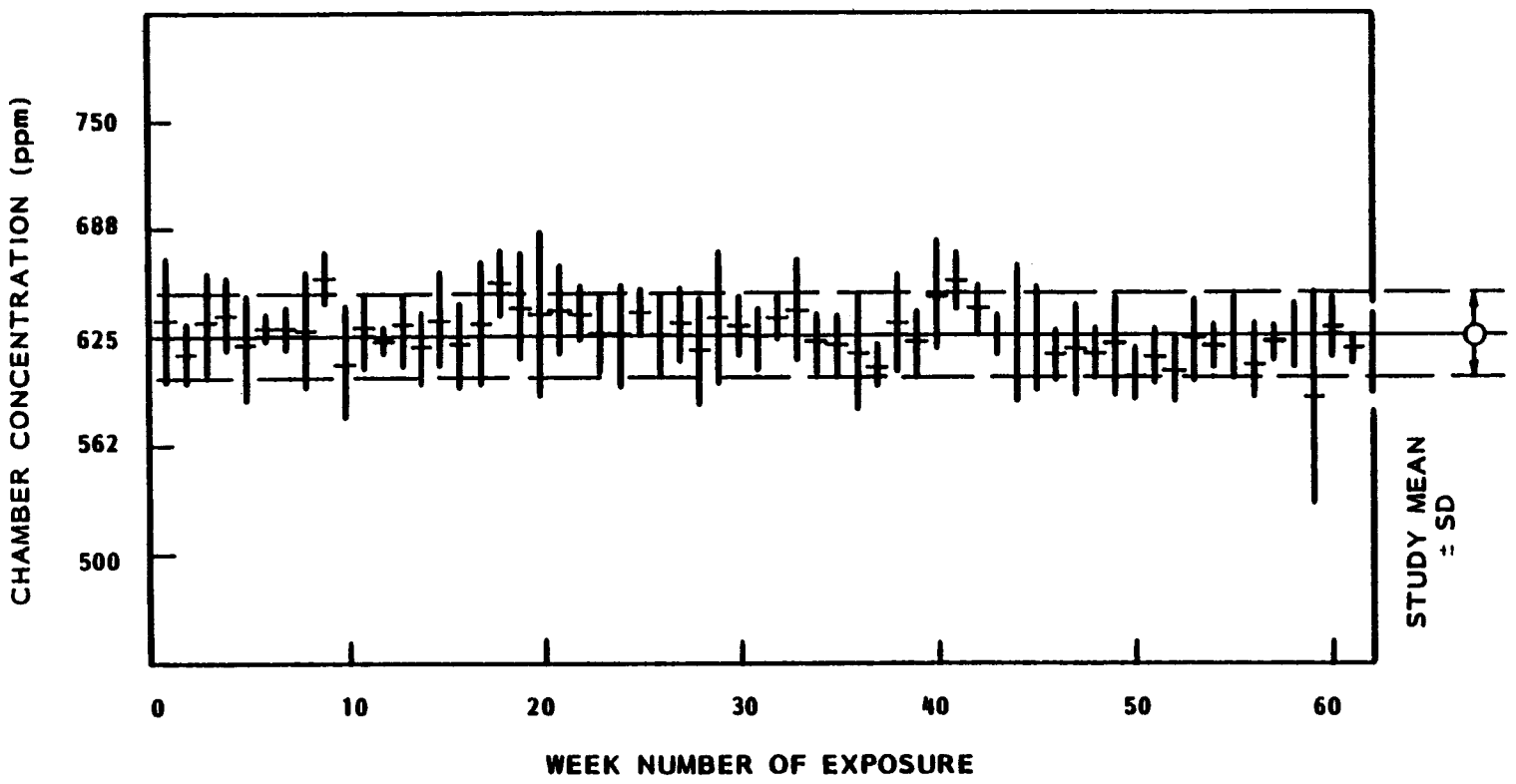

FIGURE 6. WEEKLY MEAN CONCENTRATION AND STANDARD DEVIATION (bars) IN 625-PPM MOUSE EXPOSURE CHAMBER FOR ENTIRE SIXTY-ONE-WEEK STUDIES

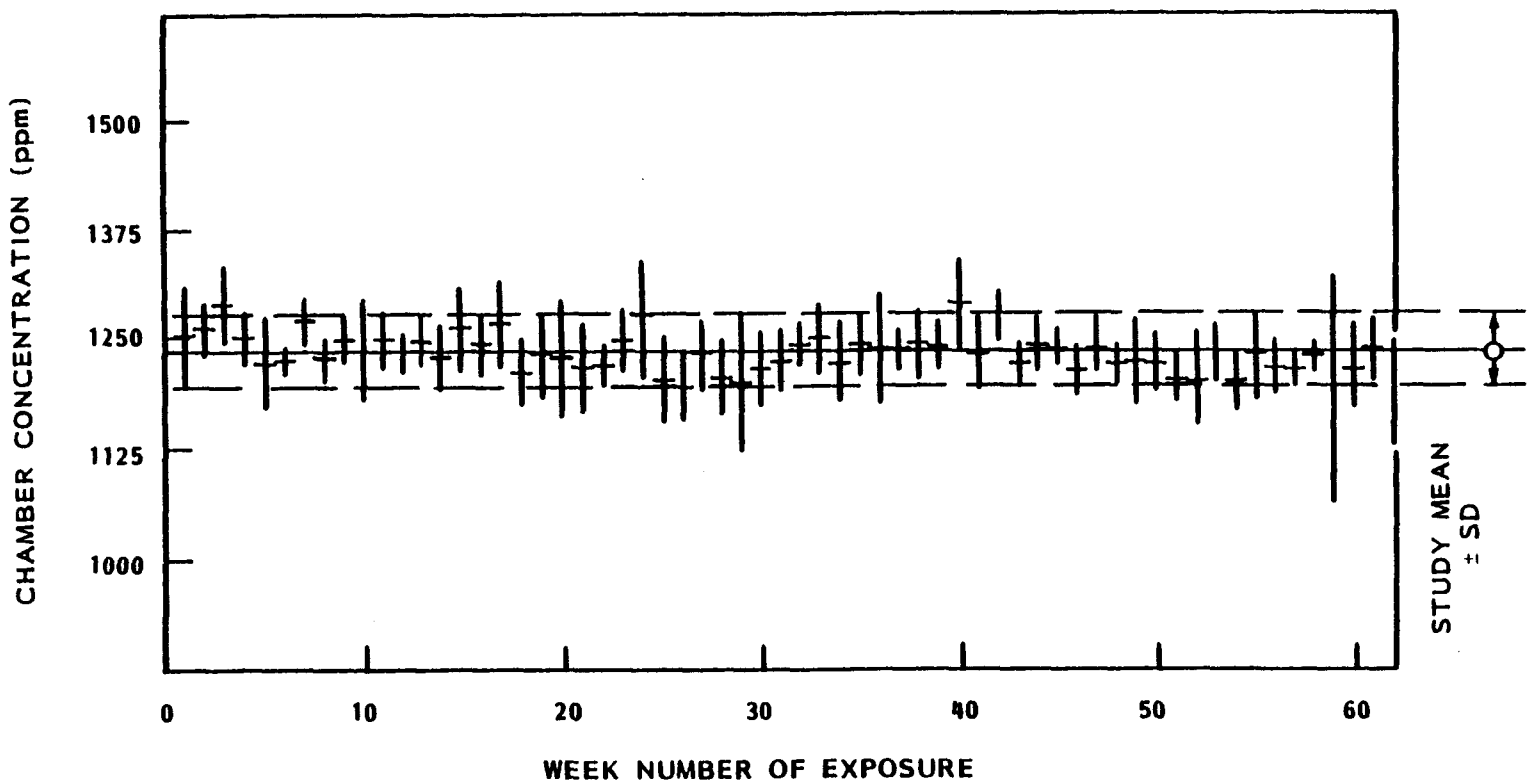

FIGURE 7. WEEKLY MEAN CONCENTRATION AND STANDARD DEVIATION (bars) IN 1,250-PPM MOUSE EXPOSURE CHAMBER FOR ENTIRE SIXTY-ONE-WEEK STUDIES

APPENDIX F

RESULTS OF SEROLOGIC ANALYSES

APPENDIX F. SEROLOGIC ANALYSES

A. METHODS

Data from animals surviving to the end of the studies (60 or 61 weeks) were collected from 5/50 randomly selected control animals of each sex. The blood from each animal was collected and clotted, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests were performed:

Hemagglutination Inhibition	Complement Fixation	ELISA
PVM (pneumonia virus of mice)	M.Ad. (mouse adenovirus)	MHV (mouse hepatitis virus)
Reo 3 (reovirus type 3)	LCM (lymphocytic choriomeningitis virus)	
GDVII (Theiler's encephalomyelitis virus)		
Poly (polyoma virus)		
MVM (minute virus of mice)		
Ectro (infectious ectromelia)		
Sendai		

B. RESULTS

TABLE F1. MURINE VIRUS ANTIBODY DETERMINATIONS IN MICE IN THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

	Interval (weeks)	No. of Animals	Positive Serologic Reaction for
MALE			
	60		None
FEMALE			
	61	1/5	MHV

APPENDIX G

INGREDIENTS, NUTRIENT COMPOSITION, AND MEASURED CONTAMINANT LEVELS OF NIH 07 DIET

Pelleted Diet: February 1981 to May 1982

(Manufactured by Zeigler Bros., Inc.)
(Gardners, PA)

TABLE G1. INGREDIENTS OF NIH 07 RAT AND MOUSE DIET (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Pre-mixes (vitamins and minerals) (c)	0.25

(a) Prepared according to NIH, 1978; NCI, 1976
(b) Ingredients should be ground to pass through a U.S. Standard Screen #16 before mixing.
(c) Details given in Table G2

TABLE G2. VITAMINS AND MINERALS IN THE NIH 07 DIET (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D activated animal sterol
K_3	2.8 g	Menadione activity
d-A-tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	d-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	d-biotin
Minerals		
Cobalt	0.4 g	Cobalt carbonate
Copper	4.0 g	Copper sulfate
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Iodine	1.4 g	Calcium iodate

(a) Per ton (2,000 lb) of finished product

TABLE G3. NUTRIENT COMPOSITION OF NIH 07 DIET: PELLETS

Nutrient (percent by weight)	Mean	Range	Number of Samples
Crude protein	24.18 ± 0.83	22.7 - 25.1	14
Crude fat	4.88 ± 0.37	4.2 - 5.5	14
Crude fiber	3.35 ± 0.23	2.9 - 3.6	14
Ash	6.41 ± 0.45	5.8 - 7.43	14
Vitamins			
Vitamin A (IU/kg)	11,088 ± 1,872	880 - 1,500	14
Vitamin D (IU/kg)	6,300		(a) 1
A-tocopherol (ppm)	37.6	31.1 - 44.0	(a) 2
Thiamine (ppm)	17.3 ± 1.44	15.0 - 19.0	13
Riboflavin (ppm)	6.9	6.1 - 7.4	(a) 2
Niacin (ppm)	75	65 - 85	2
Pantothenic acid (ppm)	30.2	29.8 - 30.5	(a) 2
Pyridoxine (ppm)	7.2	5.6 - 8.8	(a) 2
Folic acid (ppm)	2.1	1.8 - 2.4	(a) 2
Biotin (ppm)	0.13	0.21 - 0.27	(a) 2
Vitamin B_{12} (ppm)	12.8	10.6 - 15.0	(a) 2
Choline (ppm)	3,315	3,200 - 3,430	(a) 2
Minerals			
Calcium	1.24 ± 0.17	1.08 - 1.69	14
Phosphorous	0.99 ± 0.06	0.88 - 1.10	14
Potassium	0.809	0.772 - 0.846	(a) 2
Chloride	0.557	0.479 - 0.635	(a) 2
Sodium	0.304	0.258 - 0.349	(a) 2
Magnesium	0.172	0.166 - 0.177	(a) 2
Sulfur	0.278	0.270 - 0.285	(a) 2
Iron (ppm)	418	409 - 426	(a) 2
Manganese (ppm)	90.8	86.0 - 95.5	(a) 2
Zinc (ppm)	55.1	54.2 - 56.0	(a) 2
Copper (ppm)	12.68	9.65 - 15.70	(a) 2
Iodine (ppm)	2.58	1.52 - 3.64	(a) 2
Chromium (ppm)	1.86	1.79 - 1.93	(a) 2
Cobalt (ppm)	0.57	0.49 - 0.65	(a) 2
Essential Amino Acids			
Arginine	1.260	1.21 - 1.31	(a) 2
Cystine	0.395	0.39 - 0.40	(a) 2
Glycine	1.175	1.15 - 1.20	(a) 2
Histadine	0.553	0.530 - 0.576	(a) 2
Isoleucine	0.908	0.881 - 0.934	(a) 2
Leucine	1.905	1.85 - 1.96	(a) 2
Lysine	1.250	1.20 - 1.30	(a) 2
Methionine	0.310	0.306 - 0.314	(a) 2
Phenylalanine	0.967	0.960 - 0.974	(a) 2
Threonine	0.834	0.827 - 0.840	(a) 2
Tryptophan	0.175	0.171 - 0.178	(a) 2
Tyrosine	0.587	0.566 - 0.607	(a) 2
Valine	1.085	1.05 - 1.12	(a) 2
Essential Fatty Acids			
Linoleic	2.37		(a) 1
Linolenic	0.308		(a) 1
Arachidonic	0.008		(a) 1

(a) Analyses were done on batches of diet manufactured in January and/or April 1983.

TABLE G4. CONTAMINANT LEVELS OF NIH 07 DIET: PELLETS

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.45 ± 0.19	<0.29 - 1.06	14
Cadmium (ppm)	(a) <0.1		14
Lead (ppm)	0.92 ± 0.62	0.50 - 1.02	14
Mercury (ppm)	(a) <0.05		14
Selenium (ppm)	0.30 ± 0.07	0.14 - 0.40	14
Aflatoxins (ppb)	(a)(b) <10		14
Nitrate nitrogen (c) (ppm)	9.11 ± 2.37	4.7 - 13.0	14
Nitrite nitrogen (c) (ppm)	2.27 ± 1.81	0.4 - 6.9	14
BHA (d) (ppm)	6.40 ± 3.65	(e) <0.4 - 13.0	14
BHT (d) (ppm)	3.04 ± 1.56	0.9 - 5.9	14
Aerobic plate count (CFU/g)	43,607 ± 31,877	4,900 - 88,000	14
Coliform (MPN/g) (f)	17 ± 26	<3 - 93	14
E. coli (MPN/g)	(g) <3		14
Total nitrosamines (ppb)	(h) 2.61 ± 1.02	0.8 - 5.0	12
	(i) 33.36 ± 81.13	0.8 - 273.2	14
N-Nitrosodimethylamine (ppb)	(j) 1.35 ± 0.386	0.8 - 2.0	12
	(k) 31.87 ± 80.74	0.8 - 272	14
N-Nitrosopyrrolidine (ppb)	1.26 ± 0.92	0.0 - 3.5	14
Pesticides (ppm)			
Alpha BHC (l)	(a) <0.01		14
Beta BHC	(a) <0.02		14
Gamma BHC-Lindane	(a) <0.01		14
Delta BHC	(a) <0.01		14
Heptachlor	(a) <0.01		14
Aldrin	(a) <0.01		14
Heptachlor epoxide	(a) <0.01		14
DDE	(a) <0.01		14
DDD	(a) <0.01		14
DDT	(a) <0.01		14
HCB	(a) <0.01		14
Mirex	(a) <0.01		14
Methoxychlor	(a) <0.05	(m) (n) 0.09 (8/26/81)	14
Dieldrin	(a) <0.01		14
Endrin	(a) <0.01		14
Telodrin	(a) <0.01		14
Chlordane	(a) <0.05		14
Toxaphene	(a) <0.1		14
Estimated PCB's	(a) <0.2		14
Ronnel	(a) <0.01		14
Ethion	(a) <0.02		14
Trithion	(a) <0.05		14
Diazinon	(a) <0.01	(m) 0.2 (4/27/81)	14
Methyl parathion	(a) <0.02		14
Ethyl parathion	(a) <0.02		14
Malathion	0.09 ± 0.06	(o) <0.05-0.27	14
Endosulfan I	(a) <0.01		14
Endosulfan II	(a) <0.01		14
Endosulfan sulfate	(a) <0.03		14

(a) All values were less than the detection limit given.
(b) Detection limit reduced from 10 ppb to 5 ppb after 7/81
(c) Source of contamination: alfalfa, grains, and fish meal
(d) Source of contamination: soy oil and fish meal
(e) One batch contained less than 0.4 ppm.
(f) MPN = most probable number
(g) All values were less than 3 MPN/g
(h) All values were corrected for percent recovery; mean, standard deviation, and range exclude 2 very high values of 162.5 and 273.2 ppb in batches produced on 2/23/81 and 4/27/81.
(i) All values were corrected for percent recovery; mean, standard deviation, and range include the very high values given in (h).
(j) All values were corrected for percent recovery; mean, standard deviation, and range exclude 2 very high values of 158 and 272 ppb in batches produced on 2/23/81 and 4/27/81.
(k) All values were corrected for percent recovery; mean, standard deviation, and range include the very high values given in (j).
(l) BHC is hexachlorocyclohexane or benzene hexachloride.
(m) The value of the one observation above the detection limit and the date it was obtained
(n) The detection limit increased from 0.01 to 0.05 ppm after 5/81.
(o) Six batches contained more than 0.05 ppm.

APPENDIX H

EXPERIMENTAL DATA AUDIT OF THE SIXTY-ONE-WEEK INHALATION STUDIES OF 1,3-BUTADIENE

APPENDIX H. EXPERIMENTAL DATA AUDIT

The experimental data and tables of the draft Technical Report on the toxicology and carcinogenesis studies of 1,3-butadiene were examined for Good Laboratory Practices compliance and scientific procedures by the following persons: National Toxicology Program--Ms. C. Davies, Dr. C. Lingeman, Dr. M. Powers, Dr. B.A. Schwetz, Dr. C. Whitmire, and Dr. M. Wolfe; Experimental Pathology Laboratories, Inc.--Dr. W. Busey, Ms. H. Cook, and Dr. M. Hamlin; Tracor Jitco, Inc.--Ms. P. Errico and Ms. K. Rascigno.

The report of the audit of the 1,3-butadiene studies and the response to the National Toxicology Program audit report by Battelle Pacific Northwest Laboratories are on file in the National Toxicology Program. The main discrepancies or problems and their resolution were as follows:

1. Individual animal identification: A number of mice lost their eartags during the study (35/300). Missing eartags were replaced at one time during the last month of the 61-week studies. Mice were housed in individual cages inside the chambers continuously throughout the studies. In addition to eartags, animal identification was maintained by an animal/cage map. During the course of the studies, five mice were noted to have escaped from the chamber to the room floor. Two other chemicals (epoxybutane and ethylene oxide) were under test in the same room during part of the 1,3-butadiene studies. No mice from these other two studies were noted to be outside their chambers simultaneously with any of the five butadiene mice. Also, none of these five mice was identified as having missing eartags. Thus, it is not likely that mice were mixed up between dose levels of butadiene or that mice of the butadiene studies were mixed up with those of other chemicals under test at the same time. The wet tissue bags of 12 animals did not contain eartags (3 controls, 7 low exposure and 2 high exposure group mice). Ten of these died prior to the time the mice were retagged. Even if these mice were excluded from the studies, the final conclusions would be unaffected.

2. Potential exposure to other test chemicals: The daily exposure records mention leaks of butadiene and ethylene oxide into the room air in the room containing the chambers for these two chemicals and for epoxybutane. Further examination of the records by the laboratory personnel revealed that test animals in the chambers would not have been exposed to these chemicals even if they were in the room air because the leaks occurred when the chambers were closed and the intake air for chambers passed through filters to remove organic chemicals from the air.

3. Stress to certain animals: The male mice were without feed for 3 days during the 16th week of the study. Four of these mice died in the following week. Also, the cage mesh sizes were such that some female mice sometimes got their head stuck in the openings of the cage walls. The eyes of these mice were often traumatized when the mice were freed. Although such stress is clearly undesirable, these animals are considered to be adequately representative of their respective groups.

4. Correspondence of clinical signs between observation periods: Individual animal observations made at any one time were not necessarily consistent with observations at later time periods. This may have been a function of loose terminology used to describe clinical observations, especially those of the eyes, or may have been due to animals' being in the wrong cages. Since mice are not likely to have been mixed up between groups, since separate chambers housed single dose groups, and since these observations during the studies are not a critical determinant of the final conclusions, this problem was not considered critical to the interpretations of the data.

5. Body weight data: Errors in the body weight data which were not adequately explained or dated were found. This was partially related to implementation of a new semi-automatic weighing system during the study. Although there were inconsistencies with GLP's, this problem was considered not to weaken the conclusions made in these studies.

These findings and comments are based on the NTP audit and information obtained from Battelle Pacific Northwest Laboratory personnel. Any discrepancies that may have significantly influenced the final interpretation of these inhalation studies on male and female B6C3F$_1$ mice were resolved. Minor problems not mentioned here which were not considered to affect the outcome of the study were not necessarily pursued to final resolution but are identified in the NTP audit report. In conclusion, the data examined in this audit are considered adequate to meet the objectives of these studies.

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