NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 406

TOXICOLOGY AND CARCINOGENESIS STUDIES OF γ-BUTYROLACTONE (CAS NO. 96-48-0) IN F344/N RATS AND B6C3F$_1$ MICE (GAVAGE STUDIES)

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

FOREWORD

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

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

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

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

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF γ-BUTYROLACTONE

(CAS NO. 96-48-0)

IN F344/N RATS AND B6C3F$_1$ MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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CONTRIBUTORS

National Toxicology Program

Evaluated and interpreted results and reported findings

C.J. Alden, Ph.D.
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J. Cirvello, B.S.
S.L. Eustis, D.V.M., Ph.D.
T.J. Goehl, Ph.D.
R.A. Griesemer, D.V.M., Ph.D.
J.K. Haseman, Ph.D.
R.D. Irwin, Ph.D.
G.N. Rao, D.V.M., Ph.D.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Southern Research Institute

Conducted studies, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
D.R. Farnell, D.V.M., Ph.D.
J.E. Heath, D.V.M.
R.B. Thompson, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.

Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
B.F. Hamilton, D.V.M., Ph.D.
K. Yoshitomi, D.V.M., Ph.D.

Integrated Laboratory Systems

Prepared quality assurance audits

S.L. Smith, J.D., Principal Investigator

NTP Pathology Working Group

Evaluated slides, prepared pathology report for rats (16 February 1990)

J.C. Seely, D.V.M., Chair
PATHCO, Inc.
R. Cattley, V.M.D., Ph.D.
North Carolina State University
B.F. Hamilton, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
S. Imoto, D.V.M., Ph.D.
Shin Nippon Biomedical Laboratories, Ltd., Japan
M.P. Jokinen, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program

Evaluated slides, prepared pathology report for mice (9 February 1988)

D.G. Goodman, V.M.D., Chair
PATHCO, Inc.
J.E. Heath, D.V.M.
Southern Research Institute
G. Hottendorf, D.V.M., Ph.D.
University of South Carolina
M.P. Jokinen, D.V.M.
National Toxicology Program
M.M. McDonald, D.V.M., Ph.D.
National Toxicology Program
K.T. Morgan, Ph.D.
CIIT
K. Yoshitomi, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.

Biotechnical Services, Inc.

Prepared Technical Report

L.G. Cockerham, Ph.D., Principal Investigator
G.F. Corley, D.V.M.
T.A. King-Hunter, B.S.
D.D. Lambright, Ph.D.
W.D. Sharp, B.A., B.S.

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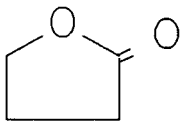
ABSTRACT



γ-BUTYROLACTONE

CAS No. 96-48-0

Chemical Formula: $C_4H_6O_2$ Molecular Weight: 86.09

Synonyms: Dihydro-2(3H)-furanone (8CI) (9CI), 1,2-butanolide, butyrolactone, 1,4-butanolide, 4-butyrolactone, 4-hydroxybutanoic acid lactone, γ-hydroxybutyric acid cyclic ester, γ-hydroxybutyric acid lactone, γ-lactone 4-hydroxy-butanoic acid, butyric acid lactone, butyryl lactone, 4-hydroxybutyric acid lactone, tetrahydro-2-furanone, 4-butanolide, 4-deoxytetronic acid, γ-hydroxybutyrolactone

γ-Butyrolactone is an intermediate in the synthesis of polymers used as film formers in hair sprays, blood plasma extenders, and clarifying agents in beer and wine. Toxicology and carcinogenesis studies were conducted by administering γ-butyrolactone (greater than 97% pure) in corn oil by gavage to groups of F344/N rats and $B6C3F_1$ mice of each sex, 5 days per week for 16 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, *Drosophila melanogaster*, and Chinese hamster ovary cells.

16-Day Studies

Groups of five rats of each sex received doses of 0, 75, 150, 300, 600, or 1,200 mg of γ-butyrolactone per kg of body weight and groups of five mice of each sex received doses of 0, 87, 175, 350, 700, or 1,400 mg/kg. All male and female rats given 1,200 mg/kg and one male rat given 600 mg/kg died within 3 days. The mean body weight gain of female rats given 600 mg/kg was significantly lower than that of the controls. Mean body weight gains of the other female dose groups and all male dose groups were similar to those of the controls. All of the male and four female mice receiving 1,400 mg/kg died during the studies. Mean body weight gains of dosed mice were generally similar to those of the controls. Rats receiving 600 or 1,200 mg/kg and mice receiving 350 mg/kg or more became inactive or recumbent with irregular respiration following dosing.

13-Week Studies

Groups of 10 rats of each sex received doses of 0, 56, 112, 225, 450, or 900 mg of γ-butyrolactone per kg of body weight and groups of 10 mice of each sex received doses of 0, 65, 131, 262, 525, or 1,050 mg/kg. One female and all male rats given 900 mg/kg died during the studies. The final mean body weight and mean body weight gain of male rats receiving 450 mg/kg were significantly lower than those of the controls; final mean body weights and body weight gains of all female rat dose groups were similar to those of the controls. There was an increased incidence of focal inflammation of the nasal mucosa in rats administered γ-butyrolactone. Three male mice and one female receiving 1,050 mg/kg died from γ-butyrolactone toxicity during the studies. The mean body weight gain and final mean body weight of high-dose male mice were lower than those of the controls; the mean body weight gains and final mean body weights of dosed female mice were similar to those of the controls. No lesions related to the administration of γ-butyrolactone occurred in mice of either sex.

2-Year Studies
The doses administered to groups of 50 animals per sex were 0, 112, and 225 mg of γ-butyrolactone per kg of body weight for male rats; 0, 225, and 450 mg/kg for female rats; and 0, 262, and 525 mg/kg for male and female mice.

Body Weight and Survival in the 2-Year Studies
The mean body weights of male rats administered γ-butyrolactone were similar to those of the controls throughout the study. The mean body weight of high-dose females was lower than that of the controls after week 5 and was 10% to 20% lower than that of the controls throughout the second year. The survival of high-dose male rats was slightly higher than that of the controls (control, 24/50; low-dose, 27/50, high-dose, 32/50) due primarily to a lower incidence of mononuclear cell leukemia in the high-dose group (16/50, 15/50, 9/50). The survival of dosed females was similar to that of the controls (28/50, 27/50, 28/50).

The mean body weights of dosed male mice were lower than those of the controls throughout the study, but the differences in mean body weights decreased when male mice were housed individually at week 67. The final mean body weights of dosed male mice were 6% lower than that of the controls. Mean body weights of dosed female mice were also lower than those of the controls throughout the study, and the final mean body weights were from 14% to 17% lower than that of the controls. The survival in high-dose male mice was significantly lower than that of the controls (35/50, 30/50, 12/50) due to bite wounds and fighting in high-dose males recovering from the sedative effects of γ-butyrolactone. The survival of female dosed mice was similar to that of the controls (38/50, 34/50, 38/50).

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies
No increased incidences of neoplasms or nonneoplastic lesions in male rats were related to the administration of γ-butyrolactone for 2 years. In female rats, negative trends were observed in the incidences of cysts (42/50, 35/50, 23/50) and fibroadenomas of the mammary gland (22/50, 14/50, 6/50) and in cysts of the pituitary pars distalis (25/49, 13/37, 11/48). These decreases were considered to be related to γ-butyrolactone administration.

Increased incidences of proliferative lesions, primarily hyperplasia, of the adrenal medulla in low-dose male mice were associated with γ-butyrolactone administration (pheochromocytoma, benign or malignant: 2/48, 6/50, 1/50; hyperplasia: 2/48, 9/50, 4/50). The incidence of hepatocellular neoplasms in both dose groups of male mice was lower than the incidence in the controls (hepatocellular adenoma or carcinoma: 24/50, 8/50, 9/50).

Genetic Toxicology
γ-Butyrolactone was not mutagenic, with or without exogenous metabolic activation (S9), in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537, nor did it induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* when administered in feed or by injection. Positive results were obtained, however, in cytogenetic tests with Chinese hamster ovary cells; γ-butyrolactone induced sister chromatid exchanges and chromosomal aberrations in trials conducted in the presence of S9 activation.

Conclusions
Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of γ-butyrolactone in male F344/N rats given 112 or 225 mg/kg or in female F344/N rats given 225 or 450 mg/kg in corn oil. There was *equivocal evidence of carcinogenic activity* of γ-butyrolactone in male B6C3F$_1$ mice based on marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia in the low-dose group. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high-dose group associated with fighting. There was *no evidence of carcinogenic activity* of γ-butyrolactone in female B6C3F$_1$ mice given 262 or 525 mg/kg in corn oil.

A decreased incidence of hepatocellular neoplasms in dosed male mice and decreased incidences of mammary gland fibroadenomas and cysts and pituitary cysts in female rats were associated with the administration of γ-butyrolactone.

* Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of γ-Butyrolactone

Variable	Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses	0, 112, or 225 mg/kg in corn oil by gavage	0, 225, or 450 mg/kg in corn oil by gavage	0, 262, or 525 mg/kg in corn oil by gavage	0, 262, or 525 mg/kg in corn oil by gavage
Body weights	Dosed groups similar to controls	High-dose group lower than controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates	24/50, 27/50, 32/50	28/50, 27/50, 28/50	35/50, 30/50, 12/50	38/50, 34/50, 38/50
Nonneoplastic effects	None	Decreased incidences of mammary gland cysts (42/50, 35/50, 23/50) and pituitary gland cysts (25/49, 13/37, 11/48)	Adrenal medulla: hyperplasia (2/48, 9/50, 4/50)	None
Neoplastic effects	None	Decreased incidence of mammary gland fibroadenomas (22/50, 14/50, 6/50)	Decreased incidence of hepatocellular neoplasms (24/50, 8/50, 9/50)	None
Uncertain findings	Decreased incidences of mononuclear cell leukemia (16/50, 15/15, 9/50)	None	Adrenal medulla: benign or malignant pheochromocytoma (2/48, 6/50, 1/50)	None
Level of evidence of carcinogenic activity	No evidence	No evidence	Equivocal evidence	No evidence

Genetic toxicology

Salmonella typhimurium gene mutation:	Negative with and without S9 in strains TA98, TA100, TA1535, or TA1537
Sister chromatid exchanges	
Chinese hamster ovary cells *in vitro*:	Positive with S9
Chromosomal aberrations	
Chinese hamster ovary cells *in vitro*:	Positive with S9
Sex-linked recessive lethal mutations	
Drosophila melanogaster:	Negative administered by injection or in feed

[a] Number with lesion/total evaluated

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on γ-butyrolactone on July 9, 1991, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

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

Daniel S. Longnecker, M.D., Chair
Department of Pathology
Dartmouth Medical School
Hanover, NH

Paul T. Bailey, Ph.D.
Toxicology Division
Mobil Oil Corporation
Princeton, NJ

Louis S. Beliczky, M.S., M.P.H.
Department of Industrial Hygiene
United Rubber Workers International Union
Akron, OH

Gary P. Carlson, Ph.D., Principal Reviewer
Department of Pharmacology and Toxicology
Purdue University
West Lafayette, IN

Harold Davis, D.V.M., Ph.D.
School of Aerospace Medicine
Brooks Air Force Base, TX

Robert H. Garman, D.V.M.
Consultants in Veterinary Pathology
Murrysville, PA

Jay I. Goodman, Ph.D., Principal Reviewer
Department of Pharmacology and Toxicology
Michigan State University
East Lansing, MI

David W. Hayden, D.V.M., Ph.D., Principal Reviewer
Department of Veterinary Pathobiology
College of Veterinary Medicine
University of Minnesota
St. Paul, MN

Curtis D. Klaassen, Ph.D.
Department of Pharmacology and Toxicology
University of Kansas Medical Center
Kansas City, KS

Barbara McKnight, Ph.D.
Department of Biostatistics
University of Washington
Seattle, WA

Ellen K. Silbergeld, Ph.D.*
University of Maryland Medical School
Baltimore, MD

Lauren Zeise, Ph.D.
California Department of Health Services/RCHAS
Berkeley, CA

*Did not attend

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On July 9, 1991, the draft Technical Report on the toxicology and carcinogenesis studies of γ-butyrolactone received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. S.L. Eustis, NIEHS, introduced the toxicology and carcinogenesis studies of γ-butyrolactone by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on nonneoplastic lesions in mice. The proposed conclusions were *no evidence of carcinogenic activity* in male or female rats or female mice, and *equivocal evidence of carcinogenic activity* in male mice.

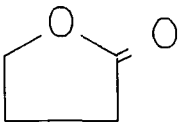
Dr. Carlson, a principal reviewer, agreed with the proposed conclusions. However, he said he could be convinced that the study in male mice was inadequate due to poor survival. He said the conclusions should note that the incidence of mononuclear cell leukemia in male rats occurred with a significant negative trend.

Dr. Goodman, the second principal reviewer, agreed in principle with the proposed conclusions. He thought the sentence in the conclusions should specify that the level of evidence in male mice was based on an increased incidence of adrenal tumors in only the low-dose group. Dr. Eustis said the definition of *equivocal evidence* presumes a lack of statistical significance. Dr. Goodman asked that a rationale be given for using the *Drosophila* protocol involving the sex-linked recessive lethal test.

Dr. Hayden, the third principal reviewer, agreed in principle with the conclusions. He noted the low survival rate for high-dose male mice and the resultant lower sensitivity for detecting a carcinogenic effect. He wondered at what point low numbers of surviving animals rendered a study group inadequate, versus only less sensitive, for evaluating carcinogenic potential. Dr. Eustis said there are no hard-and-fast rules about deciding when survival is adequate, and this was certainly a borderline case. Dr. R.A. Griesemer, NIEHS, said this could be viewed as a one-dose study because survival was certainly adequate in the low-dose male mice group.

Dr. Carlson moved that the Technical Report on γ-butyrolactone be accepted with the revisions discussed and with the conclusions as written for male and female rats and female mice, *no evidence of carcinogenic activity,* and for male mice, *equivocal evidence of carcinogenic activity.* Dr. Goodman seconded the motion, which was accepted unanimously with 10 votes.

INTRODUCTION



γ-BUTYROLACTONE

CAS No. 96-48-0

Chemical Formula: $C_4H_6O_2$ Molecular Weight: 86.09

Synonyms: Dihydro-2(3H)-furanone (8CI) (9CI), 1,2-butanolide, butyrolactone, 1,4-butanolide, 4-butyrolactone, 4-hydroxybutanoic acid lactone, γ-hydroxybutyric acid cyclic ester, γ-hydroxybutyric acid lactone, γ-lactone 4-hydroxy-butanoic acid, butyric acid lactone, butyryl lactone, 4-hydroxybutyric acid lactone, tetrahydro-2-furanone, 4-butanolide, 4-deoxytetronic acid, γ-hydroxybutyrolactone

PHYSICAL AND CHEMICAL PROPERTIES

γ-Butyrolactone is an oily liquid with a boiling point of 206° C at 760 mm Hg, a density of 1.1441 g/mL, and a specific gravity of 1.1286 at 15° C. It is completely miscible with water and common organic solvents. γ-Butyrolactone undergoes the usual reactions of γ-lactones such as ring openings and reactions in which oxygen is replaced by another ring heteroatom. It is rapidly hydrolyzed by bases and slowly hydrolyzed by acids (*Kirk-Othmer*, 1981, 1985; *Merck Index*, 1989).

PRODUCTION, USE, AND HUMAN EXPOSURE

γ-Butyrolactone is produced commercially by the dehydrogenation of 1,4-butanediol and by the hydrogenation of maleic anhydride to tetrahydrofuran and butyrolactone (*Kirk-Othmer*, 1981; *Merck Index*, 1989). γ-Butyrolactone has been used principally as an intermediate in the synthesis of 2-pyrrolidone, an intermediate for vinyl-pyrrolidone; the latter compound is used in the manufacture of homo- and copolymers. These polymers are used as film formers in hair sprays, as blood plasma extenders, and as clarifying agents in beer and wine. γ-Butyrolactone is also used as a solvent in the textile and petroleum industries and as an intermediate in the preparation of the herbicide 4-(2,4-dichlorophenoxy) butyric acid (IARC, 1976). γ-Butyrolactone is a constituent of paint removers, textile aids, and drilling oils. γ-Butyrolactone and its hydrolytic product, γ-hydroxybutyrate, have been used in humans as anesthetic agents or anesthetic adjuvants due to their sedative-hypnotic effects (Helrich *et al.*, 1964; Vickers, 1969). Moreover, alkyl derivatives of γ-butyrolactone substituted on the α- and γ-positions are neuropharmacologically active agents being investigated for their potential clinical usefulness in anticonvulsant therapy (Klunk *et al.*, 1982a,b; Levine *et al.*, 1986). Production of γ-butyrolactone in the United States in 1974 was estimated at approximately 14 million kilograms. Current production data for γ-butyrolactone are unavailable.

From a survey conducted from 1981-1983, the National Institute of Occupational Safety and Health (NIOSH) has estimated that 44,126 workers (11,013 of whom are female) are potentially exposed to γ-butyrolactone. These workers were observed in 15 different industries. Of this total number, 65% were potentially exposed in the printing and publishing and textiles mill industries (NIOSH, 1990). Additional human exposure may occur through certain food products. Residues of γ-butyrolactone have been identified in beer (2 mg/L, Spence *et al.*, 1973), apple brandy (5-31 mg/L, Rudali *et al.*, 1976), wine (Webb *et al.*, 1964), vinegar (Kahn *et al.*, 1972), cooked meats (Liebich *et al.*, 1972; Gordon, 1972), roasted filberts (Sheldon *et al.*, 1972), coffee

(Gianturco *et al.*, 1966), and tomatoes (Johnson *et al.*, 1971); they have also been detected in tobacco smoke condensate (Neurath *et al.*, 1971).

METABOLISM

γ-Butyrolactone is rapidly and completely absorbed over a wide dose range following oral administration, and the peak plasma concentration after dosing is proportional to the dose (Lettieri and Fung, 1978; Arena and Fung, 1980). When total plasma concentration of the compound (γ-butyrolactone and its principal metabolite, γ-hydroxybutyrate) is plotted against time, the area under the curve following oral administration is nearly identical to that following intravenous administration (Lettieri and Fung, 1978). It has been estimated that approximately 10% of a dose applied percutaneously is absorbed in the rat (Fung *et al.*, 1979).

γ-Butyrolactone is rapidly metabolized and eliminated primarily as respiratory CO_2 and urinary metabolites. After a single intravenous dose of ^{14}C-labeled γ-butyrolactone in the rat, traces of $^{14}CO_2$ could be detected in respiratory air after less than 4 minutes, and a maximum was reached after 15 minutes. Sixty percent of the total ^{14}C was eliminated as $^{14}CO_2$ within 2.5 hours (Roth and Giarman, 1965, 1966). The plasma half-life for intravenously administered γ-butyrolactone in rats is less than one minute. Further studies in the rat by these investigators showed that γ-butyrolactone is converted to γ-hydroxybutyrate by a lactonase enzyme present primarily in the plasma and liver (blood removed from liver by perfusion); enzymatic activity was not detected in other tissues including brain, kidney, heart, skeletal muscle, and intestine. A γ-lactonase catalyzing the formation and hydrolysis of four- to eight-carbon lactones has been purified from human blood and has similar kinetic properties to that isolated from rat liver microsomes (Fishbein and Bessman, 1966). When γ-butyrolactone is given orally, the major metabolite, γ-hydroxybutyrate, can be formed in the intestinal tract nonenzymatically by hydrolysis.

γ-Hydroxybutyrate, the principal metabolite of γ-butyrolactone, is an endogenous substance that occurs in normal mammalian brain. The metabolic pathway for γ-hydroxybutyrate has not been completely characterized, and may vary either quantitatively or qualitatively depending on the plasma levels and the organ, i.e., whether it is endogenous γ-hydroxybutyrate in the brain or exogenously administered and metabolized by the liver. Several pathways have been suggested for the catabolism of γ-hydroxybutyrate, such as its conversion into succinic acid and other Krebs cycle intermediates (Fishbein and Bessman, 1964; Doherty and Roth, 1978), interconversion into γ-aminobutyric acid (Margolis, 1969; Doherty *et al.*, 1975; Vayer *et al.*, 1985), and breakdown via β-oxidation (Walkenstein *et al.*, 1964).

It was originally suggested that γ-hydroxybutyrate is catabolyzed by entry into the Krebs cycle. However, only a very small proportion of the radioactive label from [1-^{14}C] and [4-^{14}C]-γ-hydroxybutyrate administered intravenously or intraperitoneally to rats or cats appeared in succinate (Walkenstein *et al.*, 1964; Roth and Giarman, 1966). In contrast to these findings, other investigators obtained substantial labeling of succinate and its amino acid metabolites in the brain of rats after intraventricular administration of [1-^{14}C]-labeled γ-hydroxybutyrate (Doherty *et al.*, 1975). Moreover, Möhler *et al.* (1976) demonstrated that the labeling pattern in the mouse brain after an intravenous injection of [1-^{14}C]-labeled γ-hydroxybutyrate can be explained by oxidation via succinate, but not by β-oxidation.

More recently, Vayer *et al.* (1985) have shown that γ-hydroxybutyric acid is metabolized to γ-aminobutyric acid in incubated brain slices. Further, specific inhibitors of γ-aminobutyrate-2-oxoglutarate transaminase blocked the production of labeled γ-aminobutyric acid from labeled γ-hydroxybutyric acid and of labeled 2-oxoglutarate from labeled glutamate. These findings suggested that the catabolism of γ-hydroxybutyric acid to γ-aminobutyric acid occurs via a transamination mechanism and not through the Krebs cycle. It has also been reported that brain tissue possesses some capacity to reduce succinic semi-aldehyde to γ-hydroxybutyrate as well as convert γ-aminobutyric acid to γ-hydroxybutyrate (Roth and Giarman, 1969, 1970).

Nevertheless, these findings do not preclude the possibility of alternative metabolic pathways such as β-oxidation being involved in other organs such as the liver. In other experiments, increased urinary excretion of *S*-3,4-dihydroxybutyric acid, glycolic acid, and the hydroxyepoxide tautomer of 4-hydroxy-3-oxobutyric acid was observed in humans receiving a 1 g oral dose of γ-butyrolactone (Lee, 1977).

Although these results provided evidence of metabolism by β-oxidation, the extent of contribution of the β-oxidative pathway to the turnover of endogenous γ-hydroxybutyrate was not determined.

TOXICITY

γ-Butyrolactone has relatively low toxicity with LD_{50} values in mice of 880 mg/kg by intravenous or intraperitoneal administration and 1,260 mg/kg by oral administration (Hampel and Hapke, 1968). In rats, the oral LD_{50} is 1,800 mg/kg (Kvasov, 1974). In these studies, no clinical signs of toxicity were reported other than dose-related sedative and hypnotic effects characterized by the loss of righting reflex. In rats administered γ-butyrolactone intraperitoneally, the sedative/hypnotic effects lasted for approximately 90 minutes at 200 mg/kg, for 3 hours at 400 mg/kg, and for 5 to 8 hours at 700 to 800 mg/kg (Borbély and Huston, 1972). Low doses of γ-butyrolactone (100 or 200 mg/kg) have a biphasic effect on locomotor activity in the rat (Davies, 1978). Initially, locomotor activity is reduced, followed by a period of hyperactivity. Sedative and hypnotic effects similar to those seen in animals are observed in humans (Winters and Spooner, 1965; Vickers, 1969).

The sedation and stupor produced in experimental animals by γ-butyrolactone and its hydroxy acid, γ-hydroxybutyrate, are associated with electrical seizure activity similar to the petit mal absences in humans (Winters and Spooner, 1965; Godschalk *et al.*, 1976, 1977). The electroencephalographic (EEG) phenomena, characterized by high amplitude (generally called hypersynchrony), are seen in the rat (Marcus *et al.*, 1967), cat (Winters and Spooner, 1965), rabbit, and man (Schneider *et al.*, 1963) with concomitant arrest of behavioral activity. The EEG induced by γ-butyrolactone or γ-hydroxybutyrate is similar to the EEG phenomena seen in epileptic patients and convulsing animals, but convulsions do not occur.

Other physiological effects observed in experimental animals include depression of cerebral glucose metabolism in rats (32% of controls for gray matter and 58% for white matter; Wolfson *et al.*, 1977), hyperthermia and respiratory depression in rats at hypnotic doses (Borbély and Huston, 1972) and mild metabolic acidosis as evidenced by decreased arterial pH and HCO_3 content (MacMillan, 1978). Intravenous administration of 100 mg of γ-butyrolactone to anesthetized dogs elevated blood pressure and respiratory rate, but had opposite effects in anesthetized cats (Hampel and Hapke, 1968).

The pharmacologic and toxicologic effects of γ-butyrolactone are likely attributable to its principal metabolite, γ-hydroxybutyrate, or to γ-aminobutyric acid. γ-Aminobutyric acid appears to be the major precursor of endogenous γ-hydroxybutyrate in the brain, although γ-hydroxybutyrate formation represents only a minor route of γ-aminobutyric acid metabolism (Roth and Giarman, 1969; Gold and Roth, 1977). The endogenous concentration of γ-aminobutyric acid in the substantia nigra of the rat is about 1,000 times that of γ-hydroxybutyrate (Roth and Giarman, 1970). It has been suggested that γ-hydroxybutyrate may be involved in synaptic transmission based on its low and heterogeneous distribution in the brain, extremely rapid turnover rate (Gold and Roth, 1977), the immunocytochemical localization of the γ-hydroxybutyrate synthesizing enzyme in the brain (Weissmann-Nanopoulos *et al.*, 1982), transport through membrane vesicles (Benavides *et al.*, 1982a), and high-affinity binding and release (Benavides *et al.*, 1982b; Maitre *et al.*, 1983a,b,c). Administration of "anesthetic" doses of γ-butyrolactone or γ-hydroxybutyrate produces an acute blockade of impulse flow in the nigro-striatal dopaminergic pathway. Single unit recordings of dopamine cell neuronal activity have shown that dopamine neurophysiological activity is completely inhibited for at least one hour following a single injection of γ-butyrolactone (Walters *et al.*, 1973; Roth *et al.*, 1973). Striatal dopamine levels increase while levels of dihydroxyphenylacetic acid and homovanillic acid, the two major dopamine metabolites, decrease; dopamine synthesis rates initially increase, but later fall below normal (Gessa *et al.*, 1966; Roth and Suhr, 1970; Spano *et al.*, 1971; Walters *et al.*, 1973; Argiolas *et al.*, 1982).

REPRODUCTIVE TOXICITY

Groups of 10 pregnant Sprague-Dawley rats were given 10, 50, 125, 250, or 500 mg/kg γ-butyrolactone in soy bean oil by gavage daily on days 6 through 15 of gestation. A control group of nine rats was given 5 mL/kg soybean oil. On day 21 of gestation the rats were anesthetized by ethyl ether and the fetuses removed by caesarean section. No embryotoxic effects were seen (Kronevi *et al.*, 1988).

CARCINOGENICITY

Groups of 60 male and 60 female C3H mice were given 1 g γ-butyrolactone per kg of diet for life; lifetime studies were also conducted in groups of 36 XVII/G mice of both sexes by administering doses of 2 mg γ-butyrolactone in 0.1 mL water twice weekly. No increases in the incidences of hepatomas in males or mammary gland tumors in females were observed in treated C3H mice compared to 54 male or 61 female controls. The incidence of lung tumors in treated XVII/G mice was 55%, compared with 61% in 44 controls; the average survival was 571 days for treated mice and 595 days for controls (Rudali *et al.*, 1976).

Twelve weanling male albino rats were given four doses of γ-butyrolactone ranging from 200 to 900 mg/kg body weight by gavage over a period of 7.5 months. Six of the treated rats survived for more than a year after receiving the last dose. Of these six survivors, five developed tumors: one developed an interstitial cell tumor of the testes, two developed squamous cell carcinomas of the jaw, and two developed pituitary tumors. Similar pituitary tumors were found among the control rats. Testicular interstitial cell tumors and jaw tumors were reported by the investigators to occur occasionally in aging control rats. The γ-butyrolactone used in this study was obtained by distillation of an epoxy resin hardener consisting of 54% 4,4′-diaminodiphenylmethane in γ-butyrolactone (Schoental, 1968).

In a dermal application study, 30 eight-week-old male Swiss ICR/Ha mice received 0.1 mL of a 10% solution of γ-butyrolactone in benzene on the dorsal skin three times per week for life. Two of the animals developed skin tumors and one of these animals had a skin carcinoma; the median survival time was 292 days. Among 150 benzene vehicle controls, 11 mice developed skin tumors, one of which was a carcinoma. Mean survival in the four control groups ranged from 262 to 412 days (Van Duuren *et al.*, 1965). No compound-related increases in tumor incidences were observed in a separate lifetime study of 30 female Swiss ICR/Ha mice painted with 0.1 mL of a 10% solution of γ-butyrolactone in acetone three times a week; mean survival was 495 days (Van Duuren *et al.*, 1965).

No skin tumors were observed among groups of 30 male and female XVII/G mice given repeated skin applications of a 1% solution of γ-butyrolactone in acetone twice per week for life. The incidence of lung tumors was 21/30 (70%) compared with 9/17 (53%) in the acetone vehicle controls; the average survival was 601 days for the treated mice versus 499 days for the controls (Rudali *et al.*, 1976).

Sixteen female Swiss/Webster mice were given 12 subcutaneous injections of 0.005 mg γ-butyrolactone in 0.1 mL tricaprylin three times per week for four weeks. No tumors were observed at the injection site; 11 mice survived 18 months (Swern *et al.*, 1970).

Five 8-week-old male Wistar rats were given 2 mg γ-butyrolactone in *Arachis* oil subcutaneously, twice per week for 61 weeks and were observed up to 100 weeks. All rats survived and no tumors were observed at the injection site (Dickens and Jones, 1961).

Of 34 XVII/G mice given subcutaneous injections of 1 μg γ-butyrolactone on the first, fourth, and eighth days of life, 18 (54%) developed lung tumors compared with 27/44 (61%) of the controls. Average survival of the treated animals was 590 days versus 595 days for the controls (Rudali *et al.*, 1976).

GENETIC TOXICITY

γ-Butyrolactone has been extensively studied for mutagenicity as part of the International Collaborative Program's (ICP) evaluation of the use of short-term tests for chemical carcinogens (*Progress In Mutation Research*, 1981). All results from bacterial, yeast, insect, or mammalian test systems conducted for this collaborative study were negative, as were most results from the few independent mutagenicity studies conducted with this chemical. A thorough discussion of the performance of this chemical in bacterial mutation assays is presented by Bridges *et al.* (1981). Briefly, γ-butyrolactone did not cause DNA damage (Green, 1981; Ichinotsubo *et al.*, 1981; Tweats, 1981) or gene mutation in *Escherichia coli* (Gatehouse, 1981; Matsushima *et al.*, 1981; Venitt and Crofton-Sleigh, 1981; Kuroda *et al.*, 1986) or *Salmonella typhimurium* (Baker and Bonin, 1981; Brooks and Dean, 1981; Loquet *et al.*, 1981; Richold and Jones, 1981; Rowland and Severn, 1981; Simmon and Shepherd, 1981; Trueman, 1981; Haworth *et al.*, 1983). Tests in yeast for mitotic gene conversion and aneuploidy

induction were also negative (Jagannath *et al.*, 1981; Parry and Sharp, 1981; Sharp and Parry, 1981; Zimmermann and Scheel, 1981) and a detailed presentation of the yeast assay results from the ICP study is provided by de Serres and Hoffmann (1981). γ-Butyrolactone did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (Vogel *et al.*, 1981) or sperm head abnormalities in mice (Topham, 1980). In mammalian cells *in vitro*, negative results were obtained with γ-butyrolactone in tests for chromosome aberration induction using a rat liver epithelial cell line without supplemental S9 (Dean, 1981) and in tests for unscheduled DNA repair in HeLa cells with and without S9 (Martin and McDermid, 1981). Also, γ-butyrolactone was negative for induction of gene mutations in Chinese hamster V79 cells (Knaap *et al.*, 1981) and human fibroblasts (Gupta and Goldstein, 1981), with and without S9. Additionally, *in vivo* mammalian tests for induction of micronuclei in bone marrow cells of mice were negative (Salamone *et al.*, 1981; Tsuchimoto and Matter, 1981). In contrast to the overwhelming evidence of an absence of genetic toxicity for γ-butyrolactone, there is one recent report of induction of chromosomal aberrations and sister chromatid exchanges by high concentrations (above 2,500 μg/mL) of γ-butyrolactone in Chinese hamster ovary cells (Loveday *et al.*, 1989). In this report, both endpoints were significantly increased only in the presence of induced S9 and the authors speculated that the addition of S9 enzymes coupled with 10-fold higher concentration of γ-butyrolactone allowed detection of cytogenetic effects which were not observed in the earlier study with a rat liver cell line (Dean, 1981).

STUDY RATIONALE

γ-Butyrolactone is a representative of the five-membered ring lactones. The potential for widespread exposure exists due to its use as a chemical intermediate in the manufacture of a variety of products including polymers and herbicides. γ-Butyrolactone has also been detected in various foods and has been used as an anesthetic adjuvant.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF γ -BUTYROLACTONE

γ-Butyrolactone (commercial grade) was obtained in one lot (lot 600-BLO) from GAF Corporation (New York, NY) which was used throughout the studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI; Kansas City, MO) and confirmed by the study laboratory, Southern Research Institute (Birmingham, AL). The methods and results of these studies are given in Appendix G.

The study chemical, a clear, colorless liquid, was identified as γ-butyrolactone by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. Lot 600-BLO was greater than 97% pure, as determined by Karl Fischer water analysis, thin layer chromatography, two gas chromatography systems, titration, and elemental analysis.

Stability studies performed by the analytical chemistry laboratory using gas chromatography indicated that γ-butyrolactone was stable as a bulk chemical for at least 2 weeks at temperatures to 60° C. Throughout the studies, the bulk chemical was stored in the dark at 5° C at the study laboratory. The stability of the bulk chemical was monitored by the study laboratory using gas chromatography and infrared absorption periodically during all phases of the studies. No change in the study material was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing appropriate amounts of γ-butyrolactone and corn oil (Table G1). Studies were conducted by the analytical chemistry laboratory to determine stability of γ-butyrolactone in corn oil. Gas chromatography confirmed the stability of the dose formulations when stored 14 days in the dark at temperatures to 25° C. During the studies, the dose formulations were stored in sealed amber serum vials in the dark at 5° C for no longer than 2 weeks.

The study laboratory conducted periodic analyses of the γ-butyrolactone dose formulations using gas chromatography as described in Appendix G. During the 13-week studies, the dose formulations were analyzed twice and 9 of 10 dose formulations for rats and 7 of 10 dose formulations for mice were within 10% of the target dose (Table G3). During the 2-year studies, the dose formulations were analyzed at approximately 8-week intervals; 98% (41/42) of the dose formulations for rats and 96% (27/28) of the dose formulations for mice were within 10% of the target concentrations (Table G4). Results of periodic referee analyses of the dose formulations performed by the analytical chemistry laboratory were in agreement with the results from the study laboratory (Table G5).

16-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and observed for 16 days before the studies began. The rats averaged 48 days old and the mice averaged 55 days old when placed on the studies. Groups of 5 male and female rats received γ-butyrolactone in 5 mL corn oil by gavage at doses of 0, 75, 150, 300, 600, or 1,200 mg/kg of body weight. Groups of five male and female mice received γ-butyrolactone in 10 mL corn oil by gavage at doses of 0, 87, 175, 350, 700, or 1,400 mg/kg of body weight (Table 1). All groups received the doses for 12 consecutive days, excluding weekends, with at least two consecutive dosing days before study end. Animals were housed five per cage, and water and feed were available *ad libitum*. Clinical observations were conducted and recorded twice daily. Animals were weighed at the start of the study, on day 8, and on day 16. Complete necropsies were performed on all animals. Further details are presented in Table 1.

13-WEEK STUDIES

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to γ-butyrolactone and to determine appropriate chemical concentrations to be used in the 2-year studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and were observed for 19 days before the studies began. The average age of rats was 51 days and mice were 58 days old at the beginning of the studies. Groups of 10 rats received γ-butyrolactone by gavage at doses of 0, 56, 112, 225, 450, or 900 mg/kg of body weight, and groups of 10 mice received γ-butyrolactone by gavage at doses of 0, 65, 131, 262, 525, or 1,050 mg/kg 5 days a week for 13 weeks (Table 1). Animals were housed five per cage, and water and feed were available *ad libitum*. Animals were observed twice a day and clinical observations were recorded once a week. Animals were weighed at the start of the study and weekly thereafter. Further experimental details are presented in Table 1.

Surviving animals were killed at the end of the 13-week studies. Necropsies were performed on all study animals. The brain, heart, right kidney, liver, lungs, and thymus of survivors were weighed at necropsy. Complete histopathology was performed on all animals killed or dying during the study, all control animals, rats receiving 900 mg/kg, male rats receiving 450 mg/kg, and mice receiving 1,050 mg/kg. The liver and nose (nasal cavity and turbinates) were examined from rats in the 56, 112, and 225 mg/kg dose groups and from female rats in the 450 mg/kg dose groups. Tissues examined for each group are listed in Table 1.

2-YEAR STUDIES

Study Design

Groups of 50 rats and mice of each sex were administered γ-butyrolactone in corn oil by gavage 5 days a week for up to 103 weeks. Male rats received 0, 112, or 225 mg/kg, female rats received 0, 225, or 450 mg/kg of body weight, and mice received 0, 262, or 525 mg/kg of body weight (Table 1).

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) for use in the 2-year studies. Rats were quarantined 18 days and mice were quarantined 19 days. Five rats and mice per sex were randomly selected and killed for parasite evaluation and gross observation of disease. Serology samples were collected for viral screens. Rats were about 61 days old at study initiation, male mice were 55 days old, and female mice were 62 days old. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix I).

Animal Maintenance

Rats were housed five per cage throughout the study. Mice also were housed five per cage until week 67 (males) or week 87 (females); after this time mice were housed individually. Feed and water were available *ad libitum*. Cage racks were rotated every 2 weeks beginning week 37. Information on feed composition and contaminants is provided in Appendix H. Further details of animal maintenance are given in Table 1.

Clinical Examinations and Pathology

Clinical observations were made twice daily; findings were recorded at the time of weighing or as necessary. Animals were weighed at study initiation, weekly for 13 weeks, and monthly thereafter. Animals found moribund or surviving to the end of the 2-year studies were killed. Necropsy was performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Complete histopathologic examinations were performed on rats that died or were killed moribund prior to day 637, on all control and high-dose rats and mice, and on all low-dose male mice. Selected tissues were examined from all low-dose rats and from low-dose female mice. Histopathology examinations were performed on all grossly visible lesions in all dose groups. The tissues and tissue groups examined are listed in Table 1.

Upon completion of the microscopic evaluation by the laboratory pathologist, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The individual animal records and pathology tables were compared for accuracy, slide and tissue counts were verified, and histotechnique was evaluated. All tissues with a diagnosis of neoplasia and all tissues from a randomly selected 10% of the control and high-dose rats and mice were reevaluated microscopically by a quality assessment pathologist. The quality assessment pathologist also examined the following organs: adrenal medulla (mice), bone and marrow (female mice), liver (rats), skin (mice), and testis and epididymis (male rats).

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides of male and female rat livers; rat testes and epididymis; male mouse skin, bones (feet and tail), urogenital tract, and adrenal medulla; and female mouse ovary and bone marrow; examples of disagreements in diagnoses between the laboratory and quality assessment pathologists; and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

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

Calculation of Incidence

The incidence of neoplastic or nonneoplastic lesions is 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 include 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 had multiple potential sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

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

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

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

Historical Control Data

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

Analysis of Continuous Variables

The multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972) were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of organ weight and body weight data. Jonckheere's test (Jonckheere, 1954) was used to evaluate the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's test).

Quality Assurance Methods

The 13-week and 2-year studies were conducted in compliance with FDA Good Laboratory Practice Regulations (CFR Part 58). In addition, as study records were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all had been resolved or were otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of γ-butyrolactone was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges, and chromosomal aberrations in Chinese hamster ovary cells, and sex-linked recessive lethal mutations in *Drosophila melanogaster*. The protocols for these studies and tabular presentations of their findings are given in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of γ-Butyrolactone

16-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)
Strain and Species		
Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$	Rats: F344/N Mice: $B6C3F_1$
Animal Source		
Charles River Breeding Laboratories (Kingston, NY)	Charles River Breeding Laboratories (Kingston, NY)	Frederick Cancer Research Facility (Frederick, MD)
Date of Birth		
Rats: 20-27 August 1980 Mice: 13-20 August 1980	Rats: 5-12 November 1980; Mice: 29 October - 5 November 1980	Rats: 9 September 1981 Mice: Males - 8 September 1981; Females - 1 September 1981
Time Held Before Study		
16 days	19 days	Rats: 18 days Mice: 19 days
Average Age When Placed on Study		
Rats: 48 days Mice: 55 days	Rats: 51 days Mice: 58 days	Rats: 61 days Mice: Males - 55 days; Females - 62 days
Date of First Dose		
11 October 1980	30 December 1980	Rats: 10 November 1981 Mice: 3 November 1981
Duration of Dosing		
Days 1-5, 8-12, 15, 16	13 weeks (5 days/week)	103 weeks (5 days/week)
Date of Last Dose		
26 October 1980	30 March 1981	Rats: 31 October 1983 Mice: 24 October 1983
Method of Sacrifice		
CO_2 asphyxiation	CO_2 asphyxiation	CO_2 asphyxiation
Necropsy Dates		
27-30 October 1980	31 March - 8 April 1981	Rats: 8-14 November 1983 Mice: 1-4 November 1983
Average Age When Killed		
Rats: 66 days Mice: 72 days	Rats: 146 days Mice: 153 days	Rats: 793 days Mice: Males - 786 days Females - 793 days
Size of Study Groups		
5 males and 5 females	10 males and 10 females	50 males and 50 females

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of γ-Butyrolactone (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Method of Animal Distribution		
Animals were grouped by weight intervals, then groups were assigned to cages. A table of random numbers was used to assign cages to treatment groups.	Same as 16-day studies	Same as 16-day studies
Animals per Cage		
5	5	5 (Male mice housed individually beginning 9 February 1983 and female mice housed individually beginning 1 July 1983)
Method of Animal Identification		
Ear punch	Ear punch	Ear punch and toe clip
Diet		
NIH-07 Rat and Mouse Ration, Open formula, pellets (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Feeders		
Rats: Stainless steel trough (Hahn Roofing and Sheet Metal Co., Birmingham, AL), changed once weekly Mice: 14-gauge aluminum cups with stainless steel cups (Sargent-Welch, Birmingham, AL), changed once weekly	Stainless steel, hanging, slotted (Lab Products, Inc., Garfield, NJ), changed once weekly	Same as 13-week studies
Water		
Tap water (Birmingham Water Works) via outside-the-cage automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 16-day studies	Same as 16-day studies
Cages		
Solid-bottom polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as 16-day studies	Same as 16-day studies
Bedding		
BetaChips® (Northeastern Products Corp., Warrensburg, NY), changed twice weekly	Same as 16-day studies	Rats: Same as 16-day studies Mice: Same as 16-day studies except changed once weekly after animals housed individually
Cage Filters		
Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as 16-day studies	Same as 16-day studies
Animal Room Environment		
Temperature: 22°-24° C Relative humidity: 43%-61% Fluorescent light: 12 hours/day Room air changes: minimum of 15/hour	Temperature: 22°-24° C Relative humidity: 35%-62% Fluorescent light: 12 hours/day Room air changes: minimum of 15/hour	Temperature: 16°-29° C Relative humidity: 31%-79% (rats) 25%-79% (mice) Fluorescent light: 12 hours/day Room air changes: minimum of 15/hour

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of γ-Butyrolactone (continued)

16-Day Studies	13-Week Studies	2-Year Studies
Doses		
Rats: 0, 75, 150, 300, 600, or 1,200 mg of γ-butyrolactone in 5 mL corn oil/kg body weight by gavage Mice: 0, 87, 175, 350, 700, or 1,400 mg of γ-butyrolactone in 10 mL corn oil/kg body weight by gavage	Rats: 0, 56, 112, 225, 450, or 900 mg of γ-butyrolactone in 5 mL corn oil/kg body weight by gavage Mice: 0, 65, 131, 262, 525, or 1,050 mg of γ-butyrolactone in 10 mL corn oil/kg body weight by gavage	Rats: Males - 0, 112, or 225 mg of γ-butyrolactone in 5 mL of corn oil/kg body weight by gavage Females - 0, 225, or 450 mg of γ-butyrolactone in 5 mL of corn oil/kg body weight by gavage Mice: 0, 262, or 525 mg of γ-butyrolactone in 10 mL of corn oil/kg body weight by gavage
Type and Frequency of Observation		
Observed twice/day; weighed initially and once/week; clinical observations recorded twice daily	Observed twice/day; weighed initially and once/week; clinical observations recorded once/week	Observed twice/day; weighed initially, once/week for 13 weeks, once/month thereafter; clinical observations recorded at each weighing period
Necropsy Examinations		
Necropsy performed on all animals.	Necropsy performed on all animals. The following organs were weighed: brain, heart, right kidney, liver, lung, and thymus.	Necropsy performed on all animals.
Histopathological Examinations		
No histopathology performed.	Complete histopathology on all animals that died or were killed moribund during the study, all controls, 900 mg/kg rats, 450 mg/kg male rats, and 1,050 mg/kg mice. Tissues examined included: adrenal gland, bone and marrow (femur), brain, clitoral gland (rats) or preputial gland, esophagus, epididymis (rats), gallbladder (mice), heart, kidney, large intestine, liver, lung with mainstem bronchi, lymph nodes (mesenteric, mandibular), mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle (thigh), skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes). Tissues examined from rats in the 56, 112, and 225 mg/kg dose groups and the 450 mg/kg female dose group included: liver (males), nasal cavity and turbinates, and gross lesions.	Complete histopathology on all rats dying or killed moribund prior to day 637, all control and high-dose rats and mice, and low-dose male mice. Tissues examined: adrenal gland, bone and marrow (femur), brain, clitoral or preputial gland (rats), epididymis, esophagus, gallbladder (mice), harderian gland (low-dose male mice), heart, kidney, large intestine, liver, lung with mainstem bronchi, lymph nodes (mandibular, mesenteric), mammary gland, nasal cavity and turbinates, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle (rats), skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, uterus, and gross lesions and tissue masses (with regional lymph nodes). Tissues examined in low-dose rats dying or killed moribund after day 636 or killed at study end: liver, mammary gland (females), spleen, testes, and gross lesions. Tissues examined for low-dose female mice: bone and marrow (femur), brain, kidney, harderian gland, liver, lung, mammary gland, ovary, pancreas, pituitary gland, stomach, thyroid gland, and gross lesions.

RESULTS

RATS

16-Day Studies

All male and female rats receiving 1,200 mg/kg γ-butyrolactone died within the first three days of the studies; one male receiving 600 mg/kg died on day 3 (Table 2). There were no significant differences between the final mean body weights of controls and of rats administered γ-butyrolactone. However, the mean body weight gain of the female 600 mg/kg group was significantly lower than that of the controls. The mean body weight gains of females given 300 mg/kg or less and of all males given γ-butyrolactone were similar to those of the controls (Table 2). Rats in the 600 or 1,200 mg/kg dose groups became recumbent or inactive with irregular and labored respiration shortly after dosing.

TABLE 2
Survival and Mean Body Weights of Rats in the 16-Day Gavage Studies of γ-Butyrolactone

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	134 ± 4	219 ± 4	85 ± 3	
75	5/5	128 ± 2	214 ± 4	85 ± 3	97
150	5/5	132 ± 2	211 ± 4	79 ± 2	96
300	5/5	124 ± 1*	206 ± 4	82 ± 4	94
600	4/5[c]	132 ± 2	213 ± 1	80 ± 1	97
1,200	0/5[d]	133 ± 3	-	-	-
Female					
0	5/5	112 ± 5	154 ± 4	42 ± 3	
75	5/5	109 ± 3	154 ± 5	44 ± 3	100
150	5/5	118 ± 2	162 ± 2	43 ± 1	105
300	5/5	105 ± 2	143 ± 3	38 ± 2	93
600	5/5	114 ± 2	146 ± 2	32 ± 1**	95
1,200	0/5[e]	107 ± 3	-	-	-

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No final mean body weight was calculated for groups with 100% mortality.
[c] Day of death: 3
[d] Day of death: 1, 3, 3, 3, 3
[e] Day of death: 1, 2, 3, 3, 3

13-Week Studies

All male rats and one female rat given 900 mg/kg γ-butyrolactone died by week 8 (Table 3). The deaths of one female in the 112 mg/kg group and one control male were attributed to improper gavage technique. The final mean body weights and mean body weight gains of males in the 450 mg/kg group were significantly lower than those of the controls; final mean body weights and weight gains for males given 56, 112, or 225 mg/kg γ-butyrolactone and for all female dose groups were similar to those of the controls (Table 3). All rats in the 900 mg/kg dose groups became recumbent within several minutes after dosing, but appeared normal at the next observation period several hours later. Rats in the 225 and 450 mg/kg dose groups exhibited slight inactivity after dosing. After 2 to 3 weeks, all animals ceased to react visibly to the

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Gavage Studies of γ-Butyrolactone

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	9/10[c]	148 ± 2	370 ± 7	223 ± 6	
56	10/10	146 ± 4	375 ± 8	229 ± 5	101
112	10/10	147 ± 3	379 ± 4	232 ± 5	102
225	10/10	147 ± 3	363 ± 4	216 ± 4	98
450	10/10	149 ± 4	345 ± 7**	196 ± 6**	93
900	0/10[d]	149 ± 3	–	–	–
Female					
0	10/10	119 ± 2	203 ± 3	84 ± 2	
56	10/10	115 ± 2	203 ± 3	87 ± 3	100
112	9/10[c]	117 ± 2	209 ± 2	90 ± 3	103
225	10/10	118 ± 2	208 ± 3	90 ± 4	103
450	10/10	116 ± 2	202 ± 4	86 ± 3	100
900	9/10[e]	115 ± 2	198 ± 3	82 ± 3	98

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No final mean body weight was calculated for groups with 100% mortality.
[c] Accidental deaths
[d] Week of death: 1, 1, 1, 1, 1, 1, 1, 5, 5, 5
[e] Week of death: 8

daily administration of γ-butyrolactone, indicating some form of adaptation or tolerance to its "anesthetic" and sedative properties. At necropsy there were no biologically significant differences in absolute or relative organ weights between dosed and control rats (Table F1), and no gross lesions were attributed to γ-butyrolactone administration. Microscopic examination of tissue specimens revealed increased incidences of inflammation of the nasal mucosa in dosed rats (males: control, 1/10; 56 mg/kg, 7/10; 112 mg/kg, 9/9; 225 mg/kg, 9/9; 450 mg/kg, 9/10; 900 mg/kg, 6/10; females: 2/10, 4/9, 6/10, 9/9, 9/10, 9/10). The lesions were focal or multifocal and consisted of small accumulations of neutrophils and macrophages in the lumen or mucosa. Similar lesions have been seen in other gavage studies with a variety of chemicals and may be related to the reflux of the gavage solution into the nasopharynx after dosing.

Dose Selection Rationale: The doses selected for the 2-year study in male rats were 0, 112, and 225 mg/kg. These doses were based on the mortality in males receiving 900 mg/kg and the depressed body weight gain in males given 450 mg/kg in the 13-week study. Because of the lower mortality in female rats receiving 900 mg/kg γ-butyrolactone, the doses selected for the 2-year study in female rats were 0, 225, and 450 mg/kg.

2-Year Studies

Body Weights and Clinical Findings

The mean body weights of male rats given γ-butyrolactone were similar to those of the control group throughout the 2-year study (Table 4). However, the mean body weight of high-dose female rats was lower than those of the controls from week 6 to the end of the 2-year study (Table 5 and Figure 1). The mean body weight of high-dose females was within 10% of the mean body weight of the controls until week 58; by the end of the 2-year studies the mean body weight was 20% lower than that of the controls. The mean body weight of low-dose female rats was similar to that of the controls. There were no clinical findings attributed to γ-butyrolactone administration.

TABLE 4
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone

Weeks on Study	Vehicle Control		112 mg/kg			225 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	191	50	191	100	50	187	98	50
2	225	50	222	99	50	221	98	50
3	243	50	240	99	50	238	98	50
4	265	50	264	100	50	262	99	50
5	284	50	280	99	50	278	98	50
6	298	50	295	99	50	293	98	50
7	308	50	305	99	50	301	98	50
8	316	50	315	100	50	310	98	50
9	330	50	327	99	50	323	98	50
10	339	50	337	99	50	331	98	50
11	343	50	340	99	50	334	98	50
12	354	50	349	99	50	346	98	50
13	360	50	359	100	50	354	98	50
17	380	50	380	100	50	376	99	50
21	402	50	406	101	50	403	100	50
26	425	50	430	101	50	425	100	50
31	443	50	446	101	50	444	100	50
34	453	50	451	100	50	449	99	50
40	465	50	467	100	50	465	100	50
43	473	50	470	99	50	468	99	50
47	482	50	482	100	50	478	99	50
51	487	50	487	100	50	480	99	50
53	489	50	490	100	50	482	99	49
58	498	50	497	100	47	487	98	49
62	500	50	497	99	47	490	98	48
66	502	50	502	100	47	496	99	48
70	501	46	499	100	47	490	98	48
74	499	45	499	100	46	491	98	48
78	498	43	497	100	44	484	97	47
82	494	42	496	100	42	489	99	45
86	493	37	494	100	39	489	99	43
90	484	35	489	101	37	487	101	42
94	480	33	479	100	35	475	99	42
98	476	27	472	99	33	462	97	37
102	466	25	467	100	30	461	99	34
Terminal sacrifice		24			27			32
Mean for weeks								
1-13	297		294	99		291	98	
14-52	446		447	100		443	99	
53-102	491		491	100		483	98	

TABLE 5
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone

Weeks on Study	Vehicle Control		225 mg/kg			450 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	139	50	139	100	50	137	98	50
2	155	50	155	101	50	151	98	50
3	162	50	162	100	50	156	96	50
4	173	50	171	99	50	166	96	50
5	180	50	181	100	50	173	96	50
6	187	50	185	99	50	177	95	50
7	190	50	187	99	50	180	95	50
8	194	50	192	99	50	182	94	50
9	196	50	195	100	50	184	94	50
10	199	50	199	100	50	189	95	50
11	202	50	199	99	50	189	94	50
12	203	50	200	99	50	189	93	50
13	206	50	204	99	50	192	93	50
17	214	50	209	98	50	200	93	50
21	220	50	216	98	50	206	94	49
26	230	50	226	98	50	215	93	48
31	238	50	235	99	50	222	93	48
34	243	50	237	98	50	223	92	46
40	252	50	245	97	50	234	93	46
43	255	50	252	99	49	236	92	46
47	268	49	260	97	49	241	90	45
51	271	49	268	99	48	246	91	45
53	276	49	274	99	47	248	90	45
58	289	49	283	98	46	254	88	45
62	300	49	294	98	45	261	87	45
66	306	49	301	98	45	268	87	44
70	313	49	305	98	45	270	86	44
74	323	46	313	97	43	275	85	44
78	326	45	316	97	43	276	85	44
82	328	42	322	98	42	277	85	43
86	331	41	319	96	42	272	82	37[a]
90	334	41	323	97	42	278	83	37
94	331	40	325	98	40	277	84	36
98	333	34	321	96	37	273	82	31
102	339	30	323	95	28	272	80	30
Terminal sacrifice		28			27			28
Mean for weeks								
1-13	184		182	99		174	95	
14-52	243		239	98		225	93	
53-102	318		309	97		269	85	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.

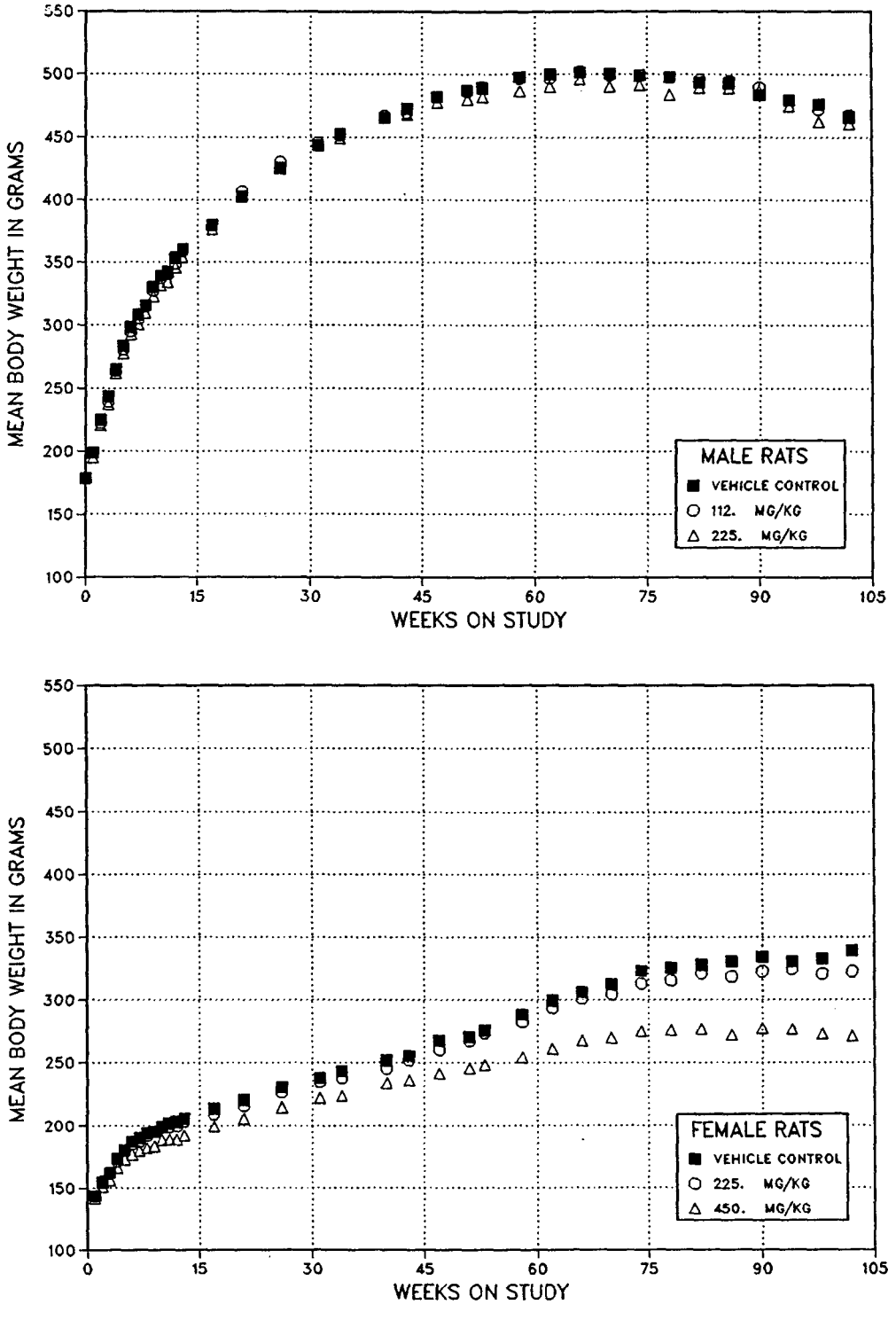


FIGURE 1
Growth Curves for Rats Administered γ-Butyrolactone by Gavage for 2 Years

Survival

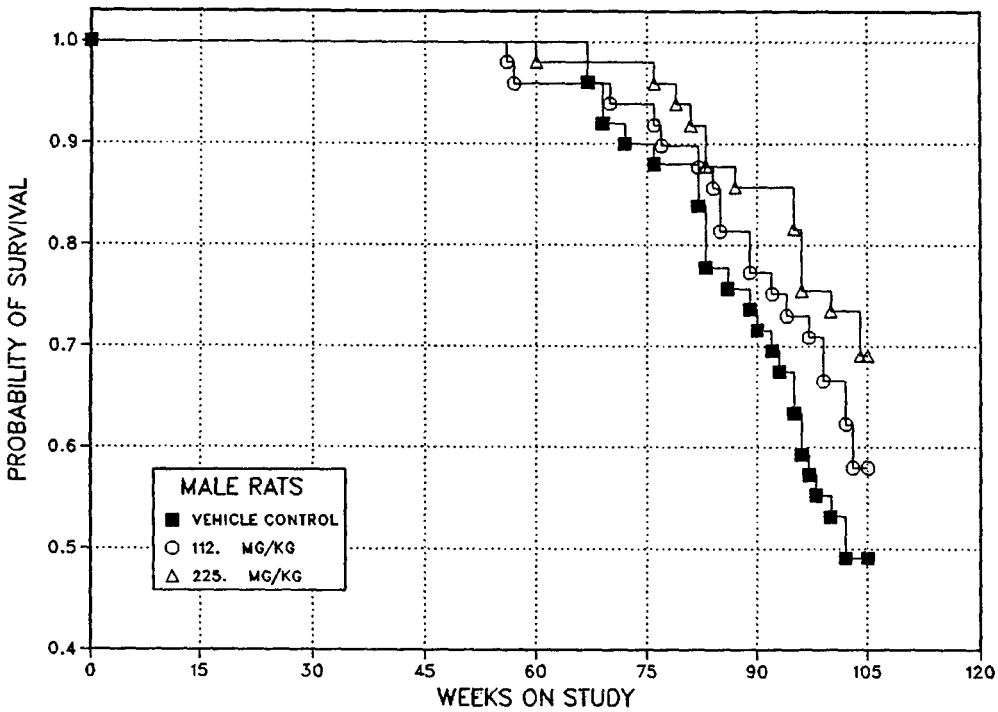
There was a marginally significant increased survival of dosed males compared to controls; however, pairwise comparisons of survival between controls and low- or high-dose groups showed no significant difference (Table 6 and Figure 2). The higher survival rates in the 225 mg/kg male dose group are due in part to the marginally decreased incidence of mononuclear cell leukemia (control, 16/50; low-dose, 15/50; high-dose, 9/50). Survival was similar in all female groups.

TABLE 6
Survival in Rats in the 2-Year Gavage Studies of γ-Butyrolactone

	Vehicle Control	112 mg/kg	225 mg/kg
Male[a]			
Animals initially in study	50	50	50
Natural deaths	6	7	3
Moribund kills	19	13	12
Accidental deaths[b]	1	3	3
Animals surviving until study termination	24	27	32
Percent survival at end of studies[c]	49	58	69
Mean survival (days)[d]	662	668	688
Survival analysis[e]	P=0.043N	P=0.415N	P=0.053N

	Vehicle Control	225 mg/kg	450 mg/kg
Female[a]			
Animals initially in study	50	50	50
Natural deaths	3	7	6
Moribund kills	19	16	14
Accidental deaths[b]	0	0	2
Animals surviving until study termination	28	27[f]	28
Percent survival at end of studies[c]	56	54	56
Mean survival (days)[d]	678	669	644
Survival analysis[e]	P=0.927N	P=0.950	P=0.969N

[a] First day of terminal sacrifice: male, 729; female, 730
[b] Censored from survival analyses
[c] Kaplan-Meier determinations. Survival rates adjusted for accidental deaths.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice).
[e] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.
[f] Includes one animal that died during the last week of the study



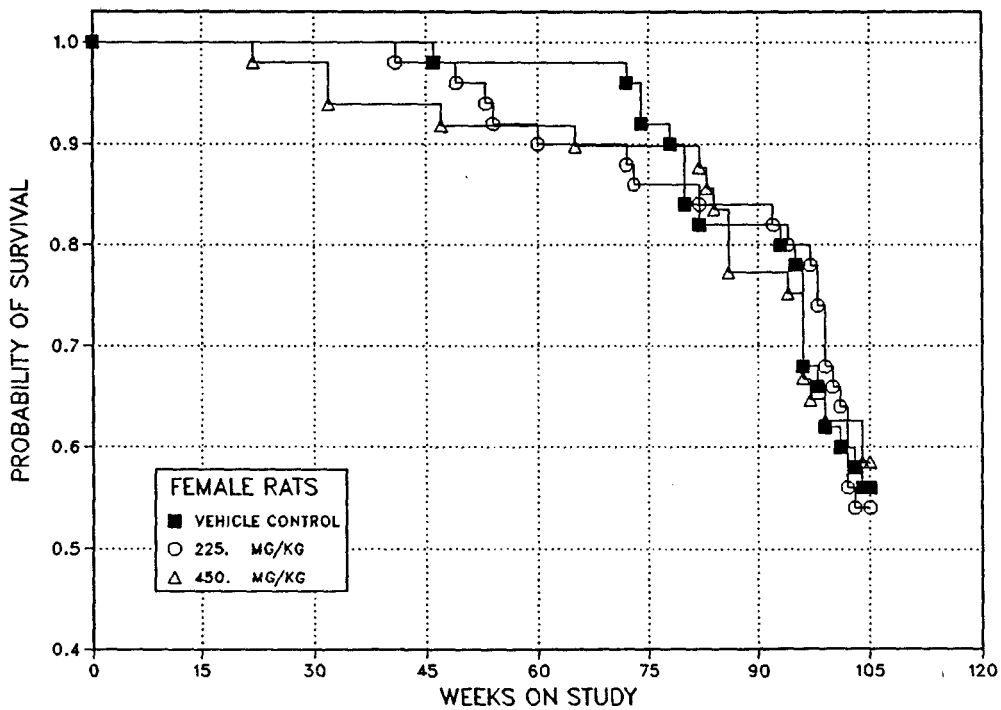


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered γ-Butyrolactone by Gavage for 2 Years

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the skin, mesothelium, mammary gland, pituitary gland, and hematopoietic system.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors occurring with an incidence of at least 5% in at least one animal group, and historical control incidences for selected neoplasms discussed in this section are presented in Appendixes A for male rats and B for female rats.

Skin: Several morphological types of epithelial neoplasms, principally benign neoplasms, occurred more frequently in dosed male rats than in controls. The incidence of keratoacanthoma was marginally increased in low- and high-dose males, but pairwise comparisons were not significant (control, 1/50; low-dose, 4/50; high-dose, 6/50). The overall historical control incidence of keratoacanthomas in NTP corn oil gavage studies with F344/N male rats is 26/770 (3.4%) with a range of 0% to 12% (Table A4a). Because the incidences of keratoacanthoma in low- and high-dose male rats are not significantly greater than the incidence in the controls (Table A3) and because the incidences are within the range for historical controls, the marginally increased incidence of keratoacanthoma is not considered related to γ-butyrolactone administration. Further, all keratoacanthomas occurred in animals killed at 2 years, and it is likely that the apparent increase in this neoplasm reflects in part the increased survival in the high-dose group relative to controls.

Basal cell adenomas occurred in four low-dose males; none were observed in high-dose or control males. One basal cell carcinoma occurred in a high-dose male rat. Although the incidence in the low-dose group was not significantly greater than the incidence in the control group, basal cell adenomas occur infrequently in male rats. The overall historical incidence of basal cell and related neoplasms in corn oil gavage controls is 13/770 (1.7%) with a range of 0% to 5% (Table A4b). The basal cell adenomas were not considered related to γ-butyrolactone administration, because they did not occur at a significantly increased incidence in the low-dose group and did not occur with an increased incidence in the high-dose group.

Mesothelium: Mesotheliomas occurred in four high-dose males and one low-dose male rat, but were not present in controls. The historical incidence of mesotheliomas in corn oil control male rats is 26/770 (3.4%) with a range of 0% to 10% (Table A4c). Thus, the apparent increased incidence reflects the low incidence in control males and is not considered to be related to γ-butyrolactone administration.

Mammary Gland: The incidence of fibroadenomas in female rats occurred with a statistically significant ($P<0.01$) negative trend, and the incidence in the high-dose group was significantly lower than that of the controls (22/50, 14/50, 6/50). The overall historical control incidence for fibroadenomas in female rats is 298/770 (38.7%) with a range of 18% to 56% (Table B4). The decreased incidence of fibro-adenomas in low- and high-dose female rats was considered related to γ-butyrolactone administration. The incidence of mammary gland cysts (markedly dilated ducts or glands lined by a single layer of epithelium) also showed a statistically significant ($P<0.01$) negative trend (42/50, 35/50, 23/50).

Pituitary Gland: There was a statistically significant ($P<0.01$) decrease in the incidence of cysts in the pars distalis of high-dose female rats (25/49, 13/37, 11/48). Cysts of the pars distalis are cavities filled with serum proteins displacing the parenchyma and often occur within focal hyperplasia or adenoma. A decreased incidence of adenomas in high-dose females was not statistically significant (22/49, 24/37, 16/48; Table B3).

Hematopoietic System: The incidence of mononuclear cell leukemia in male rats occurred with a significant negative trend, and the incidence in the high-dose males was significantly less than controls (16/50, 15/50, 9/50). Mononuclear cell leukemia is a common neoplasm in male F344/N rats with a overall historical control incidence of 164/770 (21.3%) and a range of 4% to 38% (Table A4d).

MICE

16-Day Studies

All male mice and four female mice receiving 1,400 mg/kg γ-butyrolactone died from chemical toxicity before the end of the studies. One control male, one male and two females given 175 mg/kg, and one female given 700 mg/kg died as a result of improper gavage technique. Mean body weight gains of dosed mice were generally similar to those of the controls (Table 7). Mice receiving doses of 350 mg/kg or more became recumbent or inactive shortly after dosing. Some mice also exhibited irregular respiration or dyspnea.

13-Week Studies

Nine male and 13 female mice from various dose groups died from improper gavage technique. Deaths related to γ-butyrolactone administration occurred in three males and one female from the 1,050 mg/kg dose groups (Table 8). Except for the final mean body weight of the 1,050 mg/kg male dose group, which was approximately 11% lower than that of the controls, the final mean body weights of male and female dose groups were similar to those of the controls (Table 8). Mice in the 525 and 1,050 mg/kg dose groups became recumbent several minutes after dosing, but were normal at the next observation period several hours later. Mice in the 262 mg/kg dose group exhibited moderate inactivity after dosing. In mice given 525 mg/kg or less, these acute reactions to γ-butyrolactone diminished after 3 to 4 weeks. There were no biologically significant differences in absolute or relative organ weights between dosed and control mice (Table F2). No gross or microscopic lesions related to γ-butyrolactone administration were observed.

TABLE 7
Survival and Mean Body Weights of Mice in the 16-Day Gavage Studies of γ-Butyrolactone

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	4/5[c]	24.6 ± 0.6	28.0 ± 0.6	3.3 ± 0.3	
87	5/5	24.4 ± 0.2	27.8 ± 0.4	3.4 ± 0.2	99
175	4/5[c]	24.8 ± 0.2	28.3 ± 0.5	3.5 ± 0.5	101
350	5/5	23.4 ± 0.5	26.4 ± 0.9	3.0 ± 0.5	94
700	5/5	24.8 ± 0.4	27.2 ± 0.4	2.4 ± 0.4	97
1,400	0/5[d]	24.0 ± 0.7	-	-	-
Female					
0	5/5	19.8 ± 0.5	22.4 ± 0.9	2.6 ± 0.6	
87	5/5	20.4 ± 0.2	21.4 ± 0.2	1.0 ± 0.3	96
175	3/5[c]	19.6 ± 0.4	21.7 ± 0.9	1.7 ± 0.3	96
350	5/5	18.2 ± 0.4*	19.8 ± 0.4*	1.6 ± 0.5	88
700	4/5[c]	19.4 ± 0.2	20.8 ± 0.3*	1.5 ± 0.3	93
1,400	1/5[e]	19.0 ± 0.6	20.0	2.0	89

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 16 days/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies. No final mean body weight was calculated for groups with 100% mortality. No standard error was calculated for groups with high mortality.
[c] Accidental deaths
[d] Day of death: 2, 3, 8, 8, 8
[e] Day of death: 2, 9, 10, 10

TABLE 8
Survival and Mean Body Weights of Mice in the 13-Week Gavage Studies of γ-Butyrolactone

Concentration (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	8/10[c]	25.3 ± 0.4	37.3 ± 0.8	11.8 ± 0.8	
65	6/10[c]	24.7 ± 0.5	35.2 ± 0.8	10.2 ± 0.8	94
131	8/10[c]	24.7 ± 0.5	38.1 ± 0.5	13.4 ± 0.7	102
262	9/10[c]	24.7 ± 0.5	35.7 ± 0.9	11.0 ± 0.8	96
525	10/10	24.6 ± 0.5	34.9 ± 0.8	10.3 ± 0.5	94
1,050	7/10[d]	24.5 ± 0.5	33.3 ± 1.4**	9.3 ± 1.0*	89
Female					
0	7/10[c]	18.6 ± 0.3	25.9 ± 0.7	7.0 ± 0.5	
65	7/10[c]	18.1 ± 0.4	25.3 ± 0.6	7.3 ± 0.5	98
131	7/10[c]	18.7 ± 0.3	26.0 ± 0.6	7.1 ± 0.7	101
262	10/10	19.0 ± 0.3	26.3 ± 0.4	7.3 ± 0.3	102
525	8/10[c]	18.8 ± 0.3	26.5 ± 0.7	7.8 ± 0.7	103
1,050	7/10[c,e]	18.2 ± 0.3	25.9 ± 1.0	7.9 ± 0.8	100

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Accidental deaths
[d] Week of death: 1, 1, 12.
[e] One chemical-related death week 1.

Dose Selection Rationale: Dose levels of 0, 262, and 525 mg/kg were selected for both sexes of mice in the 2-year studies based on the mortality observed in the 1,050 mg/kg dose group during the 13-week studies.

2-Year Studies

Body Weights and Clinical Findings

Mean body weights of low- and high-dose male mice followed a similar pattern throughout the study and were consistently lower than the mean body weights of the controls (Table 9). The decrement in body weight gain was evident as early as week 3 and continued to increase until approximately week 66. Mean body weights of low- and high-dose males were within 10% of the mean body weight of the controls through week 27; from week 32 to week 66 the decrement increased to a maximum of 17%. During week 67, all male mice were housed individually; thereafter, the difference between the mean body weights of dosed males and control mice decreased. By the end of the study, the final mean body weights of low- and high-dose male mice were only 6% less than that of the controls. In female mice, the mean body weights of both dose groups were within 10% of those of the controls through week 27. Thereafter, weight gains of low-and high-dose females steadily declined relative to controls, and the differences did not diminish after the females were housed individually at week 87. At the end of the study, the final mean body weights of low- and high-dose female groups were 17% and 14% lower than that of the controls (Table 10). Growth curves for mice in the 2-year studies are shown in Figure 3. High-dose male and female mice were observed to be partially sedated or lethargic and inactive shortly after dosing.

TABLE 9
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone

Weeks on Study	Vehicle Control		262 mg/kg			525 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	23.6	50	24.1	102	50	23.7	100	50
2	25.8	50	25.9	100	50	26.3	102	50
3	28.8	50	27.4	95	50	27.0	94	50
4	29.1	50	28.5	98	50	27.9	96	50
5	30.1	50	29.8	99	50	28.8	96	50
6	31.3	50	29.0	93	50	30.0	96	50
7	32.4	50	31.7	98	50	30.9	95	50
8	33.1	50	31.9	96	50	31.5	95	50
9	33.9	50	32.4	96	50	32.2	95	50
10	34.6	50	33.5	97	50	32.8	95	50
11	35.1	50	34.2	97	50	34.0	97	49
12	35.7	50	34.7	97	50	34.4	96	49
13	36.1	50	34.6	96	50	33.6	93	49
15	36.7	50	35.4	97	50	35.2	96	48
18	37.5	50	35.3	94	50	36.0	96	44
22	39.9	50	37.2	93	48	37.5	94	44
27	41.9	49	38.0	91	47	38.7	92	38
32	44.3	49	39.8	90	46	39.0	88	36
35	44.4	49	39.2	88	46	39.6	89	36
40	46.0	49	40.8	89	45	40.2	87	36
44	45.8	49	40.7	89	45	38.6	84	36
48	46.1	49	40.6	88	45	40.9	89	35
52	48.1	49	41.4	86	44	41.7	87	35
56	48.9	48	42.1	86	43	41.0	84	33
58	48.7	48	40.6	83	40	41.4	85	33
62	49.9	48	41.4	83	39	41.7	84	32
66	50.0	47	42.7	85	38	41.3	83	30
70	48.1	45	42.5	88	38	41.3	86	30[a]
74	46.9	45	41.9	89	38	40.2	86	28
78	47.5	44	42.6	90	36	41.2	87	28
82	46.5	44	43.2	93	36	42.9	92	24
86	47.4	41	43.0	91	34	40.9	86	20
90	47.4	39	43.8	92	32	41.8	88	19
94	45.3	38	42.4	94	32	41.8	92	17
98	43.9	38	42.4	97	30	42.0	96	14
102	44.2	36	41.6	94	30	41.7	94	12
Terminal sacrifice		35			30			12
Mean for weeks								
1-13	31.5		30.6	97		30.2	96	
14-52	43.1		38.8	90		38.7	90	
53-102	47.3		42.3	89		41.5	88	

[a] The number of animals weighed for this week is fewer than the number of animals surviving.

TABLE 10
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone

Weeks on Study	Vehicle Control		262 mg/kg			525 mg/kg		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	18.5	50	18.7	101	50	18.4	100	50
2	19.8	50	19.8	100	50	20.1	102	50
3	21.3	50	20.9	98	50	21.0	99	50
4	22.0	50	21.5	98	50	21.0	96	50
5	23.2	50	22.5	97	50	22.4	97	50
6	23.0	50	23.3	101	50	22.4	97	50
7	23.8	50	23.8	100	50	23.1	97	50
8	24.3	50	23.4	96	50	22.8	94	50
9	24.6	50	24.0	98	50	23.9	97	50
10	25.8	50	24.8	96	50	25.0	97	50
11	26.5	50	26.0	98	50	25.6	97	50
12	26.6	50	25.3	95	50	25.5	96	50
13	27.1	50	25.0	92	50	24.8	92	50
15	26.9	50	25.7	96	50	26.0	97	50
18	27.6	50	26.1	95	50	26.2	95	50
22	29.2	50	28.2	97	50	27.8	95	50
27	31.7	50	29.2	92	50	28.9	91	50
32	34.6	50	29.2	84	50	30.6	88	50
35	33.1	50	30.9	93	50	30.8	93	50
40	35.9	50	31.5	88	50	30.7	86	50
44	36.6	50	32.6	89	50	32.5	89	50
48	37.4	50	33.4	89	50	33.0	88	50
52	39.2	50	34.6	88	50	34.8	89	50
56	39.8	50	34.5	87	50	35.5	89	50
58	40.1	50	34.9	87	50	36.0	90	50
62	40.2	50	35.6	89	50	36.6	91	50
66	43.3	50	38.5	89	50	39.7	92	50
70	44.8	50	38.4	86	48	39.8	89	49
74	44.9	50	38.4	86	48	39.1	87	48
78	47.1	50	38.8	82	47	39.4	84	48
82	46.0	50	39.2	85	45	40.3	88	48
86	48.2	46	40.2	83	41	40.5	84	46
90	45.9	42	38.7	84	39	39.0	85	46
94	47.1	41	38.3	81	35	40.3	86	44
98	44.1	39	36.9	84	35	39.5	90	41
102	44.4	38	36.7	83	34	38.3	86	39
Terminal sacrifice		38			34			38
Mean for weeks								
1-13	23.6		23.0	97		22.8	97	
14-52	33.2		30.1	91		30.1	91	
53-102	44.3		37.6	85		38.8	88	

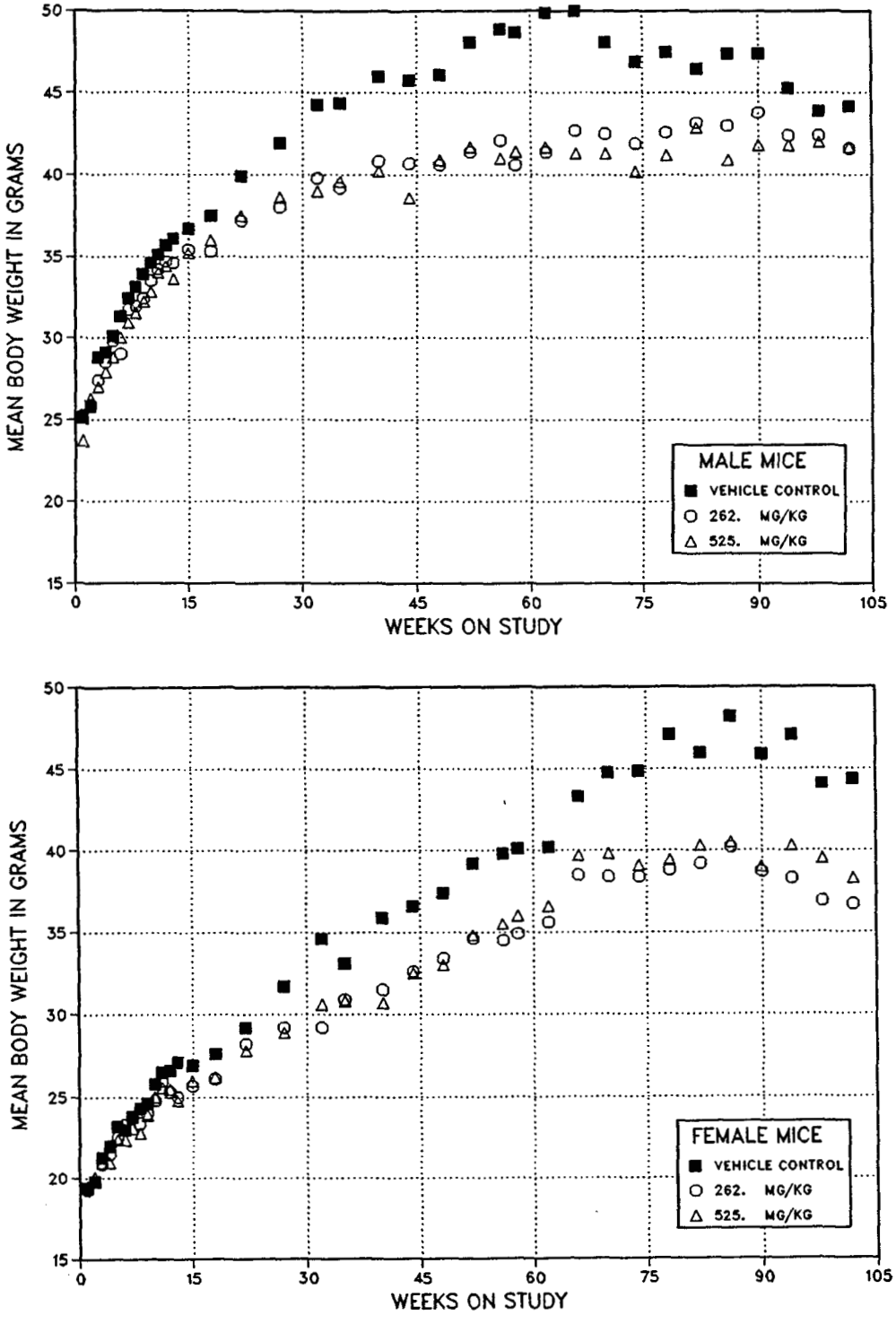


FIGURE 3
Growth Curves for Mice Administered γ-Butyrolactone by Gavage for 2 Years

Survival

The survival of high-dose male mice was significantly lower than that of the controls, whereas the survival of low-dose males and low- and high-dose females was similar to that of the controls (males: 35/50, 30/50, 12/50; females: 38/50, 34/50, 38/50) (Table 11 and Figure 4). The reduced survival of the high-dose male mice was attributed partially to fighting during the first year of the study, when the animals were housed in groups of five (males were housed individually after approximately 66 weeks on study). The increased aggression in the high-dose males seemed to be related to the sedative or anesthetic properties of γ-butyrolactone. High-dose male mice were noted to be partially sedated or lethargic and inactive after dosing. The first males to recover were observed to attack and bite those male mice still sedated. Bite wounds, scratches, and sores around the genitalia and backs of the mice were more frequently observed in the low- and high-dose mice as were a number of nonneoplastic lesions believed to be related to debilitation, stress, or ascending infections of the urogenital tract as a result of the fighting.

TABLE 11
Survival in Mice in the 2-Year Gavage Studies of γ-Butyrolactone

	Vehicle Control	262 mg/kg	525 mg/kg
Male[a]			
Animals initially in study	50	50	50
Natural deaths	2	12	13
Moribund kills	13	8	24
Accidental deaths[b]	0	0	1
Animals surviving until study termination	35	30	12
Percent survival at end of studies[c]	70	60	25
Mean survival (days)[d]	674	606	481
Survival analysis[e]	P<0.001	P=0.257	P<0.001
Female[a]			
Animals initially in study	50	50	50
Natural deaths	4	5	3
Moribund kills	8	11	9
Animals surviving until study termination	38	34	38
Percent survival at end of studies[c]	76	68	76
Mean survival (days)[d]	704	685	705
Survival analysis[e]	P=0.997N	P=0.436	P=1.000N

[a] First day of terminal sacrifice: male, 729; female, 730
[b] Censored from survival analyses
[c] Kaplan-Meier determinations. Survival rates adjusted for accidental deaths.
[d] Mean of all deaths (uncensored, censored, terminal sacrifice).
[e] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

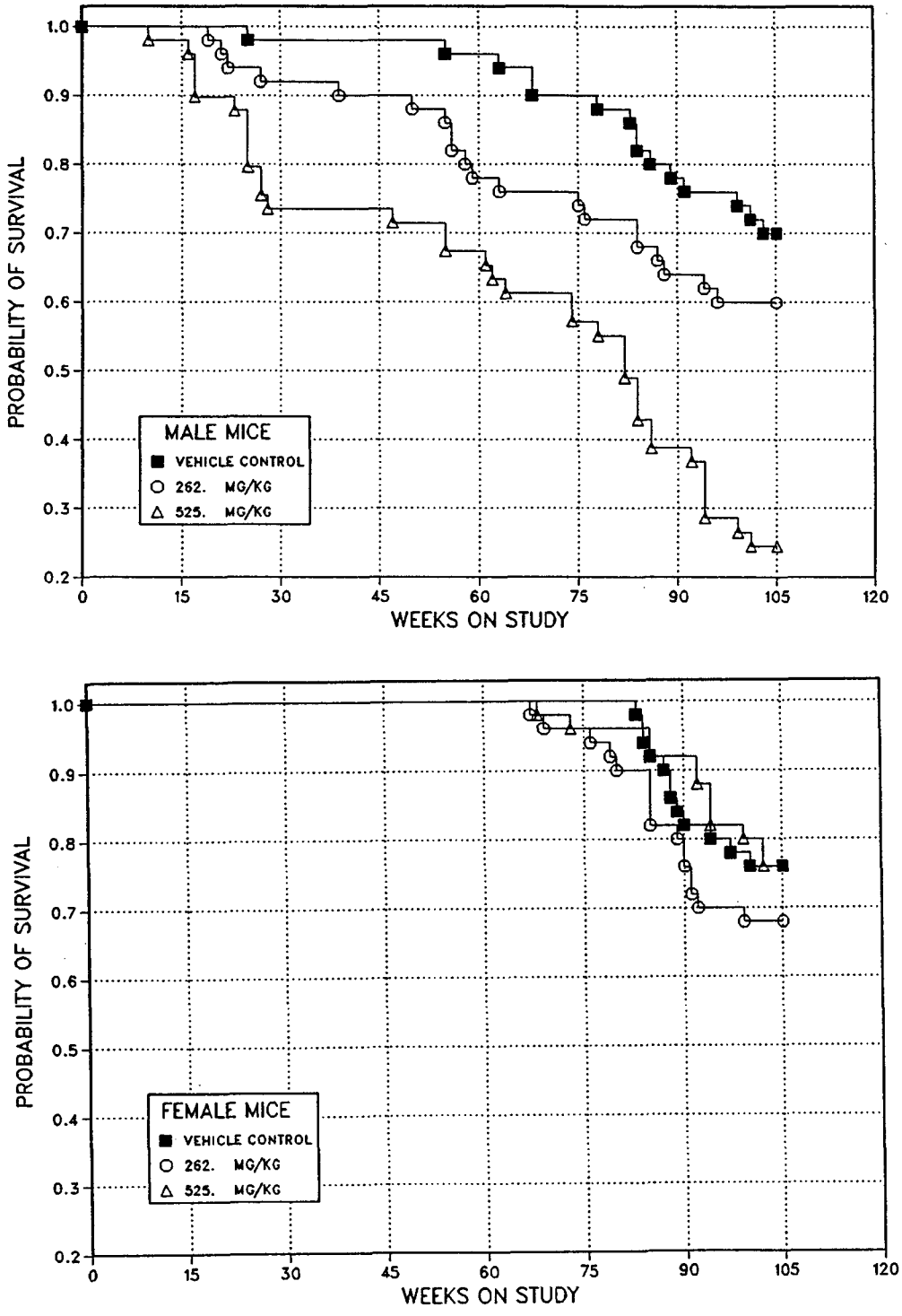


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered γ-Butyrolactone by Gavage for 2 Years

Pathology and Statistical Analysis

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms or nonneoplastic lesions of the adrenal medulla, liver, harderian gland, skin, inguinal lymph node, prostate gland, thymus, and lung in mice.

Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors occurring with an incidence of at least 5% in at least one animal group, and historical control incidences for selected neoplasms discussed in this section are presented in Appendixes C for male mice and D for female mice.

Adrenal Medulla: There was a statistically significant increase in the incidence of focal hyperplasia in low-dose male mice. Moreover, there was a marginal increase in the incidence of pheochromocytomas (benign or malignant combined) in low-dose male mice compared to controls (Table 12), although neither the trend test nor the pairwise comparison was statistically significant. Because focal hyperplasia and pheochromocytomas constitute a morphological and biological continuum, the increased incidence of these lesions, principally hyperplasia, may be related to γ-butyrolactone administration. The lack of a dose response may be related to the reduced survival in the high-dose group. In female mice, there was no apparent increase in the incidence of adrenal medulla proliferative lesions associated with the administration of γ-butyrolactone (hyperplasia: control, 3/50; high-dose, 1/49; pheochromocytoma, benign or malignant: 0/50, 2/49).

TABLE 12
Lesions of the Adrenal Medulla in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone

	Vehicle Control	262 mg/kg	525 mg/kg
Hyperplasia			
Overall rates[a]	2/48 (4%)	9/50 (18%)	4/50 (8%)
Logistic regression tests[b]	P=0.071	P=0.011	P=0.191
Benign Pheochromocytoma			
Overall rates	1/48 (2%)	5/50 (10%)	1/50 (2%)
Adjusted rates[c]	2.3%	16.7%	5.3%
Terminal rates[d]	0/34 (0%)	5/30 (17%)	0/12 (0%)
First incidence (days)	582	729 (T)	640
Logistic regression tests	P=0.352	P=0.073	P=0.760
Malignant Pheochromocytoma			
Overall rates	1/48 (2%)	1/50 (2%)	0/50 (0%)
Benign or Malignant Pheochromocytoma[e]			
Overall rates	2/48 (4%)	6/50 (12%)	1/50 (2%)
Adjusted rates	4.9%	20.0%	5.3%
Terminal rates	0/34 (0%)	6/30 (20%)	0/12 (0%)
First incidence (days)	582	729 (T)	640
Logistic regression tests	P=0.472	P=0.092	P=0.592N

(T)Terminal sacrifice
[a] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this lesion
[b] Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal. A lower incidence in a dose group is indicated by N.
[c] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[d] Observed incidence at terminal kill
[e] Historical incidence for 2-year NTP corn-oil gavage studies with vehicle control groups (mean ± standard deviation): 18/582 (3.1% ± 1.8%), range 0%–6%

Focal hyperplasia is characterized by circumscribed aggregates of hypertrophied cells with slightly enlarged nuclei and more prominent basophilic granules. Pheochromocytoma is a nodular, expansile lesion causing compression and displacement of adjacent normal tissue and consisting of similar cells. Larger pheochromocytomas may exhibit some cellular pleomorphism and atypia; those which penetrate and extend beyond the capsule of the adrenal gland are considered malignant.

Liver: Hepatocellular adenomas or carcinomas (combined) occurred with a statistically significant negative trend in male mice, and the incidences in low- and high-dose groups were significantly lower than the incidence in the controls by survival-adjusted analyses (24/50, 8/50, 9/50; Table C3). The overall incidence of hepatocellular neoplasms in NTP historical control males receiving corn oil by gavage is 210/599 (35.1%, range 14%-52%; Table C4b).

Harderian Gland: Adenomas in male mice occurred with a statistically significant negative trend, and the incidences in low- and high-dose groups were significantly less than controls by survival-adjusted analyses (8/50, 1/50, 0/50; Table C3). The overall historical control incidence of this tumor in males is 38/600 (6.3%) with a range of 0% to 16% (Table C4c). Thus, the significance of the decrease may be due to the rather high incidence in controls in this study, rather than to the administration of γ-butyrolactone. The incidence of harderian gland neoplasms in low- and high-dose female mice was not decreased (2/50, 2/50, 4/50).

Miscellaneous Nonneoplastic Lesions: Decreases in a number of miscellaneous spontaneous nonneoplastic lesions in low- and high-dose male mice were attributed to decreased survival and were not considered related to γ-butyrolactone administration. The observed dose-related increases in several nonneoplastic lesions in male mice were considered to be associated with fighting or bite wounds (Table 13). The skin lesions were primarily located around the genitalia and backs, and the lymphoid hyperplasia of the inguinal lymph node was considered to be an immunological response to superficial bacterial infections of the bite wounds. Prostatitis is frequently seen in group-housed male mice and is believed to be the result of ascending bacterial infections resulting from bite wounds on and around the genitalia. Depletion of lymphocytes from the thymus (also called thymic atrophy) often accompanies debilitation and stress and was usually seen in mice dying early from fight wounds. The leukocytosis, hemorrhage, and congestion of the lung were also seen principally in males dying early from fight wounds.

GENETIC TOXICITY

γ-Butyrolactone (100-10,000 μg/plate) was tested for induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 using a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no significant increase in mutant colonies was seen after treatment with γ-butyrolactone (Table E1; Haworth *et al.*, 1983). Also, no induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* was observed following exposure of adult males to γ-butyrolactone by feeding (20,000 or 28,000 ppm) or by injection (15,000 ppm) (Table E4). In cytogenetic tests with Chinese hamster ovary cells, γ-butyrolactone induced sister chromatid exchanges (Table E2) and chromosomal aberrations (Table E3) in trials conducted with Aroclor 1254-induced male Sprague-Dawley rat liver S9; neither endpoint was elevated in the absence of S9 (Loveday *et al.*, 1989). In the sister chromatid exchange test, concentrations of 3,010 to 5,010 μg/mL yielded positive results, and a delayed harvest protocol was used at the 5,010 μg/mL dose level to offset chemical-induced cell cycle delay. Significant increases in chromosomal aberrations were seen at concentrations of 2,580 to 3,990 μg/mL γ-butyrolactone at standard harvest times.

TABLE 13
Selected Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	262 mg/kg	525 mg/kg
Inguinal Lymph Node			
Lymphoid hyperplasia	0/50	5/50*	5/49*
Lung			
Congestion	1/50	1/50	4/50
Hemorrhage	0/50	1/50	7/50**
Leukocytosis	1/50	2/50	5/50
Prostate Gland			
Inflammation, suppurative	1/49	5/48	8/48*
Skin			
Acanthosis	12/50	36/50**	39/50**
Inflammation, chronic	4/50	17/50**	19/50**
Pigmentation	3/50	12/50*	19/50**
Ulcer	4/50	15/50**	17/50**
Hair follicle, atrophy	1/50	11/50**	16/50**
Thymus			
Depletion	0/42	5/39*	6/38**
Epithelial hyperplasia	0/42	4/39*	4/38*

* Significantly different ($P<0.05$) from the control group by the logistic regression tests
** $P<0.01$
[a] Number of lesion-bearing animals/number of tissues examined

DISCUSSION AND CONCLUSIONS

γ-Butyrolactone is an intermediate in the synthesis of polymers used as film formers in hair sprays, as blood plasma extenders, and as clarifying agents in beer and wine. It is used as a solvent in the textile and petroleum industries and is a constituent of paint removers, textile aids, and drilling oils. It is also an intermediate in the preparation of the herbicide 4-(2,4-dichlorophenoxy) butyric acid. γ-Butyrolactone was nominated for 2-year toxicology and carcinogenesis studies because there is potential for widespread exposure from its use in the manufacture of a variety of products and its presence in various foods. The NTP studies were conducted by administering γ-butyrolactone in corn oil by gavage to F344/N rats and B6C3F$_1$ mice of each sex.

The acute toxicity associated with the administration of γ-butyrolactone to rats and mice in the 16-day and 13-week studies is consistent with data reported in the literature. In the 16-day studies, all male and female rats receiving 1,200 mg/kg γ-butyrolactone and all male mice and four of five female mice receiving 1,400 mg/kg died before the end of the studies. Male rats were slightly more susceptible to the lethal effects of γ-butyrolactone; all male rats given 900 mg/kg died, whereas only one of 10 females given the same dose died in the 13-week studies. No sex difference was observed in mice.

The clinical findings of sedation, recumbency, and inactivity observed in rats and mice in these 16-day and 13-week studies are also consistent with previous reports. The inactivity and recumbency were evident within minutes after dosing, but the animals were apparently normal several hours later. In the 13-week studies, rats and, to a lesser extent, mice developed tolerance to these effects after several weeks of dosing. Tolerance to both the behavioral depression and to the dopaminergic actions of γ-butyrolactone has been previously shown to develop (Gianutsos and Moore, 1978; Nowycky and Roth, 1979).

The precise biochemical basis for the central nervous system effects has not been clearly established. It has been suggested that the central nervous system depressant properties of γ-butyrolactone and dopamine accumulation might be causally related because a) a temporal relation exists between the sedative action and the accumulation of brain dopamine after γ-butyrolactone administration, particularly in rabbits (Gessa *et al.*, 1966), b) the striatum, an area high in dopamine, is the most sensitive to the actions of γ-butyrolactone, c) only butyric acid congeners with anesthetic activity selectively increase dopamine, d) α-methyl-*p*-tyrosine, an agent which interferes with catecholamine biosynthesis, potentiates the "sleep" time of γ-butyrolactone, but not that of pentobarbital, and e) amphetamine, a drug causing the release of central catecholamines, produces a significant reduction in γ-butyrolactone-induced sleep time (Roth and Suhr, 1970). On the other hand, the "anesthetic" effect of γ-butyrolactone in humans is reversed by physostigmine (Henderson and Holmes, 1976) and acetylcholine levels are elevated in certain brain regions in rats (Giarman and Schmidt, 1963), suggesting that impaired cholinergic as well as dopaminergic neurotransmission may occur.

The administration of γ-butyrolactone by gavage to rats and mice at levels up to and including lethal doses did not produce any major histopathologic lesions. The inflammatory lesions observed in the nose of dosed rats in the 13-week studies may be related to reflux of gavage material into the nasopharynx immediately following removal of the gavage needle, rather than to any particular susceptibility of the nasal mucosa. Similar nasal lesions have been observed in other NTP gavage studies of a variety of chemicals. The lack of any histologically evident degenerative lesions may be attributed in part to the rapid absorption and metabolism of the chemical. γ-Butyrolactone may undergo non-enzymatic hydrolysis in the intestinal tract, although it is uncertain to what extent this might

have occurred in these gavage studies. Moreover, γ-butyrolactone is rapidly converted to γ-hydroxybutyrate in the liver and blood by a lactonase enzyme; the half-life of intravenously administered γ-butyrolactone is less than a minute (Roth and Giarman, 1965, 1966).

The doses selected for the NTP 2-year rat studies were 112 and 225 mg/kg for males and 225 and 450 mg/kg for females. Higher doses in males were excluded because of the chemical-related mortality (10/10) observed in rats given 900 mg/kg and the 12% reduction in weight gain in rats given 450 mg/kg in the 13-week study. The high dose selected for female rats was twice that selected for males because no reduction in weight gain and the death of only one female rat receiving 900 mg/kg was attributed to chemical toxicity.

In the 2-year rat studies, the survival of high-dose males was slightly increased compared with controls; survival of female rats was similar among dosed and control groups (males: 24/50, 27/50, 32/50; females: 28/50, 27/50, 28/50). The increased survival of high-dose male rats may be related in part to the lower incidence of mononuclear cell leukemia (16/50, 15/50, 9/50) in this group. The trends for improved survival and lower incidence of mononuclear cell leukemia in dosed male were statistically significant ($P<0.05$), but it is uncertain if these trends are related to the administration of γ-butyrolactone. Although there are no clear indications a maximum tolerated dose was achieved in male rats, a consistent, chemical-related reduction in group mean body weight was evident in high-dose female rats by week 15 of the studies, and the mean body weight of high-dose females was 20% lower than that of the controls by the end of the studies. Nevertheless, male rats were more susceptible than females to the toxic effects, including body weight effects, of γ-butyrolactone in the 13-week studies. Based on these data, a doubling of the high dose to 450 mg/kg in male rats would likely have produced lower group mean body weights than those exhibited by females. Thus, although male rats may have been able to tolerate slightly higher doses, the doses used were considered adequate for determining the potential carcinogenicity of γ-butyrolactone.

In the 2-year studies in rats, there were no nonneoplastic toxic lesions or increased incidences in neoplasms in dosed male rats that were attributed to the administration of γ-butyrolactone. There were marginal numerical increases in keratoacanthomas in dosed males (1/50, 4/50, 6/50), but the incidences in the dosed groups were not significantly higher than that of concurrent controls and were within the range of NTP historical controls. Basal cell adenomas of the skin occurred in four low-dose males, whereas none occurred in the control and high-dose groups. A basal cell carcinoma occurred in a single high-dose male. Although basal cell neoplasms are relatively uncommon in NTP historical controls, the incidence of basal cell neoplasms in the low-dose group was not significantly higher than controls and there was no corresponding increase in the high-dose group. Therefore, lacking stronger evidence, it cannot be concluded that the overall numerical increase in epithelial neoplasms of the skin is related to exposure to γ-butyrolactone. Moreover, the majority of the chemicals studied by the NTP which have induced neoplasms of the skin after oral administration are mutagens in the *Salmonella typhimurium* assay, in contrast to γ-butyrolactone.

In dosed groups of female rats, the incidences of fibroadenoma of the mammary gland occurred with a statistically significant negative trend. Moreover, the incidence of fibroadenoma in the high-dose group was significantly lower than that in controls by the pairwise comparison. The incidence of mammary gland cysts in the high-dose group was also significantly lower than that in controls. The lower incidences of these lesions in dosed female rats may be related to the decreased body weights or to alterations in the secretion of prolactin from the pituitary gland. From the sixth week until the end of the study, there was a consistent depression of weight gain among the high-dose females, and the final mean body weight for this group was 20% lower than that of the controls. Rao *et al.* (1987) found a direct association between maximum mean body weight and the incidence of mammary gland fibroadenomas in control groups of female F344/N rats in NTP 2-year studies. In addition, there are a number of reports showing a clear relationship between reduced body weight resulting from diet restriction and reduced tumor incidence (Sylvester *et al.*, 1981; Gross and Dreyfuss, 1984). On the other hand, a relationship between the decreased incidence of fibroadenoma and prolactin secretion may exist based on a) the well established inhibitory action of dopamine on prolactin secretion (MacLeod, 1976), b) the demonstrated role of

prolactin in enhancing the growth of spontaneous and chemically induced mammary gland neoplasms (Meites, 1980), and c) the demonstrated effects of γ-butyrolactone on the impulse flow of dopaminergic neurons, levels of dopamine in various regions of the brain, and dopamine receptor sensitivity (Roth and Suhr, 1970; Menon *et al.*, 1974; Roth *et al.*, 1973; Andén *et al.*, 1983).

The doses selected for the NTP 2-year mouse studies were 262 and 525 mg/kg. Higher doses were excluded because of chemical-related deaths (3/10 males and 1/10 females) in mice given 1,050 mg/kg during the 13-week studies. Although the mean body weight and survival of high-dose male mice were significantly lower than that of controls, these effects were only indirectly related to γ-butyrolactone administration and are not clear indications that a maximum tolerated dose was achieved. High-dose mice were partially sedated or lethargic and inactive shortly after dosing; this seemed to contribute to an increase in fighting-related trauma in dosed males and the lower body weights and excess mortality. After the male mice were individually housed (week 67), the difference between mean body weights of dosed and control groups decreased. Body weights of low- and high-dose female mice were lower than that of the controls throughout much of the study, but there was no improvement following the change to individual housing. Survival of dosed and control female mice was similar.

Although male mice might have been able to tolerate slightly higher doses, it is clear from the mortality in the 13-week study that a doubling of the dose from 525 mg/kg to 1,050 mg/kg could not have been tolerated. Thus, the doses in the 2-year studies were considered adequate for determining the potential carcinogenicity of γ-butyrolactone. The lower survival of high-dose male mice, however, was believed to reduce the sensitivity of this study to detect a carcinogenic effect.

The administration of γ-butyrolactone to mice for 2 years was associated with a statistically significant increased incidence of focal hyperplasia of the adrenal medulla in low-dose males. There was a corresponding numerical increased incidence of pheochromocytoma (benign or malignant) in the same group. Although it was not statistically significant, the incidence of pheochromocytomas in low-dose male mice fell outside the historic range for control male mice receiving corn oil by gavage. Because focal hyperplasia and pheochromocytomas constitute a morphological continuum, the increased incidence of these proliferative lesions in the low-dose males, principally hyperplasia, may have been related to administration of γ-butyrolactone. Despite the significant increase in proliferative lesions in low-dose males, the survival-adjusted analyses show no increase in high-dose males, even though 12 mice survived until the end of the study. Nevertheless, the lack of a similar increase in high-dose males may have been related to the reduced survival, lower body weights, or perhaps other physiological effects associated with fighting-related stress in that group. The association of adrenal medulla proliferative lesions with γ-butyrolactone is plausible in view of the histogenesis of the adrenal medulla and of the demonstrated effects of this chemical on dopaminergic and cholinergic neurons in the brain. The adrenal medulla is a sympathetic ganglion that is modified to be a neuroendocrine organ. The chromaffin cells of the adrenal medulla develop from ectodermal cells of the neural crest comprising the sympathomedullary anlage. The chromaffin cells are capable of producing catecholamines, including dopamine, although in the adult, the cells contain primarily epinephrine or norepinephrine. The remarkable plasticity of medullary chromaffin cells is demonstrated by their ability *in vitro* to assume the morphological and metabolic characteristics of neurons in response to changing levels of glucocorticoids and nerve growth factor (Doupe *et al.*, 1985). The chromaffin cell-derived neurons were also capable of developing cholinergic properties including acetylcholine synthesis and storage and choline acetyltransferase activity. Moreover, the medulla is innervated by cholinergic preganglionic sympathetic nerve endings which synapse on chromaffin cells and stimulate catecholamine synthesis. Thus, it is plausible to expect γ-butyrolactone or its metabolite, γ-hydroxybutyrate, to interact with adrenal medulla chromaffin cells and affect catecholamine synthesis or other metabolic functions.

There were no nonneoplastic degenerative lesions associated with the administration of γ-butyrolactone to male or female mice for up to 2 years. Decreased incidences of a number of miscellaneous spontaneous nonneoplastic lesions in dosed male mice were attributed to decreased survival and were not considered chemical-related. The observed dose-related increased incidences in several lesions in the

lung, prostate gland, skin, lymph node and thymus of male mice were believed to be associated with fighting or bite wounds.

There was a statistically significant negative trend for hepatocellular neoplasms in dosed male mice, and the lower incidences in the low- and high-dose groups compared to the controls were significant by survival-adjusted analyses (hepatocellular adenoma or carcinoma combined: 24/50, 8/50, 9/50). Although the lower incidence of hepatocellular neoplasms is associated with the administration of γ-butyrolactone, it may also be related to the lower body weights of dosed mice. Rao *et al.* (1990) have shown a positive correlation between body weight and the incidence of hepatocellular neoplasms in control mice in NTP studies. The incidences of harderian gland adenoma in the dosed groups of male mice were also significantly lower than the incidence in the controls. The incidence of this tumor in controls equalled the highest rate seen in historical groups of mice in NTP studies, and thus, the apparent decreased incidences in mice receiving γ-butyrolactone may not be related to administration of γ-butyrolactone.

In 1984, the NTP initiated a project to develop a database that would permit evaluation of the ability of four of the most commonly used *in vitro* short-term genetic toxicity tests to predict rodent carcinogenicity. The four tests included induction of mutations in *Salmonella* and mouse lymphoma L5178Y cells, and induction of sister chromatid exchanges and chromosome aberrations in Chinese hamster ovary cells. Subsequently, the NTP has evaluated the effectiveness of these four tests for predicting rodent carcinogenicity results of 114 chemicals (Tennant *et al.*, 1987; Zeiger *et al.*, 1990). In this evaluation, the *Salmonella* assay was shown to have the lowest sensitivity (0.48 = proportion of carcinogens positive in *Salmonella*), the highest specificity (0.91 = proportion of noncarcinogens negative in *Salmonella*), and have the highest positive predictivity for carcinogenicity (89% of the chemicals mutagenic in *Salmonella* were carcinogenic in rodents) of the four *in vitro* tests. Positive tests for chromosomal aberrations or sister chromatid exchanges were less predictive of carcinogenicity; 73% of chemicals inducing chromosomal aberrations and 63% of chemicals inducing sister chromatid exchanges were carcinogenic in rodents. In the NTP genetic toxicity studies, γ-butyrolactone was negative for gene mutations in four strains of *Salmonella typhimurium*, but induced sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells at very high concentrations in the presence of S9 activation enzymes. These positive genotoxicity test results are not predictive of the results of the rodent bioassay where no evidence of carcinogenicity was observed. Another consideration is that the clastogenic effects observed with γ-butyrolactone in Chinese hamster ovary cells have not been demonstrated in other cytogenetic studies, either *in vitro* or *in vivo*. Thus, the positive tests for the latter two endpoints by γ-butyrolactone and lack of definitive evidence of carcinogenic activity in male and female rats and mice in these 2-year studies is consistent with the overall findings reported by Tennant *et al.* (1987) and Zeiger *et al.* (1990).

Conclusions: Under the conditions of these 2-year gavage studies, there was *no evidence of carcinogenic activity** of γ-butyrolactone in male F344/N rats given 112 or 225 mg/kg or in female F344/N rats given 225 or 450 mg/kg in corn oil. There was *equivocal evidence of carcinogenic activity* of γ-butyrolactone in male B6C3F$_1$ mice based on marginally increased incidences of adrenal medulla pheochromocytomas and hyperplasia in the low-dose group. The sensitivity of the study in male mice to detect a carcinogenic effect was reduced by the low survival of the high-dose group associated with fighting. There was *no evidence of carcinogenic activity* of γ-butyrolactone in female B6C3F$_1$ mice given 262 or 525 mg/kg in corn oil.

A decreased incidence of hepatocellular neoplasms in dosed male mice and decreased incidences of mammary gland fibroadenomas and cysts and pituitary cysts in female rats were associated with the administration of γ-butyrolactone.

* Explanation of Levels of Evidence of Carcinogenic Activity appears on page 8. A summary of Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR GAVAGE STUDY OF γ-BUTYROLACTONE

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	112 mg/kg	225 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	6	7	3
Moribund	19	13	12
Dosing accident	1	3	3
Survivors			
Terminal sacrifice	24	27	32
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(48)	(17)	(49)
Intestine large, colon	(47)	(21)	(50)
Polyp adenomatous		1 (5%)	
Intestine large, rectum	(47)	(19)	(49)
Intestine small, ileum	(46)	(18)	(49)
Intestine small, jejunum	(46)	(17)	(50)
Adenocarcinoma	1 (2%)		
Liver	(50)	(50)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Hepatocellular carcinoma		1 (2%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Mesentery	(11)	(10)	(19)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (5%)
Osteosarcoma, metastatic, uncertain primary site	1 (9%)		
Pancreas	(50)	(22)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Acinar cell, adenoma	4 (8%)		4 (8%)
Acinar cell, adenoma, multiple	3 (6%)		1 (2%)
Salivary glands	(49)	(23)	(50)
Schwannoma malignant		1 (4%)	
Stomach	(50)	(29)	(50)
Forestomach, papilloma squamous	1 (2%)		1 (2%)
Tongue	(3)	(4)	
Papilloma squamous		1 (25%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Cardiovascular System			
Heart	(50)	(25)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Endocrine System			
Adrenal gland, cortex	(48)	(24)	(49)
Adenoma	1 (2%)		1 (2%)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Adrenal gland, medulla	(48)	(23)	(49)
Pheochromocytoma malignant		1 (4%)	5 (10%)
Pheochromocytoma benign	10 (21%)	6 (26%)	7 (14%)
Bilateral, pheochromocytoma benign	5 (10%)	4 (17%)	7 (14%)
Islets, pancreatic	(49)	(22)	(50)
Adenoma	3 (6%)		2 (4%)
Adenoma, multiple			1 (2%)
Carcinoma	2 (4%)	1 (5%)	
Parathyroid gland	(46)	(22)	(48)
Adenoma			1 (2%)
Pituitary gland	(48)	(28)	(49)
Pars distalis, adenoma	11 (23%)	11 (39%)	16 (33%)
Pars distalis, carcinoma	1 (2%)	1 (4%)	
Thyroid gland	(50)	(25)	(50)
Bilateral, C-cell, adenoma	1 (2%)		
C-cell, adenoma	6 (12%)	1 (4%)	5 (10%)
C-cell, carcinoma	4 (8%)	1 (4%)	2 (4%)
Follicular cell, adenoma	1 (2%)		
Follicular cell, carcinoma	1 (2%)	1 (4%)	
General Body System			
None			
Genital System			
Epididymis	(50)	(23)	(50)
Preputial gland	(48)	(24)	(50)
Adenoma	6 (13%)	1 (4%)	2 (4%)
Carcinoma	1 (2%)	3 (13%)	3 (6%)
Bilateral, carcinoma		1 (4%)	
Prostate	(49)	(24)	(49)
Seminal vesicle	(50)	(25)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Testes	(50)	(50)	(50)
Bilateral, interstitial cell, adenoma	37 (74%)	36 (72%)	35 (70%)
Interstitial cell, adenoma	7 (14%)	10 (20%)	9 (18%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Hematopoietic System			
Blood	(2)	(2)	
Bone marrow	(50)	(23)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Lymph node	(50)	(25)	(50)
Inguinal, fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Mediastinal, fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Lymph node, mandibular	(48)	(21)	(49)
Lymph node, mesenteric	(48)	(23)	(50)
Spleen	(50)	(45)	(50)
Fibrosarcoma	1 (2%)		
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Hemangiosarcoma	1 (2%)		
Osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Thymus	(43)	(20)	(49)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Thymoma benign			1 (2%)
Integumentary System			
Mammary gland	(44)	(23)	(48)
Adenocarcinoma		1 (4%)	
Fibroadenoma	4 (9%)	1 (4%)	3 (6%)
Skin	(50)	(37)	(50)
Basal cell adenoma		4 (11%)	
Basal cell carcinoma			1 (2%)
Keratoacanthoma	1 (2%)	4 (11%)	5 (10%)
Keratoacanthoma, multiple			1 (2%)
Subcutaneous tissue, fibroma	3 (6%)	4 (11%)	4 (8%)
Subcutaneous tissue, fibroma, multiple		2 (5%)	
Subcutaneous tissue, fibrosarcoma	4 (8%)		
Subcutaneous tissue, lipoma			2 (4%)
Subcutaneous tissue, myxosarcoma		1 (3%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Musculoskeletal System			
Bone	(50)	(23)	(50)
Cranium, carcinoma, metastatic, Zymbal's gland	1 (2%)		
Skeletal muscle	(1)	(1)	(3)
Fibroma		1 (100%)	
Abdominal, osteosarcoma, metastatic, uncertain primary site	1 (100%)		
Back, fibrous histiocytoma, metastatic, uncertain primary site			1 (33%)
Diaphragm, osteosarcoma, metastatic, uncertain primary site	1 (100%)		
Neck, carcinoma, extension, metastatic, thyroid gland			1 (33%)
Nervous System			
Brain	(50)	(24)	(50)
Astrocytoma malignant		1 (4%)	
Meningioma malignant	1 (2%)		
Meninges, carcinoma, metastatic, Zymbal's gland	1 (2%)		
Nerve, carcinoma, metastatic, Zymbal's gland	1 (2%)		
Spinal cord	(2)		(1)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (100%)
Respiratory System			
Lung	(50)	(29)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (3%)	3 (6%)
Alveolar/bronchiolar carcinoma			1 (2%)
Carcinoma, metastatic, multiple, thyroid gland			1 (2%)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Osteosarcoma, metastatic		1 (3%)	
Artery, pheochromocytoma malignant, metastatic, adrenal gland			1 (2%)
Mediastinum, osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Special Senses System			
Ear	(2)		(2)
Schwannoma malignant			1 (50%)
Zymbal's gland	(1)		(1)
Carcinoma	1 (100%)		1 (100%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Urinary System			
Kidney	(50)	(23)	(50)
Fibrous histiocytoma, metastatic, uncertain primary site			1 (2%)
Lipoma		1 (4%)	
Transitional epithelium, carcinoma	1 (2%)	1 (4%)	
Urinary bladder	(48)	(22)	(50)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Leukemia mononuclear	16 (32%)	15 (30%)	9 (18%)
Mesothelioma malignant		1 (2%)	4 (8%)
Tumor Summary			
Total animals with primary neoplasms[c]	50	49	50
Total primary neoplasms	141	120	138
Total animals with benign neoplasms	46	48	50
Total benign neoplasms	106	89	111
Total animals with malignant neoplasms	29	29	22
Total malignant neoplasms	35	31	27
Total animals with secondary neoplasms[d]	2	1	3
Total secondary neoplasms	10	1	18
Total animals with malignant neoplasms of uncertain primary site	1		1

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control

Number of Days on Study	4 6 5	4 6 9	4 8 1	4 8 1	4 9 8	5 2 9	5 4 3	5 7 0	5 7 4	5 7 5	5 7 7	5 8 0	5 9 6	6 1 8	6 2 4	6 4 1	6 5 1	6 6 3	6 6 4	6 6 8	6 6 8	6 7 4	6 8 1	7 0 0	7 0 8
Carcass ID Number	0 2 1	0 9 1	0 6 1	0 8 1	0 8 2	0 4 1	0 3 1	0 5 1	0 4 2	0 1 1	0 6 2	0 1 2	0 6 3	0 2 2	0 7 1	0 9 2	0 6 4	0 7 2	1 0 2	0 5 2	0 7 3	0 1 3	0 4 3	0 2 3	0 8 4
Alimentary System																									
Esophagus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, ileum	+	A	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	A	+	+	M	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+
Adenocarcinoma														X											
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, uncertain primary site																				X					
Mesentery					+	+							+					+		+			+	+	
Osteosarcoma, metastatic, uncertain primary site																				X					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, uncertain primary site																				X					
Acinar cell, adenoma																									X
Acinar cell, adenoma, multiple																									
Pharynx															+										
Salivary glands	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, papilloma squamous													X												
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+		+		+	+	+	+	+		+	+		+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Tooth	+																								
Cardiovascular System																									
Blood vessel																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 1 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 8 3	0 1 4	0 1 5	0 2 4	0 2 5	0 3 2	0 3 3	0 3 4	0 3 5	0 4 4	0 4 5	0 5 3	0 5 4	0 5 5	0 6 5	0 7 4	0 7 5	0 8 5	1 0 1	0 9 3	0 9 4	0 9 5	1 0 3	1 0 4	1 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenocarcinoma																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, uncertain primary site																										1
Mesentery				+											+		+			+						11
Osteosarcoma, metastatic, uncertain primary site																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, uncertain primary site																										1
Acinar cell, adenoma	X									X									X							4
Acinar cell, adenoma, multiple					X	X									X											3
Pharynx																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Forestomach, papilloma squamous																										1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	44
Stomach, glandular	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue										+					+	+										3
Tooth																										1
Cardiovascular System																										
Blood vessel																	+									1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																	X									1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	4 6 5	4 6 9	4 6 1	4 8 1	4 9 8	5 2 9	5 4 3	5 7 0	5 7 4	5 7 5	5 7 7	5 8 0	5 9 6	6 1 8	6 2 4	6 4 1	6 5 1	6 6 3	6 6 4	6 6 8	6 6 8	6 7 4	6 8 1	7 0 3	7 0 8
Carcass ID Number	0 2 1	0 9 1	0 6 1	0 8 1	0 8 2	0 4 1	0 3 1	0 5 1	0 4 2	0 1 1	0 6 2	0 1 2	0 6 3	0 2 2	0 7 1	0 9 2	0 6 4	0 7 2	1 0 2	0 5 2	0 7 3	0 1 3	0 4 3	0 2 3	0 8 4
Endocrine System (continued)																									
Adrenal gland, medulla	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																							X		
Bilateral, pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	
Adenoma													X												
Carcinoma																X	X								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	
Pituitary gland	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Pars distalis, adenoma											X							X	X			X			
Pars distalis, carcinoma										X															
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bilateral, C-cell, adenoma																									
C-cell, adenoma													X												
C-cell, carcinoma												X							X						
Follicular cell, adenoma																							X		
Follicular cell, carcinoma																									
General Body System																									
Tissue NOS																			+						
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Preputial gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	
Adenoma			X											X							X			X	
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Bilateral, interstitial cell, adenoma			X			X	X		X		X			X	X	X			X	X			X	X	X
Interstitial cell, adenoma	X				X			X					X				X	X			X				
Hematopoietic System																									
Blood																							+		
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 1 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 8 3	0 1 4	0 1 5	0 2 4	0 2 5	0 3 2	0 3 3	0 3 4	0 3 5	0 4 4	0 4 5	0 5 3	0 5 4	0 5 5	0 6 5	0 7 4	0 7 5	0 8 5	1 0 1	0 9 3	0 9 4	0 9 5	1 0 3	1 0 4	1 0 5	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign		X	X						X		X				X			X	X			X			X	10
Bilateral, pheochromocytoma benign					X							X	X								X		X			5
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma												X											X			3
Carcinoma																										2
Parathyroid gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma								X			X	X				X		X					X	X		11
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, c-cell, adenoma																				X						1
C-cell, adenoma		X			X			X					X			X										6
C-cell, carcinoma									X									X								4
Follicular cell, adenoma																										1
Follicular cell, carcinoma																	X									1
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma	X										X															6
Carcinoma						X																				1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		37
Interstitial cell, adenoma																										7
Hematopoietic System																										
Blood	+																									2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	4	4	4	4	4	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7
	6	6	8	8	9	2	4	7	7	7	7	8	9	1	2	4	5	6	6	6	7	8	0	0
	5	9	1	1	8	9	3	0	4	5	7	0	6	8	4	1	1	3	4	8	4	1	0	8
Carcass ID Number	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0
	2	9	6	8	8	4	3	5	4	1	6	1	6	2	7	9	6	7	0	5	7	1	4	2	8
	1	1	1	1	2	1	1	1	2	1	2	2	3	2	1	2	4	2	2	2	3	3	3	3	4
Hematopoietic System (continued)																									
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma																									
Hemangiosarcoma																									
Osteosarcoma, metastatic, uncertain primary site																				X					
Thymus	+	M	+	M	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	I	M	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Fibroadenoma																					X	X			
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma																									X
Subcutaneous tissue, fibrosarcoma									X						X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cranium, carcinoma, metastatic, Zymbal's gland	X																								
Skeletal muscle																				+					
Abdominal, osteosarcoma, metastatic, uncertain primary site																				X					
Diaphragm, osteosarcoma, metastatic, uncertain primary site																				X					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Meningioma malignant												X													
Meninges, carcinoma, metastatic, Zymbal's gland	X																								
Nerve, carcinoma, metastatic, Zymbal's gland	X																								
Spinal cord												+					+								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 1 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 8 3	0 1 4	0 1 5	0 2 4	0 2 5	0 3 2	0 3 3	0 3 4	0 3 5	0 4 4	0 4 5	0 5 3	0 5 4	0 5 5	0 6 5	0 7 4	0 7 5	0 8 5	1 0 1	0 9 3	0 9 4	0 9 5	1 0 3	1 0 4	1 0 5	**Total Tissues/ Tumors**
Hematopoietic System (continued)																										
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma						X																				1
Hemangiosarcoma												X														1
Osteosarcoma, metastatic, uncertain primary site																										1
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	43
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Fibroadenoma					X						X															4
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma				X																						1
Subcutaneous tissue, fibroma										X													X			3
Subcutaneous tissue, fibrosarcoma																				X					X	4
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cranium, carcinoma, metastatic, Zymbal's gland																										1
Skeletal muscle																										1
Abdominal, osteosarcoma, metastatic, uncertain primary site																										1
Diaphragm, osteosarcoma, metastatic, uncertain primary site																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Meningioma malignant																										1
Meninges, carcinoma, metastatic, Zymbal's gland																										1
Nerve, carcinoma, metastatic, Zymbal's gland																										1
Spinal cord																										2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	4 6 5	4 6 9	4 8 1	4 8 1	4 9 8	5 2 9	5 4 3	5 7 0	5 7 4	5 7 5	5 7 7	5 8 0	5 9 6	6 1 8	6 2 4	6 4 1	6 5 1	6 6 3	6 6 4	6 6 8	6 6 8	6 7 4	6 8 1	7 0 0	7 0 8
Carcass ID Number	0 2 1	0 9 1	0 6 1	0 8 1	0 8 2	0 4 1	0 3 1	0 5 1	0 4 2	0 1 1	0 6 2	0 1 2	0 6 3	0 2 2	0 7 1	0 9 2	0 6 4	0 7 2	1 0 2	0 5 2	0 7 3	0 1 3	0 4 3	0 2 3	0 8 4
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																X	X								
Mediastinum, osteosarcoma, metastatic, uncertain primary site																				X					
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear												+													+
Eye	+									+							+						+		+
Harderian gland	+																								
Zymbal's gland	+																								
Carcinoma	X																								
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, carcinoma																								X	
Urinary bladder	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X		X		X	X		X												X	X	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 1 4	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	0 8 3	0 1 4	0 1 5	0 2 4	0 2 5	0 3 2	0 3 3	0 3 4	0 3 5	0 4 4	0 4 5	0 5 3	0 5 4	0 5 5	0 6 5	0 7 4	0 7 5	0 8 5	1 0 1	0 9 3	0 9 4	0 9 5	1 0 3	1 0 4	1 0 5	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										2
Mediastinum, osteosarcoma, metastatic, uncertain primary site																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										2
Eye				+															+							7
Harderian gland																										1
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, carcinoma																										1
Urinary bladder	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X	X				X				X		X			X		X						16

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg

Number of Days on Study	3 8 1	3 9 2	3 9 9	4 8 9	5 2 9	5 3 3	5 5 5	5 7 1	5 8 8	5 9 0	5 9 3	6 1 7	6 1 8	6 4 0	6 4 5	6 5 8	6 7 4	6 8 9	6 9 0	7 0 8	7 1 2	7 1 8	7 2 1	7 2 9	7 2 9
Carcass ID Number	2 1 1	2 1 2	2 4 1	2 8 1	2 5 1	3 0 1	2 6 1	2 6 2	2 6 3	2 7 1	2 8 2	2 1 3	2 6 4	2 2 1	2 2 2	2 2 3	2 9 1	2 4 2	2 7 3	2 7 2	2 4 3	2 9 2	3 0 2	2 1 4	2 1 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Intestine large	A	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+		
Intestine large, cecum	A	+	A	+	M	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+		
Intestine large, colon	A	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+		
Polyp adenomatous																									
Intestine large, rectum	A	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+		
Intestine small	+	+	A	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+		
Intestine small, duodenum	+	+	A	+	A	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+		
Intestine small, ileum	+	+	A	+	+	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	A		
Intestine small, jejunum	A	+	A	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	A	+	+		
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Mesentery		+							+		+	+		+		+									
Pancreas	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+		
Schwannoma malignant														X											
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+		+	+		+	+		+	+	+			+	+	+	+	+	+			+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Papilloma squamous																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, medulla	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Pheochromocytoma malignant										X															
Pheochromocytoma benign				X				X			X	X		X						X					
Bilateral, pheochromocytoma benign																X			X				X		
Islets, pancreatic	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Carcinoma																					X				
Parathyroid gland	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Pars distalis, adenoma				X					X				X		X				X		X		X		
Pars distalis, carcinoma			X																						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 4 4	2 4 5	2 5 2	2 5 3	2 5 4	2 5 5	2 6 5	2 7 4	2 7 5	2 8 3	2 8 4	2 8 5	2 9 3	2 9 4	2 9 5	3 0 3	3 0 4	3 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus																										23
Intestine large																			+							21
Intestine large, cecum																										17
Intestine large, colon																			+							21
Polyp adenomatous																			X							1
Intestine large, rectum																										19
Intestine small																										20
Intestine small, duodenum																										19
Intestine small, ileum																										18
Intestine small, jejunum																										17
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma			X																							1
Mesentery		+					+							+			+									10
Pancreas			+																							22
Salivary glands	+																									23
Schwannoma malignant																										1
Stomach	+	+	+																	+						29
Stomach, forestomach	+	+	+																	+						22
Stomach, glandular	+	+	+																							28
Tongue									+				+				+	+								4
Papilloma squamous																		X								1
Cardiovascular System																										
Heart			+													+										25
Endocrine System																										
Adrenal gland														+												24
Adrenal gland, cortex														+												24
Adrenal gland, medulla														+												23
Pheochromocytoma malignant																										1
Pheochromocytoma benign																										6
Bilateral, pheochromocytoma benign														X												4
Islets, pancreatic																										22
Carcinoma																										1
Parathyroid gland																						+				22
Pituitary gland					+		+						+							+	+					28
Pars distalis, adenoma					X		X						X								X					11
Pars distalis, carcinoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg (continued)

Number of Days on Study	3 8 1	3 9 2	3 9 9	4 8 9	5 2 9	5 3 3	5 5 5	5 7 1	5 8 8	5 9 0	5 9 3	6 1 7	6 1 8	6 4 0	6 4 5	6 5 8	6 7 4	6 8 9	6 9 0	7 0 8	7 1 2	7 1 8	7 2 1	7 2 9	7 2 9
Carcass ID Number	2 1 1	2 1 2	2 4 1	2 8 1	2 5 1	3 0 1	2 6 1	2 6 2	2 6 3	2 7 1	2 8 2	2 1 3	2 6 4	2 2 1	2 2 2	2 2 3	2 9 1	2 4 2	2 7 3	2 7 2	2 4 3	2 9 2	3 0 2	2 1 4	2 1 5
Endocrine System (continued)																									
Thyroid gland	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
C-cell, adenoma																									
C-cell, carcinoma																									
Follicular cell, carcinoma																									
General Body System																									
Tissue NOS																		+							
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Preputial gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adenoma																									
Carcinoma											X		X												
Bilateral, carcinoma																				X					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X	X					X	X	X	X			X	X	X	X		X		X	X
Interstitial cell, adenoma	X						X	X	X	X					X	X							X		
Hematopoietic System																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+		
Lymph node, mesenteric	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Spleen	A	+	A	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+		
Integumentary System																									
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+		
Adenocarcinoma																		X							
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Basal cell adenoma																									X
Keratoacanthoma																									
Subcutaneous tissue, fibroma							X															X			
Subcutaneous tissue, fibroma, multiple																									
Subcutaneous tissue, myxosarcoma								X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 2 4	2 2 5	2 3 1	2 3 2	2 3 3	2 3 4	2 3 5	2 4 4	2 4 5	2 5 2	2 5 3	2 5 4	2 5 5	2 6 5	2 7 4	2 7 5	2 8 3	2 8 4	2 8 5	2 9 3	2 9 4	2 9 5	3 0 3	3 0 4	3 0 5	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Thyroid gland									+								+		+							25
C-cell, adenoma																	X									1
C-cell, carcinoma																			X							1
Follicular cell, carcinoma									X																	1
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis																										23
Preputial gland											+						+									24
Adenoma											X															1
Carcinoma																	X									3
Bilateral, carcinoma																										1
Prostate																									+	24
Seminal vesicle															+											25
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X		X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	36
Interstitial cell, adenoma						X							X													10
Hematopoietic System																										
Blood												+					+									2
Bone marrow																										23
Lymph node							+																			25
Lymph node, mandibular																										21
Lymph node, mesenteric							+																			23
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Thymus																										20
Integumentary System																										
Mammary gland			+		+																		+			23
Adenocarcinoma																										1
Fibroadenoma																							X			1
Skin	+		+	+		+	+	+					+	+	+	+			+				+	+		37
Basal cell adenoma													X	X					X							4
Keratoacanthoma				X		X									X									X		4
Subcutaneous tissue, fibroma						X	X																			4
Subcutaneous tissue, fibroma, multiple			X											X												2
Subcutaneous tissue, myxosarcoma																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg (continued)

Number of Days on Study	3 8 1	3 9 2	3 9 9	4 8 9	5 2 9	5 3 3	5 5 5	5 7 1	5 8 8	5 9 0	5 9 3	6 1 7	6 1 8	6 4 0	6 4 5	6 5 8	6 7 4	6 8 9	6 9 0	7 0 8	7 1 2	7 1 8	7 2 1	7 2 9	7 2 9
Carcass ID Number	2 1 1	2 1 2	2 4 1	2 8 1	2 5 1	3 0 1	2 6 1	2 6 2	2 6 3	2 7 1	2 8 2	2 1 3	2 6 4	2 2 1	2 2 2	2 2 3	2 9 1	2 4 2	2 7 3	2 7 2	2 4 3	2 9 2	3 0 2	2 1 4	2 1 5
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Skeletal muscle																									
Fibroma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Astrocytoma malignant																									
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Alveolar/bronchiolar adenoma																									
Osteosarcoma, metastatic		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Special Senses System																									
Eye							+				+				+								+		
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Lipoma																				X					
Transitional epithelium, carcinoma					X																				
Urinary bladder	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+		
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X						X				X	X	X				X		X	X
Mesothelioma malignant									X																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 112 mg/kg (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	22 4	22 5	23 1	23 2	23 3	23 4	23 5	24 4	24 5	25 2	25 3	25 4	25 5	25 5	26 5	27 4	27 5	28 3	28 4	28 5	29 3	29 4	29 5	30 3	30 4	30 5	**Total Tissues/ Tumors**
Musculoskeletal System																											
Bone																											23
Skeletal muscle	+																										1
Fibroma	X																										1
Nervous System																											
Brain																						+					24
Astrocytoma malignant																						X					1
Respiratory System																											
Lung					+											+	+					+	+				29
Alveolar/bronchiolar adenoma					X																						1
Osteosarcoma, metastatic																											1
Nose																											23
Trachea			+																								24
Special Senses System																											
Eye												+															5
Urinary System																											
Kidney																											23
Lipoma																											1
Transitional epithelium, carcinoma																											1
Urinary bladder																											22
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X									X	X				X		X					X	X		15
Mesothelioma malignant																											1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg

Number of Days on Study	3 6 5	4 1 4	5 2 9	5 5 0	5 6 1	5 7 7	5 8 1	6 0 9	6 6 1	6 6 3	6 6 7	6 6 8	6 6 9	6 9 5	7 0 3	7 0 4	7 2 6	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 3 1	1 4 5	1 7 1	1 9 1	1 6 1	1 5 1	1 8 1	1 4 1	1 4 2	1 9 2	1 7 2	1 6 2	1 9 3	1 5 2	1 2 1	1 7 3	1 5 3	1 2 2	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 2 3	1 2 4
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Mesentery			+		+					+	+	+		+	+		+	+	+			+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Acinar cell, adenoma														X								X		
Acinar cell, adenoma, multiple																								
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Forestomach, papilloma squamous																								
Stomach, forestomach	+			+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Stomach, glandular	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Endocrine System																								
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Fibrous histiocytoma, metastatic, uncertain primary site					X																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 2 5	1 3 2	1 3 3	1 3 4	1 3 5	1 4 3	1 4 4	1 5 4	1 5 5	1 6 3	1 6 4	1 6 5	1 7 4	1 7 5	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Mesentery			+							+			+		+				+						+	19
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Acinar cell, adenoma			X				X																			4
Acinar cell, adenoma, multiple																									X	1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Forestomach, papilloma squamous								X																		1
Stomach, forestomach	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	44
Stomach, glandular	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X																								1
Fibrous histiocytoma, metastatic, uncertain primary site																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	3 6 5	4 1 4	5 2 9	5 5 0	5 6 1	5 7 7	5 8 1	6 0 9	6 6 1	6 6 3	6 6 7	6 6 8	6 6 9	6 9 5	7 0 3	7 0 4	7 2 6	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 3 1	1 4 5	1 7 1	1 9 1	1 6 1	1 5 1	1 8 1	1 4 1	1 4 2	1 9 2	1 7 2	1 6 2	1 9 3	1 5 2	1 2 1	1 7 3	1 5 3	1 2 2	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 2 3	1 2 4
Endocrine System (continued)																								
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																X						X		
Pheochromocytoma benign	X			X											X						X			
Bilateral, pheochromocytoma benign								X		X									X					X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X				X												
Adenoma, multiple																							X	
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma		X					X	X		X				X				X	X	X		X	X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X					X									X						
C-cell, carcinoma																								
General Body System																								
Tissue NOS														+	+									
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X
Carcinoma				X			X				X													
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma					X	X			X		X		X		X	X	X	X	X	X	X	X		X
Interstitial cell, adenoma	X		X									X											X	
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inguinal, fibrous histiocytoma, metastatic, uncertain primary site					X																			
Mediastinal, fibrous histiocytoma, metastatic, uncertain primary site					X																			

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 2 5	1 3 2	1 3 3	1 3 4	1 3 5	1 4 3	1 4 4	1 5 4	1 5 5	1 6 3	1 6 4	1 6 5	1 7 4	1 7 5	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Adrenal gland, medulla	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																							X	X	X	5
Pheochromocytoma benign																X			X			X				7
Bilateral, pheochromocytoma benign						X		X													X					7
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										2
Adenoma, multiple																										1
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma										X																1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X					X			X				X								X				16
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																	X								X	5
C-cell, carcinoma																						X			X	2
General Body System																										
Tissue NOS																										2
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X																		2
Carcinoma																										3
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X		X		X	X	X		X	X	X	X	X		X	X	X	X		35
Interstitial cell, adenoma								X		X				X						X					X	9
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Inguinal, fibrous histiocytoma, metastatic, uncertain primary site																										1
Mediastinal, fibrous histiocytoma, metastatic, uncertain primary site																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	3 6 5	4 1 4	5 2 9	5 5 0	5 6 1	5 7 7	5 8 1	6 0 9	6 6 1	6 6 3	6 6 7	6 6 8	6 6 9	6 9 5	7 0 3	7 0 4	7 2 6	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 3 1	1 4 5	1 7 1	1 9 1	1 6 1	1 5 1	1 8 1	1 4 1	1 4 2	1 9 2	1 7 2	1 6 2	1 9 3	1 5 2	1 2 1	1 7 3	1 5 3	1 2 2	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 2 3	1 2 4
Hematopoietic System (continued)																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																				
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																				
Thymoma benign																								X	
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma														X										X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Keratoacanthoma																									
Keratoacanthoma, multiple																									
Subcutaneous tissue, fibroma										X			X												
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+		+																				
Back, fibrous histiocytoma, metastatic, uncertain primary site					X																				
Neck, carcinoma, extension, metastatic, thyroid gland																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord					+																				
Fibrous histiocytoma, metastatic, uncertain primary site					X																				

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 2 5	1 3 2	1 3 3	1 3 4	1 3 5	1 4 3	1 4 4	1 5 4	1 5 5	1 6 3	1 6 4	1 6 5	1 7 4	1 7 5	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	**Total Tissues/ Tumors**
Hematopoietic System (continued)																										
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Thymoma benign																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibroadenoma																					X					3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																	X									1
Keratoacanthoma	X								X					X							X	X				5
Keratoacanthoma, multiple		X																								1
Subcutaneous tissue, fibroma		X																				X				4
Subcutaneous tissue, lipoma								X								X										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																						+				3
Back, fibrous histiocytoma, metastatic, uncertain primary site																										1
Neck, carcinoma, extension, metastatic, thyroid gland																						X				1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1
Fibrous histiocytoma, metastatic, uncertain primary site																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	3 6 5	4 1 4	5 2 9	5 5 0	5 6 1	5 7 7	5 8 1	6 0 9	6 6 1	6 6 3	6 6 7	6 6 8	6 6 9	6 9 5	7 0 3	7 0 4	7 2 6	7 2 7	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	1 3 1	1 4 5	1 7 1	1 9 1	1 6 1	1 5 1	1 8 1	1 4 1	1 4 2	1 9 2	1 7 2	1 6 2	1 9 3	1 5 2	1 2 1	1 7 3	1 5 3	1 2 2	1 1 1	1 1 2	1 1 3	1 1 4	1 1 5	1 2 3	1 2 4
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																					X			
Alveolar/bronchiolar carcinoma																								
Carcinoma, metastatic, multiple, thyroid gland																								
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Artery, pheochromocytoma malignant, metastatic, adrenal gland																								
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																								
Ear											+													
Schwannoma malignant											X													
Eye												+												
Zymbal's gland											+													
Carcinoma											X													
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrous histiocytoma, metastatic, uncertain primary site					X																			
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X			X			X		X				X						
Mesothelioma malignant			X														X	X						

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	1 2 5	1 3 2	1 3 3	1 3 4	1 3 5	1 4 3	1 4 4	1 5 4	1 5 5	1 6 3	1 6 4	1 6 5	1 7 4	1 7 5	1 8 2	1 8 3	1 8 4	1 8 5	1 9 4	1 9 5	2 0 1	2 0 2	2 0 3	2 0 4	2 0 5	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma													X					X								3
Alveolar/bronchiolar carcinoma												X														1
Carcinoma, metastatic, multiple, thyroid gland																						X				1
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Artery, pheochromocytoma malignant, metastatic, adrenal gland																							X			1
Nose	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear				+																						2
Schwannoma malignant																										1
Eye				+						+																3
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrous histiocytoma, metastatic, uncertain primary site																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X							X	X														9
Mesothelioma malignant																				X						4

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone

	Vehicle Control	112 mg/kg	225 mg/kg
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	15/48 (31%)	10/23 (43%)[e]	14/49 (29%)
Adjusted rates[b]	59.8%		36.9%
Terminal rates[c]	14/24 (58%)		9/31 (29%)
First incidence (days)	681		365
Life table tests[d]			P=0.216N
Logistic regression tests[d]			P=0.373N
Fisher exact test[d]			P=0.473N
Adrenal Medulla: Malignant Pheochromocytoma			
Overall rates	0/48 (0%)	1/23 (4%)[e]	5/49 (10%)
Adjusted rates	0.0%		15.4%
Terminal rates	0/24 (0%)		4/31 (13%)
First incidence (days)	–[f]		704
Life table tests			P=0.061
Logistic regression tests			P=0.056
Fisher exact test			P=0.030
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	15/48 (31%)	10/23 (43%)[e]	19/49 (39%)
Adjusted rates	59.8%		49.9%
Terminal rates	14/24 (58%)		13/31 (42%)
First incidence (days)	681		365
Life table tests			P=0.578N
Logistic regression tests			P=0.406
Fisher exact test			P=0.287
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	2/50 (4%)	1/29 (3%)[e]	3/50 (6%)
Adjusted rates	5.7%		9.4%
Terminal rates	0/24 (0%)		3/32 (9%)
First incidence (days)	641		729 (T)
Life table tests			P=0.612
Logistic regression tests			P=0.541
Fisher exact test			P=0.500
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	2/50 (4%)	1/29 (3%)[e]	4/50 (8%)
Adjusted rates	5.7%		12.5%
Terminal rates	0/24 (0%)		4/32 (13%)
First incidence (days)	641		729 (T)
Life table tests			P=0.461
Logistic regression tests			P=0.391
Fisher exact test			P=0.339
Mammary Gland: Fibroadenoma			
Overall rates	4/50 (8%)	1/50 (2%)	3/50 (6%)
Adjusted rates	14.3%	3.7%	8.8%
Terminal rates	2/24 (8%)	1/27 (4%)	2/32 (6%)
First incidence (days)	668	729 (T)	695
Life table tests	P=0.293N	P=0.148N	P=0.354N
Logistic regression tests	P=0.335N	P=0.158N	P=0.415N
Cochran-Armitage test[d]	P=0.414N		
Fisher exact test		P=0.181N	P=0.500N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Mammary Gland: Fibroadenoma or Adenocarcinoma			
Overall rates	4/50 (8%)	2/50 (4%)	3/50 (6%)
Adjusted rates	14.3%	6.7%	8.8%
Terminal rates	2/24 (8%)	1/27 (4%)	2/32 (6%)
First incidence (days)	668	690	695
Life table tests	P=0.289N	P=0.283N	P=0.354N
Logistic regression tests	P=0.340N	P=0.309N	P=0.415N
Cochran-Armitage test	P=0.418N		
Fisher exact test		P=0.339N	P=0.500N
Pancreas: Adenoma			
Overall rates	7/50 (14%)	0/22 (0%)[e]	5/50 (10%)
Adjusted rates	26.9%		14.9%
Terminal rates	5/24 (21%)		4/32 (13%)
First incidence (days)	708		695
Life table tests			P=0.200N
Logistic regression tests			P=0.203N
Fisher exact test			P=0.380N
Pancreatic Islets: Adenoma			
Overall rates	3/49 (6%)	0/22 (0%)[e]	3/50 (6%)
Adjusted rates	10.7%		7.8%
Terminal rates	2/24 (8%)		1/32 (3%)
First incidence (days)	596		609
Life table tests			P=0.550N
Logistic regression tests			P=0.653N
Fisher exact test			P=0.651N
Pancreatic Islets: Adenoma or Carcinoma			
Overall rates	5/49 (10%)	1/22 (5%)[e]	3/50 (6%)
Adjusted rates	15.8%		7.8%
Terminal rates	2/24 (8%)		1/32 (3%)
First incidence (days)	596		609
Life table tests			P=0.249N
Logistic regression tests			P=0.355N
Fisher exact test			P=0.346N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	11/48 (23%)	11/28 (39%)[e]	16/49 (33%)
Adjusted rates	37.4%		41.7%
Terminal rates	7/24 (29%)		10/31 (32%)
First incidence (days)	577		414
Life table tests			P=0.425
Logistic regression tests			P=0.234
Fisher exact test			P=0.200
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	12/48 (25%)	12/28 (43%)[e]	16/49 (33%)
Adjusted rates	38.9%		41.7%
Terminal rates	7/24 (29%)		10/31 (32%)
First incidence (days)	575		414
Life table tests			P=0.514
Logistic regression tests			P=0.299
Fisher exact test			P=0.272

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Preputial Gland: Adenoma			
Overall rates	6/48 (13%)	1/24 (4%)[e]	2/50 (4%)
Adjusted rates	18.3%		6.3%
Terminal rates	1/24 (4%)		2/32 (6%)
First incidence (days)	481		729 (T)
Life table tests			P=0.081N
Logistic regression tests			P=0.125N
Fisher exact test			P=0.121N
Preputial Gland: Carcinoma			
Overall rates	1/48 (2%)	4/24 (17%)[e]	3/50 (6%)
Adjusted rates	4.2%		6.7%
Terminal rates	1/24 (4%)		0/32 (0%)
First incidence (days)	729 (T)		550
Life table tests			P=0.379
Logistic regression tests			P=0.280
Fisher exact test			P=0.324
Preputial Gland: Adenoma or Carcinoma			
Overall rates	7/48 (15%)	5/24 (21%)[e]	5/50 (10%)
Adjusted rates	21.9%		12.6%
Terminal rates	2/24 (8%)		2/32 (6%)
First incidence (days)	481		550
Life table tests			P=0.251N
Logistic regression tests			P=0.388N
Fisher exact test			P=0.351N
Skin: Basal Cell Adenoma			
Overall rates	0/50 (0%)	4/50 (8%)	0/50 (0%)
Adjusted rates	0.0%	14.8%	0.0%
Terminal rates	0/24 (0%)	4/27 (15%)	0/32 (0%)
First incidence (days)	-	729 (T)	-
Life table tests	P=0.526N	P=0.077	-
Logistic regression tests	P=0.526N	P=0.077	-
Cochran-Armitage test	P=0.619N		
Fisher exact test		P=0.059	-
Skin: Keratoacanthoma			
Overall rates	1/50 (2%)	4/50 (8%)	6/50 (12%)
Adjusted rates	4.2%	14.8%	18.8%
Terminal rates	1/24 (4%)	4/27 (15%)	6/32 (19%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.088	P=0.213	P=0.112
Logistic regression tests	P=0.088	P=0.213	P=0.112
Cochran-Armitage test	P=0.042		
Fisher exact test		P=0.181	P=0.056

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Skin: Basal Cell Adenoma or Carcinoma			
Overall rates	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted rates	0.0%	14.8%	3.1%
Terminal rates	0/24 (0%)	4/27 (15%)	1/32 (3%)
First incidence (days)	–	729 (T)	729 (T)
Life table tests	P=0.499	P=0.077	P=0.557
Logistic regression tests	P=0.499	P=0.077	P=0.557
Cochran-Armitage test	P=0.393		
Fisher exact test		P=0.059	P=0.500
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	3/50 (6%)	6/50 (12%)	4/50 (8%)
Adjusted rates	11.9%	19.6%	10.9%
Terminal rates	2/24 (8%)	4/27 (15%)	2/32 (6%)
First incidence (days)	708	555	663
Life table tests	P=0.547N	P=0.308	P=0.643
Logistic regression tests	P=0.511	P=0.273	P=0.586
Cochran-Armitage test	P=0.431		
Fisher exact test		P=0.243	P=0.500
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted rates	13.0%	0.0%	0.0%
Terminal rates	2/24 (8%)	0/27 (0%)	0/32 (0%)
First incidence (days)	574	–	–
Life table tests	P=0.011N	P=0.058N	P=0.043N
Logistic regression tests	P=0.015N	P=0.063N	P=0.066N
Cochran-Armitage test	P=0.015N		
Fisher exact test		P=0.059N	P=0.059N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	7/50 (14%)	6/50 (12%)	4/50 (8%)
Adjusted rates	24.0%	19.6%	10.9%
Terminal rates	4/24 (17%)	4/27 (15%)	2/32 (6%)
First incidence (days)	574	555	663
Life table tests	P=0.114N	P=0.422N	P=0.149N
Logistic regression tests	P=0.171N	P=0.472N	P=0.219N
Cochran-Armitage test	P=0.215N		
Fisher exact test		P=0.500N	P=0.262N
Testes: Adenoma			
Overall rates	44/50 (88%)	46/50 (92%)	44/50 (88%)
Adjusted rates	97.7%	100.0%	100.0%
Terminal rates	23/24 (96%)	27/27 (100%)	32/32 (100%)
First incidence (days)	465	381	365
Life table tests	P=0.039N	P=0.422N	P=0.047N
Logistic regression tests	P=0.388N	P=0.325	P=0.437N
Cochran-Armitage test	P=0.564N		
Fisher exact test		P=0.370	P=0.620N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Thyroid Gland (C-cell): Adenoma			
Overall rates	7/50 (14%)	1/25 (4%)[e]	5/50 (10%)
Adjusted rates	27.0%		13.1%
Terminal rates	6/24 (25%)		2/32 (6%)
First incidence (days)	596		550
Life table tests			P=0.221N
Logistic regression tests			P=0.311N
Fisher exact test			P=0.380N
Thyroid Gland (C-cell): Carcinoma			
Overall rates	4/50 (8%)	1/25 (4%)[e]	2/50 (4%)
Adjusted rates	13.5%		6.3%
Terminal rates	2/24 (8%)		2/32 (6%)
First incidence (days)	580		729 (T)
Life table tests			P=0.232N
Logistic regression tests			P=0.299N
Fisher exact test			P=0.339N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	11/50 (22%)	2/25 (8%)[e]	6/50 (12%)
Adjusted rates	38.7%		16.0%
Terminal rates	8/24 (33%)		3/32 (9%)
First incidence (days)	580		550
Life table tests			P=0.055N
Logistic regression tests			P=0.102N
Fisher exact test			P=0.143N
All Organs: Mononuclear Cell Leukemia			
Overall rates	16/50 (32%)	15/50 (30%)	9/50 (18%)
Adjusted rates	44.9%	43.9%	23.2%
Terminal rates	7/24 (29%)	9/27 (33%)	4/32 (13%)
First incidence (days)	469	533	577
Life table tests	P=0.023N	P=0.383N	P=0.033N
Logistic regression tests	P=0.063N	P=0.492N	P=0.096N
Cochran-Armitage test	P=0.071N		
Fisher exact test		P=0.500N	P=0.083N
All Organs: Malignant Mesothelioma			
Overall rates	0/50 (0%)	1/50 (2%)	4/50 (8%)
Adjusted rates	0.0%	2.4%	10.7%
Terminal rates	0/24 (0%)	0/27 (0%)	1/32 (3%)
First incidence (days)	-	588	529
Life table tests	P=0.044	P=0.520	P=0.104
Logistic regression tests	P=0.023	P=0.510	P=0.062
Cochran-Armitage test	P=0.026		
Fisher exact test		P=0.500	P=0.059

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
All Organs: Benign Tumors			
Overall rates	46/50 (92%)	48/50 (96%)	50/50 (100%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	24/24 (100%)	27/27 (100%)	32/32 (100%)
First incidence (days)	465	381	365
Life table tests	P=0.135N	P=0.409N	P=0.153N
Logistic regression tests	P=0.017	P=0.174	P=0.062
Cochran-Armitage test	P=0.037		
Fisher exact test		P=0.339	P=0.059
All Organs: Malignant Tumors			
Overall rates	30/50 (60%)	30/50 (60%)	23/50 (46%)
Adjusted rates	69.7%	65.9%	51.5%
Terminal rates	12/24 (50%)	12/27 (44%)	11/32 (34%)
First incidence (days)	465	392	529
Life table tests	P=0.032N	P=0.412N	P=0.037N
Logistic regression tests	P=0.136N	P=0.555	P=0.159N
Cochran-Armitage test	P=0.095N		
Fisher exact test		P=0.581N	P=0.115N
All Organs: Benign or Malignant Tumors			
Overall rates	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	24/24 (100%)	27/27 (100%)	32/32 (100%)
First incidence (days)	465	381	365
Life table tests	P=0.048N	P=0.309N	P=0.055N
Logistic regression tests	[g]	-	-
Cochran-Armitage test	-		
Fisher exact test		P=1.000N	P=1.000N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.
[f] Not applicable; no tumors in animal group
[g] Value of statistic cannot be computed

TABLE A4a
Historical Incidence of Keratoacanthomas in Male F344/N Rats Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
Benzaldehyde	1/50
Dichlorvos	3/50
Furan	0/50
Furfural	2/50
γ-Butyrolactone	1/50
Total	7/250 (2.8%)
Standard deviation	2.3%
Range	0%-6%
Overall Historical Incidence	
Total	26/770 (3.4%)
Standard deviation	2.9%
Range	0%-12%

[a] Data as of 17 September 1990.

TABLE A4b
Historical Incidence of Skin Tumors in Male F344/N Rats Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls			
	Tricoepithelioma	Basal Cell Adenoma	Basal Cell Carcinoma	Tricoepithelioma, Basal Cell Adenoma, or Carcinoma
Historical Incidence at Southern Research Institute				
Benzaldehyde	0/50	0/50	0/50	0/50
Dichlorvos	1/50	0/50	0/50	1/50
Furan	0/50	1/50	0/50	1/50
Furfural	0/50	0/50	2/50	2/50
γ-Butyrolactone	0/50	0/50	0/50	0/50
Total	1/250 (0.4%)	1/250 (0.4%)	2/250 (0.8%)	4/250 (1.6%)
Standard deviation	0.9%	0.9%	1.8%	1.7%
Range	0%-2%	0%-2%	0%-4%	0%-4%
Overall Historical Incidence				
Total	5/770 (0.6%)	4/770 (0.5%)	4/770 (0.5%)	13/770 (1.7%)
Standard deviation	1.1%	0.9%	1.2%	1.7%
Range	0%-3%	0%-2%	0%-4%	0%-5%

[a] Data as of 17 September 1990

TABLE A4c
Historical Incidence of Mesothelioma in Male F344/N Rats Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence of Mesothelioma[b] in Controls
Historical Incidence at Southern Research Institute	
Benzaldehyde	0/50
Dichlorvos	3/50
Furan	1/50
Furfural	3/50
γ-Butyrolactone	0/50
Total	7/250 (2.8%)
Standard deviation	3.0%
Range	0%-6%
Overall Historical Incidence	
Total	26/770 (3.4%)
Standard deviation	2.8%
Range	0%-10%

[a] Data as of 17 September 1990
[b] Includes mesothelioma benign, malignant, and NOS

TABLE A4d
Historical Incidence of Leukemias in Male F344/N Rats Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls[b]
Historical Incidence at Southern Research Institute	
Benzaldehyde	10/50
Dichlorvos	11/50
Furan	8/50
Furfural	13/50
γ-Butyrolactone	16/50
Total	58/250 (23.2%)
Standard deviation	6.1%
Range	16%-32%
Overall Historical Incidence	
Total	164/770 (21.3%)
Standard deviation	8.9%
Range	4%-38%

[a] Data as of 17 September 1990
[b] Includes occurrences of mononuclear, lymphocytic, monocytic, or undifferentiated leukemias

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	112 mg/kg	225 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Dead	6	7	3
Moribund	19	13	12
Dosing accident	1	3	3
Survivors			
Terminal sacrifice	24	27	32
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large	(49)	(21)	(50)
Parasite metazoan	1 (2%)		
Intestine large, cecum	(48)	(17)	(49)
Parasite metazoan	1 (2%)		
Intestine large, colon	(47)	(21)	(50)
Fibrosis, focal			1 (2%)
Parasite metazoan	6 (13%)	1 (5%)	8 (16%)
Ulcer			1 (2%)
Intestine large, rectum	(47)	(19)	(49)
Parasite metazoan		2 (11%)	1 (2%)
Intestine small, ileum	(46)	(18)	(49)
Hyperplasia, lymphoid	2 (4%)		5 (10%)
Intestine small, jejunum	(46)	(17)	(50)
Hyperplasia, lymphoid	1 (2%)		
Metaplasia, osseous		1 (6%)	
Liver	(50)	(50)	(50)
Basophilic focus	7 (14%)	2 (4%)	5 (10%)
Basophilic focus, multiple	13 (26%)	20 (40%)	18 (36%)
Clear cell focus	4 (8%)		2 (4%)
Clear cell focus, multiple			3 (6%)
Congestion		1 (2%)	1 (2%)
Degeneration, cystic	1 (2%)	1 (2%)	4 (8%)
Eosinophilic focus	4 (8%)		1 (2%)
Eosinophilic focus, multiple			1 (2%)
Fibrosis		1 (2%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)	1 (2%)
Hemorrhage	1 (2%)	1 (2%)	
Hepatodiaphragmatic nodule	5 (10%)	4 (8%)	7 (14%)
Hyperplasia, nodular	4 (8%)	4 (8%)	6 (12%)
Inflammation, granulomatous, multiple	1 (2%)	1 (2%)	3 (6%)
Mixed cell focus	3 (6%)		4 (8%)
Mixed cell focus, multiple	1 (2%)	2 (4%)	
Necrosis, focal		1 (2%)	
Vacuolization cytoplasmic	6 (12%)	8 (16%)	11 (22%)
Bile duct, hyperplasia	45 (90%)	35 (70%)	38 (76%)
Centrilobular, degeneration	2 (4%)	1 (2%)	3 (6%)
Centrilobular, necrosis	1 (2%)		1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Alimentary System (continued)			
Mesentery	(11)	(10)	(19)
Inflammation, chronic		2 (20%)	
Metaplasia, osseous	1 (9%)		
Polyarteritis			1 (5%)
Fat, inflammation, granulomatous, focal		1 (10%)	2 (11%)
Fat, mineralization, focal	1 (9%)	1 (10%)	
Fat, necrosis, focal	9 (82%)	9 (90%)	13 (68%)
Pancreas	(50)	(22)	(50)
Polyarteritis	3 (6%)		6 (12%)
Acinar cell, atrophy	18 (36%)	7 (32%)	21 (42%)
Acinar cell, hyperplasia	2 (4%)	2 (9%)	4 (8%)
Acinar cell, hyperplasia, multiple			1 (2%)
Pharynx	(1)		
Palate, hyperplasia, squamous	1 (100%)		
Stomach	(50)	(29)	(50)
Forestomach, cyst		1 (3%)	
Forestomach, edema	1 (2%)	3 (10%)	1 (2%)
Forestomach, fibrosis, chronic		1 (3%)	
Forestomach, hyperkeratosis		1 (3%)	
Forestomach, inflammation, chronic	2 (4%)	3 (10%)	1 (2%)
Forestomach, mineralization	1 (2%)		
Forestomach, polyarteritis	1 (2%)		1 (2%)
Forestomach, ulcer	1 (2%)	2 (7%)	1 (2%)
Forestomach, epithelium, hyperplasia	1 (2%)	3 (10%)	2 (4%)
Glandular, mineralization	2 (4%)		
Glandular, polyarteritis			2 (4%)
Tongue	(3)	(4)	
Hemorrhage, focal		1 (25%)	
Hyperkeratosis		1 (25%)	
Inflammation, focal	1 (33%)		
Cardiovascular System			
Blood vessel	(1)		
Mesenteric artery, polyarteritis	1 (100%)		
Heart	(50)	(25)	(50)
Congestion		1 (4%)	
Fibrosis, focal			1 (2%)
Inflammation, chronic	46 (92%)	21 (84%)	43 (86%)
Mineralization		1 (4%)	
Atrium, congestion		1 (4%)	4 (8%)
Atrium, fibrosis			1 (2%)
Atrium, thrombus		1 (4%)	4 (8%)
Valve, bacterium	1 (2%)		
Valve, thrombus	1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Endocrine System			
Adrenal gland, cortex	(48)	(24)	(49)
Accessory adrenal cortical nodule			2 (4%)
Degeneration, cystic			4 (8%)
Hyperplasia, focal	5 (10%)		3 (6%)
Hypertrophy, focal	2 (4%)	1 (4%)	
Vacuolization cytoplasmic	11 (23%)	5 (21%)	5 (10%)
Adrenal gland, medulla	(48)	(23)	(49)
Hemorrhage		1 (4%)	
Hyperplasia, focal	5 (10%)	1 (4%)	1 (2%)
Hyperplasia, focal, multiple			2 (4%)
Mineralization			1 (2%)
Thrombus			1 (2%)
Vacuolization cytoplasmic	1 (2%)		
Parathyroid gland	(46)	(22)	(48)
Hyperplasia	1 (2%)	1 (5%)	
Pituitary gland	(48)	(28)	(49)
Pars distalis, angiectasis	3 (6%)	1 (4%)	2 (4%)
Pars distalis, cyst	4 (8%)	2 (7%)	1 (2%)
Pars distalis, cyst, multiple	1 (2%)	1 (4%)	
Pars distalis, hemorrhage		1 (4%)	1 (2%)
Pars distalis, hyperplasia, focal	8 (17%)	4 (14%)	3 (6%)
Pars intermedia, hemorrhage		1 (4%)	
Thyroid gland	(50)	(25)	(50)
Hyperplasia, cystic	1 (2%)		
Ultimobranchial cyst			1 (2%)
C-cell, hyperplasia	9 (18%)	5 (20%)	13 (26%)
Follicle, cyst	2 (4%)		1 (2%)
Follicle, hyperplasia, cystic			1 (2%)
Follicular cell, hyperplasia	1 (2%)		
General Body System			
None			
Genital System			
Epididymis	(50)	(23)	(50)
Edema			1 (2%)
Preputial gland	(48)	(24)	(50)
Atrophy	12 (25%)	3 (13%)	13 (26%)
Fibrosis	1 (2%)	2 (8%)	
Hyperplasia		1 (4%)	1 (2%)
Inflammation, chronic	4 (8%)		1 (2%)
Inflammation, suppurative	8 (17%)	9 (38%)	2 (4%)
Necrosis			1 (2%)
Duct, cyst	10 (21%)	5 (21%)	7 (14%)
Prostate	(49)	(24)	(49)
Cyst		1 (4%)	
Cyst, multiple		2 (8%)	
Fibrosis			1 (2%)
Inflammation, chronic		2 (8%)	2 (4%)
Inflammation, suppurative	16 (33%)	11 (46%)	17 (35%)
Epithelium, hyperplasia			1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Genital System (continued)			
Seminal vesicle	(50)	(25)	(50)
Atrophy		1 (4%)	
Dilatation		2 (8%)	
Inflammation, chronic		1 (4%)	
Epithelium, hyperplasia		1 (4%)	
Testes	(50)	(50)	(50)
Atrophy	15 (30%)	19 (38%)	22 (44%)
Mineralization			1 (2%)
Necrosis	1 (2%)		
Bilateral, atrophy			1 (2%)
Bilateral, interstitial cell, hyperplasia	8 (16%)	2 (4%)	2 (4%)
Interstitial cell, atrophy	2 (4%)		
Interstitial cell, hyperplasia	7 (14%)	8 (16%)	14 (28%)
Hematopoietic System			
Blood	(2)	(2)	
Leukocytosis	1 (50%)		
Bone marrow	(50)	(23)	(50)
Atrophy	1 (2%)		
Myelofibrosis	1 (2%)		
Myeloid cell, hyperplasia	1 (2%)	1 (4%)	1 (2%)
Lymph node	(50)	(25)	(50)
Axillary, hyperplasia, lymphoid			1 (2%)
Inguinal, cyst		1 (4%)	
Inguinal, hyperplasia, lymphoid	1 (2%)		1 (2%)
Mediastinal, hemorrhage		2 (8%)	
Mediastinal, hyperplasia, histiocyte		1 (4%)	
Mediastinal, hyperplasia, lymphoid	1 (2%)	1 (4%)	
Mediastinal, necrosis		1 (4%)	
Mediastinal, pigmentation			1 (2%)
Pancreatic, hyperplasia, lymphoid	1 (2%)		
Lymph node, mandibular	(48)	(21)	(49)
Cyst		1 (5%)	1 (2%)
Cyst, multiple			2 (4%)
Hyperplasia, lymphoid	5 (10%)	1 (5%)	2 (4%)
Lymphocyte, necrosis			1 (2%)
Lymph node, mesenteric	(48)	(23)	(50)
Angiectasis	1 (2%)	1 (4%)	
Cyst, multiple	1 (2%)		
Hyperplasia, lymphoid	1 (2%)		1 (2%)
Lymphocyte, necrosis			1 (2%)
Spleen	(50)	(45)	(50)
Atrophy		1 (2%)	1 (2%)
Atrophy, focal			1 (2%)
Congestion		1 (2%)	
Developmental malformation			1 (2%)
Fibrosis	1 (2%)		
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)
Hemorrhage		1 (2%)	
Hyperplasia, lymphoid	2 (4%)		
Necrosis		2 (4%)	
Pigmentation		1 (2%)	
Thymus	(43)	(20)	(49)
Hyperplasia, lymphoid			1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Integumentary System			
Mammary gland	(44)	(23)	(48)
Hyperplasia, lobular	1 (2%)	1 (4%)	1 (2%)
Duct, cyst	6 (14%)	8 (35%)	10 (21%)
Skin	(50)	(37)	(50)
Cyst	1 (2%)		
Cyst epithelial inclusion		1 (3%)	4 (8%)
Fibrosis	1 (2%)	1 (3%)	
Ulcer	1 (2%)		
Epidermis, hyperplasia	1 (2%)		
Epidermis, hyperplasia, focal	1 (2%)	3 (8%)	1 (2%)
Subcutaneous tissue, hemorrhage, chronic	1 (2%)		
Subcutaneous tissue, inflammation, granulomatous			1 (2%)
Subcutaneous tissue, necrosis	1 (2%)		1 (2%)
Musculoskeletal System			
Skeletal muscle	(1)	(1)	(3)
Abdominal, metaplasia, osseous	1 (100%)		
Nervous System			
Brain	(50)	(24)	(50)
Compression	1 (2%)	2 (8%)	1 (2%)
Hemorrhage	5 (10%)	1 (4%)	1 (2%)
Hippocampus, vacuolization cytoplasmic	1 (2%)		
Respiratory System			
Lung	(50)	(29)	(50)
Congestion	6 (12%)	9 (31%)	5 (10%)
Edema	1 (2%)		
Foreign body	2 (4%)		
Hemorrhage	1 (2%)	1 (3%)	2 (4%)
Inflammation, granulomatous		1 (3%)	
Inflammation, suppurative	1 (2%)		
Alveolar epithelium, hyperplasia	1 (2%)	1 (3%)	1 (2%)
Alveolus, edema		1 (3%)	
Alveolus, foreign body		1 (3%)	2 (4%)
Alveolus, pigmentation		2 (7%)	
Artery, embolus tumor			1 (2%)
Artery, embolus tumor, multiple		1 (3%)	
Artery, mineralization, multiple		1 (3%)	
Lymphatic, foreign body		3 (10%)	3 (6%)
Perivascular, foreign body			1 (2%)
Subpleura, hemorrhage			1 (2%)
Nose	(50)	(23)	(49)
Lumen, foreign body	1 (2%)		
Lumen, fungus	7 (14%)	2 (9%)	9 (18%)
Lumen, inflammation, suppurative	8 (16%)	2 (9%)	8 (16%)
Nasolacrimal duct, inflammation, suppurative	2 (4%)		3 (6%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	112 mg/kg	225 mg/kg
Special Senses System			
Eye	(7)	(5)	(3)
Cataract	3 (43%)	3 (60%)	3 (100%)
Hemorrhage	1 (14%)		
Inflammation, suppurative	1 (14%)		
Retina, degeneration	4 (57%)		3 (100%)
Harderian gland	(1)		
Pigmentation	1 (100%)		
Urinary System			
Kidney	(50)	(23)	(50)
Bacterium	1 (2%)		
Hydronephrosis		2 (9%)	
Hydronephrosis, multiple		1 (4%)	
Infarct	1 (2%)		
Inflammation, suppurative	1 (2%)		
Nephropathy, chronic	41 (82%)	15 (65%)	46 (92%)
Medulla, necrosis		1 (4%)	
Pelvis, hemorrhage		1 (4%)	
Pelvis, necrosis		1 (4%)	
Renal tubule, mineralization	1 (2%)	1 (4%)	2 (4%)
Renal tubule, pigmentation	1 (2%)	1 (4%)	4 (8%)
Urinary bladder	(48)	(22)	(50)
Inflammation, chronic		1 (5%)	

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR GAVAGE STUDY OF γ-BUTYROLACTONE

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	225 mg/kg	450 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	3	7	6
Moribund	19	16	14
Dosing accident			2
Survivors			
Terminal sacrifice	28	26	28
Died last week of study		1	
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large	(48)	(22)	(49)
Mixed tumor malignant, metastatic, mammary gland		1 (5%)	
Intestine large, cecum	(48)	(18)	(46)
Intestine large, colon	(48)	(20)	(49)
Intestine small, ileum	(47)	(20)	(45)
Intestine small, jejunum	(47)	(20)	(47)
Liver	(50)	(50)	(50)
Mixed tumor malignant, metastatic, mammary gland		1 (2%)	
Mesentery	(5)	(3)	(4)
Pancreas	(49)	(24)	(50)
Acinar cell, adenoma	2 (4%)		
Pharynx		(2)	
Palate, papilloma squamous		1 (50%)	
Palate, squamous cell carcinoma		1 (50%)	
Salivary glands	(50)	(24)	(50)
Carcinoma, metastatic			1 (2%)
Stomach	(49)	(24)	(50)
Cardiovascular System			
Heart	(50)	(25)	(50)
Fibrosarcoma, metastatic, lung	1 (2%)		
Endocrine System			
Adrenal gland, cortex	(50)	(25)	(50)
Adenoma	1 (2%)		2 (4%)
Adrenal gland, medulla	(50)	(25)	(49)
Pheochromocytoma malignant		1 (4%)	
Pheochromocytoma benign	1 (2%)		4 (8%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Endocrine System (continued)			
Islets, pancreatic	(49)	(24)	(50)
Adenoma	1 (2%)		
Carcinoma			1 (2%)
Parathyroid gland	(48)	(23)	(47)
Carcinoma, metastatic, thyroid gland	1 (2%)		
Pituitary gland	(49)	(37)	(48)
Pars distalis, adenoma	22 (45%)	24 (65%)	16 (33%)
Thyroid gland	(50)	(27)	(50)
C-cell, adenoma	7 (14%)	2 (7%)	3 (6%)
C-cell, carcinoma	1 (2%)	1 (4%)	1 (2%)
Follicular cell, carcinoma	1 (2%)	1 (4%)	2 (4%)
General Body System			
None			
Genital System			
Clitoral gland	(48)	(22)	(46)
Adenoma	5 (10%)	4 (18%)	5 (11%)
Carcinoma			1 (2%)
Bilateral, adenoma	1 (2%)	1 (5%)	
Ovary	(50)	(24)	(50)
Uterus	(50)	(32)	(50)
Adenocarcinoma		1 (3%)	
Adenoma			1 (2%)
Hemangiosarcoma			1 (2%)
Polyp stromal	10 (20%)	7 (22%)	12 (24%)
Polyp stromal, multiple		2 (6%)	1 (2%)
Sarcoma stromal	1 (2%)		
Hematopoietic System			
Blood	(2)		(4)
Bone marrow	(49)	(24)	(50)
Lymph node	(50)	(26)	(50)
Axillary, mixed tumor malignant, metastatic, mammary gland		1 (4%)	
Lymph node, mandibular	(49)	(23)	(50)
Carcinoma, metastatic			1 (2%)
Lymph node, mesenteric	(49)	(24)	(49)
Spleen	(48)	(49)	(50)
Sarcoma		1 (2%)	
Thymus	(48)	(21)	(47)
Fibrosarcoma, metastatic, lung	1 (2%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Integumentary System			
Mammary gland	(50)	(50)	(50)
Adenocarcinoma	4 (8%)		
Adenoma			1 (2%)
Fibroadenoma	16 (32%)	10 (20%)	5 (10%)
Fibroadenoma, multiple	6 (12%)	4 (8%)	1 (2%)
Mixed tumor malignant		1 (2%)	
Skin	(50)	(28)	(50)
Lip, papilloma squamous	1 (2%)		
Subcutaneous tissue, carcinoma, metastatic			1 (2%)
Subcutaneous tissue, fibroma		1 (4%)	
Subcutaneous tissue, fibroma, multiple	1 (2%)		
Subcutaneous tissue, fibrosarcoma		1 (4%)	1 (2%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)
Subcutaneous tissue, myxosarcoma	1 (2%)		
Subcutaneous tissue, sarcoma		1 (4%)	1 (2%)
Subcutaneous tissue, squamous cell carcinoma, multiple			1 (2%)
Musculoskeletal System			
Bone	(50)	(28)	(50)
Vertebra, osteosarcoma		1 (4%)	
Skeletal muscle	(2)		(1)
Hindlimb, hemangiosarcoma, extension			1 (100%)
Nervous System			
Brain	(50)	(24)	(50)
Astrocytoma malignant			1 (2%)
Meninges, carcinoma, metastatic, Zymbal's gland		1 (4%)	
Respiratory System			
Lung	(50)	(30)	(50)
Alveolar/bronchiolar adenoma		1 (3%)	2 (4%)
Alveolar/bronchiolar carcinoma	1 (2%)		
Carcinoma, metastatic			1 (2%)
Mixed tumor malignant, metastatic, mammary gland		1 (3%)	
Mediastinum, fibrosarcoma	1 (2%)		
Mediastinum, mixed tumor malignant, metastatic, mammary gland		1 (3%)	
Mediastinum, squamous cell carcinoma	1 (2%)		
Trachea	(50)	(24)	(50)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Special Senses System			
Zymbal's gland		(2)	(1)
Carcinoma		2 (100%)	1 (100%)
Urinary System			
Kidney	(49)	(24)	(49)
Urinary bladder	(50)	(24)	(50)
Transitional epithelium, papilloma, multiple	1 (2%)		
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Leukemia mononuclear	13 (26%)	9 (18%)	11 (22%)
Tumor Summary			
Total animals with primary neoplasms[c]	46	46	41
Total primary neoplasms	99	78	77
Total animals with benign neoplasms	38	42	33
Total benign neoplasms	75	57	53
Total animals with malignant neoplasms	22	19	19
Total malignant neoplasms	24	21	24
Total animals with secondary neoplasms[d]	2	2	1
Total secondary neoplasms	3	6	4

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control

Number of Days on Study	3 1 6	4 9 8	5 1 2	5 1 2	5 4 2	5 5 6	5 5 6	5 5 9	5 7 4	6 5 1	6 6 3	6 6 6	6 6 7	6 6 7	6 6 7	6 6 8	6 8 3	6 8 8	6 9 0	7 0 2	7 1 6	7 2 6	7 3 0	7 3 0	7 3 0
Carcass ID Number	3 1 1	3 1 2	3 6 1	3 7 1	3 8 1	3 5 1	3 8 2	3 7 2	3 5 2	3 2 1	3 3 1	3 4 1	3 4 2	3 8 3	4 0 1	3 5 3	4 0 2	3 8 4	3 6 2	3 4 3	3 9 1	3 2 2	3 1 3	3 1 4	3 1 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+			+										+						+					
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Acinar cell, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Stomach, forestomach	+	+		+	+	+	+		+		+	+	+	+	+	+	+	+	+		+	A	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, lung										X															
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																					X				
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland																									
Pituitary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X							X				X		X	X				X		X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma													X										X		
C-cell, carcinoma																									
Follicular cell, carcinoma																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 2 3	3 2 4	3 2 5	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	3 4 5	3 5 4	3 5 5	3 6 3	3 6 4	3 6 5	3 7 3	3 7 4	3 7 5	3 8 5	3 9 2	3 9 3	3 9 4	3 9 5	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																						+				5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinar cell, adenoma				X	X																					2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, lung																										1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																								X		1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma																					X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Carcinoma, metastatic, thyroid gland										X																1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma			X		X			X	X	X		X		X			X	X	X			X	X	X	X	22
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X				X								X				X	X				7
C-cell, carcinoma										X																1
Follicular cell, carcinoma																X										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	3 1 6	4 9 8	5 1 2	5 1 2	5 4 2	5 5 6	5 5 6	5 5 9	5 7 4	6 5 1	6 6 3	6 6 6	6 6 7	6 6 7	6 6 7	6 6 8	6 8 3	6 8 8	7 9 0	7 0 2	7 1 6	7 2 6	7 3 0	7 3 0	7 3 0
Carcass ID Number	3 1 1	3 1 2	3 6 1	3 7 1	3 8 1	3 5 1	3 8 2	3 7 2	3 5 2	3 2 1	3 3 1	3 4 1	3 4 2	3 4 3	3 8 1	4 0 2	3 5 3	4 0 2	3 8 4	3 6 2	3 4 3	3 9 1	3 2 2	3 1 3	3 1 4	3 1 5
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Adenoma																	X								
Bilateral, adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal						X										X		X					X		X
Sarcoma stromal																									
Vagina							+					+													
Hematopoietic System																									
Blood																								+	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Thymus	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Fibrosarcoma, metastatic, lung										X															
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma									X												X				
Fibroadenoma							X		X					X	X	X					X	X	X		X
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lip, papilloma squamous																					X				
Subcutaneous tissue, fibroma, multiple																					X				
Subcutaneous tissue, myxosarcoma				X																					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																			+					

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 2 3	3 2 4	3 2 5	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	3 4 5	3 5 4	3 5 5	3 6 3	3 6 4	3 6 5	3 7 3	3 7 4	3 7 5	3 8 5	3 9 2	3 9 3	3 9 4	3 9 5	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma	X												X			X				X						5
Bilateral, adenoma																		X								1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal													X					X		X	X		X			10
Sarcoma stromal															X											1
Vagina																										2
Hematopoietic System																										
Blood																			+							2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Fibrosarcoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma							X																		X	4
Fibroadenoma	X		X	X			X					X					X		X							16
Fibroadenoma, multiple									X		X					X		X			X	X				6
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lip, papilloma squamous																										1
Subcutaneous tissue, fibroma, multiple																										1
Subcutaneous tissue, myxosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	3 1 6	4 9 8	5 1 2	5 1 2	5 4 2	5 5 6	5 5 6	5 5 9	5 7 4	6 5 1	6 6 3	6 6 6	6 6 7	6 6 7	6 6 8	6 6 8	6 8 3	6 8 8	6 9 0	7 0 2	7 1 6	7 2 6	7 3 0	7 3 0	7 3 0
Carcass ID Number	3 1 1	3 1 2	3 6 1	3 7 1	3 8 1	3 5 1	3 8 2	3 7 2	3 5 2	3 2 1	3 3 1	3 4 1	3 4 2	3 8 3	4 0 1	3 5 3	4 0 2	3 8 4	3 6 2	3 4 3	3 9 1	3 2 2	3 1 3	3 1 4	3 1 5
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									X
Mediastinum, fibrosarcoma										X															
Mediastinum, squamous cell carcinoma										X															
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma, multiple																								X	
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X			X				X	X		X			X	X	X				X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 2 3	3 2 4	3 2 5	3 3 2	3 3 3	3 3 4	3 3 5	3 4 4	3 4 5	3 5 4	3 5 5	3 6 3	3 6 4	3 6 5	3 7 3	3 7 4	3 7 5	3 8 5	3 9 2	3 9 3	3 9 4	3 9 5	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																										1
Mediastinum, fibrosarcoma																										1
Mediastinum, squamous cell carcinoma																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye																		+								1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma, multiple																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																			X		X					13

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg

Number of Days on Study	2 8 7	3 3 7	3 6 6	3 7 6	4 1 7	5 0 1	5 1 1	5 6 9	6 4 1	6 5 2	6 7 8	6 8 2	6 8 2	6 8 9	6 9 0	6 9 0	7 0 0	7 0 2	7 0 8	7 0 8	7 1 0	7 1 1	7 1 6	7 3 1	7 3 1
Carcass ID Number	5 5 1	5 8 1	5 9 1	6 0 1	5 7 1	5 9 2	5 5 2	5 3 1	5 1 1	5 5 3	5 6 1	5 6 2	5 7 2	5 3 3	5 9 3	5 3 4	5 5 4	5 3 2	5 7 3	5 8 2	5 8 3	5 2 1	5 4 1	5 1 2	5 1 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Intestine large	+	+	+	+	M	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+		
Mixed tumor malignant, metastatic, mammary gland																						X			
Intestine large, cecum	+	+	+	A	M	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	A	+		
Intestine large, colon	+	+	+	+	M	+	+	+	+	M	+	A	M	+	+	+	+	+	+	+	+	+	+		
Intestine large, rectum	+	+	+	+	M	+	+	+	+	+	+	A	+	+	+	+	A	+	+	+	+	A	+		
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Intestine small, duodenum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	A	+		
Intestine small, jejunum	+	+	+	+	M	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	A	+		
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mixed tumor malignant, metastatic, mammary gland																						X			
Mesentery					+		+																		
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Pharynx														+						+					
Palate, papilloma squamous														X											
Palate, squamous cell carcinoma																				X					
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Stomach, forestomach	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+		+	+	+	+		
Stomach, glandular	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+		
Cardiovascular System																									
Blood vessel					+																				
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Pheochromocytoma malignant																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M		
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X			X		X	X		X	X	X		X	X	X			X	X			X	X		X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
C-cell, adenoma									X											X					
C-cell, carcinoma																									
Follicular cell, carcinoma																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 1 4	5 1 5	5 2 2	5 2 3	5 2 4	5 2 5	5 3 5	6 0 2	5 4 2	5 4 3	5 4 4	5 4 5	5 5 5	5 6 3	5 6 4	5 6 5	5 7 4	5 7 5	5 8 4	5 8 5	5 9 4	5 9 5	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus								+																		24
Intestine large								+																		22
Mixed tumor malignant, metastatic, mammary gland																										1
Intestine large, cecum								A																		18
Intestine large, colon								+																		20
Intestine large, rectum								+																		20
Intestine small								+																		24
Intestine small, duodenum								+																		23
Intestine small, ileum								A																		20
Intestine small, jejunum								A																		20
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mixed tumor malignant, metastatic, mammary gland																										1
Mesentery										+																3
Pancreas								+																		24
Pharynx																										2
Palate, papilloma squamous																										1
Palate, squamous cell carcinoma																										1
Salivary glands								+																		24
Stomach								+																		24
Stomach, forestomach								+																		21
Stomach, glandular								+																		22
Cardiovascular System																										
Blood vessel																										1
Heart								+																		25
Endocrine System																										
Adrenal gland						+		+																		25
Adrenal gland, cortex						+		+																		25
Adrenal gland, medulla						+		+																		25
Pheochromocytoma malignant						X																				1
Islets, pancreatic								+																		24
Parathyroid gland								+																		23
Pituitary gland			+		+	M		+		+	+			+			+	+	+	+	+	+			+	37
Pars distalis, adenoma			X		X						X			X				X		X	X	X			X	24
Thyroid gland				+				+			+							+								27
C-cell, adenoma																										2
C-cell, carcinoma																		X								1
Follicular cell, carcinoma											X															1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	2 8 7	3 3 7	3 6 6	3 7 6	4 1 7	5 0 1	5 1 1	5 6 9	6 4 1	6 5 2	6 7 8	6 8 2	6 8 2	6 8 9	6 8 9	6 9 0	7 0 0	7 0 2	7 0 8	7 0 8	7 1 0	7 1 1	7 1 6	7 3 1	7 3 1
Carcass ID Number	5 5 1	5 8 1	5 9 1	6 0 1	5 7 1	5 9 2	5 5 2	5 3 1	5 1 1	5 5 3	5 6 1	5 6 2	5 7 2	5 3 3	5 9 3	5 3 4	5 5 4	5 3 2	5 7 3	5 8 2	5 8 3	5 2 1	5 4 1	5 1 2	5 1 3
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	M	M	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+		
Adenoma																				X					
Bilateral, adenoma										X															
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Adenocarcinoma																									
Polyp stromal						X														X			X		
Polyp stromal, multiple													X												
Vagina																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Axillary, mixed tumor malignant, metastatic, mammary gland																							X		
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+		
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Spleen	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma			X																						
Thymus	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+			
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma												X				X	X		X		X		X		
Fibroadenoma, multiple																		X							
Mixed tumor malignant																							X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma														X											
Subcutaneous tissue, sarcoma											X														

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 1 4	5 1 5	5 2 2	5 2 3	5 2 4	5 2 5	5 3 5	6 0 2	5 4 2	5 4 3	5 4 4	5 4 5	5 5 5	5 6 3	5 6 4	5 6 5	5 7 4	5 7 5	5 8 4	5 8 5	5 9 4	5 9 5	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Clitoral gland								+	+				+	+												22
Adenoma									X				X	X												4
Bilateral, adenoma																										1
Ovary								+																		24
Uterus		+		+				+					+	+							+		+		+	32
Adenocarcinoma								X																		1
Polyp stromal		X		X									X	X												7
Polyp stromal, multiple																							X			2
Vagina				M																						
Hematopoietic System																										
Bone marrow								+																		24
Lymph node							+	+													+					26
Axillary, mixed tumor malignant, metastatic, mammary gland																										1
Lymph node, mandibular								+																		23
Lymph node, mesenteric								+																		24
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Sarcoma																										1
Thymus								M																		21
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma							X							X			X								X	10
Fibroadenoma, multiple	X																		X				X			4
Mixed tumor malignant																										1
Skin								+						+			+		+							28
Subcutaneous tissue, fibroma								X																		1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, sarcoma																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	2 8 7	3 3 7	3 6 6	3 7 6	4 1 7	5 0 1	5 1 1	5 6 9	6 4 1	6 5 2	6 7 8	6 8 2	6 8 2	6 8 9	6 8 9	6 9 0	7 0 0	7 0 2	7 0 8	7 0 8	7 1 0	7 1 1	7 1 6	7 3 1	7 3 1
Carcass ID Number	5 5 1	5 8 1	5 9 1	6 0 1	5 7 1	5 9 2	5 5 2	5 3 1	5 1 1	5 5 3	5 6 1	5 6 2	5 7 2	5 3 3	5 9 3	5 3 4	5 5 4	5 3 2	5 7 3	5 8 2	5 8 3	5 2 1	5 4 1	5 1 2	5 1 3
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Vertebra, osteosarcoma										X															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Meninges, carcinoma, metastatic, zymbal's gland		X																							
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+
Alveolar/bronchiolar adenoma										X															
Mixed tumor malignant, metastatic, mammary gland																						X			
Mediastinum, mixed tumor malignant, metastatic, mammary gland																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Special Senses System																									
Ear																+									
Eye																									
Zymbal's gland		+														+									
Carcinoma		X														X									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear								X	X				X				X								

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 225 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 1 4	5 1 5	5 2 2	5 2 3	5 2 4	5 2 5	5 3 5	6 0 2	5 4 2	5 4 3	5 4 4	5 4 5	5 5 5	5 5 3	5 6 4	5 6 5	5 7 4	5 7 5	5 8 4	5 8 5	5 9 4	5 9 5	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
Musculoskeletal System																										
Bone						+	+	+														+				28
Vertebra, osteosarcoma																										1
Nervous System																										
Brain								+																		24
Meninges, carcinoma, metastatic, zymbal's gland																										1
Respiratory System																										
Lung			+				+	+	+	+												+				30
Alveolar/bronchiolar adenoma																										1
Mixed tumor malignant, metastatic, mammary gland																										1
Mediastinum, mixed tumor malignant, metastatic, mammary gland																										1
Nose								+																		24
Trachea								+																		24
Special Senses System																										
Ear																										1
Eye								+																		1
Zymbal's gland																										2
Carcinoma																										2
Urinary System																										
Kidney								+																		24
Urinary bladder								+																		24
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X						X										X			X				X	9

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg

Number of Days on Study	1 1 2 2 3 4 5 5 5 5 5 5 6 6 6 6 6 6 7 7 7 7 7 4 4 2 2 2 5 6 7 7 8 9 9 9 5 6 6 7 7 7 8 2 2 3 3 3 1 8 0 1 5 2 9 4 8 4 6 6 9 3 8 9 0 0 7 7 6 6 0 0 1
Carcass ID Number	4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 1 7 2 6 2 4 7 9 6 5 2 4 6 2 6 3 3 4 6 3 1 2 1 1 1 1 1 1 1 2 1 2 1 2 1 3 2 3 4 4 1 2 3 5 3 2 5 3 4 5
Alimentary System	
Esophagus	+ +
Intestine large	+ + + + + + A + + + + + + + + + + + + + + + + + +
Intestine large, cecum	+ + + A + + A + + + + + + A + + + + + + + + A + + +
Intestine large, colon	+ + + + + + A + + + + + + + + + + + + + + + + + +
Intestine large, rectum	M + + + A + + + + + A + + + + + + + + A + + +
Intestine small	+ +
Intestine small, duodenum	A + + + + + + + + + + + + + + + + + + + M + + +
Intestine small, ileum	+ + + A + + A + + + + + A A + + + + + + + A + + +
Intestine small, jejunum	A + + + + + A + + + + + + A + + + + + + + + + + +
Liver	+ +
Mesentery	
Pancreas	+ +
Salivary glands	+ +
Carcinoma, metastatic	X
Stomach	+ +
Stomach, forestomach	+ +
Stomach, glandular	+ +
Tongue	+
Tooth	+
Cardiovascular System	
Blood vessel	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adenoma	X
Adrenal gland, medulla	+ + M +
Pheochromocytoma benign	X
Islets, pancreatic	+ +
Carcinoma	
Parathyroid gland	+ M + + +
Pituitary gland	+ + + + + + A + + + + + + + + + + + + + + + + + +
Pars distalis, adenoma	X X X X X X X X X
Thyroid gland	+ +
C-cell, adenoma	
C-cell, carcinoma	
Follicular cell, carcinoma	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 3 4	4 3 5	4 4 4	4 4 5	4 5 2	4 5 3	4 5 4	4 5 5	4 7 3	4 7 4	4 7 5	4 8 1	4 8 2	4 8 3	4 8 4	4 8 5	4 9 2	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery							+									+						+			+	4
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Stomach, glandular	+	+	+	+			+	+	+	+		+	+	+	+	+		+	+	+	+	+	+	+	+	44
Tongue																										1
Tooth																										1
Cardiovascular System																										
Blood vessel							+																			1
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma										X																2
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign															X	X		X								4
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma								X																		1
Parathyroid gland	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Pars distalis, adenoma			X		X						X	X		X			X							X	X	16
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X				X														X					3
C-cell, carcinoma						X																				1
Follicular cell, carcinoma	X																									2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg (continued)

Number of Days on Study	1 4 1	1 4 8	2 2 0	2 2 1	3 2 5	4 5 2	5 6 9	5 7 4	5 7 8	5 8 4	5 9 6	5 9 6	5 9 9	6 5 3	6 6 8	6 6 9	6 7 0	6 7 0	6 7 7	6 8 7	7 2 6	7 2 6	7 3 0	7 3 0	7 3 1
Carcass ID Number	4 1 1	4 7 1	4 2 1	4 6 1	4 2 2	4 4 1	4 7 2	4 9 1	4 6 2	4 5 1	4 2 3	4 4 2	4 6 3	4 2 4	4 6 4	4 3 1	4 3 2	4 4 3	4 6 5	4 3 3	4 1 2	4 2 5	4 1 3	4 1 4	4 1 5
General Body System																									
Tissue NOS																+		+							
Genital System																									
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenoma																					X				
Carcinoma																	X								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Hemangiosarcoma																									
Polyp stromal					X												X								X
Polyp stromal, multiple																									
Vagina							+	+	+			+													
Hematopoietic System																									
Blood												+	+								+				
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic								X																	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma															X										
Fibroadenoma									X					X	X							X			
Fibroadenoma, multiple																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, carcinoma, metastatic								X																	
Subcutaneous tissue, fibrosarcoma																			X						
Subcutaneous tissue, hemangiosarcoma						X																			
Subcutaneous tissue, sarcoma													X												
Subcutaneous tissue, squamous cell carcinoma, multiple																X									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 3 4	4 3 5	4 4 4	4 4 5	4 5 2	4 5 3	4 5 4	4 5 5	4 7 3	4 7 4	4 7 5	4 8 1	4 8 2	4 8 3	4 8 4	4 8 5	4 9 2	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
General Body System																										
Tissue NOS																										2
Genital System																										
Clitoral gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Adenoma		X										X	X									X				5
Carcinoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma	X																									1
Hemangiosarcoma														X												1
Polyp stromal		X		X				X	X	X	X		X	X				X								12
Polyp stromal, multiple	X																									1
Vagina																										4
Hematopoietic System																										
Blood							+																			4
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Fibroadenoma		X																								5
Fibroadenoma, multiple															X											1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, carcinoma, metastatic																										1
Subcutaneous tissue, fibrosarcoma																										1
Subcutaneous tissue, hemangiosarcoma																										1
Subcutaneous tissue, sarcoma																										1
Subcutaneous tissue, squamous cell carcinoma, multiple																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg (continued)

Number of Days on Study	1 4 1	1 4 1	2 2 0	2 2 1	3 2 5	4 5 2	5 6 9	5 7 4	5 7 8	5 8 4	5 9 6	5 9 6	5 9 9	6 5 3	6 6 8	6 6 9	6 7 0	6 7 0	6 7 7	7 8 7	7 2 6	7 2 6	7 3 0	7 3 0	7 3 1
Carcass ID Number	4 1 1	4 7 1	4 2 1	4 6 1	4 2 2	4 4 1	4 7 2	4 9 1	4 6 2	4 5 1	4 2 3	4 4 2	4 6 3	4 2 4	4 6 4	4 3 1	4 3 2	4 4 3	4 6 5	4 3 3	4 1 2	4 2 5	4 1 3	4 1 4	4 1 5
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle						+																			
Hindlimb, hemangiosarcoma, extension						X																			
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																					X				
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Carcinoma, metastatic								X																	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear						+		+																+	
Eye					M														+						
Zymbal's gland								+																	
Carcinoma								X																	
Urinary System																									
Kidney	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear											X	X				X		X		X	X	X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Gavage Study of γ-Butyrolactone: 450 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 3 4	4 3 5	4 4 4	4 4 5	4 5 2	4 5 3	4 5 4	4 5 5	4 7 3	4 7 4	4 7 5	4 8 1	4 8 2	4 8 3	4 8 4	4 8 5	4 9 2	4 9 3	4 9 4	4 9 5	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Hindlimb, hemangiosarcoma, extension																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X															X							2
Carcinoma, metastatic																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear							+										+									5
Eye							+										+									3
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X			X		X													X				11

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone

	Vehicle Control	225 mg/kg	450 mg/kg
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	1/50 (2%)	0/25 (0%)[e]	4/49 (8%)
Adjusted rates[b]	3.3%		13.7%
Terminal rates[c]	0/28 (0%)		3/28 (11%)
First incidence (days)	716		726
Life table tests[d]			P=0.185
Logistic regression tests[d]			P=0.169
Fisher exact test[d]			P=0.175
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	1/50 (2%)	1/25 (4%)[e]	4/49 (8%)
Adjusted rates	3.3%		13.7%
Terminal rates	0/28 (0%)		3/28 (11%)
First incidence (days)	716		726
Life table tests			P=0.185
Logistic regression tests			P=0.169
Fisher exact test			P=0.175
Clitoral Gland: Adenoma			
Overall rates	6/48 (13%)	5/22 (23%)[e]	5/46 (11%)
Adjusted rates	20.3%		17.7%
Terminal rates	5/28 (18%)		4/27 (15%)
First incidence (days)	683		726
Life table tests			P=0.525N
Logistic regression tests			P=0.537N
Fisher exact test			P=0.530N
Clitoral Gland: Adenoma or Carcinoma			
Overall rates	6/48 (13%)	5/22 (23%)[e]	6/46 (13%)
Adjusted rates	20.3%		20.1%
Terminal rates	5/28 (18%)		4/27 (15%)
First incidence (days)	683		670
Life table tests			P=0.598
Logistic regression tests			P=0.573
Fisher exact test			P=0.590
Mammary Gland: Adenocarcinoma			
Overall rates	4/50 (8%)	0/50 (0%)	0/50 (0%)
Adjusted rates	12.4%	0.0%	0.0%
Terminal rates	2/28 (7%)	0/27 (0%)	0/28 (0%)
First incidence (days)	574	-[f]	-
Life table tests	P=0.016N	P=0.071N	P=0.065N
Logistic regression tests	P=0.016N	P=0.064N	P=0.068N
Cochran-Armitage test[d]	P=0.015N		
Fisher exact test		P=0.059N	P=0.059N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Mammary Gland: Fibroadenoma			
Overall rates	22/50 (44%)	14/50 (28%)	6/50 (12%)
Adjusted rates	62.0%	41.4%	17.1%
Terminal rates	15/28 (54%)	8/27 (30%)	2/28 (7%)
First incidence (days)	556	682	578
Life table tests	P<0.001N	P=0.093N	P<0.001N
Logistic regression tests	P<0.001N	P=0.064N	P<0.001N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.072N	P<0.001N
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall rates	24/50 (48%)	14/50 (28%)	6/50 (12%)
Adjusted rates	67.9%	41.4%	17.1%
Terminal rates	17/28 (61%)	8/27 (30%)	2/28 (7%)
First incidence (days)	556	682	578
Life table tests	P<0.001N	P=0.045N	P<0.001N
Logistic regression tests	P<0.001N	P=0.025N	P<0.001N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.032N	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	22/49 (45%)	24/37 (65%)	16/48 (33%)
Adjusted rates	63.7%	77.1%	44.1%
Terminal rates	16/28 (57%)	10/15 (67%)	8/27 (30%)
First incidence (days)	512	287	578
Life table tests	P=0.208N	P=0.057	P=0.202N
Logistic regression tests	P=0.211N	P=0.036	P=0.237N
Cochran-Armitage test	P=0.153N		
Fisher exact test		P=0.052	P=0.169N
Skin (Subcutaneous Tissue): Fibroma, Fibrosarcoma, or Sarcoma			
Overall rates	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted rates	3.3%	8.6%	5.5%
Terminal rates	0/28 (0%)	1/27 (4%)	0/28 (0%)
First incidence (days)	716	678	599
Life table tests	P=0.385	P=0.326	P=0.491
Logistic regression tests	P=0.386	P=0.302	P=0.500
Cochran-Armitage test	P=0.399		
Fisher exact test		P=0.309	P=0.500
Thyroid Gland (C-cell): Adenoma			
Overall rates	7/50 (14%)	2/27 (7%)[e]	3/50 (6%)
Adjusted rates	23.5%		10.7%
Terminal rates	6/28 (21%)		3/28 (11%)
First incidence (days)	667		730 (T)
Life table tests			P=0.157N
Logistic regression tests			P=0.168N
Fisher exact test			P=0.159N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	8/50 (16%)	3/27 (11%)[e]	4/50 (8%)
Adjusted rates	27.0%		14.3%
Terminal rates	7/28 (25%)		4/28 (14%)
First incidence (days)	667		730 (T)
Life table tests			P=0.174N
Logistic regression tests			P=0.187N
Fisher exact test			P=0.178N
Uterus: Stromal Polyp			
Overall rates	10/50 (20%)	9/50 (18%)	13/50 (26%)
Adjusted rates	30.9%	28.4%	42.4%
Terminal rates	7/28 (25%)	6/27 (22%)	11/28 (39%)
First incidence (days)	556	501	325
Life table tests	P=0.263	P=0.510N	P=0.309
Logistic regression tests	P=0.223	P=0.510N	P=0.262
Cochran-Armitage test	P=0.271		
Fisher exact test		P=0.500N	P=0.318
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	11/50 (22%)	9/50 (18%)	13/50 (26%)
Adjusted rates	34.2%	28.4%	42.4%
Terminal rates	8/28 (29%)	6/27 (22%)	11/28 (39%)
First incidence (days)	556	501	325
Life table tests	P=0.349	P=0.414N	P=0.399
Logistic regression tests	P=0.303	P=0.409N	P=0.344
Cochran-Armitage test	P=0.359		
Fisher exact test		P=0.402N	P=0.408
All Organs: Mononuclear Cell Leukemia			
Overall rates	13/50 (26%)	9/50 (18%)	11/50 (22%)
Adjusted rates	31.3%	26.5%	30.7%
Terminal rates	3/28 (11%)	5/27 (19%)	4/28 (14%)
First incidence (days)	498	569	596
Life table tests	P=0.392N	P=0.252N	P=0.443N
Logistic regression tests	P=0.378N	P=0.227N	P=0.417N
Cochran-Armitage test	P=0.359N		
Fisher exact test		P=0.235N	P=0.408N
All Organs: Benign Tumors			
Overall rates	38/50 (76%)	42/50 (84%)	33/50 (66%)
Adjusted rates	92.5%	91.3%	86.5%
Terminal rates	25/28 (89%)	23/27 (85%)	23/28 (82%)
First incidence (days)	512	287	325
Life table tests	P=0.249N	P=0.292	P=0.249N
Logistic regression tests	P=0.293N	P=0.142	P=0.336N
Cochran-Armitage test	P=0.148N		
Fisher exact test		P=0.227	P=0.189N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450mg/kg
All Organs: Malignant Tumors			
Overall rates	22/50 (44%)	19/50 (38%)	19/50 (38%)
Adjusted rates	52.0%	46.2%	47.9%
Terminal rates	9/28 (32%)	7/27 (26%)	8/28 (29%)
First incidence (days)	498	337	452
Life table tests	P=0.367N	P=0.365N	P=0.396N
Logistic regression tests	P=0.316N	P=0.328N	P=0.372N
Cochran-Armitage test	P=0.305N		
Fisher exact test		P=0.342N	P=0.342N
All Organs: Benign or Malignant Tumors			
Overall rates	46/50 (92%)	46/50 (92%)	41/50 (82%)
Adjusted rates	95.8%	93.9%	91.1%
Terminal rates	26/28 (93%)	24/27 (89%)	24/28 (86%)
First incidence (days)	498	287	325
Life table tests	P=0.287N	P=0.532	P=0.300N
Logistic regression tests	P=0.187N	P=0.612	P=0.297N
Cochran-Armitage test	P=0.078N		
Fisher exact test		P=0.643N	P=0.117N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy; thus statistical comparisons with the controls are not appropriate.
[f] Not applicable; no tumors in animal group

TABLE B4
Historical Incidence of Mammary Gland Fibroadenoma in Female F344/N Rats Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls
Historical Incidence at Southern Research Institute	
Benzaldehyde	28/50
Dichlorvos	9/50
Furan	15/50
Furfural	12/50
γ-Butyrolactone	22/50
Total	86/250 (34.4%)
Standard deviation	15.5%
Range	18%-56%
Overall Historical Incidence	
Total	298/770 (38.7%)
Standard deviation	11.0%
Range	18%-56%

[a] Data as of 17 September 1990

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	225 mg/kg	450 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	3	7	6
Moribund	19	16	14
Dosing accident			2
Survivors			
Terminal sacrifice	28	26	28
Died last week of study		1	
Animals examined microscopically	50	50	50
Alimentary System			
Esophagus	(50)	(24)	(50)
Serosa, inflammation, chronic	1 (2%)		
Intestine large, colon	(48)	(20)	(49)
Infiltration cellular, lipocyte	1 (2%)		
Parasite metazoan	6 (13%)	2 (10%)	7 (14%)
Intestine large, rectum	(48)	(20)	(43)
Parasite metazoan			1 (2%)
Intestine small, ileum	(47)	(20)	(45)
Hyperplasia, lymphoid			1 (2%)
Liver	(50)	(50)	(50)
Angiectasis	1 (2%)		1 (2%)
Basophilic focus	1 (2%)		1 (2%)
Basophilic focus, multiple	35 (70%)	40 (80%)	32 (64%)
Clear cell focus			2 (4%)
Cyst		1 (2%)	
Eosinophilic focus	1 (2%)	3 (6%)	1 (2%)
Eosinophilic focus, multiple		2 (4%)	
Fibrosis, focal			1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)
Hepatodiaphragmatic nodule	4 (8%)	5 (10%)	6 (12%)
Hyperplasia, nodular	4 (8%)	4 (8%)	3 (6%)
Inflammation, granulomatous, multiple	5 (10%)	9 (18%)	10 (20%)
Mixed cell focus	1 (2%)	2 (4%)	4 (8%)
Necrosis, focal	1 (2%)	1 (2%)	
Vacuolization cytoplasmic	4 (8%)	2 (4%)	1 (2%)
Bile duct, dilatation		1 (2%)	
Bile duct, hyperplasia	14 (28%)	15 (30%)	10 (20%)
Centrilobular, necrosis, multiple	1 (2%)		
Serosa, hemorrhage	1 (2%)		
Serosa, inflammation, suppurative		1 (2%)	
Mesentery	(5)	(3)	(4)
Fat, inflammation, chronic	3 (60%)	1 (33%)	
Fat, inflammation, granulomatous, focal			1 (25%)
Fat, necrosis, focal	4 (80%)	2 (67%)	2 (50%)
Pancreas	(49)	(24)	(50)
Polyarteritis, multiple		1 (4%)	
Acinar cell, atrophy	5 (10%)	4 (17%)	6 (12%)
Acinar cell, hyperplasia	2 (4%)		5 (10%)
Acinar cell, hyperplasia, multiple			1 (2%)
Duct, cyst			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Alimentary System (continued)			
Stomach	(49)	(24)	(50)
Forestomach, edema	1 (2%)	2 (8%)	2 (4%)
Forestomach, inflammation, chronic	1 (2%)		1 (2%)
Forestomach, ulcer	1 (2%)	2 (8%)	3 (6%)
Forestomach, ulcer, multiple			2 (4%)
Glandular, cyst, multiple			1 (2%)
Glandular, mineralization		1 (4%)	4 (8%)
Glandular, epithelium, hyperplasia			1 (2%)
Tongue			(1)
Hyperplasia, squamous			1 (100%)
Cardiovascular System			
Blood vessel		(1)	(1)
Aorta, media, hypertrophy		1 (100%)	
Heart	(50)	(25)	(50)
Congestion			1 (2%)
Fibrosis, focal		1 (4%)	
Fibrosis, multiple		1 (4%)	1 (2%)
Inflammation, chronic	35 (70%)	13 (52%)	29 (58%)
Atrium, congestion			1 (2%)
Atrium, thrombus		1 (4%)	
Endocrine System			
Adrenal gland, cortex	(50)	(25)	(50)
Accessory adrenal cortical nodule	4 (8%)	4 (16%)	2 (4%)
Angiectasis	1 (2%)		
Congestion			1 (2%)
Degeneration, cystic	4 (8%)		2 (4%)
Hematopoietic cell proliferation	1 (2%)		
Hypertrophy, focal	3 (6%)	3 (12%)	6 (12%)
Vacuolization cytoplasmic	5 (10%)	3 (12%)	3 (6%)
Adrenal gland, medulla	(50)	(25)	(49)
Angiectasis			1 (2%)
Congestion			1 (2%)
Hyperplasia, focal	4 (8%)	1 (4%)	1 (2%)
Pituitary gland	(49)	(37)	(48)
Cyst	1 (2%)		
Cyst, multiple	2 (4%)		
Pars distalis, angiectasis	4 (8%)	9 (24%)	4 (8%)
Pars distalis, cyst	9 (18%)	6 (16%)	5 (10%)
Pars distalis, cyst, multiple	16 (33%)	7 (19%)	6 (13%)
Pars distalis, hemorrhage		3 (8%)	
Pars distalis, hyperplasia, focal	7 (14%)	7 (19%)	6 (13%)
Pars distalis, inflammation, granulomatous, focal			1 (2%)
Thyroid gland	(50)	(27)	(50)
Angiectasis		1 (4%)	
Degeneration, cystic		1 (4%)	
Ultimobranchial cyst	2 (4%)		
C-cell, hyperplasia	10 (20%)	2 (7%)	3 (6%)
Follicle, cyst	1 (2%)		
Follicular cell, hyperplasia	1 (2%)	1 (4%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
General Body System			
None			
Genital System			
Clitoral gland	(48)	(22)	(46)
Atrophy		1 (5%)	1 (2%)
Fibrosis			1 (2%)
Hemorrhage	1 (2%)		
Hyperplasia	2 (4%)	1 (5%)	
Inflammation, suppurative	4 (8%)	2 (9%)	3 (7%)
Metaplasia, squamous			1 (2%)
Necrosis	1 (2%)		
Duct, cyst	19 (40%)	4 (18%)	11 (24%)
Ovary	(50)	(24)	(50)
Cyst	5 (10%)		3 (6%)
Hyperplasia, tubular			1 (2%)
Uterus	(50)	(32)	(50)
Decidual reaction	1 (2%)		1 (2%)
Dilatation	4 (8%)	1 (3%)	2 (4%)
Inflammation, suppurative	1 (2%)		
Cervix, cyst	2 (4%)	1 (3%)	
Endometrium, fibrosis	1 (2%)		
Endometrium, fibrosis, focal	1 (2%)		
Endometrium, hyperplasia, cystic	6 (12%)	3 (9%)	7 (14%)
Endometrium, hyperplasia, glandular		1 (3%)	2 (4%)
Vagina	(2)		(4)
Cyst	1 (50%)		
Hematopoietic System			
Bone marrow	(49)	(24)	(50)
Hyperplasia, reticulum cell			2 (4%)
Myelofibrosis	3 (6%)		1 (2%)
Myeloid cell, hyperplasia	1 (2%)	1 (4%)	
Lymph node	(50)	(26)	(50)
Mediastinal, hyperplasia, lymphoid		1 (4%)	
Pancreatic, inflammation, granulomatous			1 (2%)
Lymph node, mandibular	(49)	(23)	(50)
Hyperplasia, lymphoid	1 (2%)	1 (4%)	1 (2%)
Lymph node, mesenteric	(49)	(24)	(49)
Depletion lymphoid	1 (2%)	1 (4%)	
Hyperplasia, lymphoid	1 (2%)	1 (4%)	
Pigmentation	1 (2%)	1 (4%)	
Spleen	(48)	(49)	(50)
Atrophy		2 (4%)	1 (2%)
Fibrosis		1 (2%)	1 (2%)
Hematopoietic cell proliferation	3 (6%)	8 (16%)	3 (6%)
Necrosis		1 (2%)	
Pigmentation	7 (15%)	9 (18%)	3 (6%)
Thymus	(48)	(21)	(47)
Atrophy		1 (5%)	
Cyst, multiple			2 (4%)
Hemorrhage			1 (2%)
Hyperplasia, lymphoid	1 (2%)		
Mediastinum, hemorrhage			1 (2%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Integumentary System			
Mammary gland	(50)	(50)	(50)
Hyperplasia, lobular	6 (12%)	7 (14%)	3 (6%)
Duct, cyst	42 (84%)	35 (70%)	23 (46%)
Skin	(50)	(28)	(50)
Cyst epithelial inclusion	1 (2%)		
Hemorrhage	1 (2%)		
Ulcer		1 (4%)	
Subcutaneous tissue, thrombus, multiple		1 (4%)	
Musculoskeletal System			
Bone	(50)	(28)	(50)
Calvarium, hyperostosis	2 (4%)	4 (14%)	2 (4%)
Femur, fracture			1 (2%)
Skeletal muscle	(2)		(1)
Diaphragm, inflammation, chronic	1 (50%)		
Nervous System			
Brain	(50)	(24)	(50)
Compression	5 (10%)	6 (25%)	1 (2%)
Hydrocephalus		1 (4%)	
Respiratory System			
Lung	(50)	(30)	(50)
Congestion	2 (4%)	5 (17%)	6 (12%)
Hemorrhage	1 (2%)		
Alveolar epithelium, hyperplasia	3 (6%)		1 (2%)
Alveolus, pigmentation		1 (3%)	2 (4%)
Bronchus, foreign body			1 (2%)
Lymphatic, foreign body	1 (2%)	2 (7%)	1 (2%)
Mediastinum, edema			1 (2%)
Mediastinum, foreign body		1 (3%)	2 (4%)
Mediastinum, hemorrhage			2 (4%)
Peribronchial, infiltration cellular, lymphocytic			1 (2%)
Nose	(50)	(24)	(50)
Nasolacrimal duct, inflammation, suppurative	2 (4%)		1 (2%)
Trachea	(50)	(24)	(50)
Inflammation, suppurative			1 (2%)
Special Senses System			
Ear		(1)	(5)
Inflammation, suppurative		1 (100%)	
Eye	(1)	(1)	(3)
Cataract			2 (67%)
Cataract, multiple	1 (100%)		
Cornea, edema		1 (100%)	
Retina, degeneration	1 (100%)	1 (100%)	2 (67%)
Zymbal's gland		(2)	(1)
Inflammation, suppurative		1 (50%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	225 mg/kg	450 mg/kg
Urinary System			
Kidney	(49)	(24)	(49)
Fibrosis, focal	1 (2%)		
Infarct, multiple			1 (2%)
Nephropathy, chronic	23 (47%)	7 (29%)	19 (39%)
Cortex, cyst	1 (2%)		
Medulla, cyst	1 (2%)		
Renal tubule, degeneration		1 (4%)	
Renal tubule, dilatation	1 (2%)		1 (2%)
Renal tubule, mineralization	10 (20%)	2 (8%)	19 (39%)
Renal tubule, pigmentation	4 (8%)		3 (6%)
Urinary bladder	(50)	(24)	(50)
Inflammation, chronic, focal	1 (2%)		
Transitional epithelium, hyperplasia	1 (2%)		1 (2%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF γ-BUTYROLACTONE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	262 mg/kg	525 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	2	12	13
Moribund	13	8	24
Accidental death			1
Survivors			
Terminal sacrifice	35	30	12
Animals examined microscopically	50	50	50
Alimentary System			
Intestine small, jejunum	(47)	(45)	(45)
Liver	(50)	(50)	(50)
Hemangiosarcoma	2 (4%)	1 (2%)	
Hepatocellular carcinoma	16 (32%)	2 (4%)	8 (16%)
Hepatocellular adenoma	8 (16%)	6 (12%)	1 (2%)
Pancreas	(50)	(49)	(49)
Salivary glands	(48)	(50)	(48)
Fibrosarcoma, metastatic, skin	1 (2%)		
Stomach, forestomach	(50)	(49)	(49)
Papilloma squamous	7 (14%)	2 (4%)	1 (2%)
Stomach, glandular	(50)	(49)	(49)
Cardiovascular System			
Heart	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
Endocrine System			
Adrenal gland, cortex	(48)	(50)	(50)
Adenoma	3 (6%)	3 (6%)	
Spindle cell, adenoma			1 (2%)
Adrenal gland, medulla	(48)	(50)	(50)
Pheochromocytoma malignant	1 (2%)	1 (2%)	
Pheochromocytoma benign	1 (2%)	4 (8%)	1 (2%)
Bilateral, pheochromocytoma benign		1 (2%)	
Islets, pancreatic	(50)	(49)	(48)
Adenoma	1 (2%)		
Thyroid gland	(49)	(50)	(48)
Follicular cell, adenoma	1 (2%)	2 (4%)	2 (4%)
Follicular cell, carcinoma	1 (2%)		
General Body System			
Tissue NOS	(1)		(1)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Genital System			
Epididymis	(50)	(50)	(49)
Prostate	(49)	(48)	(48)
Fibrosarcoma, metastatic, skin			1 (2%)
Seminal vesicle	(8)	(5)	(9)
Testes	(50)	(50)	(50)
Interstitial cell, adenoma	1 (2%)		1 (2%)
Hematopoietic System			
Blood	(7)	(2)	(1)
Bone marrow	(50)	(50)	(49)
Lymph node	(50)	(50)	(49)
Inguinal, hemangiosarcoma	1 (2%)		
Lymph node, mandibular	(45)	(46)	(46)
Lymph node, mesenteric	(48)	(46)	(41)
Hemangiosarcoma	1 (2%)		
Spleen	(50)	(50)	(48)
Hemangiosarcoma	1 (2%)		
Integumentary System			
Skin	(50)	(50)	(50)
Adenoma			1 (2%)
Basosquamous tumor benign		1 (2%)	
Carcinoma			1 (2%)
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	
Subcutaneous tissue, fibrosarcoma	9 (18%)	6 (12%)	6 (12%)
Subcutaneous tissue, hemangiosarcoma			1 (2%)
Subcutaneous tissue, lipoma			1 (2%)
Subcutaneous tissue, schwannoma malignant		1 (2%)	1 (2%)
Musculoskeletal System			
Skeletal muscle	(1)	(4)	(2)
Schwannoma malignant		1 (25%)	
Nervous System			
None			
Respiratory System			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	10 (20%)	9 (18%)	6 (12%)
Hepatocellular carcinoma, metastatic, liver	2 (4%)		1 (2%)
Nose	(50)	(50)	(49)
Hemangiosarcoma		1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Special Senses System			
Harderian gland	(9)	(48)	
Adenoma	8 (89%)	1 (2%)	
Urinary System			
Kidney	(50)	(50)	(50)
Adenoma		1 (2%)	
Urinary bladder	(50)	(48)	(48)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Lymphoma malignant histiocytic		1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)		
Lymphoma malignant mixed	2 (4%)	2 (4%)	
Lymphoma malignant undifferentiated cell	1 (2%)		
Tumor Summary			
Total animals with primary neoplasms[c]	40	31	23
Total primary neoplasms	77	48	33
Total animals with benign neoplasms	25	26	12
Total benign neoplasms	41	31	15
Total animals with malignant neoplasms	29	12	16
Total malignant neoplasms	36	17	18
Total animals with secondary neoplasms[d]	3		2
Total secondary neoplasms	3		2

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control

Number of Days on Study	1 7 5	3 8 5	4 4 1	4 7 3	4 7 3	5 4 0	5 7 8	5 8 2	5 8 2	6 0 1	6 1 8	6 3 3	6 8 8	7 0 3	7 2 1	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 9 1	0 2 1	0 2 2	0 1 1	0 1 2	0 9 4	0 3 5	1 0 1	1 0 5	0 7 3	0 5 5	0 3 1	0 4 1	0 2 4	0 5 2	0 1 3	1 1 4	1 2 5	2 2 3	2 3 5	3 3 2	3 3 3	3 4 4	4 2 2	4 3 3
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	M	+	+	M	+	M	M	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	M	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+
Intestine small	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	M	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Intestine small, jejunum	+	M	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma						X																		
Hepatocellular carcinoma		X		X	X			X				X		X	X	X				X				
Hepatocellular adenoma									X									X	X			X	X	X
Mesentery															+									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin							X																	
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous				X														X		X		X		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth								+			+								+		+			+
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X						X	
Adrenal gland, medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant													X											
Pheochromocytoma benign								X																
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 4	0 4 5	0 5 1	0 5 3	0 5 4	0 6 1	0 6 2	0 6 3	0 6 4	0 6 5	0 7 1	0 7 2	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 2	0 9 3	0 9 5	1 0 2	1 0 3	1 0 4	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	I	46
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	I	+	+	+	+	+	42
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																				X						2
Hepatocellular carcinoma			X						X									X		X			X	X	X	16
Hepatocellular adenoma	X			X																						8
Mesentery														+									+			3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Fibrosarcoma, metastatic, skin																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous													X				X		X							7
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth	+		+	+				+															+			10
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma															X											3
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma malignant																										1
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																									X	1
Parathyroid gland	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	45

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	1 7 5	3 8 5	4 4 1	4 7 3	4 7 3	5 4 0	5 7 8	5 8 2	5 8 2	6 0 1	6 1 8	6 3 3	6 8 8	7 0 3	7 2 1	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	0 9 1	0 2 1	0 2 2	0 1 1	0 1 2	0 9 4	0 3 5	1 0 1	1 0 5	0 7 3	0 5 5	0 3 1	0 4 1	0 2 4	0 5 2	0 1 3	1 1 4	1 1 5	1 2 3	2 2 5	3 3 2	3 3 3	3 3 4	4 2 2	4 4 3
Endocrine System (continued)																								
Pituitary gland	+	M	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								X	
Follicular cell, carcinoma																									X
General Body System																									
Tissue NOS			+																						
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis																		+							
Preputial gland									+					+			+	+	+	+	+	+	+	+	+
Prostate	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+		+																	+					+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																									
Hematopoietic System																									
Blood			+														+		+	+					+
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Inguinal, hemangiosarcoma																	X								
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Lymph node, mesenteric	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																	X								
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																	X								
Thymus	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Integumentary 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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma							X					X		X			X								
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 4	0 4 5	0 5 1	0 5 3	0 5 4	0 6 1	0 6 2	0 6 3	0 6 4	0 6 5	0 7 1	0 7 2	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 2	0 9 3	0 9 5	1 0 2	1 0 3	1 0 4	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	43
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma																										1
Follicular cell, carcinoma																										1
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland		+					+			+		+	+					+						+		18
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle											+						+							+	+	8
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																					X					1
Hematopoietic System																										
Blood	+			+																						7
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Inguinal, hemangiosarcoma																										1
Lymph node, mandibular	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hemangiosarcoma																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Thymus	+	+	M	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	42
Integumentary 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	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma																					X					1
Subcutaneous tissue, fibrosarcoma				X						X						X	X			X						9
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	1 3 4 4 4 5 5 5 5 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7
	7 8 4 7 7 4 7 8 8 0 1 3 8 0 2 2 2 2 2 2 2 2 2 2 2
	5 5 1 3 3 0 8 2 2 1 8 3 8 3 1 9 9 9 9 9 9 9 9 9 9
Carcass ID Number	0 0 0 0 0 0 0 1 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	9 2 2 1 1 9 3 0 0 7 5 3 4 2 5 1 1 1 2 2 3 3 3 4 4
	1 1 2 1 2 4 5 1 5 3 5 1 1 4 2 3 4 5 3 5 2 3 4 2 3
Nervous System	
Brain	+ +
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X X X
Hepatocellular carcinoma, metastatic, liver	X
Nose	+ +
Trachea	+ +
Special Senses System	
Eye	
Harderian gland	+ + + + +
Adenoma	X X X
Urinary System	
Kidney	+ +
Urethra	+
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant lymphocytic	X
Lymphoma malignant mixed	X
Lymphoma malignant undifferentiated cell type	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	
Carcass ID Number	0 4 4	0 4 5	0 5 1	0 5 3	0 5 4	0 6 1	0 6 2	0 6 3	0 6 4	0 6 5	0 7 1	0 7 2	0 7 4	0 7 5	0 8 1	0 8 2	0 8 3	0 8 4	0 8 5	0 9 2	0 9 3	0 9 5	1 0 2	1 0 3	1 0 4	**Total Tissues/ Tumors**
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X			X				X			X						X				X				X	10
Hepatocellular carcinoma, metastatic, liver																									X	2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye								+					+					+								3
Harderian gland								+	+	+			+					+								9
Adenoma								X	X	X			X					X								8
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed							X																			2
Lymphoma malignant undifferentiated cell type																					X					1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg

Number of Days on Study	113	146	151	178	207	234	248	259	263	270	271	272	276	279	282	284	288	290	295	296	297	303	303	303	303	303
Carcass ID Number	23111	23913	23914	23994	30411	25511	26211	23412	27111	25212	27222	30226	28525	26591	28144	30444	28543	25513	25514	21115	21112	21113	21114	21115
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	M	M	A	A	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	M	A	A	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	I	+	M	A	A	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	A	A	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Hepatocellular carcinoma															X	X										
Hepatocellular adenoma						X											X		X							
Mesentery																										
Pancreas	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																										X
Stomach, glandular	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue										+																
Tooth																										
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																										
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																								X		
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																					X					
Pheochromocytoma benign																										
Bilateral, pheochromocytoma benign																										
Islets, pancreatic	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																								X		

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 2 1	2 2 2	2 2 3	2 2 4	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	2 4 4	2 4 5	2 5 4	2 6 2	2 6 3	2 6 4	2 7 3	2 7 4	2 7 5	2 8 2	2 8 3	2 9 2	2 9 5	3 0 3	3 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	41
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	42
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																	1
Hepatocellular carcinoma																										2
Hepatocellular adenoma	X	X																X								6
Mesentery								+						+								+				3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous	X																									2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																										1
Tooth								+		+		+			+					+						5
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																	1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma													X											X		3
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										1
Pheochromocytoma benign			X																		X	X			X	4
Bilateral, pheochromocytoma benign					X																					1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma								X																		2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	1 3 2	1 4 6	1 5 1	1 8 7	2 7 0	3 4 4	3 8 0	3 9 1	3 9 1	4 0 1	4 1 2	4 3 6	5 1 9	5 3 2	5 8 4	5 8 4	6 0 5	6 1 3	6 5 6	6 7 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	2 3 1	2 9 3	2 9 1	2 9 4	3 0 1	2 5 1	2 6 1	2 3 2	2 7 1	2 5 2	2 7 2	3 0 2	2 8 5	2 6 5	2 8 1	3 0 4	2 8 4	2 5 3	2 5 5	2 2 5	2 1 1	2 1 2	2 1 3	2 1 4	2 1 5
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis								M					+												
Preputial gland							+			+				M	+					+			+		+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Seminal vesicle	+		+					+				+													
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematopoietic System																									
Blood																								+	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	M	+	+	M	+	M	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+
Integumentary 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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basosquamous tumor benign																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, fibrosarcoma																	X	X						X	
Subcutaneous tissue, schwannoma malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle							+					+													
Schwannoma malignant																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 2 1	2 2 2	2 2 3	2 2 4	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	2 4 4	2 4 5	2 5 4	2 6 2	2 6 3	2 6 4	2 7 3	2 7 4	2 7 5	2 8 2	2 8 3	2 9 2	2 9 5	3 0 3	3 0 5	**Total Tissues/ Tumors**
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Penis																										1
Preputial gland		+		+	+		+		+		+		+	+			+	+	+	+	+		+			20
Prostate	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle																					+					5
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hematopoietic System																										
Blood																			+							2
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	M	M	+	+	M	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+		+	M	+	+	39
Integumentary System																										
Mammary gland	M	M	M	M	I	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basosquamous tumor benign										X																1
Subcutaneous tissue, fibroma		X																								1
Subcutaneous tissue, fibrosarcoma				X															X			X				6
Subcutaneous tissue, schwannoma malignant													X													1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle													+											+		4
Schwannoma malignant													X													1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	1 3 2	1 4 6	1 5 1	1 8 7	2 7 0	3 4 4	3 8 0	3 9 1	3 9 1	4 0 1	4 1 2	4 3 6	5 1 9	5 3 2	5 8 4	5 8 4	6 0 5	6 1 3	6 5 6	6 7 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	2 3 1	2 9 3	2 9 1	2 9 4	3 0 1	2 5 1	2 6 1	2 3 2	2 7 1	2 5 2	2 7 2	3 0 2	2 8 5	2 6 5	2 8 1	3 0 4	2 8 4	2 5 3	2 5 5	2 5 5	2 1 1	1 1 2	1 1 3	1 1 4	2 1 5
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma				X																X	X				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Trachea	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye																									
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Urethra		+					+					+													
Urinary bladder	+	+	+	+	A	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																		X							
Lymphoma malignant mixed																									X

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 2 1	2 2 2	2 2 3	2 2 4	2 3 3	2 3 4	2 3 5	2 4 1	2 4 2	2 4 3	2 4 4	2 4 5	2 5 4	2 5 5	2 6 2	2 6 3	2 6 4	2 7 3	2 7 4	2 7 5	2 8 2	2 8 3	2 9 2	2 9 5	3 0 3	3 0 5	**Total Tissues/ Tumors**
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma							X		X							X					X			X	X	9
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma									X																		1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																											
Eye																						M					
Harderian gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	48
Adenoma		X																									1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																	X										1
Urethra																				+							4
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																											1
Lymphoma malignant mixed									X																		2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg

Number of Days on Study	0 6 7	0 9 3	1 0 8	1 1 9	1 1 9	1 1 9	1 5 9	1 6 9	1 6 9	1 7 0	1 7 1	1 8 5	1 8 8	1 9 2	3 2 9	3 8 1	3 8 5	4 2 7	4 3 1	4 4 5	5 1 2	5 1 4	5 4 5	5 6 8	5 6 8
Carcass ID Number	1 5 3	1 1 1	1 7 1	1 7 2	1 7 3	1 8 1	1 5 5	2 0 1	2 0 2	2 0 3	2 0 4	1 3 1	1 3 2	1 4 1	1 8 2	1 6 1	1 2 1	1 4 2	1 2 2	1 2 3	1 3 5	1 8 5	1 4 3	2 5 5	1 5 2
Alimentary System																									
Esophagus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	M	+	+	A	+	+	M	+	+	+	M	M	+	+
Intestine large	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	M	+	+	+	+	A	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	A	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	M	+	+	+	+	A	A	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	M	+	+	+	A	A	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	A	A	+	A	+	+	+	M	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																						X	X	X	X
Hepatocellular adenoma																									
Mesentery	+							+								+									
Pancreas	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																									
Stomach, glandular	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue				+	+																				
Tooth																									
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	M	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	M	+	+	I	+	M	M	+	+	+	+	+
Pituitary gland	M	M	+	+	+	A	+	+	M	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	5 6 8	5 8 3	5 8 5	5 8 5	5 9 7	6 0 1	6 4 0	6 5 2	6 5 5	6 5 6	6 5 7	6 9 0	7 0 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	
Carcass ID Number	1 6 5	1 6 4	1 6 2	1 7 5	1 9 2	1 3 3	1 6 3	1 1 5	1 5 4	1 9 3	1 1 4	1 1 3	1 1 2	1 2 4	1 3 4	1 4 4	1 4 5	1 5 1	1 7 4	1 8 3	1 8 4	1 9 1	1 9 4	1 9 5	2 0 5	**Total Tissues/ Tumors**
Alimentary System																									
Esophagus	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	43
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma						X			X	X						X									8
Hepatocellular adenoma																			X						1
Mesentery	+			+																+				+	7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous													X												1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tongue																									2
Tooth								+		+	+														3
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spindle cell, adenoma																			X						1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign							X																		1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma										X			X												2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	0 6 7	0 9 3	1 0 8	1 1 9	1 1 9	1 1 9	1 5 9	1 6 9	1 6 9	1 7 0	1 7 1	1 8 5	1 8 8	1 9 2	3 2 9	3 8 1	3 8 5	4 2 7	4 3 1	4 4 5	5 1 2	5 1 4	5 4 5	5 6 8	5 6 8
Carcass ID Number	1 5 3	1 1 1	1 7 1	1 7 2	1 7 3	1 8 1	1 5 5	2 0 1	2 0 2	2 0 3	2 0 4	1 3 1	1 3 2	1 4 1	1 8 2	1 6 1	2 2 1	2 4 2	2 2 2	2 3 3	3 5 5	1 8 5	4 3 5	2 5 2	1 5 2
General Body System																									
Tissue NOS																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis																									
Preputial gland		+										+	+			+				+			+		
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																									
Seminal vesicle							A											+	+						
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Interstitial cell, adenoma																					X				
Hematopoietic System																									
Blood															+										
Bone marrow	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	M	+	+	+	M	M	M	M	+	+	+	+	M	+	A	M	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	M	M	+	+	M	M	+	M	+	+
Integumentary 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
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																						X			
Subcutaneous tissue, fibrosarcoma																							X		
Subcutaneous tissue, hemangiosarcoma																									
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, schwannoma malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																		+							

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	5 6 8	5 8 3	5 8 5	5 8 5	5 9 7	6 0 1	6 4 0	6 5 2	6 5 5	6 5 6	6 5 7	6 9 0	7 0 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	
Carcass ID Number	1 6 5	1 6 4	1 6 2	1 7 5	1 9 2	1 3 3	1 6 3	1 1 5	1 5 4	1 9 3	1 1 4	1 1 3	1 1 2	1 2 4	1 3 4	1 4 4	1 4 5	1 5 1	1 7 4	1 8 3	1 8 4	1 9 1	1 9 4	1 9 5	2 0 5	**Total Tissues/ Tumors**
General Body System																									
Tissue NOS																					+				1
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Penis									+		+														2
Preputial gland	+	+	+					+	+		+				+	+		+			+			+	17
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Fibrosarcoma, metastatic, skin													X												1
Seminal vesicle						+			+					+		+						+		+	9
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Interstitial cell, adenoma																									1
Hematopoietic System																									
Blood																									1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	41
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	M	+	+	+	+	+	M	+	M	+	+	+	I	I	+	+	+	+	+	38
Integumentary 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	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X																1
Carcinoma																									1
Subcutaneous tissue, fibrosarcoma	X				X			X				X										X			6
Subcutaneous tissue, hemangiosarcoma																							X		1
Subcutaneous tissue, lipoma														X											1
Subcutaneous tissue, schwannoma malignant													X												1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle				+																					2

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	0 6 7	0 9 3	1 0 8	1 1 9	1 1 9	1 1 9	1 5 9	1 6 9	1 6 9	1 7 0	1 7 1	1 8 5	1 8 8	1 9 2	1 2 9	3 8 1	3 8 5	4 2 7	4 3 1	4 4 5	5 1 2	5 1 4	5 4 5	5 6 8	5 6 8
Carcass ID Number	1 5 3	1 1 1	1 7 1	1 7 2	1 7 3	1 8 1	1 5 5	1 0 1	1 0 2	1 0 3	1 0 4	1 3 1	1 3 2	1 4 1	1 8 2	1 6 1	1 2 1	1 4 2	1 2 2	1 2 3	1 3 5	1 8 5	1 4 3	1 2 5	1 5 2
Nervous System																										
Brain	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord																			+							
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																								X		
Hepatocellular carcinoma, metastatic, liver																										X
Nose	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
Ear																			+							
Eye																		+								
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urethra							+					+												+		
Urinary bladder	+	+	+	+	+	A	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																		X								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	5 6 8	5 8 3	5 8 5	5 8 5	5 9 7	6 0 1	6 4 0	6 5 2	6 5 5	6 5 6	6 5 7	6 9 0	7 0 3	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	
Carcass ID Number	1 6 5	1 6 4	1 6 2	1 7 5	1 9 2	1 3 3	1 6 3	1 1 5	1 5 4	1 9 3	1 1 4	1 1 3	1 1 2	1 2 4	1 3 4	1 4 4	1 4 5	1 5 1	1 7 4	1 8 3	1 8 4	1 9 1	1 9 4	1 9 5	2 0 5	**Total Tissues/ Tumors**
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spinal cord		+																							2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma			X	X		X				X										X					6
Hepatocellular carcinoma, metastatic, liver																									1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																									
Ear		+																							2
Eye																									1
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urethra																									3
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																									1

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone

	Vehicle Control	262 mg/kg	525 mg/kg
Adrenal Cortex: Adenoma			
Overall rates[a]	3/48 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rates[b]	8.8%	10.0%	8.3%
Terminal rates[c]	3/34 (9%)	3/30 (10%)	1/12 (8%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests[d]	P=0.602	P=0.605	P=0.705N
Logistic regression tests[d]	P=0.602	P=0.605	P=0.705N
Cochran-Armitage test[d]	P=0.224N		
Fisher exact test[d]		P=0.641N	P=0.293N
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates	1/48 (2%)	5/50 (10%)	1/50 (2%)
Adjusted rates	2.3%	16.7%	5.3%
Terminal rates	0/34 (0%)	5/30 (17%)	0/12 (0%)
First incidence (days)	582	729 (T)	640
Life table tests	P=0.242	P=0.076	P=0.612
Logistic regression tests	P=0.352	P=0.073	P=0.760
Cochran-Armitage test	P=0.576N		
Fisher exact test		P=0.112	P=0.742N
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	2/48 (4%)	6/50 (12%)	1/50 (2%)
Adjusted rates	4.9%	20.0%	5.3%
Terminal rates	0/34 (0%)	6/30 (20%)	0/12 (0%)
First incidence (days)	582	729 (T)	640
Life table tests	P=0.335	P=0.095	P=0.716
Logistic regression tests	P=0.472	P=0.092	P=0.592N
Cochran-Armitage test	P=0.396N		
Fisher exact test		P=0.148	P=0.485N
Harderian Gland: Adenoma			
Overall rates	8/50 (16%)	1/50 (2%)	0/50 (0%)
Adjusted rates	21.9%	3.3%	0.0%
Terminal rates	7/35 (20%)	1/30 (3%)	0/12 (0%)
First incidence (days)	582	729 (T)	-[e]
Life table tests	P=0.009N	P=0.031N	P=0.081N
Logistic regression tests	P=0.006N	P=0.033N	P=0.043N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.015N	P=0.003N
Liver: Hepatocellular Adenoma			
Overall rates	8/50 (16%)	6/50 (12%)	1/50 (2%)
Adjusted rates	21.9%	17.3%	8.3%
Terminal rates	7/35 (20%)	3/30 (10%)	1/12 (8%)
First incidence (days)	582	344	729 (T)
Life table tests	P=0.185N	P=0.508N	P=0.232N
Logistic regression tests	P=0.068N	P=0.465N	P=0.144N
Cochran-Armitage test	P=0.015N		
Fisher exact test		P=0.387N	P=0.015N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Liver: Hepatocellular Carcinoma			
Overall rates	16/50 (32%)	2/50 (4%)	8/50 (16%)
Adjusted rates	37.3%	5.6%	33.8%
Terminal rates	9/35 (26%)	0/30 (0%)	1/12 (8%)
First incidence (days)	385	584	514
Life table tests	P=0.357N	P=0.002N	P=0.484
Logistic regression tests	P=0.061N	P<0.001N	P=0.180N
Cochran-Armitage test	P=0.024N		
Fisher exact test		P<0.001N	P=0.050N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	24/50 (48%)	8/50 (16%)	9/50 (18%)
Adjusted rates	55.3%	21.9%	39.8%
Terminal rates	16/35 (46%)	3/30 (10%)	2/12 (17%)
First incidence (days)	385	344	514
Life table tests	P=0.168N	P=0.008N	P=0.447N
Logistic regression tests	P=0.007N	P=0.001N	P=0.033N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P<0.001N	P=0.001N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	10/50 (20%)	9/50 (18%)	6/50 (12%)
Adjusted rates	27.7%	27.4%	28.2%
Terminal rates	9/35 (26%)	7/30 (23%)	1/12 (8%)
First incidence (days)	703	187	568
Life table tests	P=0.255	P=0.555	P=0.284
Logistic regression tests	P=0.535N	P=0.562	P=0.581
Cochran-Armitage test	P=0.174N		
Fisher exact test		P=0.500N	P=0.207N
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	9/50 (18%)	6/50 (12%)	6/50 (12%)
Adjusted rates	23.2%	18.4%	28.9%
Terminal rates	6/35 (17%)	4/30 (13%)	1/12 (8%)
First incidence (days)	578	605	545
Life table tests	P=0.274	P=0.419N	P=0.266
Logistic regression tests	P=0.545	P=0.397N	P=0.575
Cochran-Armitage test	P=0.236N		
Fisher exact test		P=0.288N	P=0.288N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	10/50 (20%)	7/50 (14%)	6/50 (12%)
Adjusted rates	25.9%	21.6%	28.9%
Terminal rates	7/35 (20%)	5/30 (17%)	1/12 (8%)
First incidence (days)	578	605	545
Life table tests	P=0.322	P=0.438N	P=0.321
Logistic regression tests	P=0.516N	P=0.425N	P=0.593N
Cochran-Armitage test	P=0.166N		
Fisher exact test		P=0.298N	P=0.207N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Stomach (Forestomach): Squamous Papilloma			
Overall rates	7/50 (14%)	2/50 (4%)	1/50 (2%)
Adjusted rates	18.9%	6.7%	7.7%
Terminal rates	6/35 (17%)	2/30 (7%)	0/12 (0%)
First incidence (days)	473	729 (T)	703
Life table tests	P=0.116N	P=0.124N	P=0.286N
Logistic regression tests	P=0.063N	P=0.121N	P=0.140N
Cochran-Armitage test	P=0.014N		
Fisher exact test		P=0.080N	P=0.030N
All Organs: Hemangiosarcoma			
Overall rates	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted rates	7.8%	3.3%	8.3%
Terminal rates	2/35 (6%)	1/30 (3%)	1/12 (8%)
First incidence (days)	540	729 (T)	729 (T)
Life table tests	P=0.464N	P=0.369N	P=0.654N
Logistic regression tests	P=0.359N	P=0.344N	P=0.507N
Cochran-Armitage test	P=0.202N		
Fisher exact test		P=0.309N	P=0.309N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rates	10.5%	9.5%	3.0%
Terminal rates	3/35 (9%)	2/30 (7%)	0/12 (0%)
First incidence (days)	441	613	427
Life table tests	P=0.404N	P=0.592N	P=0.474N
Logistic regression tests	P=0.195N	P=0.547N	P=0.205N
Cochran-Armitage test	P=0.133N		
Fisher exact test		P=0.500N	P=0.181N
All Organs: Benign Tumors			
Overall rates	25/50 (50%)	26/50 (52%)	12/50 (24%)
Adjusted rates	63.7%	73.9%	53.3%
Terminal rates	21/35 (60%)	21/30 (70%)	3/12 (25%)
First incidence (days)	473	187	512
Life table tests	P=0.213	P=0.210	P=0.333
Logistic regression tests	P=0.284N	P=0.207	P=0.276N
Cochran-Armitage test	P=0.006N		
Fisher exact test		P=0.500	P=0.006N
All Organs: Malignant Tumors			
Overall rates	29/50 (58%)	12/50 (24%)	16/50 (32%)
Adjusted rates	62.6%	34.8%	61.1%
Terminal rates	18/35 (51%)	8/30 (27%)	3/12 (25%)
First incidence (days)	385	584	427
Life table tests	P=0.435	P=0.011N	P=0.245
Logistic regression tests	P=0.087N	P=0.001N	P=0.149N
Cochran-Armitage test	P=0.005N		
Fisher exact test		P<0.001N	P=0.008N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
All Organs: Benign or Malignant Tumors			
Overall rates	40/50 (80%)	31/50 (62%)	23/50 (46%)
Adjusted rates	85.0%	81.4%	78.3%
Terminal rates	28/35 (80%)	23/30 (77%)	6/12 (50%)
First incidence (days)	385	187	427
Life table tests	P=0.107	P=0.338N	P=0.095
Logistic regression tests	P=0.117N	P=0.159N	P=0.131N
Cochran-Armitage test	P<0.001N		
Fisher exact test		P=0.038N	P=<0.001N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE C4a
Historical Incidence of Adrenal Medulla Neoplasms in Male B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Benign Pheochromocytoma	Malignant Pheochromocytoma	Benign or Malignant Pheochromocytoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	2/49	0/49	2/49
Dichlorvos	2/48	0/48	2/48
Furan	1/49	0/49	1/49
Furfural	2/50	1/50	3/50
γ-Butyrolactone	1/48	1/48	2/48
Total	8/244 (3.3%)	2/244 (0.8%)	10/244 (4.1%)
Standard deviation	1.1%	1.1%	1.4%
Range	2%–4%	0%–2%	2%–6%
Overall Historical Incidence			
Total	16/582 (2.7%)	2/582 (3.4%)	18/582 (3.1%)
Standard deviation	1.6%	0.8%	1.8%
Range	0%–4%	0%–2%	0%–6%

[a] Data as of 17 September 1990.

TABLE C4b
Historical Incidence of Hepatocellular Neoplasms in Male B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Adenoma or Neoplastic Nodule	Carcinoma	Adenoma, Neoplastic Nodule, or Carcinoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	8/50	12/50	19/50
Dichlorvos	7/50	10/50	16/50
Furan	20/50	7/50	26/50
Furfural	9/50	7/50	16/50
γ-Butyrolactone	8/50	16/50	24/50
Total	52/250 (20.8%)	52/250 (20.8%)	101/250 (40.4%)
Standard deviation	10.8%	7.6%	9.2%
Range	14%–40%	14%–32%	32%–52%
Overall Historical Incidence			
Total	123/599 (20.5%)	103/599 (17.2%)	210/599 (35.1%)
Standard deviation	10.4%	6.2%	11.0%
Range	4%–40%	10%–32%	14%–52%

[a] Data as of 17 September 1990

TABLE C4c
Historical Incidence of Harderian Gland Neoplasms in Male B6C3F$_1$ Mice Receiving Corn Oil Vehicle by Gavage[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Benzaldehyde	2/50	1/50	3/50
Dichlorvos	5/50	0/50	5/50
Furan	3/50	0/50	3/50
Furfural	0/50	0/50	0/50
γ-Butyrolactone	8/50	0/50	8/50
Total	18/250 (7.2%)	1/250 (0.4%)	19/250 (7.6%)
Standard deviation	6.1%	0.9%	5.9%
Range	0%–16%	0%–2%	0%–16%
Overall Historical Incidence			
Total	34/600 (5.7%)	4/600 (0.7%)	38/600 (6.3%)
Standard deviation	4.7%	1.3%	4.5%
Range	0%–16%	0%–4%	0%–16%

[a] Data as of 17 September 1990.

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	262 mg/kg	525 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	2	12	13
Moribund	13	8	24
Accidental death			1
Survivors			
Terminal sacrifice	35	30	12
Animals examined microscopically	50	50	50
Alimentary System			
Esophagus	(46)	(50)	(47)
Ulcer			1 (2%)
Gallbladder	(42)	(41)	(43)
Ectopic tissue		1 (2%)	
Fibrosis	1 (2%)		
Inflammation, chronic	1 (2%)		
Mineralization	1 (2%)		
Intestine large, cecum	(47)	(42)	(45)
Edema	1 (2%)		1 (2%)
Inflammation, suppurative	1 (2%)		1 (2%)
Mucosa, hyperplasia	1 (2%)		
Intestine large, rectum	(49)	(49)	(47)
Inflammation, suppurative			1 (2%)
Intestine small, duodenum	(49)	(49)	(46)
Ulcer		1 (2%)	
Intestine small, ileum	(46)	(44)	(44)
Hyperplasia, lymphoid	3 (7%)		
Intestine small, jejunum	(47)	(45)	(45)
Hyperplasia, lymphoid	1 (2%)		
Inflammation, chronic active			1 (2%)
Inflammation, suppurative	1 (2%)		
Liver	(50)	(50)	(50)
Angiectasis			1 (2%)
Basophilic focus	1 (2%)	1 (2%)	
Clear cell focus			2 (4%)
Cyst			2 (4%)
Ectopic tissue		1 (2%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)	
Inflammation, chronic	4 (8%)	1 (2%)	
Mineralization	4 (8%)		
Hepatocyte, anisokaryosis	1 (2%)		2 (4%)
Hepatocyte, atrophy	1 (2%)		
Hepatocyte, vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)
Kupffer cell, hyperplasia	1 (2%)	2 (4%)	1 (2%)
Kupffer cell, pigmentation	1 (2%)		
Lobules, necrosis	6 (12%)	6 (12%)	6 (12%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Alimentary System (continued)			
Mesentery	(3)	(3)	(7)
Artery, hypertrophy			2 (29%)
Artery, inflammation, chronic active		2 (67%)	1 (14%)
Fat, hemorrhage	1 (33%)		
Fat, inflammation, chronic			1 (14%)
Fat, inflammation, suppurative			1 (14%)
Fat, mineralization	1 (33%)		
Fat, necrosis	2 (67%)	1 (33%)	2 (29%)
Pancreas	(50)	(49)	(49)
Amyloid deposition		1 (2%)	
Atrophy	2 (4%)	1 (2%)	
Cyst	1 (2%)		1 (2%)
Fibrosis		2 (4%)	
Necrosis		1 (2%)	1 (2%)
Salivary glands	(48)	(50)	(48)
Atrophy			1 (2%)
Inflammation, chronic	9 (19%)	9 (18%)	2 (4%)
Stomach, forestomach	(50)	(49)	(49)
Diverticulum	2 (4%)	4 (8%)	1 (2%)
Inflammation, chronic			1 (2%)
Mineralization	1 (2%)		
Mucosa, hyperplasia	2 (4%)		5 (10%)
Stomach, glandular	(50)	(49)	(49)
Cyst		1 (2%)	2 (4%)
Erosion			1 (2%)
Inflammation, chronic		1 (2%)	
Inflammation, chronic active		1 (2%)	1 (2%)
Mineralization	3 (6%)		2 (4%)
Ulcer		1 (2%)	
Mucosa, hyperplasia		1 (2%)	1 (2%)
Tongue		(1)	(2)
Necrosis			1 (50%)
Tooth	(10)	(5)	(3)
Dysplasia	8 (80%)	4 (80%)	3 (100%)
Inflammation, chronic			1 (33%)
Inflammation, suppurative	2 (20%)	3 (60%)	
Cardiovascular System			
Heart	(50)	(50)	(50)
Artery, hypertrophy			1 (2%)
Artery, inflammation, chronic active			1 (2%)
Myocardium, degeneration		1 (2%)	
Myocardium, fibrosis			1 (2%)
Myocardium, inflammation, chronic	1 (2%)	1 (2%)	
Myocardium, karyomegaly		2 (4%)	

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Endocrine System			
Adrenal gland, cortex	(48)	(50)	(50)
Accessory adrenal cortical nodule		1 (2%)	2 (4%)
Basophilic focus	1 (2%)		
Cyst		1 (2%)	
Developmental malformation	1 (2%)	2 (4%)	2 (4%)
Hyperplasia, focal			1 (2%)
Hypertrophy, focal	3 (6%)	1 (2%)	
Vacuolization cytoplasmic	1 (2%)		
Spindle cell, hyperplasia	18 (38%)	10 (20%)	8 (16%)
Adrenal gland, medulla	(48)	(50)	(50)
Cyst			1 (2%)
Hyperplasia	2 (4%)	9 (18%)	4 (8%)
Infiltration cellular, mononuclear cell	1 (2%)		
Islets, pancreatic	(50)	(49)	(48)
Hyperplasia	14 (28%)	8 (16%)	2 (4%)
Parathyroid gland	(45)	(48)	(43)
Cyst	1 (2%)	1 (2%)	1 (2%)
Pituitary gland	(43)	(48)	(43)
Pars distalis, cyst	3 (7%)	5 (10%)	2 (5%)
Pars distalis, hyperplasia			2 (5%)
Thyroid gland	(49)	(50)	(48)
Cyst		5 (10%)	3 (6%)
Inflammation, chronic	1 (2%)		
Inflammation, suppurative		1 (2%)	1 (2%)
Follicular cell, hyperplasia	3 (6%)	1 (2%)	2 (4%)
General Body System			
None			
Genital System			
Epididymis	(50)	(50)	(49)
Atypical cells	1 (2%)		
Ectasia			1 (2%)
Fibrosis			1 (2%)
Granuloma sperm			2 (4%)
Inflammation, chronic		1 (2%)	1 (2%)
Penis	(1)	(1)	(2)
Inflammation, chronic active			1 (50%)
Preputial gland	(18)	(20)	(17)
Atrophy			1 (6%)
Ectasia	9 (50%)	9 (45%)	7 (41%)
Inflammation, chronic	11 (61%)	16 (80%)	13 (76%)
Inflammation, suppurative	4 (22%)	5 (25%)	6 (35%)
Prostate	(49)	(48)	(48)
Fibrosis		1 (2%)	
Inflammation, chronic		1 (2%)	
Inflammation, chronic active		1 (2%)	
Inflammation, suppurative	1 (2%)	5 (10%)	8 (17%)
Epithelium, hyperplasia		1 (2%)	

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Genital System (continued)			
Seminal vesicle	(8)	(5)	(9)
Dilatation		1 (20%)	
Fibrosis	2 (25%)	2 (40%)	4 (44%)
Inflammation, chronic	2 (25%)		1 (11%)
Inflammation, suppurative	1 (13%)	2 (40%)	
Testes	(50)	(50)	(50)
Fibrosis	1 (2%)	1 (2%)	1 (2%)
Granuloma sperm	1 (2%)		
Mineralization	5 (10%)	9 (18%)	5 (10%)
Spermatocele		1 (2%)	
Seminiferous tubule, atrophy	4 (8%)	8 (16%)	4 (8%)
Hematopoietic System			
Blood	(7)	(2)	(1)
Anemia	1 (14%)	1 (50%)	
Bone marrow	(50)	(50)	(49)
Angiectasis		1 (2%)	1 (2%)
Hyperplasia, reticulum cell		1 (2%)	
Myelofibrosis		1 (2%)	2 (4%)
Necrosis	1 (2%)		
Proliferation	6 (12%)	12 (24%)	8 (16%)
Lymph node	(50)	(50)	(49)
Iliac, hyperplasia, lymphoid		1 (2%)	
Iliac, hyperplasia, plasma cell			2 (4%)
Inguinal, hyperplasia, histiocyte		4 (8%)	
Inguinal, hyperplasia, lymphoid		5 (10%)	5 (10%)
Inguinal, hyperplasia, plasma cell		4 (8%)	2 (4%)
Mediastinal, hemorrhage	1 (2%)		
Mediastinal, inflammation, suppurative	1 (2%)		
Renal, hyperplasia, lymphoid		1 (2%)	
Renal, hyperplasia, plasma cell			1 (2%)
Lymph node, mandibular	(45)	(46)	(46)
Depletion			1 (2%)
Hyperplasia, lymphoid	1 (2%)		
Hyperplasia, plasma cell	1 (2%)		
Lymphatic, dilatation	1 (2%)		
Lymph node, mesenteric	(48)	(46)	(41)
Depletion			3 (7%)
Hematopoietic cell proliferation		1 (2%)	
Hemorrhage	11 (23%)	7 (15%)	5 (12%)
Hyperplasia, lymphoid	3 (6%)		1 (2%)
Infiltration cellular, megakaryocyte	1 (2%)		
Necrosis	1 (2%)		
Spleen	(50)	(50)	(48)
Erythrophagocytosis		1 (2%)	
Hematopoietic cell proliferation	14 (28%)	12 (24%)	18 (38%)
Hyperplasia, lymphoid	5 (10%)		2 (4%)
Hyperplasia, re cell		1 (2%)	
Pigmentation, hemosiderin		1 (2%)	
Lymphoid follicle, depletion	2 (4%)	3 (6%)	8 (17%)
Red pulp, depletion	1 (2%)	1 (2%)	

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Hematopoietic System (continued)			
Thymus	(42)	(39)	(38)
Cyst	2 (5%)	2 (5%)	1 (3%)
Depletion		5 (13%)	6 (16%)
Epithelial cell, hyperplasia		4 (10%)	4 (11%)
Integumentary System			
Skin	(50)	(50)	(50)
Acanthosis	12 (24%)	36 (72%)	39 (78%)
Cyst epithelial inclusion			1 (2%)
Dysplasia			1 (2%)
Exudate	1 (2%)	4 (8%)	5 (10%)
Fibrosis	1 (2%)	3 (6%)	
Hemorrhage		2 (4%)	
Hyperkeratosis			1 (2%)
Hyperplasia, pseudoepitheliomatous		2 (4%)	2 (4%)
Inflammation, chronic	4 (8%)	17 (34%)	19 (38%)
Inflammation, chronic active		1 (2%)	
Inflammation, suppurative	1 (2%)		
Mineralization	2 (4%)		
Pigmentation	3 (6%)	12 (24%)	19 (38%)
Ulcer	4 (8%)	15 (30%)	17 (34%)
Hair follicle, atrophy	1 (2%)	11 (22%)	16 (32%)
Lymphatic, dilatation		1 (2%)	
Subcutaneous tissue, edema	3 (6%)	2 (4%)	2 (4%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Hyperostosis	1 (2%)	1 (2%)	2 (4%)
Inflammation, chronic			1 (2%)
Coccygeal, hyperplasia		6 (12%)	1 (2%)
Coccygeal, inflammation, chronic		3 (6%)	1 (2%)
Coccygeal, inflammation, suppurative		2 (4%)	
Tarsal, hyperplasia		1 (2%)	8 (16%)
Tarsal, inflammation, chronic			3 (6%)
Tarsal, inflammation, suppurative		1 (2%)	
Skeletal muscle	(1)	(4)	(2)
Fibrosis			1 (50%)
Inflammation, chronic		1 (25%)	
Necrosis		2 (50%)	
Nervous System			
Brain	(50)	(50)	(49)
Congestion			3 (6%)
Cyst	1 (2%)		
Mineralization	27 (54%)	25 (50%)	24 (49%)
Necrosis	1 (2%)		

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Respiratory System			
Lung	(50)	(50)	(50)
Congestion	1 (2%)	1 (2%)	4 (8%)
Hemorrhage		1 (2%)	7 (14%)
Infiltration cellular, histiocytic	3 (6%)	2 (4%)	1 (2%)
Inflammation, chronic	4 (8%)		
Inflammation, suppurative		1 (2%)	
Leukocytosis	1 (2%)	2 (4%)	5 (10%)
Thrombus		1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)	2 (4%)	
Perivascular, edema		2 (4%)	
Nose	(50)	(50)	(49)
Exudate	4 (8%)		4 (8%)
Foreign body	1 (2%)	1 (2%)	
Inflammation, chronic		1 (2%)	
Special Senses System			
Eye	(3)		(1)
Cataract	1 (33%)		
Cornea, hyperplasia	1 (33%)		
Cornea, inflammation, chronic	1 (33%)		
Cornea, inflammation, granulomatous	1 (33%)		
Harderian gland	(9)	(48)	
Inflammation, chronic		1 (2%)	
Urinary System			
Kidney	(50)	(50)	(50)
Amyloid deposition		1 (2%)	
Bacterium			1 (2%)
Casts protein	4 (8%)	3 (6%)	2 (4%)
Cyst	4 (8%)	10 (20%)	7 (14%)
Glomerulosclerosis		1 (2%)	
Hemorrhage	1 (2%)		1 (2%)
Hydronephrosis	2 (4%)		2 (4%)
Infarct		2 (4%)	
Inflammation, chronic	21 (42%)	14 (28%)	5 (10%)
Inflammation, suppurative	1 (2%)	3 (6%)	2 (4%)
Metaplasia, osseous	1 (2%)	2 (4%)	
Mineralization	3 (6%)	5 (10%)	4 (8%)
Glomerulus, hyperplasia		1 (2%)	
Glomerulus, necrosis	1 (2%)		
Renal tubule, atrophy	3 (6%)	5 (10%)	8 (16%)
Renal tubule, dilatation		2 (4%)	3 (6%)
Renal tubule, dysplasia			1 (2%)
Renal tubule, necrosis	1 (2%)	1 (2%)	1 (2%)
Renal tubule, regeneration	25 (50%)	26 (52%)	24 (48%)
Renal tubule, vacuolization cytoplasmic			2 (4%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Urinary System (continued)			
Urethra	(1)	(4)	(3)
Concretion		1 (25%)	
Hemorrhage	1 (100%)		
Inflammation, suppurative		3 (75%)	3 (100%)
Necrosis		1 (25%)	
Bulbourethral gland, hyperplasia		1 (25%)	
Mucosa, hyperplasia		1 (25%)	1 (33%)
Urinary bladder	(50)	(48)	(48)
Congestion			1 (2%)
Edema			1 (2%)
Inflammation, chronic	2 (4%)	3 (6%)	
Inflammation, chronic active			1 (2%)
Inflammation, suppurative		1 (2%)	1 (2%)
Mucosa, hyperplasia	2 (4%)	1 (2%)	3 (6%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR GAVAGE STUDY OF γ-BUTYROLACTONE

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	262 mg/kg	525 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	4	5	3
Moribund	8	11	9
Survivors			
Terminal sacrifice	38	34	38
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(47)		(47)
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Intestine large, colon	(50)	(1)	(49)
Intestine small, duodenum	(49)		(49)
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Polyp			1 (2%)
Intestine small, ileum	(48)	(1)	(49)
Carcinoma			1 (2%)
Intestine small, jejunum	(49)		(49)
Liver	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin		1 (2%)	
Hepatocellular carcinoma	4 (8%)	2 (4%)	1 (2%)
Hepatocellular adenoma	5 (10%)		3 (6%)
Mesentery	(3)	(5)	(5)
Hemangiosarcoma		2 (40%)	1 (20%)
Leiomyosarcoma			1 (20%)
Pancreas	(50)	(50)	(48)
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Salivary glands	(50)		(50)
Stomach, forestomach	(50)	(49)	(50)
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Papilloma squamous	2 (4%)	5 (10%)	4 (8%)
Stomach, glandular	(50)	(50)	(50)
Cardiovascular System			
None			
Endocrine System			
Adrenal gland, cortex	(50)	(1)	(50)
Spindle cell, adenoma	1 (2%)		
Adrenal gland, medulla	(50)		(49)
Pheochromocytoma malignant			1 (2%)
Pheochromocytoma benign			1 (2%)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Endocrine System (continued)			
Islets, pancreatic	(50)	(49)	(47)
Adenoma	1 (2%)		2 (4%)
Carcinoma		1 (2%)	
Pituitary gland	(48)	(48)	(43)
Pars distalis, adenoma	3 (6%)	5 (10%)	7 (16%)
Pars intermedia, adenoma	1 (2%)		
Thyroid gland	(49)	(48)	(50)
Follicular cell, adenoma		3 (6%)	1 (2%)
General Body System			
Tissue NOS		(1)	
Genital System			
Ovary	(48)	(49)	(46)
Cystadenoma	1 (2%)	1 (2%)	4 (9%)
Granulosa-theca tumor malignant	1 (2%)		1 (2%)
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Uterus	(50)	(41)	(50)
Adenoma		1 (2%)	
Carcinoma		2 (5%)	
Deciduoma benign		1 (2%)	
Granulosa-theca tumor malignant, metastatic, ovary	1 (2%)		
Hemangioma	1 (2%)		
Hemangiosarcoma	1 (2%)		1 (2%)
Polyp stromal	1 (2%)	1 (2%)	1 (2%)
Sarcoma stromal	1 (2%)	1 (2%)	
Vagina	(2)		(1)
Leiomyosarcoma	1 (50%)		
Polyp	1 (50%)		
Hematopoietic System			
Bone marrow	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	
Lymph node	(50)	(6)	(49)
Bronchial, leiomyosarcoma, metastatic, mesentery			1 (2%)
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung		1 (17%)	
Lymph node, mandibular	(47)	(1)	(45)
Lymph node, mesenteric	(49)	(5)	(43)
Spleen	(50)	(14)	(50)
Thymus	(47)	(2)	(44)
Alveolar/bronchiolar carcinoma, metastatic, lung	1 (2%)		

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Integumentary System			
Mammary gland	(50)	(48)	(50)
Carcinoma	2 (4%)	1 (2%)	1 (2%)
Skin	(50)	(47)	(50)
Subcutaneous tissue, fibroma			1 (2%)
Subcutaneous tissue, fibrosarcoma	1 (2%)	3 (6%)	
Subcutaneous tissue, hemangiosarcoma		1 (2%)	
Subcutaneous tissue, schwannoma benign		1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Osteosarcoma	1 (2%)		
Skeletal muscle	(1)	(4)	
Alveolar/bronchiolar carcinoma, metastatic, lung		1 (25%)	
Fibrosarcoma, metastatic, skin		1 (25%)	
Hemangiosarcoma	1 (100%)	1 (25%)	
Nervous System			
Brain	(50)	(50)	(50)
Respiratory System			
Lung	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	5 (10%)	3 (6%)	3 (6%)
Alveolar/bronchiolar carcinoma	2 (4%)	1 (2%)	1 (2%)
Carcinoma, metastatic, harderian gland	1 (2%)		1 (2%)
Carcinoma, metastatic, uterus		1 (2%)	
Fibrosarcoma, metastatic, skin		1 (2%)	
Granulosa-theca tumor malignant, metastatic, ovary			1 (2%)
Osteosarcoma, metastatic, bone	1 (2%)		
Special Senses System			
Harderian gland	(2)	(43)	(4)
Adenoma	1 (50%)	2 (5%)	3 (75%)
Carcinoma	1 (50%)		1 (25%)
Urinary System			
Kidney	(50)	(50)	(50)
Fibrosarcoma, metastatic, skin		1 (2%)	
Leiomyosarcoma, metastatic, mesentery			1 (2%)
Urinary bladder	(50)	(1)	(49)
Carcinoma, metastatic, uterus		1 (100%)	

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Lymphoma malignant histiocytic	1 (2%)	1 (2%)	
Lymphoma malignant lymphocytic	2 (4%)	1 (2%)	1 (2%)
Lymphoma malignant mixed	8 (16%)	7 (14%)	8 (16%)
Tumor Summary			
Total animals with primary neoplasms[c]	33	31	35
Total primary neoplasms	50	48	51
Total animals with benign neoplasms	17	21	25
Total benign neoplasms	23	23	31
Total animals with malignant neoplasms	24	20	16
Total malignant neoplasms	27	25	20
Total animals with secondary neoplasms[d]	4	3	3
Total secondary neoplasms	4	8	9

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control

Number of Days on Study	5 8 1	5 8 4	5 8 6	5 9 2	6 0 9	6 1 0	6 1 1	6 1 7	6 3 0	6 5 5	6 7 3	6 9 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	3 9 1	3 6 1	3 7 1	3 3 1	3 9 5	3 7 5	3 6 3	3 6 2	3 2 2	3 7 3	3 2 5	3 1 2	3 1 1	3 1 3	3 1 4	3 1 5	3 2 1	3 2 3	3 3 4	3 3 2	3 3 3	3 3 4	3 4 5	3 4 1	3 4 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	M	+	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma				X																					
Hepatocellular adenoma																	X								
Mesentery																				+					+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous															X									X	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spindle cell, adenoma																									
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M
Pars distalis, adenoma											X														
Pars intermedia, adenoma																	X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 4 3	3 4 4	3 4 5	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 6 4	3 6 5	3 7 2	3 7 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 2	3 9 3	3 9 4	4 0 1	4 0 2	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma													X									X			X	4
Hepatocellular adenoma																	X			X			X		X	5
Mesentery						+																				3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous																										2
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spindle cell, adenoma																					X					1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma					X																					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X																							X		3
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	49
General Body System																										
None																										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	5 8 1	5 8 4	5 8 6	5 9 2	6 0 9	6 1 0	6 1 1	6 1 7	6 3 0	6 5 5	6 7 3	6 9 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	3 9 1	3 6 1	3 7 1	3 3 1	3 9 5	3 7 5	3 6 3	3 6 2	3 2 2	3 7 3	3 2 5	3 1 2	3 1 1	3 1 3	3 1 4	3 1 5	3 2 1	3 2 3	3 3 4	3 3 2	3 3 3	3 3 4	3 4 5	3 4 1	3 4 2
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+
Cystadenoma													X												
Granulosa-theca tumor malignant																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa-theca tumor malignant, metastatic, ovary																									
Hemangioma																									
Hemangiosarcoma								X																	
Polyp stromal											X														
Sarcoma stromal															X										
Vagina																	+								
Leiomyosarcoma																									
Polyp																	X								
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma, metastatic, lung			X																						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma												X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma		X																							
Skeletal muscle																									
Hemangiosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord		+																							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 4 3	3 4 4	3 4 5	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 6 4	3 6 5	3 7 2	3 7 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 2	3 9 3	3 9 4	4 0 1	4 0 2	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
Genital System																										
Ovary	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cystadenoma																										1
Granulosa-theca tumor malignant	X																									1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa-theca tumor malignant, metastatic, ovary	X																									1
Hemangioma																									X	1
Hemangiosarcoma																										1
Polyp stromal																										1
Sarcoma stromal																										1
Vagina													+													2
Leiomyosarcoma													X													1
Polyp																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																								X		2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle											+															1
Hemangiosarcoma											X															1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	5 8 1	5 8 4	5 8 6	5 9 2	6 0 9	6 1 0	6 1 1	6 1 7	6 3 0	6 5 5	6 7 3	6 9 7	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	3 9 1	3 6 1	3 7 1	3 3 1	3 9 5	3 7 5	3 6 3	3 6 2	3 2 2	3 7 3	3 2 5	3 1 2	3 1 1	3 1 3	3 1 4	3 1 5	3 2 1	3 2 3	3 2 4	3 3 2	3 3 3	3 3 4	3 3 5	4 4 1	4 4 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma			X																						
Carcinoma, metastatic, Harderian gland									X																
Osteosarcoma, metastatic, bone		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Harderian gland									+		+														
Adenoma											X														
Carcinoma									X																
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic																									
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed														X		X	X					X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: Vehicle Control (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	3 4 3	3 4 4	3 4 5	3 5 1	3 5 2	3 5 3	3 5 4	3 5 5	3 6 4	3 6 5	3 7 2	3 7 4	3 8 1	3 8 2	3 8 3	3 8 4	3 8 5	3 9 2	3 9 3	3 9 4	4 0 1	4 0 2	4 0 3	4 0 4	4 0 5	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma	X								X						X					X		X				5
Alveolar/bronchiolar carcinoma																							X			2
Carcinoma, metastatic, Harderian gland																										1
Osteosarcoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Harderian gland																										2
Adenoma																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic														X												1
Lymphoma malignant lymphocytic				X			X																			2
Lymphoma malignant mixed								X	X							X							X			8

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg

Number of Days on Study	4 6 4	4 7 7	5 2 7	5 5 1	5 6 0	5 9 1	5 9 1	5 9 2	5 9 2	6 1 7	6 2 4	6 3 0	6 3 3	6 3 3	6 4 1	6 9 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	5 7 1	5 9 1	5 2 1	5 1 1	5 3 1	5 8 1	5 9 2	5 4 1	5 6 1	5 6 3	5 1 5	5 8 4	5 1 4	5 4 4	5 4 2	5 8 5	5 9 4	5 1 2	5 1 3	5 2 2	5 2 3	5 2 4	5 2 5	5 3 2	5 3 3	5 3 4
Alimentary System																										
Intestine large																										
Intestine large, colon																										
Intestine small																			+							
Intestine small, ileum																			+							
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																X										
Hepatocellular carcinoma											X								X							
Mesentery					+	+							+													
Hemangiosarcoma																										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous																										
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
None																										
Endocrine System																										
Adrenal gland																										
Adrenal gland, cortex																										
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																				X						
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																										X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma										X																
General Body System																										
Tissue NOS					+																					
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																										
Uterus	+			+	+		+	+	+	+			+	+	+		+	+		+	+	+	+	+	+	+
Adenoma																										
Carcinoma				X																						
Deciduoma benign																	X									
Polyp stromal																										
Sarcoma stromal										X																

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 3 5	5 4 3	5 4 4	5 4 5	5 5 1	5 5 2	5 5 3	5 5 4	5 5 5	5 6 2	5 6 4	5 6 5	5 7 2	5 7 3	5 7 4	5 7 5	5 8 2	5 8 3	5 9 3	5 9 5	6 0 1	6 0 2	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Intestine large								+																		1
Intestine large, colon								+																		1
Intestine small																										1
Intestine small, ileum																										1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Hepatocellular carcinoma																										2
Mesentery						+											+									5
Hemangiosarcoma						X											X									2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous				X						X			X					X					X			5
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
None																										
Endocrine System																										
Adrenal gland														+												1
Adrenal gland, cortex														+												1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	48
Pars distalis, adenoma			X		X						X										X					5
Thyroid gland	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	48
Follicular cell, adenoma						X																	X			3
General Body System																										
Tissue NOS																										1
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Cystadenoma								X																		1
Uterus	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	41
Adenoma																			X							1
Carcinoma																								X		2
Deciduoma benign																										1
Polyp stromal						X																				1
Sarcoma stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	4 6 4	4 7 7	5 2 7	5 5 1	5 6 0	5 9 1	5 9 1	5 9 2	5 9 2	6 1 7	6 2 4	6 3 0	6 3 3	6 3 3	6 4 1	6 9 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	5 7 1	5 9 1	5 2 1	5 1 1	5 3 1	5 8 1	5 9 2	5 4 1	5 6 1	5 6 3	5 1 5	5 8 4	5 1 4	5 4 2	5 8 5	5 9 4	5 1 2	5 1 3	5 2 2	5 2 3	5 2 4	5 2 5	5 3 2	5 3 3	5 3 4
Hematopoietic System																									
Blood		+																							
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma																									
Lymph node					+						+		+			+									
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung											X														
Lymph node, mandibular											M		+												
Lymph node, mesenteric					+						+		+												
Spleen		+			+			+				+	+			+					+			+	
Thymus					+																				
Integumentary System																									
Mammary gland	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma					X																				
Skin		+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma		X													X										
Subcutaneous tissue, hemangiosarcoma																									
Subcutaneous tissue, schwannoma benign																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle											+				+	+									
Alveolar/bronchiolar carcinoma, metastatic, lung											X														
Fibrosarcoma, metastatic, skin															X										
Hemangiosarcoma																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X							
Alveolar/bronchiolar carcinoma											X														
Carcinoma, metastatic, uterus				X																					
Fibrosarcoma, metastatic, skin															X										

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 3 5	5 4 3	5 4 4	5 4 5	5 5 1	5 5 2	5 5 3	5 5 4	5 5 5	5 6 2	5 6 4	5 6 5	5 7 2	5 7 3	5 7 4	5 7 5	5 8 2	5 8 3	5 9 3	5 9 5	6 0 1	6 0 2	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma														X												1
Lymph node								+											+							6
Mediastinal, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Lymph node, mandibular																										1
Lymph node, mesenteric								+											+							5
Spleen				+		+		+			+										+		+			14
Thymus																				+						2
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma																										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		47
Subcutaneous tissue, fibrosarcoma																								X		3
Subcutaneous tissue, hemangiosarcoma														X												1
Subcutaneous tissue, schwannoma benign			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle														M			+									4
Alveolar/bronchiolar carcinoma, metastatic, lung																										1
Fibrosarcoma, metastatic, skin																										1
Hemangiosarcoma																	X									1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma									X					X												3
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, uterus																										1
Fibrosarcoma, metastatic, skin																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	4 6 4	4 7 7	5 2 7	5 5 1	5 6 0	5 9 1	5 9 1	5 9 2	5 9 2	6 1 7	6 2 4	6 3 0	6 3 3	6 3 3	6 4 1	6 9 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1
Carcass ID Number	5 7 1	5 9 1	5 2 1	5 1 1	5 3 1	5 8 1	5 9 2	5 4 1	5 6 1	5 6 3	5 1 5	5 8 4	5 1 4	5 4 2	5 8 5	5 9 4	5 1 2	5 1 3	5 2 2	5 2 3	5 2 4	5 2 5	5 3 2	5 3 3	5 3 4
Special Senses System																									
Eye																									
Harderian gland	M	M	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	M	+	+	+	+	+
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin															X										
Urinary bladder				+																					
Carcinoma, metastatic, uterus				X																					
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic	X																								
Lymphoma malignant lymphocytic																									
Lymphoma malignant mixed					X								X									X		X	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 262 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	5 3 5	5 4 3	5 4 4	5 4 5	5 5 1	5 5 2	5 5 3	5 5 4	5 5 5	5 6 2	5 6 4	5 6 5	5 7 2	5 7 3	5 7 4	5 7 5	5 8 2	5 8 3	5 9 3	5 9 5	6 0 1	6 0 2	6 0 3	6 0 4	6 0 5	**Total Tissues/ Tumors**
Special Senses System																										
Eye																								+		1
Harderian gland	+	+	+	+	+	+	M	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Adenoma																	X							X		2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin																										1
Urinary bladder																										1
Carcinoma, metastatic, uterus																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic											X															1
Lymphoma malignant mixed								X													X		X			7

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg

Number of Days on Study	4 7 1	5 1 1	5 9 2	5 9 2	6 4 1	6 4 3	6 5 5	6 5 6	6 5 6	6 9 3	7 0 8	7 1 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	4 7 1	4 9 1	4 2 1	4 6 1	4 4 1	4 3 4	4 9 5	4 4 2	4 8 2	4 8 4	4 6 2	4 9 2	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 3 5
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery											X														
Intestine large	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery											X														
Polyp																									
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																									
Hepatocellular adenoma			X																				X		
Mesentery	+									+	+	+	+												
Hemangiosarcoma										X															
Leiomyosarcoma											X														
Pancreas	+	A	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery											X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery											X														
Papilloma squamous										X			X										X		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue				+																					
Tooth				+																					
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant				X																					
Pheochromocytoma benign														X											
Islets, pancreatic	+	A	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																				
Parathyroid gland	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																									

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	4 4 3	4 4 4	4 4 5	4 5 1	4 5 2	4 5 3	4 5 4	4 5 5	4 6 3	4 6 4	4 6 5	4 7 2	4 7 3	4 7 4	4 7 5	4 8 1	4 8 3	4 8 5	4 9 3	4 9 4	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Gallbladder	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Leiomyosarcoma, metastatic, mesentery																										1
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma, metastatic, mesentery																										1
Polyp													X													1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																	X									1
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma															X											1
Hepatocellular adenoma																					X					3
Mesentery																										5
Hemangiosarcoma																										1
Leiomyosarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Leiomyosarcoma, metastatic, mesentery																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma, metastatic, mesentery																										1
Papilloma squamous																				X						4
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue									+																	2
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																										1
Pheochromocytoma benign																										1
Islets, pancreatic	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma					X																					2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47
Pituitary gland	I	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	43
Pars distalis, adenoma				X	X		X		X						X	X		X								7

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	4 7 1	5 1 1	5 9 2	5 9 2	6 4 1	6 4 3	6 5 5	6 5 6	6 5 6	6 9 3	7 0 8	7 1 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	4 7 1	4 9 1	4 2 1	4 6 1	4 4 1	4 3 4	4 9 5	4 4 2	4 8 2	4 8 4	4 6 2	4 9 2	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 3 5
Endocrine System (continued)																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma																		X							
General Body System																									
None																									
Genital System																									
Clitoral gland																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Cystadenoma							X																	X	
Granulosa-theca tumor malignant					X																				
Leiomyosarcoma, metastatic, mesentery											X														
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma										X															
Polyp stromal																									
Vagina																	+								
Hematopoietic System																									
Blood																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bronchial, leiomyosarcoma, metastatic, mesentery											X														
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	A	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, schwannoma malignant					X																				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	4 4 3	4 4 4	4 4 5	4 5 1	4 5 2	4 5 3	4 5 4	4 5 5	4 6 3	4 6 4	4 6 5	4 7 2	4 7 3	4 7 4	4 7 5	4 8 1	4 8 3	4 8 5	4 9 3	4 9 4	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
Endocrine System (continued)																										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Follicular cell, adenoma																										1
General Body System																										
None																										
Genital System																										
Clitoral gland																+										1
Ovary	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	I	+	+	M	+	+	+	+	46
Cystadenoma						X										X										4
Granulosa-theca tumor malignant																										1
Leiomyosarcoma, metastatic, mesentery																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangiosarcoma																										1
Polyp stromal																								X		1
Vagina																										1
Hematopoietic System																										
Blood																	+									1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bronchial, leiomyosarcoma, metastatic, mesentery																										1
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	M	+	+	+	45
Lymph node, mesenteric	+	+	I	+	+	I	M	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	43
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	I	I	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																X										1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Subcutaneous tissue, fibroma								X																		1
Subcutaneous tissue, schwannoma malignant																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	4 7 1	5 1 1	5 9 2	5 9 2	6 4 1	6 4 3	6 5 5	6 5 6	6 5 6	6 9 3	7 0 8	7 1 1	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1
Carcass ID Number	4 7 1	4 9 1	4 2 1	4 6 1	4 4 1	4 3 4	4 9 5	4 4 2	4 8 2	4 8 4	4 6 2	4 9 2	4 1 1	4 1 2	4 1 3	4 1 4	4 1 5	4 2 2	4 2 3	4 2 4	4 2 5	4 3 1	4 3 2	4 3 3	4 3 5
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																						X			
Carcinoma, metastatic, harderian gland																									
Granulosa-theca tumor malignant, metastatic, ovary					X																				
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Eye															+										
Harderian gland										+					+		+								
Adenoma										X					X		X								
Carcinoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma, metastatic, mesentery											X														
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant lymphocytic													X												
Lymphoma malignant mixed						X			X					X						X		X			

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Gavage Study of γ-Butyrolactone: 525 mg/kg (continued)

Number of Days on Study	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	4 4 3	4 4 4	4 4 5	4 5 1	4 5 2	4 5 3	4 5 4	4 5 5	4 6 3	4 6 4	4 6 5	4 7 2	4 7 3	4 7 4	4 7 5	4 8 1	4 8 3	4 8 5	4 9 3	4 9 4	5 0 1	5 0 2	5 0 3	5 0 4	5 0 5	**Total Tissues/ Tumors**
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma				X				X															X			3
Alveolar/bronchiolar carcinoma																										1
Carcinoma, metastatic, harderian gland																						X				1
Granulosa-theca tumor malignant, metastatic, ovary																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear								+								+										2
Eye																						+				2
Harderian gland																						+				4
Adenoma																										3
Carcinoma																						X				1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma, metastatic, mesentery																										1
Urinary bladder	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed	X						X				X															8

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone

	Vehicle Control	262 mg/kg	525 mg/kg
Harderian Gland: Adenoma			
Overall rates[a]	1/50 (2%)	2/50 (4%)	3/50 (6%)
Adjusted rates[b]	2.5%	5.9%	7.6%
Terminal rates[c]	0/38 (0%)	2/34 (6%)	2/38 (5%)
First incidence (days)	673	730 (T)	693
Life table tests[d]	P=0.233	P=0.459	P=0.315
Logistic regression tests[d]	P=0.229	P=0.475	P=0.305
Cochran-Armitage test[d]	P=0.222		
Fisher exact test[d]		P=0.500	P=0.309
Harderian Gland: Adenoma or Carcinoma			
Overall rates	2/50 (4%)	2/50 (4%)	4/50 (8%)
Adjusted rates	4.8%	5.9%	10.1%
Terminal rates	0/38 (0%)	2/34 (6%)	3/38 (8%)
First incidence (days)	630	730 (T)	693
Life table tests	P=0.268	P=0.653	P=0.353
Logistic regression tests	P=0.255	P=0.692N	P=0.336
Cochran-Armitage test	P=0.252		
Fisher exact test		P=0.691N	P=0.339
Liver: Hepatocellular Adenoma			
Overall rates	5/50 (10%)	0/50 (0%)	3/50 (6%)
Adjusted rates	13.2%	0.0%	7.2%
Terminal rates	5/38 (13%)	0/34 (0%)	2/38 (5%)
First incidence (days)	730 (T)	-[e]	592
Life table tests	P=0.256N	P=0.043N	P=0.356N
Logistic regression tests	P=0.250N	P=0.043N	P=0.351N
Cochran-Armitage test	P=0.253N		
Fisher exact test		P=0.028N	P=0.357N
Liver: Hepatocellular Carcinoma			
Overall rates	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted rates	9.9%	5.4%	2.6%
Terminal rates	3/38 (8%)	1/34 (3%)	1/38 (3%)
First incidence (days)	592	624	730 (T)
Life table tests	P=0.121N	P=0.384N	P=0.180N
Logistic regression tests	P=0.118N	P=0.324N	P=0.180N
Cochran-Armitage test	P=0.118N		
Fisher exact test		P=0.339N	P=0.181N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	8/50 (16%)	2/50 (4%)	4/50 (8%)
Adjusted rates	20.2%	5.4%	9.8%
Terminal rates	7/38 (18%)	1/34 (3%)	3/38 (8%)
First incidence (days)	592	624	592
Life table tests	P=0.118N	P=0.069N	P=0.179N
Logistic regression tests	P=0.115N	P=0.055N	P=0.177N
Cochran-Armitage test	P=0.115N		
Fisher exact test		P=0.046N	P=0.178N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	5/50 (10%)	3/50 (6%)	3/50 (6%)
Adjusted rates	13.2%	8.8%	7.9%
Terminal rates	5/38 (13%)	3/34 (9%)	3/38 (8%)
First incidence (days)	730 (T)	730 (T)	730 (T)
Life table tests	P=0.284N	P=0.418N	P=0.355N
Logistic regression tests	P=0.284N	P=0.418N	P=0.355N
Cochran-Armitage test	P=0.283N		
Fisher exact test		P=0.357N	P=0.357N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	7/50 (14%)	4/50 (8%)	4/50 (8%)
Adjusted rates	17.5%	11.1%	10.5%
Terminal rates	6/38 (16%)	3/34 (9%)	4/38 (11%)
First incidence (days)	586	624	730 (T)
Life table tests	P=0.204N	P=0.324N	P=0.261N
Logistic regression tests	P=0.198N	P=0.293N	P=0.252N
Cochran-Armitage test	P=0.203N		
Fisher exact test		P=0.262N	P=0.262N
Ovary: Cystadenoma			
Overall rates	1/48 (2%)	1/49 (2%)	4/46 (9%)
Adjusted rates	2.8%	3.0%	10.9%
Terminal rates	1/36 (3%)	1/33 (3%)	3/34 (9%)
First incidence (days)	730 (T)	730 (T)	655
Life table tests	P=0.098	P=0.742	P=0.172
Logistic regression tests	P=0.097	P=0.742	P=0.169
Cochran-Armitage test	P=0.092		
Fisher exact test		P=0.747N	P=0.168
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	3/48 (6%)	5/48 (10%)	7/43 (16%)
Adjusted rates	8.0%	15.2%	20.6%
Terminal rates	2/36 (6%)	5/33 (15%)	7/34 (21%)
First incidence (days)	697	730 (T)	730 (T)
Life table tests	P=0.105	P=0.306	P=0.139
Logistic regression tests	P=0.105	P=0.306	P=0.137
Cochran-Armitage test	P=0.086		
Fisher exact test		P=0.357	P=0.117
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rates	2.6%	7.6%	0.0%
Terminal rates	0/38 (0%)	1/34 (3%)	0/38 (0%)
First incidence (days)	697	477	-
Life table tests	P=0.371N	P=0.274	P=0.495N
Logistic regression tests	P=0.348N	P=0.431	P=0.500N
Cochran-Armitage test	P=0.378N		
Fisher exact test		P=0.309	P=0.500N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rates	2.6%	7.6%	2.6%
Terminal rates	0/38 (0%)	1/34 (3%)	1/38 (3%)
First incidence (days)	697	477	730 (T)
Life table tests	P=0.597N	P=0.274	P=0.757N
Logistic regression tests	P=0.605N	P=0.431	P=0.761N
Cochran-Armitage test	P=0.609N		
Fisher exact test		P=0.309	P=0.753N
Stomach (Forestomach): Squamous Papilloma			
Overall rates	2/50 (4%)	5/50 (10%)	4/50 (8%)
Adjusted rates	5.3%	14.7%	10.1%
Terminal rates	2/38 (5%)	5/34 (15%)	3/38 (8%)
First incidence (days)	730 (T)	730 (T)	693
Life table tests	P=0.289	P=0.172	P=0.342
Logistic regression tests	P=0.300	P=0.172	P=0.349
Cochran-Armitage test	P=0.283		
Fisher exact test		P=0.218	P=0.339
Thyroid Gland (Follicular Cell): Adenoma			
Overall rates	0/49 (0%)	3/48 (6%)	1/50 (2%)
Adjusted rates	0.0%	8.5%	2.6%
Terminal rates	0/37 (0%)	2/32 (6%)	1/38 (3%)
First incidence (days)	-	617	730 (T)
Life table tests	P=0.394	P=0.104	P=0.505
Logistic regression tests	P=0.388	P=0.114	P=0.505
Cochran-Armitage test	P=0.386		
Fisher exact test		P=0.117	P=0.505
All Organs: Hemangiosarcoma			
Overall rates	2/50 (4%)	3/50 (6%)	1/50 (2%)
Adjusted rates	4.9%	8.8%	2.4%
Terminal rates	1/38 (3%)	3/34 (9%)	0/38 (0%)
First incidence (days)	617	730 (T)	693
Life table tests	P=0.394N	P=0.455	P=0.486N
Logistic regression tests	P=0.398N	P=0.474	P=0.495N
Cochran-Armitage test	P=0.399N		
Fisher exact test		P=0.500	P=0.500N
All Organs: Hemangioma or Hemangiosarcoma			
Overall rates	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rates	7.5%	8.8%	2.4%
Terminal rates	2/38 (5%)	3/34 (9%)	0/38 (0%)
First incidence (days)	617	730 (T)	693
Life table tests	P=0.239N	P=0.615	P=0.298N
Logistic regression tests	P=0.237N	P=0.631	P=0.305N
Cochran-Armitage test	P=0.238N		
Fisher exact test		P=0.661N	P=0.309N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, or Mixed)			
Overall rates	11/50 (22%)	9/50 (18%)	9/50 (18%)
Adjusted rates	28.9%	23.1%	22.1%
Terminal rates	11/38 (29%)	6/34 (18%)	7/38 (18%)
First incidence (days)	730 (T)	464	643
Life table tests	P=0.348N	P=0.502N	P=0.393N
Logistic regression tests	P=0.348N	P=0.440N	P=0.375N
Cochran-Armitage test	P=0.352N		
Fisher exact test		P=0.402N	P=0.402N
All Organs: Benign Tumors			
Overall rates	17/50 (34%)	21/50 (42%)	25/50 (50%)
Adjusted rates	42.5%	59.8%	59.1%
Terminal rates	15/38 (39%)	20/34 (59%)	21/38 (55%)
First incidence (days)	673	617	592
Life table tests	P=0.067	P=0.134	P=0.086
Logistic regression tests	P=0.073	P=0.127	P=0.085
Cochran-Armitage test	P=0.064		
Fisher exact test		P=0.268	P=0.078
All Organs: Malignant Tumors			
Overall rates	24/50 (48%)	20/50 (40%)	16/50 (32%)
Adjusted rates	54.1%	46.4%	35.9%
Terminal rates	18/38 (47%)	12/34 (35%)	10/38 (26%)
First incidence (days)	584	464	592
Life table tests	P=0.082	P=0.440N	P=0.091N
Logistic regression tests	P=0.063N	P=0.214N	P=0.077N
Cochran-Armitage test	P=0.063N		
Fisher exact test		P=0.273N	P=0.076N
All Organs: Benign or Malignant Tumors			
Overall rates	33/50 (66%)	31/50 (62%)	35/50 (70%)
Adjusted rates	73.1%	73.2%	75.9%
Terminal rates	26/38 (68%)	23/34 (68%)	27/38 (71%)
First incidence (days)	584	464	592
Life table tests	P=0.420	P=0.480	P=0.454
Logistic regression tests	P=0.380	P=0.473N	P=0.422
Cochran-Armitage test	P=0.376		
Fisher exact test		P=0.418N	P=0.415

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone[a]

	Vehicle Control	262 mg/kg	525 mg/kg
Disposition Summary			
Animals initially in study	50	50	50
Early deaths			
Natural death	4	5	3
Moribund	8	11	9
Survivors			
Terminal sacrifice	38	34	38
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(47)		(47)
Dilatation			1 (2%)
Inflammation, chronic	3 (6%)		1 (2%)
Intestine large, cecum	(48)		(48)
Edema	2 (4%)		
Hyperplasia, lymphoid	1 (2%)		
Intestine small, ileum	(48)	(1)	(49)
Hyperplasia, lymphoid	1 (2%)	1 (100%)	2 (4%)
Intestine small, jejunum	(49)		(49)
Hyperplasia, lymphoid			1 (2%)
Liver	(50)	(50)	(50)
Basophilic focus			1 (2%)
Cyst		1 (2%)	
Eosinophilic focus	1 (2%)		
Focal cellular change			1 (2%)
Hematopoietic cell proliferation		2 (4%)	2 (4%)
Inflammation, chronic	14 (28%)	7 (14%)	6 (12%)
Mineralization	1 (2%)		1 (2%)
Hepatocyte, vacuolization cytoplasmic	8 (16%)	2 (4%)	2 (4%)
Kupffer cell, hyperplasia	1 (2%)	8 (16%)	3 (6%)
Kupffer cell, pigmentation	1 (2%)		
Lobules, necrosis	1 (2%)	3 (6%)	3 (6%)
Mesentery	(3)	(5)	(5)
Accessory spleen	1 (33%)		
Cyst			1 (20%)
Inflammation, suppurative		1 (20%)	1 (20%)
Fat, inflammation, granulomatous	1 (33%)		
Fat, necrosis	2 (67%)	1 (20%)	1 (20%)
Pancreas	(50)	(50)	(48)
Atrophy		1 (2%)	4 (8%)
Cyst	1 (2%)		1 (2%)
Hyperplasia, nodular			1 (2%)
Inflammation, chronic	7 (14%)	3 (6%)	5 (10%)
Necrosis	2 (4%)		
Pigmentation	2 (4%)		
Salivary glands	(50)		(50)
Hyperplasia, lymphoid			1 (2%)
Inflammation, chronic	7 (14%)		10 (20%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Alimentary System (continued)			
Stomach, forestomach	(50)	(49)	(50)
Diverticulum	1 (2%)	1 (2%)	4 (8%)
Inflammation, chronic		1 (2%)	
Mucosa, dysplasia			1 (2%)
Mucosa, hyperplasia	10 (20%)	2 (4%)	7 (14%)
Stomach, glandular	(50)	(50)	(50)
Cyst		1 (2%)	1 (2%)
Dysplasia			1 (2%)
Erosion	1 (2%)		2 (4%)
Inflammation, chronic active		1 (2%)	1 (2%)
Mineralization	2 (4%)		3 (6%)
Ulcer	1 (2%)		
Cardiovascular System			
Heart	(50)		(50)
Myocardium, mineralization			2 (4%)
Endocrine System			
Adrenal gland, cortex	(50)	(1)	(50)
Accessory adrenal cortical nodule	1 (2%)		2 (4%)
Angiectasis		1 (100%)	
Cyst			1 (2%)
Developmental malformation			2 (4%)
Inflammation, chronic	1 (2%)		
Spindle cell, hyperplasia	43 (86%)		46 (92%)
Zona fasciculata, hyperplasia, focal	1 (2%)		
Adrenal gland, medulla	(50)		(49)
Hyperplasia	3 (6%)		1 (2%)
Islets, pancreatic	(50)	(49)	(47)
Amyloid deposition		1 (2%)	
Hyperplasia	5 (10%)	4 (8%)	6 (13%)
Inflammation, chronic	1 (2%)		
Parathyroid gland	(47)		(47)
Cyst			1 (2%)
Pituitary gland	(48)	(48)	(43)
Pars distalis, angiectasis	4 (8%)	5 (10%)	2 (5%)
Pars distalis, cyst		1 (2%)	1 (2%)
Pars distalis, hyperplasia	13 (27%)	12 (25%)	7 (16%)
Thyroid gland	(49)	(48)	(50)
Cyst	2 (4%)	9 (19%)	1 (2%)
Inflammation, chronic	3 (6%)	2 (4%)	1 (2%)
Follicular cell, hyperplasia	5 (10%)	7 (15%)	1 (2%)
General Body System			
None			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Genital System			
Clitoral gland			(1)
Ectasia			1 (100%)
Inflammation, chronic			1 (100%)
Ovary	(48)	(49)	(46)
Cyst	11 (23%)	14 (29%)	14 (30%)
Hemorrhage	2 (4%)	5 (10%)	1 (2%)
Inflammation, chronic	1 (2%)	1 (2%)	
Inflammation, suppurative		6 (12%)	1 (2%)
Uterus	(50)	(41)	(50)
Angiectasis		1 (2%)	
Exudate	8 (16%)	14 (34%)	3 (6%)
Hemorrhage	2 (4%)		
Hydrometra	9 (18%)	4 (10%)	5 (10%)
Hyperplasia, cystic	46 (92%)	37 (90%)	48 (96%)
Hyperplasia, glandular		2 (5%)	
Inflammation, suppurative		1 (2%)	1 (2%)
Metaplasia, squamous	1 (2%)	2 (5%)	2 (4%)
Serosa, cyst	1 (2%)		
Vagina	(2)		(1)
Granuloma			1 (100%)
Hematopoietic System			
Blood		(1)	(1)
Anemia		1 (100%)	1 (100%)
Leukocytosis		1 (100%)	1 (100%)
Bone marrow	(50)	(50)	(50)
Myelofibrosis	41 (82%)	41 (82%)	42 (84%)
Necrosis		1 (2%)	
Proliferation	1 (2%)	6 (12%)	2 (4%)
Lymph node	(50)	(6)	(49)
Iliac, angiectasis			1 (2%)
Iliac, hyperplasia, lymphoid	1 (2%)		
Iliac, hyperplasia, plasma cell			1 (2%)
Iliac, infiltration cellular, polymorphonuclear	1 (2%)		
Inguinal, hyperplasia, lymphoid			1 (2%)
Renal, hyperplasia, lymphoid	1 (2%)		
Renal, hyperplasia, plasma cell		1 (17%)	1 (2%)
Lymph node, mandibular	(47)	(1)	(45)
Hyperplasia, lymphoid			1 (2%)
Lymph node, mesenteric	(49)	(5)	(43)
Ectasia		1 (20%)	
Hemorrhage	2 (4%)		2 (5%)
Hyperplasia, reticulum cell		1 (20%)	
Spleen	(50)	(14)	(50)
Angiectasis			1 (2%)
Developmental malformation	1 (2%)		
Hematopoietic cell proliferation	6 (12%)	7 (50%)	8 (16%)
Hyperplasia, lymphoid	3 (6%)	2 (14%)	5 (10%)
Lymphoid follicle, depletion	2 (4%)		1 (2%)
Red pulp, depletion	2 (4%)		
Sinusoid, hyperplasia			1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Hematopoietic System (continued)			
Thymus	(47)	(2)	(44)
Cyst			1 (2%)
Hyperplasia, lymphoid		1 (50%)	2 (5%)
Epithelial cell, hyperplasia	1 (2%)		
Integumentary System			
Mammary gland	(50)	(48)	(50)
Hyperplasia, cystic	17 (34%)	19 (40%)	18 (36%)
Hyperplasia, lobular		2 (4%)	2 (4%)
Infiltration cellular, histiocytic	1 (2%)		
Inflammation, chronic		1 (2%)	
Skin	(50)	(47)	(50)
Acanthosis	3 (6%)	1 (2%)	3 (6%)
Cyst epithelial inclusion			1 (2%)
Fibrosis	1 (2%)		1 (2%)
Hemorrhage			1 (2%)
Inflammation, chronic	1 (2%)		1 (2%)
Ulcer			1 (2%)
Sebaceous gland, hyperplasia			1 (2%)
Subcutaneous tissue, edema		1 (2%)	1 (2%)
Musculoskeletal System			
Bone	(50)	(50)	(50)
Coccygeal, fibrosis		1 (2%)	
Coccygeal, hyperplasia		1 (2%)	
Skeletal muscle	(1)	(4)	
Inflammation, suppurative		1 (25%)	
Nervous System			
Brain	(50)	(50)	(50)
Compression	2 (4%)		
Hemorrhage	1 (2%)		
Infiltration cellular, histiocytic	1 (2%)	1 (2%)	
Inflammation, chronic	1 (2%)		
Mineralization	36 (72%)	28 (56%)	31 (62%)
Necrosis	1 (2%)		
Pigmentation	1 (2%)		
Vacuolization cytoplasmic	1 (2%)		
Spinal cord	(1)		
Degeneration	1 (100%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of γ-Butyrolactone (continued)

	Vehicle Control	262 mg/kg	525 mg/kg
Respiratory System			
Lung	(50)	(50)	(50)
Congestion	1 (2%)		2 (4%)
Hemorrhage	1 (2%)	1 (2%)	3 (6%)
Infiltration cellular, histiocytic	4 (8%)	3 (6%)	2 (4%)
Inflammation, chronic	6 (12%)	10 (20%)	7 (14%)
Inflammation, suppurative		1 (2%)	1 (2%)
Mineralization	2 (4%)		1 (2%)
Thrombus		2 (4%)	
Alveolar epithelium, hyperplasia	2 (4%)		
Mediastinum, necrosis			2 (4%)
Nose	(50)		(50)
Exudate	2 (4%)		3 (6%)
Fungus			1 (2%)
Trachea	(50)		(50)
Inflammation, chronic active			1 (2%)
Special Senses System			
Eye		(1)	(2)
Cataract		1 (100%)	1 (50%)
Exudate			1 (50%)
Cornea, hyperplasia		1 (100%)	1 (50%)
Cornea, inflammation, chronic		1 (100%)	2 (100%)
Cornea, mineralization			1 (50%)
Urinary System			
Kidney	(50)	(50)	(50)
Casts protein	9 (18%)	15 (30%)	2 (4%)
Cyst	2 (4%)	2 (4%)	1 (2%)
Glomerulosclerosis		2 (4%)	1 (2%)
Infarct		2 (4%)	
Inflammation, chronic	28 (56%)	26 (52%)	24 (48%)
Metaplasia, osseous	3 (6%)	1 (2%)	2 (4%)
Mineralization	1 (2%)	1 (2%)	2 (4%)
Renal tubule, atrophy	5 (10%)	3 (6%)	5 (10%)
Renal tubule, dilatation	1 (2%)	1 (2%)	
Renal tubule, necrosis	2 (4%)	1 (2%)	1 (2%)
Renal tubule, regeneration	2 (4%)	3 (6%)	7 (14%)
Urinary bladder	(50)	(1)	(49)
Inflammation, chronic	35 (70%)		29 (59%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA PROTOCOL

Testing was performed as reported by Haworth *et al.* (1983). γ-Butyrolactone was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strain (TA98, TA100, TA1535, or TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of γ-butyrolactone. High dose was limited to 10 mg/plate. All assays were repeated.

A positive response in this assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which is not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A response is considered negative when no increase in revertant colonies was observed after chemical treatment.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Loveday *et al.* (1989) and is briefly described as follows. γ-Butyrolactone was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCE) and chromosomal aberrations (Abs) both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each test consisted of concurrent solvent and positive controls and of at least three doses of γ-butyrolactone; the high dose was limited to 5 mg/mL.

In the SCE test without S9, CHO cells were incubated for 26 hours with γ-butyrolactone in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (2mM), and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing γ-butyrolactone was removed and replaced with fresh medium containing BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with γ-butyrolactone, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no γ-butyrolactone, and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 hours. Harvesting and staining were the same as for cells treated without S9.

In the Abs test without S9, cells were incubated in McCoy's 5A medium with γ-butyrolactone for 8 hours; Colcemid was added and incubation continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with γ-butyrolactone and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 10 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the the same manner as for the treatment without S9.

In the SCE test, because significant cell-cycle delay caused by chemical administration was seen, at the high dose in the second trial with S9, incubation time was lengthened to ensure a sufficient number of scorable cells were present. The harvest time for the Abs test was based on the cell-cycle information

obtained in the SCE test: if cell-cycle delay was anticipated, the incubation period was extended approximately 5 hours.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, usually 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose; 100 first-division metaphase cells were scored at each dose for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Abs data are presented as percentages of cells with aberrations. As with the SCE data, both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P<0.05$) difference for one dose point and a significant trend ($P<0.015$) was considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

DROSOPHILA PROTOCOL

The assay for gene mutation induction was performed as described in Zimmering *et al.* (1985). γ-Butyrolactone was supplied as a coded aliquot from Radian Corporation (Austin, TX). Initially, γ-butyrolactone was assayed in the sex-linked recessive lethal (SLRL) test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. If no clearly positive response was obtained, γ-butyrolactone was retested by injection into adult males. Because no positive response was obtained by either route of administration, the chemical was not assayed for induction of reciprocal translocations.

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

Toxicity tests were performed to set concentrations of γ-butyrolactone at a level which would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, oral exposure was achieved by allowing Canton-S males (10 to 20 flies/vial) to feed for 72 hours on a solution of γ-butyrolactone in 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of γ-butyrolactone dissolved in 0.7% saline and were allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and were given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; sample sperm from successive matings were treated at successively earlier post-meiotic stages. F_1 heterozygous females were allowed to mate with their siblings and were then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution). If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as occurring in vials containing no wild-type

males after 17 days; these were retested. The experiments, utilizing feed and injection, resulted in the testing of approximately 5,000 treated and 5,000 control chromosomes.

Recessive lethal data were analyzed by the normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater that 0.10%, or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if a) the P value was between 0.01 and 0.05 but the frequency in the treatment group was between 0.10% and 0.15%, or b) the P value was between 0.05 and 0.10 but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

RESULTS

γ-Butyrolactone (100 to 10,000 μg/plate) was tested for induction of gene mutations in *Salmonella typhimurium* strains TA100, TA1535, TA1537, and TA98 using a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; no significant increase in mutant colonies was seen (Table E1; Haworth *et al.*, 1983). Also, no induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* was observed following exposure of adult males to γ-butyrolactone by feeding (20,000 or 28,000 ppm) or by injection (15,000 ppm) (Table E4). In cytogenetic tests with CHO cells, γ-butyrolactone induced SCE (Table E2) and Abs (Table E3) in trials conducted with Aroclor 1254-induced male Sprague-Dawley rat liver S9; neither endpoint was elevated in the absence of S9 (Loveday *et al.*, 1989). In the SCE test, concentrations of 3,010 to 5,010 μg/mL yielded positive results; a delayed harvest protocol was used at the 5,010 μg/mL dose level to offset cell-cycle delay induced by chemical administration. In the Abs test, concentrations of 2,580 to 3,990 μg/mL γ-butyrolactone caused significant increases in aberrations, with no evidence of cell cycle delay.

TABLE E1
Mutagenicity of γ-Butyrolactone in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	120 ± 5.1	105 ± 7.7	143 ± 8.5	121 ± 4.5	116 ± 10.2	118 ± 4.7
	100	125 ± 8.1	109 ± 7.5	142 ± 4.5	115 ± 13.6	129 ± 13.2	134 ± 8.2
	333	125 ± 6.9	115 ± 6.4	143 ± 1.9	122 ± 5.0	130 ± 16.6	136 ± 8.1
	1,000	112 ± 9.0	125 ± 6.4	147 ± 0.9	117 ± 2.5	114 ± 13.3	140 ± 9.3
	3,333	123 ± 11.6	116 ± 4.2	136 ± 5.5	119 ± 8.4	122 ± 13.8	111 ± 3.5
	10,000	109 ± 2.3	108 ± 5.5	137 ± 6.2	120 ± 4.8	118 ± 11.4	121 ± 10.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		277 ± 18.4	419 ± 12.6	1,100 ± 18.7	778 ± 10.2	688 ± 39.0	495 ± 23.2
TA1535	0	28 ± 0.7	24 ± 4.7	12 ± 2.3	8 ± 1.9	19 ± 0.6	11 ± 2.7
	100	17 ± 1.9	28 ± 4.2	11 ± 2.9	11 ± 5.0	16 ± 1.5	15 ± 3.8
	333	24 ± 3.9	23 ± 3.7	8 ± 2.3	9 ± 5.2	15 ± 1.2	20 ± 2.0
	1,000	23 ± 3.2	27 ± 1.5	8 ± 1.0	6 ± 1.3	12 ± 2.6	18 ± 6.1
	3,333	24 ± 2.9	24 ± 2.0	12 ± 1.7	11 ± 3.5	9 ± 2.3	20 ± 3.2
	10,000	28 ± 2.2	29 ± 6.7	10 ± 5.1	17 ± 0.7	16 ± 2.0	23 ± 2.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		315 ± 14.6	379 ± 22.3	357 ± 17.6	356 ± 53.3	260 ± 7.7	120 ± 13.2
TA1537	0	6 ± 1.2	8 ± 1.5	7 ± 2.3	6 ± 0.3	16 ± 2.6	16 ± 2.5
	100	3 ± 0.6	8 ± 3.0	3 ± 1.2	9 ± 2.4	18 ± 1.8	13 ± 3.4
	333	6 ± 1.5	7 ± 0.9	5 ± 0.7	6 ± 1.0	11 ± 2.5	11 ± 2.1
	1,000	5 ± 1.5	10 ± 1.7	8 ± 3.2	11 ± 4.2	9 ± 3.0	13 ± 0.6
	3,333	4 ± 0.7	8 ± 3.3	6 ± 1.3	12 ± 2.6	14 ± 1.5	13 ± 2.6
	10,000	4 ± 1.2	12 ± 2.7	7 ± 0.7	12 ± 2.4	12 ± 2.0	16 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		110 ± 6.9	277 ± 25.1	446 ± 16.1	454 ± 17.6	217 ± 5.3	204 ± 14.8
TA98	0	18 ± 0.9	15 ± 1.5	29 ± 0.9	27 ± 3.4	24 ± 2.2	32 ± 4.1
	100	21 ± 3.3	22 ± 3.3	33 ± 3.9	26 ± 3.6	25 ± 3.0	36 ± 4.3
	333	17 ± 0.3	17 ± 4.7	31 ± 1.2	27 ± 0.9	29 ± 6.8	33 ± 3.2
	1,000	17 ± 1.8	17 ± 1.5	29 ± 6.2	27 ± 3.3	31 ± 5.4	33 ± 4.0
	3,333	16 ± 0.9	22 ± 4.4	35 ± 4.0	32 ± 2.3	29 ± 0.9	34 ± 2.6
	10,000	21 ± 3.8	15 ± 3.4	28 ± 1.8	28 ± 0.3	29 ± 1.5	37 ± 4.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		654 ± 54.9	730 ± 18.6	926 ± 12.5	477 ± 29.8	462 ± 37.8	401 ± 33.1

[a] Study performed at SRI, International. The detailed protocol and these data are presented in Haworth *et al.* (1983). Cells and γ-butyrolactone or solvent (distilled water) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose did not exceed 10 mg/plate. 0 μg/plate dose is the solvent control.

[b] Revertants are presented as mean ± standard error from three plates.

[c] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by γ-Butyrolactone[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)[b]
-S9[c]								
Trial 1								
Summary: Negative								
Medium		50	1,045	464	0.44	9.3	26.5	
Mitomycin-C	0.002	50	1,043	841	0.80	16.8	26.5	81.60
	0.010	10	208	350	1.68	35.0	26.5	278.98
γ-Butyrolactone	148	50	1,040	528	0.50	10.6	26.5	14.34
	494	50	1,040	439	0.42	8.8	26.5	-4.93
	1,480	50	1,045	515	0.49	10.3	26.5	10.99
								P=0.274[d]
+S9[e]								
Trial 1								
Summary: Weak positive								
Medium		50	1,043	478	0.45	9.6	26.0	
Cyclophosphamide	0.500	50	1,046	684	0.65	13.7	26.0	42.68
	2.500	10	209	307	1.46	30.7	26.0	220.52
γ-Butyrolactone	494	50	1,043	484	0.46	9.7	26.0	1.25
	1,480	50	1,046	509	0.48	10.2	26.0	6.18
	4,940	50	1,039	797	0.76	15.9	26.0	67.38*
								P<0.001[d]
Trial 2								
Summary: Positive								
Medium		50	1,027	470	0.45	9.4	26.0	
Cyclophosphamide	0.500	50	1,043	852	0.81	17.0	26.0	78.50
	2.500	10	210	400	1.90	40.0	26.0	316.21
γ-Butyrolactone	3,010	50	1,041	693	0.66	13.9	26.0	45.46*
	4,010	100	2,076	1,401	0.67	14.0	26.0	47.46*
	5,010	50	1,033	932	0.90	18.6	30.0[f]	97.15*
								P<0.001[d]

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by γ-Butyrolactone (continued)

* Positive (≥20% increase over solvent control)
[a] Study performed at Bioassay Systems Corporation. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol and these data are presented by Loveday *et al.* (1989). Briefly, Chinese hamster ovary cells were incubated with γ-butyrolactone or solvent (medium) as described in [c] and [e] below, and cultured for sufficient time to reach second metaphase division. Cells were then collected by mitotic shake-off, fixed, air-dried, and stained.
[b] Percent increase in SCEs/chromosome of culture exposed to γ-butyrolactone relative to those of culture exposed to solvent.
[c] In the absence of S9, cells were incubated with γ-butyrolactone or solvent for 2 hours at 37° C. Then BrdU was added and incubation was continued for 24 hours. Cells were washed, fresh medium containing BrdU and Colcemid was added, and incubation was continued for 2 to 3 hours.
[d] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose
[e] In the presence of S9, cells were incubated with γ-butyrolactone or solvent for 2 hours at 37° C. The cells were then washed, and medium containing BrdU was added. Cells were incubated for a further 26 hours, with Colcemid present for the final 2 to 3 hours. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.
[f] Because γ-butyrolactone induced a delay in the cell division cycle, harvest time was extended to maximize the proportion of second-division cells available for analysis.

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by γ-Butyrolactone[a]

-S9[b]					+S9[c]				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 10.5 hours Summary: Negative					**Trial 1** – Harvest time: 12.0 hours Summary: Positive				
Medium					Medium				
	100	2	0.02	2.0		100	1	0.01	1.0
Mitomycin-C					Cyclophosphamide				
5	100	31	0.31	22.0	50	100	79	0.79	41.0
γ-Butyrolactone					γ-Butyrolactone				
500	100	3	0.03	3.0	400	100	0	0.00	0.0
1,500	100	2	0.02	2.0	1,200	100	0	0.00	0.0
4,990	100	2	0.02	2.0	1,500	100	2	0.02	2.0
					2,990	100	84	0.84	61.0*
				P=0.559[d]	3,990	93	87	0.94	78.0*
									P<0.001
					Trial 2 – Harvest time: 12.0 hours Summary: Positive				
					Medium				
						100	0	0.00	0.0
					Cyclophosphamide				
					50	100	58	0.58	37.0
					γ-butyrolactone				
					2,210	100	4	0.04	3.0
					2,580	100	7	0.07	7.0*
					2,950	100	83	0.83	58.0*
									P<0.001

* Positive (P<0.05)

[a] Study performed at Bioassay Systems Corporation. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations and these data are found in Loveday *et al.* (1989). Briefly, Chinese hamster ovary cells were incubated with γ-butyrolactone or solvent (medium) as described in [b] and [c]. Cells were arrested in first metaphase by addition of Colcemid and harvested by mitotic shake-off, fixed, and stained in 6% Giemsa.

[b] In the absence of S9, cells were incubated with γ-butyrolactone or solvent for 8 hours at 37° C. Cells were then washed and fresh medium containing Colcemid was added for an additional 2 hours followed by harvest.

[c] In the presence of S9, cells were incubated with γ-butyrolactone or solvent for 2 hours at 37° C. Cells were then washed, medium was added, and incubation was continued for 10 hours. Colcemid was added for the last 2 hours of incubation before harvest. S9 was from the livers of Aroclor 1254-induced male Sprague-Dawley rats.

[d] Significance of % cells with Abs. tested by the linear regression trend test vs. log of the dose

TABLE E4
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by γ-Butyrolactone[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (percent)	Incidence of Sterility (percent)	No. of Lethals/No. of X Chromosomes Tested			
				Mating 1	Mating 2	Mating 3	Total[b]
Feeding	20,000	20	13	0/427	1/411	1/311	2/1,149 (0.17%)
	0			0/321	1/299	0/227	1/847 (0.12%)
Feeding	28,000	38	2	2/1,491	0/1,405	0/1,270	2/4,166 (0.05%)
	0			1/1,799	1/1,548	0/1,322	2/4,669 (0.04%)
Injection	15,000	26	13	0/2,156	1/1,634	0/1,156	1/4,946 (0.02%)
	0			0/1,960	1/1,671	1/1,400	2/5,031 (0.04%)

[a] Study performed at University of Wisconsin, Madison. A detailed protocol of the sex-linked recessive lethal assay is presented in Zimmering *et al.* (1985). In the feed exposure experiments, 24-hour-old Canton-S males were allowed to feed for 3 days on a solution of γ-butyrolactone dissolved in 5% sucrose. In the injection experiments, 24-hour-old Canton-S males were treated with a solution of γ-butyrolactone dissolved in 0.7% saline and allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three broods of 3, 2, and 2 days; sample sperm from successive matings were treated as spermatozoa (mating 1), spermatids (mating 2), and spermatocytes (mating 3). F_1 heterozygous females were crossed to their siblings and placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters; no clusters were found. After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; these were retested. Results were not significant at the 5% level by normal approximation to the binomial test (Margolin *et al.*, 1983).

[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials.

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

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Gavage Studies of γ-Butyrolactone[a]

	Vehicle Control	56 mg/kg	112 mg/kg	225 mg/kg	450 mg/kg	900 mg/kg
Male						
n	9	10	10	9	10	0
Necropsy body wt	371 ± 6	378 ± 7	381 ± 4	364 ± 5	345 ± 7**	–[b]
Brain						
Absolute	1.96 ± 0.02	1.93 ± 0.02	1.90 ± 0.06	1.92 ± 0.01	1.57 ± 0.16**	–
Relative	5.29 ± 0.06	5.12 ± 0.07	4.99 ± 0.16	5.31 ± 0.09	4.56 ± 0.45	–
Heart						
Absolute	1.00 ± 0.02	1.02 ± 0.02	1.02 ± 0.02	1.00 ± 0.02	1.04 ± 0.06	–
Relative	2.69 ± 0.04	2.71 ± 0.04	2.68 ± 0.03	2.75 ± 0.09	3.01 ± 0.16*	–
R. Kidney						
Absolute	1.23 ± 0.04	1.35 ± 0.03	1.33 ± 0.03	1.25 ± 0.03	1.24 ± 0.03	–
Relative	3.30 ± 0.07	3.57 ± 0.06	3.49 ± 0.06	3.40 ± 0.07	3.58 ± 0.06	–
Liver						
Absolute	13.55 ± 0.37	14.51 ± 0.43	15.02 ± 0.45	13.94 ± 0.50	14.17 ± 0.41	–
Relative	36.5 ± 0.7	38.4 ± 0.8	39.4 ± 1.1	38.3 ± 1.3	41.1 ± 1.1**	–
Lungs						
Absolute	1.43 ± 0.11	1.46 ± 0.03	1.55 ± 0.05	1.40 ± 0.04[c]	1.35 ± 0.04	–
Relative	3.85 ± 0.27	3.86 ± 0.05	4.06 ± 0.11	3.77 ± 0.05[c]	3.92 ± 0.12	–
Thymus						
Absolute	0.44 ± 0.03	0.44 ± 0.03	0.41 ± 0.04	0.37 ± 0.01	0.43 ± 0.03	–
Relative	1.18 ± 0.08	1.16 ± 0.08	1.07 ± 0.09	0.99 ± 0.04	1.26 ± 0.10	–
Female						
n	10	10	9	10	10	9
Necropsy body wt	205 ± 3	202 ± 3	211 ± 3	209 ± 2	203 ± 4	199 ± 3
Brain						
Absolute	1.81 ± 0.02[c]	1.77 ± 0.02	1.75 ± 0.02	1.77 ± 0.02	1.79 ± 0.01	1.76 ± 0.03
Relative	8.83 ± 0.22[c]	8.80 ± 0.09	8.31 ± 0.13	8.46 ± 0.17	8.82 ± 0.14	8.84 ± 0.11
Heart						
Absolute	0.61 ± 0.01[d]	0.65 ± 0.01	0.66 ± 0.01	0.63 ± 0.02	0.63 ± 0.01	0.66 ± 0.01
Relative	2.98 ± 0.05[d]	3.23 ± 0.04	3.10 ± 0.05	3.03 ± 0.09	3.09 ± 0.04	3.29 ± 0.05**
R. Kidney						
Absolute	0.72 ± 0.03	0.71 ± 0.02[d]	0.74 ± 0.01	0.73 ± 0.01	0.73 ± 0.02	0.72 ± 0.01
Relative	3.48 ± 0.10	3.52 ± 0.08[d]	3.50 ± 0.04	3.47 ± 0.05	3.60 ± 0.05	3.63 ± 0.05
Liver						
Absolute	7.09 ± 0.20	6.23 ± 0.10*	6.81 ± 0.34	6.86 ± 0.24	6.76 ± 0.19	6.77 ± 0.18
Relative	34.6 ± 0.9	31.0 ± 0.6*	32.3 ± 1.6	32.9 ± 1.2	33.3 ± 0.7	34.0 ± 0.6
Lungs						
Absolute	0.99 ± 0.03[c]	1.02 ± 0.02	0.98 ± 0.02	0.95 ± 0.02	1.03 ± 0.05	1.02 ± 0.01
Relative	4.84 ± 0.17[c]	5.04 ± 0.09	4.66 ± 0.09	4.53 ± 0.11	5.10 ± 0.27	5.13 ± 0.09
Thymus						
Absolute	0.32 ± 0.02	0.30 ± 0.02	0.33 ± 0.02	0.26 ± 0.02	0.29 ± 0.02	0.25 ± 0.02*
Relative	1.57 ± 0.10	1.47 ± 0.09	1.54 ± 0.11	1.25 ± 0.08	1.44 ± 0.09	1.28 ± 0.09*

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] No weights or organ-weight-to-body-weight ratios were calculated due to 100% mortality in this group.
[c] n=8
[d] n=9

TABLE F2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Gavage Studies of γ-Butyrolactone[a]

	Vehicle Control	65 mg/kg	131 mg/kg	262 mg/kg	525 mg/kg	1,050 mg/kg
Male						
n	8	6	8	9	10	7
Necropsy body wt	37.3 ± 0.8	35.8 ± 0.8	38.1 ± 0.4	35.4 ± 1.0	34.9 ± 0.8	32.9 ± 1.3**
Brain						
Absolute	0.451 ± 0.007[b]	0.442 ± 0.005	0.436 ± 0.005	0.447 ± 0.006	0.430 ± 0.007	0.443 ± 0.006
Relative	12.2 ± 0.4[b]	12.4 ± 0.3	11.4 ± 0.1	12.7 ± 0.4	12.4 ± 0.3	13.6 ± 0.4**
Heart						
Absolute	0.173 ± 0.006	0.168 ± 0.008	0.165 ± 0.002	0.157 ± 0.005	0.153 ± 0.007*	0.136 ± 0.012**
Relative	4.68 ± 0.25	4.70 ± 0.17	4.33 ± 0.07	4.44 ± 0.14	4.38 ± 0.18	4.12 ± 0.33
R. Kidney						
Absolute	0.284 ± 0.012	0.317 ± 0.007	0.305 ± 0.013	0.313 ± 0.007	0.311 ± 0.006	0.288 ± 0.011
Relative	7.67 ± 0.39	8.86 ± 0.25	8.02 ± 0.37	8.85 ± 0.16**	8.93 ± 0.16**	8.80 ± 0.35**
Liver						
Absolute	1.52 ± 0.05	1.52 ± 0.05	1.49 ± 0.03	1.46 ± 0.07	1.48 ± 0.05	1.46 ± 0.05
Relative	40.7 ± 1.0	42.4 ± 1.5	39.0 ± 0.7	41.1 ± 1.0	42.5 ± 1.2	44.4 ± 0.5*
Lungs						
Absolute	0.191 ± 0.006	0.191 ± 0.013	0.180 ± 0.008	0.193 ± 0.010	0.180 ± 0.012	0.185 ± 0.012
Relative	5.13 ± 0.12	5.31 ± 0.30	4.73 ± 0.21	5.45 ± 0.25	5.17 ± 0.38	5.67 ± 0.41
Thymus[c]						
Absolute	53.75 ± 5.81	43.33 ± 3.80	50.00 ± 3.66	42.22 ± 3.55	36.50 ± 2.59*	50.00 ± 5.12
Relative	1.43 ± 0.14	1.21 ± 0.11	1.31 ± 0.09	1.22 ± 0.13	1.05 ± 0.07	1.52 ± 0.13
Female						
n	7	7	7	10	8	7
Necropsy body wt	25.9 ± 0.5	25.3 ± 0.5	26.4 ± 0.6	26.6 ± 0.5	26.3 ± 0.5	25.1 ± 1.3
Brain						
Absolute	0.439 ± 0.008	0.448 ± 0.018[d]	0.459 ± 0.006	0.459 ± 0.006	0.445 ± 0.007	0.439 ± 0.008
Relative	17.0 ± 0.2	17.9 ± 0.7[d]	17.4 ± 0.5	17.3 ± 0.3	17.0 ± 0.5	17.7 ± 0.7
Heart						
Absolute	0.114 ± 0.003	0.123 ± 0.007[d]	0.115 ± 0.003	0.124 ± 0.004	0.118 ± 0.002	0.109 ± 0.006
Relative	4.43 ± 0.14	4.84 ± 0.29[d]	4.35 ± 0.08	4.65 ± 0.13	4.51 ± 0.09	4.33 ± 0.13
R. Kidney						
Absolute	0.168 ± 0.008	0.185 ± 0.004	0.197 ± 0.006*[d]	0.197 ± 0.009*[e]	0.193 ± 0.005	0.178 ± 0.007
Relative	6.50 ± 0.30	7.33 ± 0.18	7.37 ± 0.11*[d]	7.36 ± 0.26*[e]	7.34 ± 0.15*	7.11 ± 0.19
Liver						
Absolute	1.10 ± 0.04	1.04 ± 0.02	1.24 ± 0.04	1.21 ± 0.04	1.08 ± 0.02	1.07 ± 0.06
Relative	42.6 ± 1.1	41.0 ± 0.7	46.8 ± 0.8*	45.3 ± 1.0	41.2 ± 0.7	42.5 ± 1.5
Lungs						
Absolute	0.160 ± 0.005[d]	0.181 ± 0.007[d]	0.172 ± 0.005	0.191 ± 0.014	0.191 ± 0.016	0.162 ± 0.005
Relative	6.13 ± 0.26	7.24 ± 0.28	6.52 ± 0.19	7.17 ± 0.55	7.29 ± 0.57	6.51 ± 0.23
Thymus[c]						
Absolute	45.71 ± 6.85	61.43 ± 7.21	59.29 ± 2.77	47.50 ± 2.81	50.63 ± 4.27	49.29 ± 5.05
Relative	1.78 ± 0.27	2.42 ± 0.28	2.24 ± 0.09	1.78 ± 0.10	1.94 ± 0.17	1.95 ± 0.17

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams unless otherwise noted; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=7
[c] Weights are given in milligrams.
[d] n=6
[e] n=9

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF γ-BUTYROLACTONE

γ-Butyrolactone was obtained from GAF Corporation in one lot (lot number 600-BLO), which was used throughout the studies. Identity, purity and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (MRI), Kansas City, MO, and confirmed by the study laboratory. Reports on analyses performed in support of the γ-butyrolactone studies are on file at the National Institute of Environmental Health Sciences.

The chemical, a clear, colorless liquid, was identified as γ-butyrolactone by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of γ-butyrolactone, as shown in Figures G1 and G2 (*Sadtler Standard Spectra*; *The Merck Index*, 1983).

The purity was determined by elemental analysis, Karl Fischer water analysis, titration, thin-layer chromatography (TLC), and gas chromatography. Titration by hydrolysis of lactone was performed by refluxing with alcoholic potassium hydroxide and back titrating with sulfuric acid. TLC was performed on silica gel 60 F-254 plates with two solvent systems: 1) 100% diethyl ether and 2) 100% chloroform. After the plates were sprayed with hydroxylamine-ferric chloride, visualization was accomplished with short wave (254 nm) ultraviolet light. 6-Methylcoumarin (1 μL of a 10mg/mL diethyl ether) was used as the reference standard. Free acid was checked with TLC on a silica gel plate using solvent system 2, but with methyl red-bromothymol blue used for visualization and γ-hydroxybutyric acid as the standard. Gas chromatography was performed with a flame ionization detector (FID) and a nitrogen carrier gas at 70 mL/minute with chloroform as a solvent, with two systems:

1) 20% SP-2100 / 0.1% Carbowax 1500 on 100/120 mesh Supelcoport, oven temperature program of 50° C for 5 minutes, then 50° to 170° C at 10° C/minute, and
2) 10% Carbowax 20M-TPA on 80/100 mesh Chromasorb W(AW), oven temperature program of 50° C for 5 minutes, then 50° to 200° C at 10° C/minute.

Elemental analysis for carbon and hydrogen were slightly low. Karl Fischer water analysis indicated the presence of 0.049% ± 0.002% water. Hydrolysis and back titration indicated a purity of 100.9% ± 0.5% after subtracting the free acid content of 0.12%. TLC by the two solvent systems indicated one major spot. Gas chromatography with the first system indicated 11 impurities, three before and eight after the major peak. The two largest impurities had a combined area of 1.8% relative to the major peak; the remaining nine impurities had a combined area of 0.28% of the major peak area. The second gas system indicated four impurities, three before and one after the major peak. The largest impurity had an area of 0.62% relative to the major peak; the remaining three impurities had a combined area of 0.11%.

Stability studies were performed with gas chromatography with the second system described for the purity analyses, but with an oven temperature of 160° C, isothermal. 0.2% pentadecane in methanol was used as the internal standard. The results indicated that γ-butyrolactone was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. During the 2-year studies, the stability of the bulk chemical was monitored by the study laboratory using gas chromatography, with the system above but with an oven temperature program of 50° C for 5 minutes, then 50° to 200° C at 10° C/minute, and held at 200° C for 5 minutes. Infrared spectrometry was also performed at each analysis period. One sample analyzed at 16 months contained an impurity of 2% of the total area; this sample was believed to have been contaminated by a sample bottle or a pipette. Within 30 days of the start of the 2-year studies, the spectrum of the reference sample had increased absorption of the band at 3520 cm^{-1} which was not considered significant. No degradation of the study material was seen throughout the studies.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing appropriate quantities of γ-butyrolactone and corn oil to give the required concentrations (Table G1). The dose formulations, which were stored at 5° C, were hand agitated before administration. Dose formulations were prepared weekly and discarded 2 weeks after the date of preparation.

Stability analyses of the corn oil suspensions were conducted by the analytical chemistry laboratory. Gas chromatography was employed with the second system used in the bulk stability analyses, but with a carrier gas flow rate of 30 mL/minute, an oven temperature program of 135° C, isothermal, and an internal standard of 478 mg n-decanol/100 mL methanol. Stability of the formulation was established for at least 2 weeks when stored in sealed containers in the dark at temperatures up to 25° C.

Periodic analyses of the dose formulations of γ-butyrolactone were conducted at the study laboratory and at the analytical chemistry laboratory with the same gas chromatography method as that used in the stability studies, but with a carrier gas flow rate of 35 mL/minute. Dose formulations were analyzed twice during the 13-week studies. During the 13-week studies, 9 of 10 dose formulations for rats and 7 of 10 dose formulations for mice were within 10% of the target concentrations Table G2). The dose formulation for rats and two of the dose formulations for mice which were outside acceptable limits were used for dosing due to lack of time for remixing. During the 2-year studies, the dose formulations were analyzed at least once every 8 weeks; 41 of 42 dose formulations for rats and 27 of 28 dose formulations for mice were within 10% of the target concentrations. Results of the dose formulation analyses for the 2-year studies are presented in Table G3. Periodic analyses of the corn oil vehicle by the study laboratory demonstrated peroxide levels within the acceptable limit of 10 mEq/kg. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained for mice by the study laboratory, and results were within acceptable limits for rats Table G4).

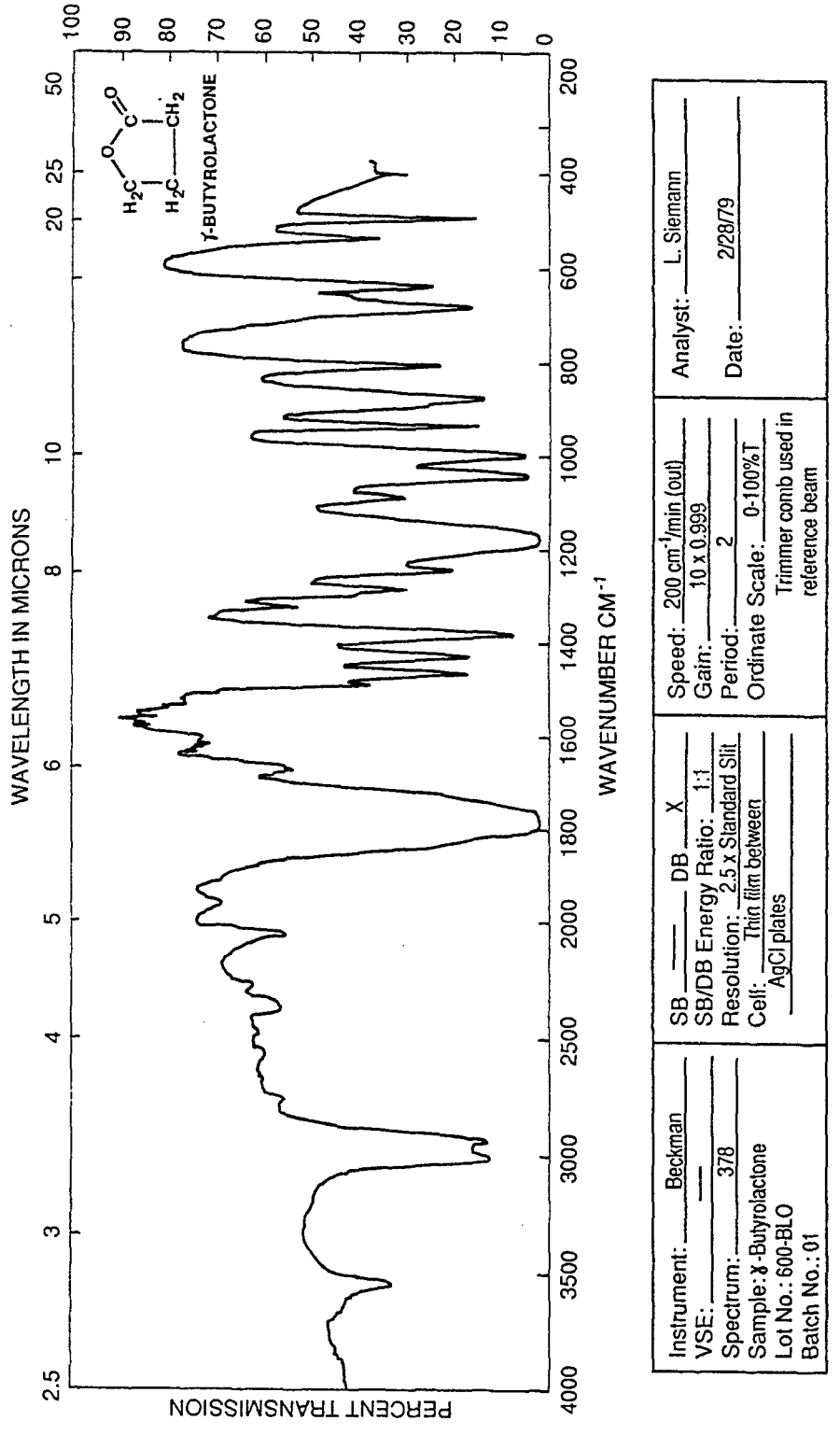

FIGURE G1
Infrared Absorption Spectrum of γ-Butyrolactone

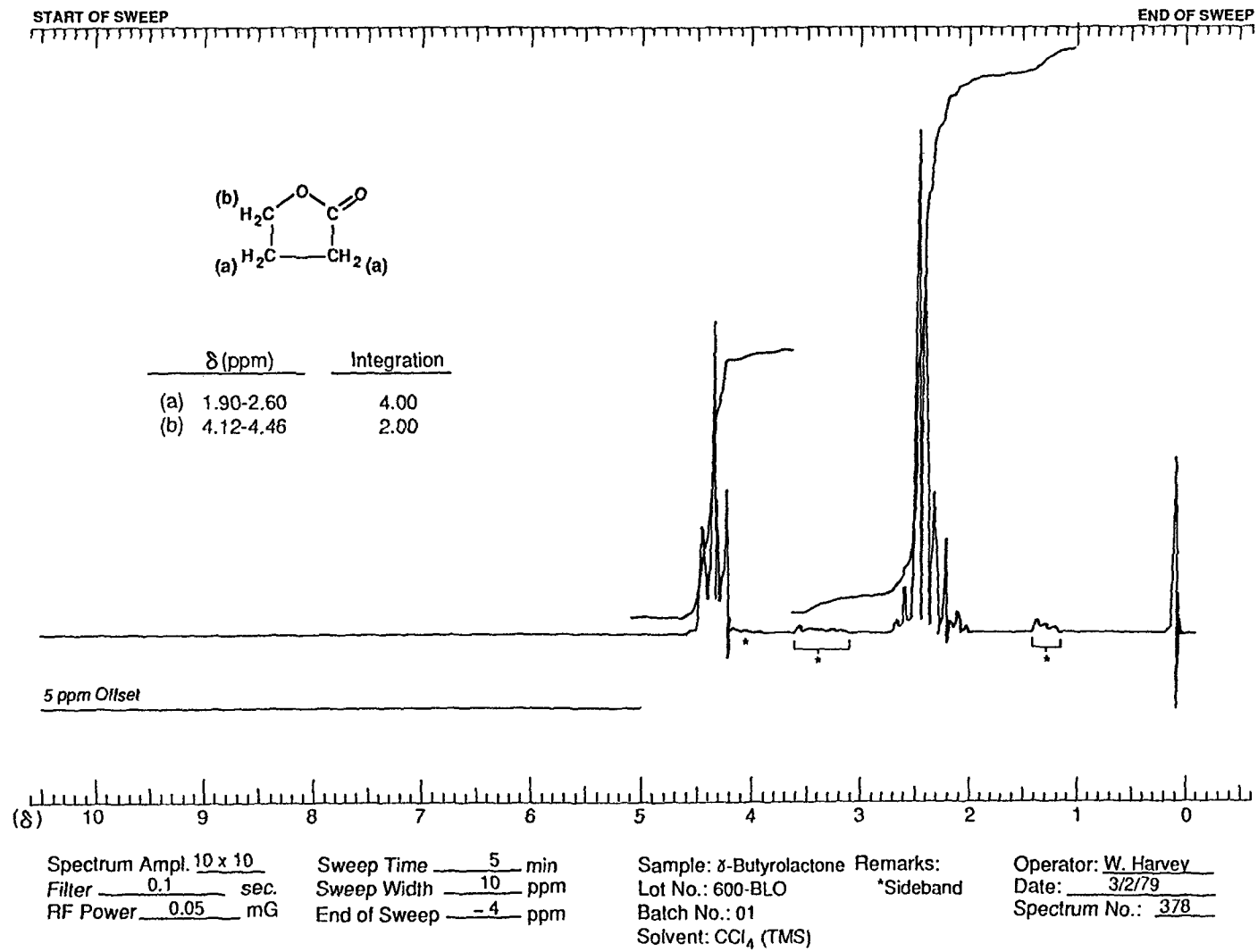

FIGURE G2
Nuclear Magnetic Resonance Spectrum of γ-Butyrolactone

TABLE G1
Preparation and Storage of Dose Formulations in the Gavage Studies of γ-Butyrolactone

16-Day Studies	13-Week Studies	2-Year Studies
Preparation		
γ-Butyrolactone was allowed to come to room temperature, then mixed with corn oil with a magnetic stirrer in appropriate concentrations (wt/vol) in a covered beaker. Formulations were hand agitated for 15 seconds before administration.	Same as 16-day studies	Same as 16-day studies
Chemical Lot Number		
600-BLO	Same as 16-day studies	Same as 16-day studies
Maximum Storage Time		
7 days from date of preparation	14 days from date of preparation	14 days from date of preparation
Storage Conditions		
Sealed in labeled serum vials and stored in the dark at 5° C	Same as 16-day studies	Same as 16-day studies
Study Laboratory		
Southern Research Institute, Birmingham, AL	Same as 16-day studies	Same as 16-day studies
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 16-day studies	Same as 16-day studies

TABLE G2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Gavage Studies of γ-Butyrolactone

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
Rats				
7 January 1981	8 January 1981	11.2	28.2	+152
		22.4	22.2	-1
		45.0	49.5	+10
		90.0	93.9	+4
		180.0	193.8	+8
11 February 1981	11 February 1981	11.2	12.2	+9
		22.4	22.6	+1
		45.0	45.1	0
		90.0	85.1	-5
		180.0	167.3	-7
Mice				
7 January 1981	8 January 1981	6.5	6.1	-6
		13.1	13.3	+2
		26.2	26.3	0
		52.5	56.7	+8
		105.0	117.2	+12
11 February 1981	11 February 1981	6.5	3.4	-48[c]
		13.1	7.8	-40[c]
		26.2	23.7	-10
		52.5	49.5	-6
		105.0	107.0	+2
13 February 1981[d]	13 February 1981	6.5	8.6	+32[c]
		13.1	20.7	+58[c]
16 February 1981[d]	16 February 1981	6.5	6.7[e]	+3
		13.1	11.6[e]	-11

[a] Rats: Dosing volume = 5 mL/kg; 11.2 mg/mL = 56 mg/kg; 22.4 mg/mL = 112 mg/kg; 45.0 mg/mL = 225 mg/kg; 90.0 mg/mL = 450 mg/kg; 180 mg/mL = 900 mg/kg
Mice: Dosing volume = 10 mL/kg; 6.5 mg/mL = 65 mg/kg; 13.1 mg/mL = 131 mg/kg; 26.2 mg/mL = 262 mg/kg; 52.5 mg/mL = 525 mg/kg; 105.0 mg/mL = 1,050 mg/kg
[b] Results of duplicate analyses
[c] Sample remixed
[d] Analysis results of remix
[e] Results of single analysis

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of γ-Butyrolactone

Date Prepared	Date Analyzed	Target Concentration[a] (mg/mL)	Determined Concentration[b] (mg/mL)	% Difference from Target
Rats				
30 October 1981	30 October 1981	22.4	22.1	-1
		45.0	45.1	0
		90.0	90.8	+1
13 November 1981	13 November 1981	22.4	22.7	+1
		90.0	91.1	+1
11 December 1981	17 December 1981	45.0	32.8	-27[c]
18 December 1981[d]	18 December 1981	45.0	44.5	-1
12 February 1982	15 February 1982	22.4	22.2	-1
		45.0	44.3	-2
		90.0	85.0	-6
9 April 1982	13 April 1982	22.4	22.0	-2
		45.0	43.5	-3
		90.0	82.1	-9
4 June 1982	9 June 1982	22.4	23.1	+3
		45.0	46.3	+3
		90.0	92.0	+2
30 July 1982	19 August 1982	22.4	23.1	+3
		45.0	46.8	+4
		90.0	95.5	+6
30 July 1982[e]	20 August 1982	22.4	22.5	0
		45.0	46.0	+2
		90.0	93.8	+4
24 September 1982	27 September 1982	22.4	21.9	-2
		45.0	44.5	-1
		90.0	94.8	+5
19 November 1982	19 November 1982	22.4	22.4	0
		45.0	45.1	0
		90.0	90.3	0
10 January 1983	11 January 1983	22.4	23.5	+5
		45.0	47.5	+6
		90.0	92.9	+3
10 January 1983[e]	19 January 1983	22.4	23.1	+3
		45.0	46.4	+3
		90.0	95.3	+6
7 March 1983	8 March 1983	22.4	22.6	+1
		45.0	46.6	+4
		90.0	92.6	+3

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of γ-Butyrolactone (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Rats (continued)				
2 May 1983	3 May 1983	22.4	22.1	-1
		45.0	45.2	0
		90.0	92.1	+2
27 June 1983	28 June 1983	22.4	21.9	-2
		45.0	46.6	+4
		90.0	97.5	+8
27 June 1983[e]	6 July 1983	22.4	22.3	0
		45.0	45.4	+1
		90.0	94.8	+5
22 August 1983	24 August 1983	22.4	23.8	+6
		45.0	45.7	+2
		90.0	92.9	+3
17 October 1983	17 October 1983	22.4	23.1	+3
		45.0	44.3	-2
		90.0	89.8	0
Mice				
23 October 1981	23 October 1981	26.2	26.1	0
		52.5	52.6	0
13 November 1981	13 November 1981	52.5	52.0	-1
11 December 1981	17 December 1981	26.2	19.3	-26[c]
18 December 1981[d]	18 December 1981	26.2	25.6	-2
12 February 1982	15 February 1982	26.2	28.5	+9
		52.5	52.1	-1
9 April 1982	13 April 1982	26.2	25.4	-3
		52.5	49.2	-6
4 June 1982	9 June 1982	26.2	26.7	+2
		52.5	53.2	+1
30 July 1982	19 August 1982	26.2	26.4	+1
		52.5	53.6	+2
30 July 1982[e]	20 August 1982	26.2	26.7	+2
		52.5	52.9	+1
24 September 1982	27 September 1982	26.2	25.7	-2
		52.5	52.9	+1
19 November 1982	19 November 1982	26.2	26.1	0
		52.5	52.3	0

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of γ-Butyrolactone (continued)

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	% Difference from Target
Mice (continued)				
10 January 1983	11 January 1983	26.2	26.8	+2
		52.5	53.5	+2
10 January 1983[e]	19 January 1983	26.2	27.0	+3
		52.5	53.5	+2
7 March 1983	8 March 1983	26.2	26.3	0
		52.5	51.8	-1
2 May 1983	3 May 1983	26.2	25.9	-1
		52.5	53.5	+2
27 June 1983	28 June 1983	26.2	26.7	+2
		52.5	54.0	+3
27 June 1983[e]	6 July 1983	26.2	25.7	-2
		52.5	54.6	+4
22 August 1983	24 August 1983	26.2	25.7	-2
		52.5	51.7	-2
17 October 1983	17 October 1983	26.2	25.9	-1
		52.5	51.9	-1

[a] Rats: Dosing volume = 5 mL/kg; 22.4 mg/mL = 112 mg/kg; 45.0 mg/mL = 225 mg/kg; 90.0 mg/mL = 450 mg/kg
Mice: Dosing volume = 10 mL/kg; 26.2 mg/mL = 262 mg/kg; 52.5 mg/mL = 525 mg/kg
[b] Results of duplicate analyses
[c] Sample remixed
[d] Analysis results of remix
[e] Animal room samples

TABLE G4
Results of Referee Analysis of Dose Formulations in the 2-Year Gavage Studies of γ-Butyrolactone

Date Mixed	Target Concentration[a] (mg/mL)	Determined Concentration (mg/mL)	
		Study Laboratory[b]	Referee Laboratory[c]
Rats			
30 July 1982	45.0	46.8	43.9 ± 0.82
27 June 1983	90.0	97.5	93.8 ± 2.70
Mice			
11 December 1981	26.2	19.3	26.1 ± 0.09
10 January 1983	52.5	53.5	51.4 ± 0.18

[a] Rats: Dosing volume = 5 mL/kg; 45.0 mg/mL = 225 mg/kg; 90.0 mg/mL = 450 mg/kg
Mice: Dosing volume = 10 mL/kg; 26.2 mg/mL = 262 mg/kg; 52.5 mg/mL = 525 mg/kg
[b] Results of duplicate analysis
[c] Results of triplicate analysis. Mean ± standard deviation

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE H1
Ingredients of NIH-07 Rat and Mouse Ration[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
Dried brewer's yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

[a] NCI, 1976; NIH, 1978
[b] Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE H2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[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
d-α-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		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

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

TABLE H3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	23.5 ± 0.7	22.2–24.9	25
Crude fat (% by weight)	4.9 ± 0.5	3.3–5.7	25
Crude fiber (% by weight)	3.3 ± 0.3	2.9–3.8	25
Ash (% by weight)	6.5 ± 0.5	5.7–7.3	25
Amino Acids[a] (% of total diet)			
Arginine	1.323 ± 0.830	1.21–1.39	4
Cystine	0.310 ± 0.099	0.218–0.400	4
Glycine	1.155 ± 0.069	1.06–1.21	4
Histidine	0.572 ± 0.030	0.530–0.603	4
Isoleucine	0.910 ± 0.033	0.881–0.944	4
Leucine	1.949 ± 0.065	1.85–1.99	4
Lysine	1.279 ± 0.075	1.20–1.37	4
Methionine	0.422 ± 0.187	0.306–0.699	4
Phenylalanine	0.909 ± 0.167	0.665–1.04	4
Threonine	0.844 ± 0.029	0.824–0.886	4
Tryptophan	0.187	0.171–0.211	3
Tyrosine	0.631 ± 0.094	0.566–0.769	4
Valine	1.11 ± 0.05	1.05–1.17	4
Essential Fatty Acids[a] (% of total diet)			
Linoleic	2.44	2.37–2.52	3
Linolenic	0.274	0.256–0.308	3
Arachidonic	0.008		1
Vitamins[a]			
Vitamin A (IU/kg)	12,052 ± 4,522	4,100–24,000	25
Vitamin D (IU/kg)	3,650	3,000–6,300	2
α–Tocopherol (ppm)	41.53 ± 7.52	31.1–48.9	4
Thiamine (ppm)	16.4 ± 2.2	13.0–21.0	25
Riboflavin (ppm)	7.5 ± 1.0	6.1–8.2	4
Niacin (ppm)	85.0 ± 14.2	65.0–97.0	4
Pantothenic acid (ppm)	29.3 ± 4.6	23.0–34.0	4
Pyridoxine (ppm)	7.6 ± 1.5	5.6–8.8	4
Folic acid (ppm)	2.8 ± 0.9	1.8–3.7	4
Biotin (ppm)	0.27 ± 0.05	0.21–0.32	4
Vitamin B_{12} (ppb)	21.0 ± 11.9	11.0–38.0	4
Choline (ppm)	3,302.0 ± 120.0	3,200–3,430	4

TABLE H3
Nutrient Composition of NIH-07 Rat and Mouse Ration (continued)

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Minerals[a]			
Calcium (%)	1.27 ± 0.11	1.11–1.44	25
Phosphorus (%)	0.98 ± 0.05	0.9–1.1	25
Potassium (%)	0.86 ± 0.10	0.772–0.970	3
Chloride (%)	0.55 ± 0.10	0.442–0.635	4
Sodium (%)	0.311 ± 0.038	0.258–0.350	4
Magnesium (%)	0.169 ± 0.133	0.151–0.181	4
Sulfur (%)	0.316 ± 0.070	0.270–0.420	4
Iron (ppm)	447.0 ± 57.3	409–523	4
Manganese (ppm)	90.6 ± 8.2	81.7–95.5	4
Zinc (ppm)	53.6 ± 5.3	46.1–58.6	4
Copper (ppm)	10.77 ± 3.19	8.09–15.39	4
Iodine (ppm)	2.95 ± 1.05	1.52–3.82	4
Chromium (ppm)	1.81 ± 0.28	1.44–2.09	4
Cobalt (ppm)	0.68 ± 0.14	0.49–0.80	4

[a] One to four batches of feed were manufactured during 1983-1985.

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.53 ± 0.13	0.27–0.77	25
Cadmium (ppm)	<0.1	<0.1–0.1	25
Lead (ppm)	0.80 ± 0.64	0.33–3.37	25
Mercury (ppm)	<0.05		25
Selenium (ppm)	0.29 ± 0.06	0.14–0.38	25
Aflatoxins (ppb)[b]	<10	<5–<10	25
Nitrate nitrogen (ppm)[c]	9.2 ± 4.7	<0.1–22.0	25
Nitrite nitrogen (ppm)[c]	2.3 ± 1.9	<0.1–7.2	25
BHA (ppm)[d]	5.1 ± 4.9	<20–17	25
BHT (ppm)[d]	2.9 ± 2.7	<1.0–12.0	25
Aerobic plate count (CFU/g)[e]	44,180 ± 35,870	5,500–130,000	25
Coliform (MPN/g)[f]	11.5 ± 20.1	<3–93	24
Coliform (MPN/g)[g]	32.8 ± 91.7	<3–460	25
E. coli (MPN/g)[h]	<3		25
Total nitrosoamines (ppb)[i]	4.0 ± 2.6	0.8–9.3	25
N-Nitrosodimethylamine (ppb)[i]	3.1 ± 2.5	0.8–8.3	25
N-Nitrosopyrrolidine (ppb)	1.14 ± 0.47	<0.9–2.9	25
Pesticides (ppm)			
α-BHC[j]	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor[k]	<0.05	0.06 (26 July 1983)	25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.1		25
Estimated PCBs	<0.2		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.1		25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion[l]	0.10 ± 0.10	<0.05–0.45	25
Endosulfan I[m]	<0.01		23
Endosulfan II[m]	<0.01		23
Endosulfan sulfate[m]	<0.03		23

TABLE H4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] The detection limit was reduced from 10 ppb to 5 ppb after July 1981.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] CFU = colony-forming unit
[f] MPN = most probable number. Excludes one high value of 460 MPN/g obtained from the lot milled on 23 September 1982.
[g] Includes the high value obtained from the lot milled on 23 September 1982.
[h] All values were less than 3 MPN/g.
[i] All values were corrected for percent recovery.
[j] BHC = hexachlorocyclohexane or benzene hexachloride
[k] The value and date of one observation which was above the detection limit is given under the range. All other values were less than the detection limit.
[l] Twelve lots contained more than 0.05 ppm.
[m] Two batches milled on (26 October 1981 and 25 November 1981) were not analyzed.

APPENDIX I
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Rats

Fifteen F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	6, 12, 18, and 24 months
Sendai	6, 12, 18, and 24 months
KRV (Kilham rat virus)	6, 12, 18, and 24 months
H-1 (Toolan's H-1 virus)	6, 12, 18, and 24 months
Complement Fixation	
RCV (rat corona virus)	6 and 12 months
ELISA	
RCV/SDA (sialodacryoadenitis virus)	18 and 24 months
Mycoplasma pulmonis	18 and 24 months

Mice

Fifteen B6C3F$_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6, 12, 18, and 24 months
Reovirus 3	6, 12, 18, and 24 months
GDVII (mouse encephalomyelitis virus)	6, 12, 18, and 24 months
Polyoma virus	6, 12, 18, and 24 months
Sendai	6, 18, and 24 months
MVM (minute virus of mice)	6, 12, 18, and 24 months
Ectromelia virus (mouse pox)	6, 12, 18, and 24 months
Complement Fixation	
Mouse adenoma virus	6, 12, 18, and 24 months
LCM (lymphocytic choriomeningitis virus)	6, 12, 18, and 24 months
Sendai	12 months
ELISA	
MHV (mouse hepatitis virus)	6, 12, 18, and 24 months
Mycoplasma pulmonis	24 months

RESULTS

The serology results for sentinel animals are presented in Table I1.

TABLE I1
Murine Virus Antibody Determinations for Rats and Mice in the 2-Year Gavage Studies of γ-Butyrolactone

	Interval (months)	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
Rats			
	6	0/10	none positive
	12	0/10	none positive
	18	4/9[a]	*M. pulmonis*
	24	0/10	none positive
Mice			
	6	0/5	none positive
	12	0/10	none positive
	18	0/9	none positive
	24	0/10	none positive

[a] Further evaluation of this assay indicated that is was not specific for *Mycoplasma pulmonis*, and these results were considered to be false positive.

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichloroethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone

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338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Roxarsone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
386	Tetranitromethane
387	Amphetamine Sulfate
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
393	Sodium Fluoride
395	Probenecid
396	Monochloroacetic Acid
399	Titanocene Dichloride
405	C.I. Acid Red 114
415	Polysorbate 80

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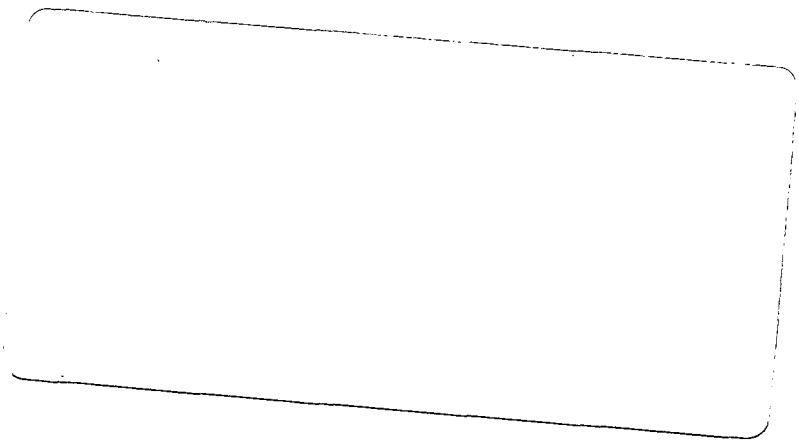

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