NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 458

TOXICOLOGY AND CARCINOGENESIS STUDIES OF BUTYL BENZYL PHTHALATE (CAS NO. 85-68-7) IN F344/N RATS (FEED 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. The interpretive conclusions presented in this Technical Report are based only on the results of these NTP studies. Extrapolation of these results to other species and quantitative risk analyses for humans require wider analyses beyond the purview of these studies. Selection *per se* is not an indicator of a chemical's carcinogenic potential.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge while supplies last from NTP Central Data Management, NIEHS, P.O. Box 12233, MD E1-02, Research Triangle Park, NC 27709 (919-541-3419). Listings of all published NTP reports and ongoing studies are also available from NTP Central Data Management. The Abstracts and other study information for 2-year studies are also available at the NTP's World Wide Web site: http://ntp-server.niehs.nih.gov.

NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF BUTYL BENZYL PHTHALATE

(CAS NO. 85-68-7)

IN F344/N RATS

(FEED STUDIES)

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

September 1997

NTP TR 458

NIH Publication No. 97-3374

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

CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings

F.W. Kari, Ph.D., Study Scientist
G.A. Boorman, D.V.M., Ph.D.
D.A. Bridge, B.S.
J.R. Bucher, Ph.D.
L.T. Burka, Ph.D.
M.R. Elwell, D.V.M., Ph.D.
J.R. Hailey, D.V.M.
J.K. Haseman, Ph.D.
G.N. Rao, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
G.S. Travlos, D.V.M.
D.B. Walters, Ph.D.
K.L. Witt, M.S., Oak Ridge Associated Universities

Hazleton Laboratories America, Inc.
Conducted 10-week modified mating study and 26-week study, evaluated pathology findings

G.W. Wolfe, Ph.D., Principal Investigator
M.R. Moore, Ph.D.
M. Steinberg, Ph.D.

Southern Research Institute
Conducted 2-year study, evaluated pathology findings

J.D. Prejean, Ph.D., Principal Investigator
H.D. Giles, Ph.D., D.V.M.
C. Lindamood, Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology quality assurance

J.F. Hardisty, D.V.M., Principal Investigator
E.T. Gaillard, M.S., D.V.M.,
C.C. Shackelford, D.V.M., M.S., Ph.D.

NTP Pathology Working Group
Evaluated slides, prepared pathology report on rats (10 January 1995)

P.K. Hildebrandt, D.V.M., Chairperson
PATHCO, Inc.
M.R. Elwell, D.V.M., Ph.D.
National Toxicology Program
E.T. Gaillard, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
J.R. Hailey, D.V.M.
National Toxicology Program
A. Radovsky, D.V.M., Ph.D.
National Toxicology Program
C.C. Shackelford, D.V.M., M.S., Ph.D.
Experimental Pathology Laboratories, Inc.
B.G. Short, D.V.M., Ph.D.
SmithKline Beecham

Analytical Sciences, Inc.
Provided statistical analyses

R.W. Morris, M.S., Principal Investigator
S.R. Lloyd, M.S.
N.G. Mintz, B.S.

Dynamac Corporation
Prepared quality assurance audits

S. Brecher, Ph.D., Principal Investigator

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
J.R. Carlton, B.A.
G. Gordon, M.A.
L.M. Harper, B.S.
M.J. Nicholls, B.S.
D.C. Serbus, Ph.D.

CONTENTS

ABSTRACT 5

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY 9

TECHNICAL REPORTS REVIEW SUBCOMMITTEE 10

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS 11

INTRODUCTION 13

MATERIALS AND METHODS 21

RESULTS 33

DISCUSSION AND CONCLUSIONS 51

REFERENCES 55

APPENDIX A Summary of Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 63

APPENDIX B Summary of Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 103

APPENDIX C Genetic Toxicology 137

APPENDIX D Organ Weights and Organ-Weight-to-Body-Weight Ratios 155

APPENDIX E Hematology and Hormone Assay Results 159

APPENDIX F Reproductive Tissue Evaluations and Modified Mating Study Results 167

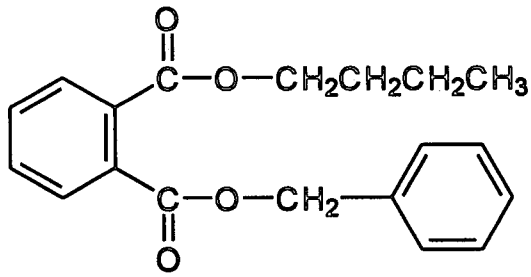
APPENDIX G Chemical Characterization and Dose Formulation Studies 171

APPENDIX H Feed and Compound Consumption in the 2-Year Feed Study of Butyl Benzyl Phthalate 183

APPENDIX I Ingredients, Nutrient Composition, and Contaminant Levels in NIH-07 Rat and Mouse Ration 187

APPENDIX J Sentinel Animal Program 191

ABSTRACT



BUTYL BENZYL PHTHALATE

CAS No. 85-68-7

Chemical Formula: $C_{19}H_{20}O_4$ Molecular Weight: 312.39

Synonyms: A13-14777; BBP; 1,2-benzenedicarboxylic acid butyl phenylmethyl ester (9CI); benzyl *n*-butyl phthalate; *n*-butyl benzyl phthalate; butyl phenylmethyl 1,2-benzenedicarboxylate; phthalic acid benzyl butyl ester (8CI)
Trade names: Palatinol BB; Santicizer 160; Sicol 160; Unimoll BB

Butyl benzyl phthalate is a plasticizer added to polymers to give flexibility and softness. It is used extensively in polyvinyl chloride and in cellulose plastics, polyvinyl acetate, polysulfides, and polyurethane. Butyl benzyl phthalate was nominated as part of a class study of phthalates. Previous studies of butyl benzyl phthalate by the NTP (1982a) resulted in chemical-related mortality in male rats beginning at about 14 weeks of exposure and, thus, were inadequate for evaluating carcinogenicity in male rats. The companion studies revealed a marginal increase in leukemia in female rats and no evidence of carcinogenicity in $B6C3F_1$ mice. Consequently, the present evaluations were conducted only in F344/N rats. Male and female F344/N rats were given butyl benzyl phthalate (at least 97% pure) in feed for 10 weeks, 26 weeks, or 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, L5178Y mouse lymphoma cells, cultured Chinese hamster ovary cells, mouse bone marrow cells, and *Drosophila melanogaster*.

10-Week Modified Mating Study in Rats

Groups of 15 male F344/N rats were given 0, 300, 2,800, or 25,000 ppm butyl benzyl phthalate (equivalent to average daily doses of approximately 20, 200, or 2,200 mg butyl benzyl phthalate/kg body weight) in feed for 10 weeks. All rats survived to the end of the study. The final mean body weight and body weight gain of the 25,000 ppm group were significantly less than those of the controls. Feed consumption by the 25,000 ppm group was less than that by the controls at the end of the study. A few minimal hematology changes occurred in the 25,000 ppm male rats. There was some evidence of a minimal anemia characterized by a decreased erythrocyte count and increases in mean cell hemoglobin and platelet count. The absolute and relative prostate gland weights of the 25,000 ppm males were significantly less than those of the controls. Degeneration of the seminiferous

tubule germinal epithelium was observed in all males from the 25,000 ppm group. The absolute right cauda, right epididymis, and right testis weights of the 25,000 ppm males were significantly less than those of the controls. The epididymal spermatozoal concentrations in 2,800 and 25,000 ppm males were significantly less than that in the controls. Although 10 females mated to 25,000 ppm males were initially found to be sperm positive, none of these females were pregnant at necropsy. The fertility indices of males and females in the 25,000 ppm group were significantly lower than those of the controls. The maternal body weights of females mated to 300 and 2,800 ppm males were similar to those of females mated to control males. There were no significant differences in litter data between the controls and the 300 and 2,800 ppm groups.

26-Week Study in Rats

Groups of 15 male F344/N rats were given 0, 300, 900, 2,800, 8,300, or 25,000 ppm butyl benzyl phthalate in feed for 26 weeks. Dietary levels of 300, 900, 2,800, and 8,300 ppm delivered average daily doses of approximately 30, 60, 180, and 550 mg butyl benzyl phthalate/kg body weight. The final mean body weight and body weight gain of the 25,000 ppm males were significantly less than those of the controls. Except for the 25,000 ppm males, feed consumption by all exposed groups was similar to that by the controls. An exposure-related macrocytic responsive anemia was present in the 25,000 ppm group at all time points. Additionally, minimal erythrocyte count decreases occurred sporadically in the 2,800 and 8,300 ppm groups at various time points. Reticulocyte counts were increased on days 60 and 90. Increases in mean cell hemoglobin and mean cell hemoglobin concentrations occurred in the 8,300 and 25,000 ppm rats. The absolute right cauda, right epididymis, and right testis weights and the sperm concentration of 25,000 ppm males were significantly less than those of the controls. The incidences of hypospermia and of atrophy of the seminiferous tubule in the testis and of hypospermia in the epididymis in 25,000 ppm males were significantly greater than those in the controls. Degenerative changes of the testis and epididymis in the 25,000 ppm males were qualitatively and quantitatively similar to those observed in males in the 10-week modified mating study.

2-Year Study in Rats

Groups of 60 male F344/N rats were given 0, 3,000, 6,000, or 12,000 ppm butyl benzyl phthalate (equivalent to average daily doses of approximately 120, 240, or 500 mg butyl benzyl phthalate/kg body weight), and groups of 60 female F344/N rats were given 0, 6,000, 12,000, or 24,000 ppm butyl benzyl phthalate (equivalent to average daily doses of approximately 300, 600, or 1,200 mg/kg) in feed for 2 years.

Survival, Body Weights, and Feed Consumption

Survival of all exposed groups of male and female rats was similar to that of the controls. Mean body weights of the 12,000 ppm males and 24,000 ppm females were less than those of the controls throughout most of the study. Feed consumption by the females exposed to 24,000 ppm was less than that by the controls at the beginning of the study, but was similar to that by the controls by week 6.

Hematology and Hormone Assays

In general, hematology changes were sporadic and minor. At 6 months, a minimal decrease in erythrocyte count and an increase in mean cell hemoglobin, similar to that which occurred in the 26-week study, occurred in male rats in the 12,000 ppm group. In female rats, a decreased hematocrit value occurred at 15 months in the 24,000 ppm group. There was also a mild decrease in triiodothyronine concentrations in the 24,000 ppm females at 6 and 15 months and at the end of the study.

Pathology Findings

At 2 years, the incidences of pancreatic acinar cell adenoma and adenoma or carcinoma (combined) in 12,000 ppm males were significantly greater than those in the controls. The incidences of adenoma and of adenoma or carcinoma (combined) in 12,000 ppm males exceeded the ranges of historical controls from NTP 2-year feed studies. One carcinoma was observed in one 12,000 ppm male, and two adenomas were observed in 24,000 ppm females. At 2 years,

the incidence of focal hyperplasia of the pancreatic acinar cell in 12,000 ppm males was significantly greater than that in the controls.

At 2 years, transitional epithelial papillomas in the urinary bladder were observed in one control female and in two 24,000 ppm females. The incidence of this neoplasm exceeded the range of historical controls from NTP 2-year feed studies. The incidence of transitional epithelial hyperplasia in 24,000 ppm females was significantly greater than that in the controls.

The absolute right kidney weight of 12,000 ppm females and the relative right kidney weights of all exposed groups of males and of 24,000 ppm females were significantly greater than those of the controls at the 15-month interim evaluation. The severities of renal tubule pigmentation in 12,000 ppm males and in 24,000 ppm females were greater than those in the controls at 15 months and 2 years. At 2 years, the incidences of kidney mineralization in 6,000 and 24,000 ppm females were significantly less than that in the controls, and the severity was decreased in exposed females. The incidence of preputial gland adenoma or carcinoma (combined) in 12,000 ppm male rats was significantly less than in the controls, and the incidences occurred with a negative trend.

Genetic Toxicology

Results from *in vitro* mutagenicity tests with butyl benzyl phthalate were uniformly negative. No mutagenic response was obtained in any of several strains of *Salmonella typhimurium* treated with up to 11,550 μg/plate butyl benzyl phthalate, with or without S9 metabolic activation enzymes. Negative results were also obtained in *in vitro* studies of mammalian cell systems with and without S9. No induction of trifluorothymidine resistance in L5178Y mouse lymphoma cells or sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells were observed. These assays also were conducted with and without S9.

No significant increase in sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* after administration of butyl benzyl phthalate either in feed or by injection.

In contrast to the negative results obtained *in vitro* and in *Drosophila*, butyl benzyl phthalate gave positive responses in two *in vivo* studies with mice. Results of a mouse bone marrow sister chromatid exchange test were positive at sample times of 23 and 42 hours, but no confirmatory test was conducted. Chromosomal aberrations were induced in bone marrow cells of male mice sampled 17 hours after intraperitoneal injection of 5,000 mg/kg butyl benzyl phthalate.

Conclusions

Under the conditions of this 2-year feed study, there was *some evidence of carcinogenic activity** of butyl benzyl phthalate in male F344/N rats based on the increased incidences of pancreatic acinar cell adenoma and of acinar cell adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of butyl benzyl phthalate in female 344/N rats based on the marginally increased incidences of pancreatic acinar cell adenoma and of transitional epithelial papilloma of the urinary bladder.

Exposure of rats to butyl benzyl phthalate in feed for 2 years resulted in focal hyperplasia in the pancreas in male rats and in transitional epithelial hyperplasia in the urinary bladder of female rats.

---

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Butyl Benzyl Phthalate

	Male F344/N Rats	Female F344/N Rats
Doses	0, 3,000, 6,000, or 12,000 ppm in feed	0, 6,000, 12,000, or 24,000 ppm in feed
Body weights	12,000 ppm group less than control group	24,000 ppm group less than control group
2-Year survival rates	28/50, 20/50, 22/50, 22/50	25/50, 29/50, 29/50, 29/50
Nonneoplastic effects	Pancreas: focal hyperplasia (4/50, 7/49, 9/50, 12/50)	Urinary bladder: transitional epithelial hyperplasia (4/50, 0/50, 1/50, 10/50)
Neoplastic effects	Pancreas: acinar cell adenoma (3/50, 2/49, 3/50, 10/50); acinar cell adenoma or carcinoma (3/50, 2/49, 3/50, 11/50)	None
Uncertain findings	None	Pancreas: acinar cell adenoma (0/50, 0/50, 0/50, 2/50) Urinary bladder: transitional epithelial papilloma (1/50, 0/50, 0/50, 2/50)
Level of evidence of carcinogenic activity	Some evidence	Equivocal evidence
Genetic toxicology		
Salmonella typhimurium gene mutations:	Negative in strains TA98, TA100, TA1535, and TA1537 with and without S9	
L5178Y Mouse lymphoma gene mutations:	No induction of trifluorothymidine resistance	
Sister chromatid exchanges		
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9	
Mouse bone marrow *in vivo*:	Weakly positive at 23 and 42 hours	
Chromosomal aberrations		
Cultured Chinese hamster ovary cells *in vitro*:	Negative with and without S9	
Mouse bone marrow *in vivo*:	Positive at 17 hours; negative at 36 hours	
Sex-linked recessive lethal mutations		
Drosophila melanogaster:	No induction of sex-linked recessive lethal mutations	

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 butyl benzyl phthalate on 20 June 1995 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing NTP studies:

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

Arnold L. Brown, M.D., Chairperson
University of Wisconsin Medical School
Madison, WI

Thomas L. Goldsworthy, Ph.D.
Department of Experimental Pathology and Toxicology
Chemical Industry Institute of Toxicology
Research Triangle Park, NC

Meryl H. Karol, Ph.D.
Department of Environmental Occupational Health
University of Pittsburgh
Pittsburgh, PA

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

Claudia S. Miller, M.D.
University of Texas Health Sciences Center
San Antonio, TX

Janardan K. Reddy, M.D., Principal Reviewer
Department of Pathology
Northwestern University Medical School
Chicago, IL

Irma Russo, M.D.
Fox Chase Cancer Center
Philadelphia, PA

Louise Ryan, Ph.D., Principal Reviewer
Division of Biostatistics
Dana-Farber Cancer Institute
Boston, MA

Robert E. Taylor, M.D., Ph.D.
Department of Pharmacology
Howard University College of Medicine
Washington, DC

Mary Jo Vodicnik, Ph.D.
Lilly MSG Development Center
Belgium

Jerrold M. Ward, D.V.M., Ph.D.
National Cancer Institute
Frederick, MD

SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 20 June 1995, the draft Technical Report on the toxicology and carcinogenesis studies of butyl benzyl phthalate received public review by the National Toxicology Program's 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. F.W. Kari, NIEHS, introduced the toxicology and carcinogenesis studies of butyl benzyl phthalate by describing the uses of the chemical and the rationale for study, describing the experimental design, reporting on survival and body weight effects, and commenting on chemical-related neoplastic lesions in male rats and uncertain neoplastic findings in female rats as well as chemical-related nonneoplastic lesions in male and female rats. Dr. Kari noted that there had been an earlier 2-year NTP study with butyl benzyl phthalate in which there was no evidence of adverse effects in mice and a marginally increased incidence of mononuclear cell leukemia in female rats. This study was inadequate in male rats due to high mortality beginning around week 14. Thus, the design of the subchronic phase of the current study in male rats was more elaborate in an attempt to determine if early mortality would be a problem. The design included a 10-week modified mating trial as well as other indices of reproductive toxicity. The proposed conclusions for the 2-year study were *some evidence of carcinogenic activity* in male F344/N rats and *equivocal evidence of carcinogenic activity* in female F344/N rats.

Dr. Reddy, a principal reviewer, agreed with the proposed conclusions although he thought that the proposed conclusions from the previous 2-year study in mice should be cited. Dr. Kari responded that this information would be added to the Abstract. Dr. Reddy noted the 20% incidence of pancreatic acinar cell adenoma versus only a 2% incidence of carcinoma in 12,000 ppm male rats. He asked for definition of the criteria for distinguishing adenomas from carcinomas since, based on his experience, he wondered if carcinomas were underrepresented. Dr. J.R. Hailey, NIEHS, said more would be added concerning how the distinction was made between adenomas and carcinomas (p. 43). While metastasis was an easy marker for a carcinoma, other features were used such as cellular pleomorphism or tremendous heterogeneity in the growth pattern, cellular atypia, and high mitotic index.

Dr. Klaassen, the second principal reviewer, agreed with the proposed conclusions. He said that reference to the mouse studies should be made earlier in the Technical Report, preferably in the Abstract, and that more information should be included on phthalate carcinogenicity in regard to the rationale (p. 20). Because of the known effects of phthalates on male reproduction, Dr. Klaassen was pleased to see reproduction studies in this Technical Report.

Dr. Ryan, the third principal reviewer, agreed with the proposed conclusions. She wondered whether there should also be a conclusion regarding the reproductive toxicity. Dr. Ryan found it worrisome that so many animals died in the 26-week study during anesthesia prior to blood sampling and asked whether there was any bias here whereby weaker or sicker animals were more likely to die during the procedure. Dr. Kari said he could only speculate that the higher ratio of carbon dioxide to oxygen than generally used may have contributed to the excessive mortality. It did not appear to be due to chemical interaction, because the numbers of deaths in control animals were similar to those in exposed groups of animals.

Dr. Miller noted the National Institute for Occupational Safety and Health (NIOSH) reference that over 300,000 workers were potentially exposed to butyl benzyl phthalate and asked whether there were data about occupational exposure in terms of airborne concentrations. Dr. J. Haartz, NIOSH, commented that their database does not have such quantitative information. She reported that the Occupational Safety and Health Administration does not have an exposure database and offered to follow up on that for Dr. Kari.

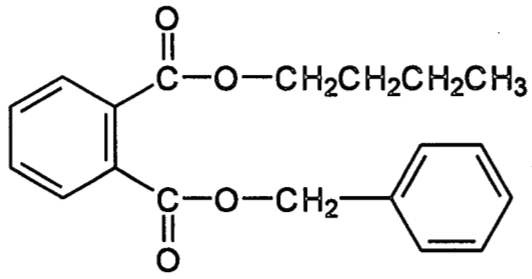
Dr. M. Stevens, Manager of Toxicology Projects, Monsanto Business Services, stated that the biggest

concern of the Monsanto Company, a primary maker of butyl benzyl phthalate, was that this study not be considered in isolation from theirs and studies of others. He pointed out that survival and neoplasm findings in the earlier NTP study were not repeated in the current study, and, further, when diet restriction was employed, the increased incidences of pancreatic neoplasms seen in the current study were eliminated. Thus, looking at the multiple studies with no consistent reported findings, Dr. Stevens thought a decision could not be made about the potential carcinogenicity of butyl benzyl phthalate.

Dr. Miller asked the industry representatives for information on occupational and consumer exposures. Dr. R. Hogue, Monsanto Company, said occupational exposure is quite low, as is consumer exposure in vinyl flooring, because the butyl benzyl phthalate is bound into a polymeric system. He said exposure data would be provided to the NTP. Dr. R.W. Hart, NCTR, returned to the effects of feed restriction on neoplasm incidence, noting that in 30-month feed restriction male rats, three exposed rats developed pancreatic acinar cell adenomas. In feed-restricted exposed females at 30 months, there was a statistically significant increased incidence of neoplasms of the urinary bladder.

Dr. Ryan moved that the Technical Report on butyl benzyl phthalate be accepted with the revisions discussed and with the conclusions as written for male rats, *some evidence of carcinogenic activity*, and for female rats, *equivocal evidence of carcinogenic activity*. Dr. Reddy seconded the motion, which was accepted unanimously with 10 votes.

INTRODUCTION



BUTYL BENZYL PHTHALATE

CAS No. 85-68-7

Chemical Formula: $C_{19}H_{20}O_4$ Molecular Weight: 312.39

Synonyms: A13-14777; BBP; 1,2-benzenedicarboxylic acid butyl phenylmethyl ester (9CI); benzyl *n*-butyl phthalate; *n*-butyl benzyl phthalate; butyl phenylmethyl 1,2-benzenedicarboxylate; phthalic acid benzyl butyl ester (8CI)
Trade names: Palatinol BB; Santicizer 160; Sicol 160; Unimoll BB

Chemical and Physical Properties

Butyl benzyl phthalate is a clear, oily liquid with a slight odor and a bitter taste. It has a melting point of -35° C, a boiling point of 370° C, and a specific gravity of 1.116 at 25° C. (Sax and Lewis, 1989). Phthalate acid esters generally have a low volatility at standard temperature and pressure, particularly those of the long-chain and branched compounds such as butyl benzyl phthalate and di(2-ethylhexyl)phthalate (Kluwe, 1982). Butyl benzyl phthalate has a vapor pressure of 8.6×10^{-6} mm Hg at 20° C and a vapor density of 10.8. It is combustible when exposed to heat or flame, with a flash point of 199° C and an autoignition temperature of 233° C; when heated to decomposition, it emits acrid smoke and irritating fumes. Butyl benzyl phthalate can react with oxidizers, and it is soluble in most organic solvents and in dimethylsulfoxide, acetone, and 95% ethanol (less than 100 mg/mL at 23° C) (Verschueren, 1983; *Hawley's*, 1987; Sax and Lewis, 1989).

Phthalate esters with short alkyl groups are appreciably soluble in water. However, most other dialkyl phthalates are relatively insoluble in aqueous media because of their lipophilic structures; butyl benzyl phthalate is almost insoluble in distilled water (2.69 mg/L at 20° C; Howard *et al.*, 1985) and in deionized well water (2.9 mg/L at 20° C; Gledhill *et al.*, 1980). The lipophilicity of butyl benzyl phthalate is also suggested by its octanol/water partition coefficient (log K_{ow}), which has been reported as 4.91 (Leyder and Boulanger, 1983) and 4.77 (Gledhill *et al.*, 1980).

Production, Use, and Human Exposure

Phthalate acid esters are synthesized commercially by condensation of an appropriate alcohol with phthalic anhydride in the presence of an acid catalyst (Kluwe, 1982). Butyl benzyl phthalate has been commercially

produced in the United States since 1946 (U.S. Tariff Commission, 1948). The annual production of butyl benzyl phthalate in the United States was estimated as 80 million pounds per year (USITC, 1978).

Butyl benzyl phthalate is a plasticizer added to polymers (generally at concentrations of 50 to 75 parts per 100 parts of resin) to provide flexibility and softness. It is used extensively in polyvinyl chloride for vinyl floor tile, vinyl foams, carpet backings, and Astroturf®. Other polymers plasticized with butyl benzyl phthalate include cellulose plastics, polyvinyl acetate, polysulfides, and polyurethane. Butyl benzyl phthalate is an organic intermediate and a component of synthetic leather, biodegradable tampon applicators, automotive paint and upholstery, acrylic caulks, plastic filler for automobile body repair, air conditioning filters, inks, and tacky adhesives used to secure medical devices such as ostomy bags and compresses to the skin. It is also used as a carrier and dispersant for pesticides, colorants, solvents, catalysts, munitions, industrial oils, insect repellents, and perfumes and as a plasticizer in aerosol hairsprays at concentrations of less than 1% (Statsek, 1974; Singmaster and Crosby, 1976; Lawrence, 1979; USEPA, 1981; Eigenberg *et al.*, 1986; CIRP, 1992).

The U.S. Food and Drug Administration (FDA) has approved butyl benzyl phthalate, provided that it contains no more than 1% by weight of dibenzylphthalate, for use in packaging and wrapping materials and polyvinyl acetate emulsion adhesives in contact with food products (21 CFR, §§ 175.105, 175.300, 175.320, 176.170, 176.180, 177.2420, 178.3740). Regenerated cellulose film is coated with plasticized nitrocellulose containing butyl benzyl phthalate to provide flexibility and heat sealability; it is used primarily to wrap confectionery products, pastries, cakes, and sandwiches. Studies have shown that the flexibility of these films results from dispersion of the various phthalate plasticizers in the matrix of the polymer chains, which decreases the interactive forces of adjacent chains (Autian, 1973). However, the lack of chemical bonding with the polymer allows plasticizers to migrate into foods at a rate of less than 0.05% to 1.8% (less than 0.05% to 0.8% for butyl benzyl phthalate) on a total film-weight basis from nitrocellulose film containing 30% to 40% plasticizer. Food products have been shown to contain 0.5 to 53 mg butyl benzyl phthalate/kg, individually or in combination with other plasticizers (Castle *et al.*, 1988).

Retail samples of Canadian butter and margarine wrapped in aluminum foil-paper laminate were found to contain butyl benzyl phthalate, dibutylphthalate, and/or di(2-ethylhexyl)phthalate at concentrations up to 47.8, 10.6, and 11.9 μg/g, respectively. Analyses of the food wrapping materials indicated that these phthalate esters migrated from the protective washcoat on the exterior surface of the wrappers and through the food-contacting surface into the food (Page and Lacroix, 1992). Migration into food of plasticizers used in ink on the outer surface of commercially printed polypropylene films has also been demonstrated; this migration increases with storage time of the wrapped product (Castle *et al.*, 1989). Concern regarding the potential toxicity of phthalate esters as contaminants of food led the Japanese Ministry of Health and Welfare to establish regulations to limit the migration of these compounds into foodstuffs and beverages (Omori, 1976).

The U.S. Environmental Protection Agency (EPA) has exempted butyl benzyl phthalate from tolerance requirements when used as an inert ingredient in laminated dispensers for controlled release of Glossypure®, a synthetic pheromone used on cotton to disrupt the mating of the pink bollworm (USEPA, 1981).

Occupational exposure to butyl benzyl phthalate was examined in a National Occupational Hazard Survey conducted by the National Institute of Occupational Safety and Health (NIOSH) covering the years from 1972 to 1974. During this time, an estimated 68,488 workers were potentially exposed to butyl benzyl phthalate (NIOSH, 1976). In a second survey of workplace exposure (National Occupational Exposure Survey) covering the years from 1981 to 1983, an estimated 331,840 workers were potentially exposed to butyl benzyl phthalate (NIOSH, 1990). However, there is no Occupational Safety and Health Administration permissible exposure limit for butyl benzyl phthalate, nor is there an American Conference of Governmental Industrial Hygienists recommended threshold limit value-time weighted average reported in the current literature.

Butyl benzyl phthalate can enter the environment as a consequence of its manufacture, use, and disposal. Humans may be exposed to butyl benzyl phthalate in occupational surroundings and from environmental contamination. Based on use patterns for vinyl flooring and food packaging materials including adhesives, almost all consumers are exposed to butyl benzyl phthalate to some extent.

Regulatory Status

Butyl benzyl phthalate is classified by the FDA as a migratory, indirect food additive (21 CFR, § 170.3). It has been designated by the EPA as a base-neutral priority pollutant as well as a hazardous waste to be disposed of according to the federal hazardous waste management system (40 CFR, § 261.33). Butyl benzyl phthalate is a regulated substance under the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA). Under CERCLA, releases of butyl benzyl phthalate into the environment in quantities exceeding 100 pounds must be reported to the EPA (40 CFR, Part 302).

Environmental Impact

STORET, the EPA water quality database, was used to determine the concentrations of butyl benzyl phthalate throughout the United States in all types of water samples (mean aqueous concentration of 9 μg/L), sediment (median concentration of less than 500 μg/kg dry, 6% detectable), and biota (median concentration of less than 2.5 μg/kg wet, 3% detectable) (Staples *et al.*, 1985). In studies by several groups of investigators, butyl benzyl phthalate was identified in drinking water in New Orleans (0.08 to 1.8 μg/L) and Philadelphia (0.3 to 1 μg/L), surface water in Illinois, Michigan, Missouri, New Jersey, and Pennsylvania (0.2 to 2.4 μg/L), and industrial wastewater effluents near Philadelphia (40 μg/L). These effluents and others were treated at a local wastewater treatment plant which discharged effluents containing 100 μg butyl benzyl phthalate/L into the Delaware river (Keith *et al.*, 1976; Shackelford and Keith, 1976; Sheldon and Hites, 1978, 1979). Sediment samples collected from the Saginaw River in Michigan and the Missouri River at Weldon Springs, Missouri, contained butyl benzyl phthalate at concentrations of 400 to 567 ng/g and 100 ng/g, respectively (Gledhill *et al.*, 1980). Butyl benzyl phthalate was identified at a concentration of 0.8 ng/g in sediment from Lake Pontchartrain in New Orleans (McFall *et al.*, 1985).

Studies performed in water treatment plants have demonstrated that biodegradation of phthalates is temperature dependent and can occur under aerobic or anaerobic conditions. A wide range of organisms in soil, river and sea water, sediments, and water treatment plants are capable of biodegrading phthalates. Longer alkyl chain phthalates biodegrade more slowly than do those with shorter chains. Elimination from natural waters could be by biodegradation, photodegradation, volatilization, or adsorption onto sediments; phthalate concentrations in sediments are markedly higher than those in the water above the sediment strata. In the opinion of the ECETOC Task Force, the widespread disposition of low concentrations of phthalate esters in surface water may reflect a balance between continuous input to the aquatic environment and elimination of the compounds by biodegradation (ECETOC, 1985).

Absorption, Distribution, Metabolism, and Excretion

Experimental Animals

Phthalic acid esters are generally well absorbed from the gastrointestinal tract. Due to the lipophilic nature of these compounds, absorption also occurs via dermal and pulmonary routes (Kluwe, 1982).

Both ester linkages of phthalate esters can be hydrolyzed, leaving phthalic acid as a product. Esterases that rapidly hydrolyze a single ester group to generate the monoester metabolite are present in several mammalian tissues, including intestinal mucosal cells, and extracellular enzymes present in the intestinal contents are also capable of hydrolyzing phthalate esters (Rowland *et al.*, 1977). Butyl benzyl phthalate is partially hydrolyzed by intestinal esterases, primarily to monobutylphthalate and benzyl alcohol with monobenzylphthalate and *n*-butanol as minor products of hydrolysis (Agarwal *et al.*, 1985).

Tissue distribution and pharmacokinetic studies by Eigenberg *et al.* (1986) demonstrated that butyl benzyl phthalate is rapidly cleared from the body of male F344 rats administered gavage (2 to 2,000 mg/kg) or

intravenous (20 mg/kg) doses of [^{14}C]-butyl benzyl phthalate. Excretion of 75% to 86% of a gavage dose occurred within 24 hours, and greater than 92% was eliminated by the end of 4 days. The major route of elimination of butyl benzyl phthalate in rats is excretion of metabolites into the bile. At gavage doses from 2 to 200 mg/kg, extensive enterohepatic reabsorption of these metabolites or their degradation products resulted in urinary excretion of 75% of the dose; another 20% was eliminated in the feces. At 2,000 mg/kg, however, 72% of the dose was eliminated in the feces, with only 22% excreted in the urine. This shift from urinary to fecal elimination may be due to incomplete absorption of butyl benzyl phthalate or its metabolites during enterohepatic recirculation. The half-lives of butyl benzyl phthalate and its metabolites in all tissues are approximately 6 hours; although lipophilic, butyl benzyl phthalate is not sequestered in fat due to its rapid metabolism.

Pharmacokinetic studies in four beagle dogs administered a divided oral dose equivalent to 5 g butyl benzyl phthalate/kg body weight over a period of 4 hours demonstrated recovery of 88% (male) and 91% (female) of unchanged butyl benzyl phthalate from the feces; approximately 4% was excreted as urinary phthalic acid (Erickson, 1965).

The major urinary metabolites of butyl benzyl phthalate in rats are monophthalate derivatives (10% to 40% of the dose) and monophthalate glucuronides (2% to 21%) (Eigenberg *et al.*, 1986). Since butyl benzyl phthalate is an asymmetric diester, two different monophthalates are produced, monobutylphthalate or monobenzylphthalate. There is a preference for hydrolysis of the benzyl ester, resulting in a preponderance (approximately 3:1) of monobutylphthalate in the urine. The urinary half esters of other phthalates, such as diethylphthalate and dibutylphthalate, have been shown to be four times more toxic than their parent compounds (International Labor Office, 1983).

Dermal absorption of [^{14}C]-radiolabeled butyl benzyl phthalate and other phthalate esters was evaluated in male F344 rats using urinary and fecal excretion as an index of relative percutaneous absorption (Elsisi *et al.*, 1989). By day 7, 30% of a 157 μmol butyl benzyl phthalate/kg dose was excreted in the urine and feces, while 44.9% and 6.3% remained in the skin of the application site and in the occlusive plastic cap over the site, respectively. Five percent of the applied dose was retained in the tissues after 7 days, 4.6% in muscle and 0.5% in the brain, spinal cord, and testes.

Recovery of radioactivity from fetal tissues following treatment of maternal Sprague-Dawley rats with [^{14}C]-di(2-ethylhexyl)phthalate or [^{14}C]-diethylphthalate demonstrated that maternal-fetal transfer of dialkyl phthalate esters occurs across the placenta (Singh *et al.*, 1975). Since lipophilic chemicals readily partition into high fat materials such as breast milk, excretion of phthalates into maternal milk is also probable.

Humans

The most common route of exposure to phthalate esters in humans is ingestion of food or liquids (Kluwe, 1982). However, no information related to the pharmacokinetics and metabolism of butyl benzyl phthalate in humans has been reported in the literature.

TOXICITY

Experimental Animals

Phthalate acid esters have very low acute toxicity by oral and parenteral routes; the acceptable daily oral intake values for phthalates are of the same order of magnitude as some chemicals approved for use as direct food additives (Krauskopf, 1973). Absorption through the skin occurs to some extent, with LD_{50} values in rabbits ranging from 3.4 mL/kg for diallylphthalate to 20 mL/kg for dibutylphthalate and dihexylphthalate (*Patty's*, 1967). However, due to their low volatility, inhalation of most phthalates is not associated with acute toxic effects (Autian, 1973).

The acute lethal toxicity of phthalate esters, administered orally or parenterally, is inversely proportional to molecular weight; esters with the shorter alkyl groups are the most water soluble and exhibit the greatest toxicity (Autian, 1973). Butyl benzyl phthalate has a relatively high molecular weight and is almost insoluble in water. The gavage LD_{50} values for butyl benzyl phthalate in a corn oil vehicle, calculated 14 days after administration of a single dose, were 2.33 g/kg for male and female Fisher 344/N rats, 6.16 g/kg for male B6C3F$_1$ mice, and 4.17 g/kg for female B6C3F$_1$ mice (NTP, 1982a). The oral LD_{50} in Sprague-Dawley rats was 20.4 g/kg

(Shibko and Blumenthal, 1973; Hammond *et al.*, 1987). The intraperitoneal LD_{50} of butyl benzyl phthalate in Swiss Webster mice was 3.16 g/kg (Calley *et al.*, 1966).

In a 14-day feed study of butyl benzyl phthalate in F344/N rats, exposure to 25,000 ppm or more resulted in lower body weight gains than those in the controls. Thymic atrophy occurred in all 100,000 ppm rats, and testicular degeneration was observed in all 50,000 and 100,000 ppm males. No compound-related effects were observed in a companion study in $B6C3F_1$ mice administered concentrations up to 25,000 ppm in feed. In similar 13-week feed studies, lower body weight gains and testicular degeneration, characterized by loss of the germinal epithelium of the seminiferous tubules, were observed in male rats receiving concentrations of 25,000 ppm, whereas compound-related effects in mice were limited to lower body weight gains in male mice exposed to concentrations of 1,600 ppm or more and in 12,500 and 25,000 ppm females (NTP, 1982a). The testicular degeneration observed in rats might have been related to conversion of butyl benzyl phthalate to its monobutylphthalate metabolite, which has been shown to produce testicular atrophy (Foster *et al.*, 1981; Gangolli, 1982).

Sprague-Dawley rats exposed to concentrations of 2,000 to 4,000 mg butyl benzyl phthalate/kg in feed for 2 weeks exhibited dose-related posterior body stiffness and incoordination of the hind limbs, which was more severe in males. These signs generally disappeared by the end of a 1-week recovery period (Hammond *et al.*, 1987). In a follow-up 6-week neurological study, body weight gains of Charles River CD rats exposed to 1,500 or 3,000 mg butyl benzyl phthalate/kg body weight were lower than those of the controls, and transient hind limb stiffness was observed in the 3,000 mg/kg group, predominantly in males. Histopathologic examination of tissues from the central and peripheral nervous systems of these animals failed to reveal compound-related pathologic changes (Robinson, 1991).

The no-observable-effect-level (NOEL) in a 90-day study in rats administered feed containing up to 2% butyl benzyl phthalate by weight was 0.5%. Body weight gains of rats exposed to 1.5% and 2% were slightly lower than those of the controls, and liver weights were greater than those of the controls at concentrations of 1% and above (Erickson, 1965). In a similar 90-day feed study in dogs, the NOEL of butyl benzyl phthalate was 2%. Dogs exposed to 5% butyl benzyl phthalate gained less weight initially due to refusal to eat; feed consumption returned to normal after capsule dosing was instituted on day 40 of the study (Erickson, 1965).

After 14 weeks of a 2-year study in F344/N rats exposed to 6,000 or 12,000 ppm butyl benzyl phthalate in feed, compound-related mortality in males resulted from unexplained internal hemorrhaging; all surviving male rats were sacrificed at week 29 or 30 (NTP, 1982a).

The comparative parenteral toxicity of a series of phthalate esters was evaluated by Calley *et al.* (1966). In a 6-week study, Swiss Webster mice given daily intraperitoneal injections of 500 mg butyl benzyl phthalate/kg body weight exhibited a slight retardation in weight gain compared to controls, extramedullary hematopoiesis in the liver and spleen, and periportal hepatitis in addition to an acute peritonitis caused by the daily intraperitoneal injections. As in acute studies, the relative toxicity of the phthalate esters tested was directly related to water solubility and molecular weight. In the same series of studies, the order of potentiation of hexobarbital narcosis also followed the water solubility of the compounds. A 500 mg/kg intraperitoneal injection of butyl benzyl phthalate emulsified in 3% acacia, given 30 minutes prior to a 60 mg/kg intraperitoneal injection of sodium hexobarbital, resulted in a sleep time of 62 minutes, which was significantly greater than that of the controls (46 minutes; $P>0.05$); this was interpreted by the authors to be an indication of acute depression of the central nervous system.

Intradermal irritation caused by phthalate esters in rabbits was evaluated following injection of 1 mL/kg of 1% trypan blue into the marginal ear vein. Mild to moderate inflammatory responses occurred with butyl benzyl, dibutyl, diisobutyl, di-(methoxyethyl), and dicapryl phthalates, as indicated by leakage of dye at the injection site 15 to 26 minutes after the injection, whereas dimethyl, diethyl, and di(2-ethylhexyl) phthalates elicited marked inflammatory responses, indicated by rapid and intense concentration of extravasated Trypan Blue at the injection sites. With the

exception of di(2-ethylhexyl)phthalate, the irritant activity of this series of esters was related to molecular weight (Calley *et al.*, 1966).

Although some of the most water soluble phthalate esters [dimethyl, diethyl, and di(methoxyethyl) phthalates] cause *in vitro* cytotoxicity in strain L 929 mouse fibroblasts, 0.05 mL of a 50 mg/mL emulsion of butyl benzyl phthalate was not toxic to mouse fibroblasts. None of the phthalate esters tested at the same concentration were toxic to chick embryo cells (Calley *et al.*, 1966). In another *in vitro* test, a butyl benzyl phthalate concentration of 1.8×10^{-4} M slightly inhibited the growth of cultured neonatal rat cerebellar cells, and cellular toxicity was observed with concentrations of 7×10^{-4} M or more (Kasuya, 1980; Teranishi and Kasuya, 1980). Butyl benzyl phthalate has been shown to be cytotoxic to HeLa cells in a 24-hour metabolic inhibition test (Ekwall *et al.*, 1982).

Humans

Butyl benzyl phthalate is a slight skin, eye, and mucous membrane irritant as well as a central nervous system depressant (Gosselin *et al.*, 1984). Occupational studies have indicated that exposure to high concentrations of mixed phthalates in workplace air may produce transient irritation of the nose and throat (*Patty's*, 1967). Prolonged contact with the liquid caused irritation of the eyes and skin (CHRIS, 1992). Other symptoms include respiratory irritation, respiratory distress, lightheadedness, nausea, pharyngitis, sleepiness, and erythema (Hazardline, 1983). Repeated exposure to phthalate vapors mixed with other plasticizers may lead to neurotoxic effects such as pain, numbness, and weakness of the extremities; a higher incidence of toxic polyneuritis; and a lowering of the excitability of the vestibular and olfactory receptors (Milkov *et al.*, 1973).

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Experimental Animals

Butyl benzyl phthalate had no adverse effect on embryo development or neonatal death rates at 21 days of age in two studies in the chick embryo system. However, compound-related lethality (as evidenced by a decrease of hatching rates) was observed in one study (Haberman *et al.*, 1968), but not in the other (Bower *et al.*, 1970). In another study, the embryotoxicity of butyl benzyl phthalate was relatively limited (Korhonen *et al.*, 1983).

In a series of feed or gavage studies, Ema *et al.* (1990, 1991, 1992a,b, 1993) administered butyl benzyl phthalate to pregnant Wistar rats throughout gestation in order to evaluate compound-related developmental deviations caused at any time during the span of development (e.g., death of the developing organism, structural abnormalities, and growth retardation) using criteria described by Wilson (1973). The NOELs for butyl benzyl phthalate administered daily in feed on gestation days 0 through 20 were 0.5% (375 mg/kg) for maternal toxicity and 1% (654 mg/kg) for embryo-fetal toxicity. Adjusted maternal body weight gain and feed consumption during pregnancy were significantly lower in the 1% and 2% (974 mg/kg) groups than those in the controls. In the 2% group, all pregnant rats exhibited complete resorption of all implanted embryos (Ema *et al.*, 1990).

A subsequent study was performed to rule out maternal toxicity (malnutrition during pregnancy) as a contributing factor in the embryolethality observed in treated rats with the addition of a feed-restricted (pair-fed) group that received approximately the same amount of feed consumed by the 2% butyl benzyl phthalate group (Ema *et al.*, 1991). As in the previous study, complete resorption of all implanted embryos occurred in the 2% butyl benzyl phthalate group but did not occur in any of the pair-fed rats, indicating that embryolethality was a direct effect of butyl benzyl phthalate and/or its metabolites.

Pregnant Wistar rats given concentrations of 0.2% butyl benzyl phthalate in feed on gestation days 0 through 20, 0 through 11, or 11 through 20 consumed less feed and gained less weight than the control and pair-fed groups. All dams exposed to butyl benzyl phthalate on days 0 through 20 or 0 through 11 exhibited complete resorption of all implanted embryos. No increase in post-implantation loss occurred in rats exposed on days 11 through 20. However, the incidence of fetal malformations in this

group was significantly higher than in the control and pair-fed groups, consisting predominantly of cleft palate (72/134 fetuses) and fusion of the sternebrae. The results of this study suggested that the susceptibility to the teratogenic effect of butyl benzyl phthalate varies with the developmental stage at the time of administration. Butyl benzyl phthalate exposure during the first half of pregnancy resulted in embryolethality; similar exposure during the second half of pregnancy caused marked teratogenicity (Ema *et al.*, 1992a). Embryolethality in experimental animals exposed to plasticizers was also reported in an earlier Russian study (Aldyreva *et al.*, 1975).

Another study by Ema *et al.* (1993) demonstrated that the susceptibility to the teratogenic effect of butyl benzyl phthalate varies with the developmental stage at the time of administration. Butyl benzyl phthalate caused a dose-related increased incidence of malformed fetuses in rats administered gavage doses of 0.6, 0.75, or 1 g/kg on gestation days 7 through 9 or 13 through 15, but was not teratogenic when given on gestation days 10 through 12. Treatment on days 7 through 9 resulted in skeletal malformations such as fusion of the cervical vertebral arches and deformity of the thoracic vertebrae, whereas treatment on days 13 through 15 resulted in external malformations and skeletal malformations such as cleft palate and fusion of the sternebrae. In a similar feed study, cleft palate occurred in 95% of the fetuses of dams exposed to 2% butyl benzyl phthalate on days 7 through 16; fusion of the sternebrae was also a common observation in this group (Ema *et al.*, 1992b).

The maternal and developmental NOELs in Sprague-Dawley (CD) rats and Swiss (CD-1®) mice exposed to butyl benzyl phthalate in feed on gestation days 6 through 15 were 0.5% (419 mg/kg per day) and 0.1% (182 mg/kg per day), respectively (Price *et al.*, 1990). In rats exposed to 1.25% (1,102 mg/kg per day) and mice exposed to 0.5% (910 mg/kg/day), dam weight gains were lower and the incidence of fetal reabsorptions and malformations were greater than the controls. The developmental NOEL in rabbits administered butyl benzyl phthalate on gestation days 6 through 18 was 10 mg/kg (Hammond, 1981).

Other phthalate acid esters that have been shown to exhibit teratogenicity in rats and/or mice include dimethoxyethyl, diethyl, dibutyl, diisobutyl, butyl carbobutoxymethyl, dioctyl, dipropyl, dicyclohexyl, di(2-ethylhexyl), and mono(2-ethylhexyl) phthalates (Singh *et al.*, 1972, 1973; Yagi *et al.*, 1980).

Humans

Increased incidences of anovulatory reproductive cycles and low estrogen concentrations were reported among Russian women working with phthalate plasticizers; the abnormal cycles were associated with spontaneous abortion (Aldyreva *et al.*, 1975). However, the specific phthalates implicated, dose levels, and other data were not reported.

CARCINOGENICITY

Experimental Animals

Di(2-ethylhexyl)phthalate, a structurally related compound, was shown to be an hepatic carcinogen in a 2-year feed study in F344/N rats exposed to 6,000 or 12,000 ppm and B6C3F$_1$ mice exposed to 3,000 or 6,000 ppm (NTP, 1982b). The incidences of hepatocellular carcinoma were significantly greater than those of the controls in 12,000 ppm female rats, 6,000 ppm male mice, and 3,000 and 6,000 ppm female mice and occurred with positive trends in female rats, male mice, and female mice. In male rats, the combined incidences of hepatocellular carcinoma or neoplastic nodules were significantly greater than in the controls.

In a carcinogenesis bioassay of butyl benzyl phthalate administered to F344/N rats in feed containing 6,000 or 12,000 ppm for approximately 2 years (NTP, 1982a), the incidence of mononuclear cell leukemia, generally characterized by splenomegaly and often by hepatomegaly, was significantly greater in 12,000 ppm females than that in the controls and occurred with a positive trend. The incidence of mononuclear cell leukemia in the 12,000 ppm females was also significantly greater than that in historical controls from NTP 2-year feed studies, and the overall trend remained statistically significant. However, the results of the study were inadequate to evaluate carcinogenicity in male rats due to unexplained compound-related toxicity and early mortality beginning at 14 weeks; all surviving males were sacrificed at 29 to 30 weeks. There was no evidence of carcinogenicity of butyl benzyl phthalate in the companion study in B6C3F$_1$ mice. In the carcinogenesis bioassay of diallyphthalate, the incidence of

mononuclear cell leukemia was also significantly greater in female rats administered gavage doses of 100 mg/kg than that in the controls (NTP, 1985).

Butyl benzyl phthalate, along with other drinking water contaminants, was tested for carcinogenicity and failed to induce a positive pulmonary adenoma response in strain A male mice administered intraperitoneal doses up to 800 mg/kg in tricaprylin 3 times per week for a total of 24 injections (19,200 mg/kg) (Theiss *et al.*, 1977).

Humans

According to the International Agency for Research on Cancer, no evaluation could be made to determine the carcinogenic risk of butyl benzyl phthalate to humans (IARC, 1982, 1987).

GENETIC TOXICITY

There are few mutagenicity data published for butyl benzyl phthalate other than the data included in Appendix C of this Technical Report, and with the exception of the two unpublished *in vivo* studies discussed in Appendix C, all results were negative. Butyl benzyl phthalate did not induce gene mutations in *Salmonella typhimurium* tested with either a standard plate incorporation protocol (Kozumbo *et al.*, 1982) or modified preincubation protocol (Zeiger *et al.*, 1985; Table C1), with and without S9.

In mammalian cell systems, butyl benzyl phthalate did not induce mutations at the TK locus of L5178Y mouse lymphoma cells (Myhr and Caspary, 1991; Table C2), and it did not induce sister chromatid exchanges or chromosomal aberrations in cultured Chinese hamster ovary cells (Galloway *et al.*, 1987; Tables C3 and C4). These tests were conducted with and without Aroclor-induced S9.

No increases in sex-linked recessive lethal mutations were observed in germ cells of male *Drosophila melanogaster* treated with butyl benzyl phthalate by injection or in dosed feed (Valencia *et al.*, 1985; Table C5).

There is only one published mammalian *in vivo* study with butyl benzyl phthalate, an abstract by Bishop *et al.* (1987) describing results of a dominant lethal test in male mice. After treatment of male $B6C3F_1$ and CD-1 mice with three subcutaneous injections of butyl benzyl phthalate (doses up to 4,560 mg/kg per day) and matings to untreated virgin females of the same strain for a period of 49 days, no increase in fetal deaths and no decrease in various fertility parameters were found. Butyl benzyl phthalate was concluded to be negative in the mouse dominant lethal assay. Two unpublished *in vivo* studies showing induction of sister chromatid exchanges and chromosomal aberrations in bone marrow cells of male mice administered butyl benzyl phthalate by a single intraperitoneal injection are described in detail in Appendix C of this report.

In summary, all mutagenicity tests with butyl benzyl phthalate were negative, with the exception of two unpublished mouse *in vivo* tests demonstrating weak chromosomal effects.

STUDY RATIONALE

Butyl benzyl phthalate was nominated by the EPA, along with several other phthalates, as part of a class study evaluation. Diethylhexyladipate and di(2-ethylhexyl)phthalate have been implicated as rodent carcinogens (NTP, 1982b,c), and diallylphthalate had equivocal evidence of carcinogenicity in female rats (NTP, 1985) and male mice (NTP, 1983a). In an earlier study, carcinogenicity of butyl benzyl phthalate in rats could not be evaluated due to an unexplained compound-related toxicity and early mortality in males (NTP, 1982a). The oral route of exposure was chosen for the current studies to mimic the principal means of human exposure to butyl benzyl phthalate. Since the reproductive morphology and function of male rats and mice have been affected by other phthalate esters, a 10-week modified mating study was conducted in rats to assess the possibility that butyl benzyl phthalate could also adversely affect male reproduction (Gray and Gangolli, 1986; Lamb *et al.*, 1987; Heindel *et al.*, 1989).

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF BUTYL BENZYL PHTHALATE

Butyl benzyl phthalate was obtained from Chem Central (Kansas City, MO) in two lots (C090882 and L-121 1-87). Lot C090882 was used during the 10-week modified mating study and the 26-week study, and lot L-121 1-87 was used during the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO) (Appendix G). Reports on analyses performed in support of the butyl benzyl phthalate studies are on file at the National Institute of Environmental Health Sciences (NIEHS).

The chemical, a clear, colorless, viscous liquid, was identified as butyl benzyl phthalate by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. The purity of both lots was determined by elemental analyses, Karl Fischer water analysis, functional group titrations (free acid titration and ester hydrolysis), thin-layer chromatography, and gas chromatography. For lot C090882, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for butyl benzyl phthalate. Karl Fischer water analysis indicated 0.23% ± 0.01% water. Functional group titrations indicated free acid values of 0.011% ± 0.001% and 0.00158 ± 0.0004 mEq acid/g sample and purities of 99.5% ± 0.2% and 100.2% ± 0.4%. Thin-layer chromatography indicated a major spot and a slight trace impurity by one system and only a major spot by the second system. Gas chromatography using one system indicated one major peak and four impurities with a combined area of 3.0% relative to the major peak. Gas chromatography using another system indicated one major peak and four impurities with a combined area of 2.8% relative to the major peak. Another analysis of lot C090882 by gas chromatography using the second system indicated one major peak and three impurities with a combined area of 2.27% relative to the major peak. The overall purity of lot C090882 was determined to be greater than 97%.

For lot L-121 1-87, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for butyl benzyl phthalate. Karl Fischer water analysis indicated 0.11% ± 0.01% water. Functional group titrations indicated a free acid value of 0.00112 ± 0.00003 mEq acid/g sample and a purity of 99.1% ± 0.4%. Thin-layer chromatography indicated a major spot and two slight trace impurities by one system and a major spot and one trace impurity by the second system. Gas chromatography using one system indicated one major peak and five impurities with a combined area of 1.20% relative to the major peak. Gas chromatography using another system indicated one major peak and three impurities with a combined area of 1.04% relative to the major peak. The overall purity of lot L-121 1-87 was determined to be greater than or equal to 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory using gas chromatography. These studies indicated that butyl benzyl phthalate was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored at 4° C in the dark in tightly sealed 1-gallon amber-glass bottles during the 10-week modified mating study and the 26-week study and at approximately 22° C in 1-gallon amber-glass bottles with Teflon®-lined lids during the 2-year study.

Stability was monitored during the 10-week modified mating study, the 26-week study, and the 2-year study using gas chromatography. No degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared weekly by mixing butyl benzyl phthalate with feed (Table G1). Homogeneity and stability studies of 300, 3,000, 6,250, 24,000, and 25,000 ppm dose formulations were performed by the analytical chemistry laboratory and/or the study laboratory using high-performance liquid chromatography. Homogeneity was confirmed, and the stability of the dose formulations was confirmed for at least 4 weeks at 5° C when stored protected from light.

Periodic analyses of the dose formulations of butyl benzyl phthalate were conducted at the study laboratories and analytical chemistry laboratory using high-performance liquid chromatography. For the 10-week modified mating study, the 26-week study, and the 2-year study, the formulations were analyzed every 8 to 9 weeks (Tables G2 and G3). All of the dose formulations analyzed were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table G4).

10-Week Modified Mating Study

Male and female F344/N rats were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the male rats were 4 weeks old. Males were quarantined for 11 days and were 6 weeks old on the first day of the study. Females were 20 to 24 weeks old on the first day of the mating period. Before initiation of the study, five male and five female rats were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the study, serologic analyses were performed on five male sentinel rats using the protocols of the NTP Sentinel Animal Program (Appendix J).

Groups of 15 male rats were fed diets containing 0, 300, 2,800, or 25,000 ppm butyl benzyl phthalate for 10 weeks and then were allowed to recover for 2 days. Male rats were housed individually during the exposure and recovery periods, and two untreated females were housed with each male during the 7-day mating period. On the first day of vaginal plug or sperm detection (gestation day 0), females were housed individually. Feed and water were available *ad libitum*. Clinical findings were recorded daily for rats. Feed consumption by males was recorded weekly. The males were weighed initially, weekly, and at the end of the study. The females were weighed at the beginning of the 7-day mating period and on gestation days 0, 6, 9, and 13. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 10-week modified mating study, the males were necropsied and evaluated for reproductive toxicity. The parameters evaluated are listed in Table 1. Methods used were those described in the NTP's sperm morphology and vaginal cytology evaluations protocol (NTP, 1983b). The right testis and right epididymis were isolated, weighed, and fixed and preserved for histopathology. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk buffer was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of the sperm motility estimates, each right cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution, after which samples were removed for morphology evaluation, and the remainder were heat fixed at 65° C. Sperm density was then determined microscopically with the aid of a hemacytometer. Four sperm morphology slides were prepared for each animal evaluated. An aliquot of killed sperm suspension was stained in a test tube, spread on a microscope slide under a coverslip, and examined.

At the end of the 10-week modified mating study, all males were anesthetized with carbon dioxide, and blood was collected from the retroorbital sinus for hematology analyses. Blood for hematology determinations was placed in tubes containing potassium EDTA as the anticoagulant. Leukocyte, erythrocyte, and reticulocyte counts were determined from blood smears by light microscopy. The hematology parameters measured are listed in Table 1.

A necropsy was performed on all males after the 7-day mating period. The brain, heart, right kidney, liver, lung, prostate gland, seminal vesicle, right testis, and thymus were weighed. A necropsy was performed on all mated females on gestation day 13, and on all virgin females 13 days after the end of the mating period. The uterus and ovaries were stained with 10% ammonium sulfide and examined grossly. Table 1 lists the parameters examined. Histopathologic examinations were performed on 0 and 25,000 ppm male rats. In addition, the epididymis, prostate gland, seminal vesicle, and testis were examined in all other exposure groups. Table 1 lists the tissues and organs routinely examined.

26-Week Study

The 26-week study was conducted to evaluate the cumulative toxic effects of repeated exposure to butyl benzyl phthalate and to determine the appropriate doses to be used in the 2-year study.

Male F344/N rats were obtained from Simonsen Laboratories, Inc. (Gilroy, CA). On receipt, the rats were 4 weeks old. Animals were quarantined for 11 days and were 6 weeks old on the first day of the study. Before initiation of the study, five male rats were randomly selected for parasite evaluation and gross observation for evidence of disease. At 13 weeks and at the end of the study, serologic analyses were performed on five male sentinel rats using the protocols of the NTP Sentinel Animal Program (Appendix J).

Groups of 15 male rats were fed diets containing 0, 300, 900, 2,800, 8,300, or 25,000 ppm butyl benzyl phthalate. Feed and water were available *ad libitum*. Rats were housed five per cage. Clinical findings were recorded daily. Feed consumption was recorded weekly. The animals were weighed initially, weekly, and at the end of the study. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 26-week study, samples were collected from 0, 300, 8,300, and 25,000 ppm male rats for sperm morphology evaluations. The parameters evaluated are listed in Table 1. Methods used were the same as those described for males in the 10-week modified mating study.

On days 30, 60, 90, 120, 150, and at the end of the study, blood was collected from all rats (except for the 25,000 ppm males on days 120 and 150) from the retroorbital sinus for hematology analyses. The rats were anesthetized with carbon dioxide. Hematology parameters were determined using the same methods as described for males in the 10-week modified mating study. The hematology parameters measured are listed in Table 1.

A necropsy was performed on all rats. The brain, heart, right kidney, liver, lung, prostate gland, seminal vesicle, right testis, and thymus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned to a thickness of 5 to 6 μm, and stained with hematoxylin and eosin. A complete histopathologic examination was performed on all 0 and 25,000 ppm rats. In addition, the epididymis, prostate gland, seminal vesicle, and testis were examined in all other exposure groups. Table 1 lists the tissues and organs routinely examined.

2-Year Study

Study Design

Groups of 60 male rats were fed diets containing 0, 3,000, 6,000, or 12,000 ppm butyl benzyl phthalate, and groups of 60 female rats were fed diets containing 0, 6,000, 12,000, or 24,000 ppm butyl benzyl phthalate. Ten male and ten female rats from each group were bled at 6 months for hematology and at 6 and 8 months for hormone assays. After 15 months of exposure, 10 male and 10 female rats from each group were evaluated for hematology, hormone assays, organ weights, and histopathology.

Source and Specification of Animals

Male and female F344/N rats were obtained from Simonsen Laboratories, Inc. (Gilroy, CA) for use in the 2-year study. Rats were 4 weeks old upon receipt and were quarantined for 11 days before the beginning of the study. Five male and five female rats were selected for parasite evaluation and gross observation for evidence of disease. Rats were approximately 6 weeks old at the beginning of the study. The health of the animals was monitored during the study according to the protocols of the

NTP Sentinel Animal Program (Appendix J). After approximately 1 year, an additional health check was performed on sentinel animals. All animals were positive for either pinworms or pinworm eggs.

Animal Maintenance

Rats were housed five per cage. Feed and water were available *ad libitum*. Feed consumption was measured weekly for 12 weeks and monthly thereafter for controls and approximately every month for exposed groups. Cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix I.

Clinical Examinations and Pathology

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

Blood for hematology analyses was collected from all rats from the retroorbital sinus at 6 and 15 months. Blood for hormone assays was collected from all rats at 6 and 15 months and at study termination and from all males at 8 months. The rats were anesthetized with carbon dioxide or a mixture of carbon dioxide and oxygen. Blood for hematology determinations and plasma hormone assays was placed in tubes containing potassium EDTA as the anticoagulant. Blood for serum hormone assays was placed in tubes without anticoagulant, allowed to clot at room temperature, and centrifuged. Hematology parameters were measured on a Technicon H-1. Erythrocyte, reticulocyte, and differential leukocyte counts and morphologic evaluations of blood cells were determined from blood smears by light microscopy. Analyses for thyroxine, triiodothyronine, and testosterone were performed with radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA). Analyses for thyroid-stimulating hormone and prolactin were performed with radioimmunoassay methods and reagents from the National Institute of Arthritis, Diabetes, Digestive, and Kidney Disease. Adrenocorticotropic hormone levels were measured using a double-antibody technique. The hematology and hormone assay parameters measured are listed in Table 1.

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

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year study, a quality assessment pathologist reviewed the adrenal gland, kidney, liver, mammary gland (females), pancreas, pituitary gland (pars distalis), prostate gland (males), skin (males), spleen, thyroid gland (males), urinary bladder, and uterus (females) for all neoplastic and nonneoplastic lesions.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) chairperson, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the chairperson to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory

pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of quality assessment pathologists, the PWG chairperson, and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analyses of the pathology data, the diagnosed lesions for each tissue type were evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

Survival Analyses

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

Calculation of Incidence

The incidences of neoplasms or nonneoplastic lesions as presented in Tables A1, A5, B1, and B5 are given as the number of animals bearing such lesions at a specific anatomic site and the number of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A3 and B3) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., intestine, harderian gland, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A3 and B3 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm, i.e., the Kaplan-Meier estimate of the neoplasm incidence that would have been observed at the end of the study in the absence of mortality from all other competing risks (Kaplan and Meier, 1958).

Analysis of Neoplasm Incidences

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

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

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

Analysis of Nonneoplastic Lesion Incidences

Because all nonneoplastic lesions in this study were considered to be incidental to the cause of death or not rapidly lethal, the primary statistical analysis used was a logistic regression analysis in which nonneoplastic lesion prevalence was modeled as a logistic function

of chemical exposure and time. For lesions detected at the interim evaluation, the Fisher exact test was used, a procedure based on the overall proportion of affected animals.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, hormone assay, spermatid, spermatozoal, fertility, and developmental toxicity data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett's or Dunn's test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel, and implausible values were eliminated from the analysis. Average severity values were analyzed for significance using the Mann-Whitney U test (Hollander and Wolfe, 1973). Fertility and developmental toxicity data were also analyzed using the Chi-square test.

Historical Control Data

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

Quality Assurance Methods

The 26-week and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year study were submitted to the NTP Archives, the study was audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, so all comments had been resolved or were otherwise addressed during the preparation of this Technical Report.

Genetic Toxicology

The genetic toxicity of butyl benzyl phthalate was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in cultured Chinese hamster ovary cells and mouse bone marrow cells, mutations in L5178Y mouse lymphoma cells, and sex-linked recessive lethal mutations in *Drosophila melanogaster*. The protocols for these studies and the results are given in Appendix C.

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

There is a strong correlation between a chemical's potential electrophilicity (structural alert to DNA reactivity), mutagenicity in *Salmonella*, and carcinogenicity in rodents. The combination of electrophilicity and *Salmonella* mutagenicity is highly correlated with the induction of carcinogenicity in rats and mice and/or at multiple tissue sites (Ashby and Tennant, 1991). Other *in vitro* genetic toxicity tests do not correlate well with rodent carcinogenicity (Tennant *et al.*, 1987; Zeiger *et al.*, 1990), although these other tests can provide information on the types of DNA and chromosome effects that can be induced

by the chemical being investigated. Data from NTP studies show that a positive response in *Salmonella* is currently the most predictive *in vitro* test for rodent carcinogenicity (89% of the *Salmonella* mutagens were rodent carcinogens), and that there is no complementarity among the *in vitro* genetic toxicity tests. That is, no battery of tests that included the *Salmonella* test improved the predictivity of the *Salmonella* test alone. The predictivity for carcinogenicity of a positive response in bone marrow chromosome aberration or micronucleus tests is not yet defined.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Butyl Benzyl Phthalate

10-Week Modified Mating Study	26-Week Study	2-Year Study
Study Laboratory		
Hazleton Laboratories America, Inc. (Rockville, MD)	Hazleton Laboratories America, Inc. (Rockville, MD)	Southern Research Institute (Birmingham, AL)
Strain and Species		
Rats: F344/N	Rats: F344/N	Rats: F344/N
Animal Source		
Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)	Simonsen Laboratories, Inc. (Gilroy, CA)
Time Held Before Studies		
11 days (males)	11 days	11 days
Average Age When Studies Began		
6 weeks (males) 20 to 24 weeks (females at the beginning of the 7-day mating period)	6 weeks	6 weeks
Date of First Dose		
24 September 1984 (males)	24 September 1984	26 June 1989
Duration of Dosing		
10 weeks (males)	26 weeks	104 weeks (males), 105 weeks (females)
Date of Last Dose		
2 December 1984 (males)	25-26 March 1985	17 June (males) and 24 June (females) 1991
Necropsy Dates		
12-13 December (males) and 16-22 December (females) 1984	25-26 March 1985	15-Month interim evaluation - 26 September (males) and 27 September (females) 1990 Terminal - 24-26 June (males) and 1-3 July (females) 1991
Average Age at Necropsy		
16 weeks (males) and 22 to 26 weeks (females)	32 weeks	15-Month interim evaluation - 72 weeks Terminal - 110 weeks (males) and 111 weeks (females)
Size of Study Groups		
15 males and 30 untreated females	15 males	15-Month interim evaluation - 10 males and 10 females Terminal - 50 males and 50 females

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Butyl Benzyl Phthalate
(continued)

10-Week Modified Mating Study	26-Week Study	2-Year Study
Method of Distribution		
Animals were distributed randomly into groups of approximately equal mean body weights	Same as 10-week modified mating study	Same as 10-week modified mating study
Animals per Cage		
1 (except during 7-day mating period, when one male was house with two females)	5	5
Method of Animal Identification		
Toe clip and cage label	Toe clip and cage label	Tail tattoo
Diet		
NIH-07 open formula meal diet (Zeigler Brothers, Inc., Gardners, PA), available *ad libitum*	Same as 10-week modified mating study	Same as 10-week modified mating study
Water Distribution		
Tap water (Rockville municipal supply) via glass water bottles (Lab Products, Garfield, NJ; Ancare Corp., Manhasset, NY), available *ad libitum*	Same as 10-week modified mating study	Tap water (Birmingham municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*
Cages		
Polycarbonate cages (Lab Products, Inc., Garfield, NJ), changed twice weekly	Same as 10-week modified mating study	Solid-bottom, polycarbonate cages (Lab Products, Inc., Maywood, NJ), changed twice weekly
Bedding		
Sani-Chips® heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Rochelle Park, NJ), changed twice weekly	Same as 10-week modified mating study	Sani-Chips® heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed twice weekly
Cage/Rack Filters		
Nonwoven polyester cage filters (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks	Same as 10-week modified mating study	Reemay® spun-bonded polyester rack filters (Andico, Birmingham, AL), changed every 2 weeks
Racks		
Aluminum racks (Lab Products, Garfield, NJ), rotated every 2 weeks	Same as 10-week modified mating study	Stainless steel racks (Lab Products, Inc., Maywood, NJ), rotated every 2 weeks

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Butyl Benzyl Phthalate
(continued)

10-Week Modified Mating Study	26-Week Study	2-Year Study
Animal Room Environment		
Temperature: 21° to 27° C Relative humidity: 26% to 69% Fluorescent light: 12 hours/day Room air: 12 to 15 changes/hour	Temperature: 21° to 26° C Relative humidity: 24% to 69% Fluorescent light: 12 hours/day Room air: 12 to 15 changes/hour	Temperature: 13.1° to 29.2° C Relative humidity: 22.2% to 89.0% Fluorescent light: 12 hours/day Room air: minimum of 10 changes/hour
Doses		
0, 300, 2,800, or 25,000 ppm in feed, available *ad libitum* (males)	0, 300, 900, 2,800, 8,300, or 25,000 ppm in feed, available *ad libitum*	0, 3,000, 6,000, or 12,000 ppm (males) or 0, 6,000, 12,000, or 24,000 ppm (females) in feed, available *ad libitum*
Type and Frequency of Observation		
Males were observed twice daily; males were weighed initially, weekly, and at the end of the study; clinical observations were recorded daily. Feed consumption by males was recorded weekly.	Observed twice daily; animals were weighed initially, weekly, and at the end of the study; clinical observations were recorded daily. Feed consumption was recorded weekly.	Observed twice daily; animals were weighed and clinical observations were recorded initially, weekly for 13 weeks, monthly thereafter, and at the end of the study. Feed consumption was recorded weekly for 12 weeks and monthly thereafter for controls and approximately monthly for exposed groups.
Method of Sacrifice		
Carbon dioxide asphyxiation and exsanguination	Carbon dioxide asphyxiation and exsanguination	Carbon dioxide asphyxiation
Necropsy		
Necropsy performed on all males. Organs weighed were brain, heart, right kidney, liver, lung, prostate gland, seminal vesicle, right testis, and thymus.	Necropsy performed on all rats. Organs weighed were brain, heart, right kidney, liver, lung, prostate gland, seminal vesicle, right testis, and thymus.	Necropsy performed on all rats. Organs weighed at the 15-month interim evaluation were right epididymis, right kidney, liver, and right testis.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Butyl Benzyl Phthalate
(continued)

10-Week Modified Mating Study	26-Week Study	2-Year Study
Clinical Pathology		
Blood for hematology was collected from the retroorbital sinus of all males surviving to the end of the study. ***Hematology:*** hematocrit, hemoglobin, erythrocyte and reticulocyte counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet count, and total leukocyte count and differential.	Blood for hematology was collected from the retroorbital sinus on days 30, 60, 90, 120, 150, and at end of the study from all rats (except for 25,000 ppm males on days 120 and 150). ***Hematology:*** hematocrit, hemoglobin, erythrocyte and reticulocyte counts, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, platelet counts, and total leukocyte counts and differentials.	Blood was collected from all rats from the retroorbital sinus at 6 and 15 months for hematology, and from males at 6, 8, and 15 months and at study termination, and from females at 6 and 15 months and at study termination for hormone assays. ***Hematology:*** hematocrit; hemoglobin; erythrocyte, reticulocyte, and nucleated erythrocyte counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; platelet counts; total leukocyte counts and differentials. ***Hormone assays:*** thyroid-stimulating hormone, triiodothyronine (6 and 15 months and study termination only in males), thyroxine (6 and 15 months and study termination only in males), adrenocorticotropic hormone (6 and 15 months and study termination only in males), testosterone, and prolactin (15 months and study termination only in females)
Histopathology		
Complete histopathology was performed on all 0 and 25,000 ppm males. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, and urinary bladder. The testis (with epididymis and seminal vesicle) and prostate gland were also examined in all other groups of rats.	Complete histopathology was performed on all 0 and 25,000 ppm rats. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain, esophagus, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, nose, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testis (with epididymis and seminal vesicle), thymus, thyroid gland, trachea, and urinary bladder. The testis (with epididymis and seminal vesicle) and prostate gland were also examined in all other groups of rats.	Complete histopathology was performed on all groups of rats. In addition to gross lesions and tissue masses, the tissues examined included: adrenal gland, brain (three sections), clitoral gland, femur (and bone marrow), heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung (and mainstem bronchi), lymph nodes (mandibular and mesenteric), mammary gland, oral mucosa (and tongue), ovary, pancreas, pituitary gland, preputial gland, prostate gland, salivary gland, skin, spleen, stomach (forestomach and glandular), testes (with epididymis and seminal vesicle), thymus, thyroid gland, urinary bladder, and uterus.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of Butyl Benzyl Phthalate
(continued)

10-Week Modified Mating Study	26-Week Study	2-Year Study
Reproductive Tissue Evaluations		
At the end of the study, sperm samples were collected from all male rats for sperm count, motility, and morphology evaluations. The right cauda, epididymis, and testis were weighed.	At the end of the study, sperm samples were collected from male rats in the 0, 300, 8,300, and 25,000 ppm groups for sperm count, motility, and morphology evaluations. The right cauda, epididymis, and testis were weighed.	None
Fertility, Maternal Body Weight, and Developmental Toxicity Evaluations		
Females were observed twice daily during mating and gestation. Females were weighed at the beginning of the 7-day mating period and on gestation days 0, 6, 9, and 13. Necropsies were performed on mated females on gestation day 13, and on virgin females 13 days after the end of the mating period. The uterus and ovaries were examined. The parameters evaluated included: male and female fertility indexes, maternal body weights, live and dead fetuses, resorptions, corpora lutea, and gross abnormalities.	None	None

RESULTS

RATS

10-WEEK MODIFIED MATING STUDY

All rats survived to the end of the study (Table 2). The final mean body weight and body weight gain of the 25,000 ppm males were significantly less than those of the controls.

Feed consumption by the 300 and 2,800 ppm groups was similar to that by the controls. Feed consumption by the 25,000 ppm group was less than that by the controls at the end of the study. Dietary levels of 300, 2,800, and 25,000 ppm delivered average daily doses of approximately 20, 200, and 2,200 mg butyl benzyl phthalate/kg body weight. No clinical findings were considered to be related to butyl benzyl phthalate exposure.

TABLE 2
Survival, Body Weights, and Feed Consumption of Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 2	Week 12
0	15/15	110 ± 3	320 ± 4	211 ± 2		14	16
300	15/15	111 ± 3	319 ± 3	208 ± 4	100	14	16
2,800	15/15	111 ± 3	316 ± 3	205 ± 3	99	14	16
25,000	15/15	110 ± 3	226 ± 4**	116 ± 3**	71	22	11

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test.
[a] Number of animals surviving at 10 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.

A few minimal hematology changes occurred in the 25,000 ppm male rats (Table E1). There was some evidence of a minimal anemia; this was characterized by a decreased erythrocyte count. The substantially lower final mean body weight of the 25,000 ppm males suggests, however, that these rats were not eating or drinking as much as the controls, and dehydration could have occurred. Dehydration can cause a relative erythrocytosis due to decreased blood volume and hemoconcentration. If significant dehydration occurred in these rats, the severity of the anemia could have been masked. The erythrocytes were macrocytic, evidenced by an increase in mean cell volume. Increases in mean cell volume usually suggest increased numbers of larger, immature erythrocytes (reticulocytes) in the circulation. However, the reticulocyte count was not increased in 25,000 ppm males, indicating the decrease in erythrocyte count was too minimal to stimulate a strong bone marrow response. An increase in mean cell hemoglobin occurred and would be consistent with the larger erythrocyte size. The platelet count was increased and would be consistent with a reactive thrombocytosis. Reactive thrombocytosis can be caused by a variety of conditions, including bone marrow response to anemia.

The absolute and relative testis and prostate gland weights of the 25,000 ppm males were significantly less than those of the controls (Table D1). The other lower organ weights of rats in the 25,000 ppm group were attributed to the moderately lower mean body weight gain of this group. There were marked degenerative testicular and associated epididymal degenerative changes in the 25,000 ppm males. All changes were associated with damage primarily to the germinal epithelium, which resulted in necrosis (detritus) and loss (atrophy and hypospermia) of germinal epithelium and with formation of syncytia of spermatids (giant cells) (Table 3).

TABLE 3
Incidences of Selected Nonneoplastic Lesions in Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate

Dose	0 ppm	300 ppm	2,800 ppm	25,000 ppm
Testis[a]	15	15	15	15
Seminiferous Tubule, Atrophy[b]	0	1 (4.0)[c]	0	15** (4.0)
Seminiferous Tubule, Giant Cells	0	0	0	10** (1.3)
Seminiferous Tubule, Necrosis	0	0	0	3 (1.0)
Epididymis	15	15	15	15
Hypospermia	0	1 (4.0)	0	15** (4.0)
Tail, Chronic Inflammation	0	0	0	4* (1.3)
Tail, Detritus	0	0	0	11** (2.9)

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

The absolute right cauda, right epididymis, and right testis weights of the 25,000 ppm males were significantly less than those of the controls (Table F1). The epididymal spermatozoal concentrations in 2,800 and 25,000 ppm males were significantly less than that in the controls. Sperm motility and epididymal spermatozoal parameters were not calculated for males exposed to 25,000 ppm due to the absence of sperm, and those in other groups of exposed males were similar to those in the controls. Although 10 females mated to 25,000 ppm males were initially found to be sperm positive, none of these females were pregnant at necropsy. The fertility indices of males and females in the 25,000 ppm group were significantly lower than those of the controls (Table F2). The maternal body weights of females mated to 300 and 2,800 ppm males were similar to those of females mated to control males. There were no maternal clinical findings related to exposure of males to butyl benzyl phthalate. There were no significant differences in litter data between the controls and the 300 and 2,800 ppm groups.

26-Week Study

Two control males, one 300 ppm male, one 900 ppm male, one 2,800 ppm male, and four 25,000 ppm males died during the study (Table 4). All deaths occurred during anesthetization for the collection of blood from the retroorbital sinus. The final mean body weight and body weight gain of the 25,000 ppm males were significantly less than those other controls. Except for the 25,000 ppm males, feed consumption by all exposed groups was similar to that by the controls. Feed consumption was not measured for the 25,000 ppm group because there was excessive scattering of the feed at this exposure concentration. Dietary levels of 300, 900, 2,800, and 8,300 ppm delivered average daily doses of approximately 30, 60, 180, and 550 mg butyl benzyl phthalate/kg body weight. No clinical findings were related to butyl benzyl phthalate exposure.

TABLE 4
Survival, Body Weights, and Feed Consumption of Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate

Dose (ppm)	Survival[a]	Mean Body Weight[b] (g) Initial	Final	Change	Final Weight Relative to Controls (%)	Feed Consumption[c] Week 2	Week 28
0	13/15[d]	113 ± 3	365 ± 15	251 ± 14		18	16
300	14/15[e]	113 ± 3	391 ± 5	278 ± 4	107	25	17
900	14/15[f]	112 ± 2	403 ± 6	290 ± 5	110	17	16
2,800	14/15[g]	112 ± 3	374 ± 8	260 ± 6	102	16	15
8,300	15/15	113 ± 3	375 ± 8	263 ± 6	103	17	15
25,000	11/15[h]	112 ± 3	254 ± 13**	140 ± 10**	70	—[i]	—

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 26 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
[c] Feed consumption is expressed as grams of feed consumed per animal per day.
[d] Week of death: 5, 9
[e] Week of death: 5
[f] Week of death: 18
[g] Week of death: 9
[h] Week of death: 5, 9, 9, 13
[i] No feed consumption data were calculated for this exposure group.

The hematology data are listed in Table E2. An exposure-related macrocytic responsive anemia was present in the 25,000 ppm group at all time points evaluated. The anemia was characterized by a minimal to mild decrease in hematocrit values and/or erythrocyte counts. Additionally, minimal erythrocyte count decreases occurred sporadically in the 2,800 and 8,300 ppm groups at various time points. The macrocytosis, evidenced by a minimal to mild increase in mean cell volume, would suggest increased numbers of circulating reticulocytes. Reticulocyte counts were increased on days 60 and 90 and would be consistent with a hematopoietic response to the anemia. An increase in mean cell hemoglobin occurred in the 8,300 and 25,000 ppm rats at all time points. In general, increases in mean cell hemoglobin would be a reflection of the larger erythrocyte size. There were minimal increases in the mean cell hemoglobin concentration in the 8,300 and 25,000 ppm exposure groups. Increases in mean cell hemoglobin concentration have been related to erythrocyte hemolysis (*in vivo* or *in vitro*) or

alterations in hemoglobin concentrations or hematocrit values. Differences in other hematology variables did not exhibit an exposure-related response and were not considered relevant.

The absolute and relative testis weights of the 25,000 ppm males were significantly less than those of the controls (Table D2). Other organ weight differences in rats were attributed to changes in body weight gains or were not considered to be related to exposure to butyl benzyl phthalate. The absolute right cauda and right epididymis weights and the sperm concentration of 25,000 ppm males were significantly less than those of the controls (Table F3). Sperm motility and epididymal spermatozoal parameters were not calculated for males exposed to 25,000 ppm, and those in other exposed groups of males were similar to those in the controls. The incidences of hypospermia and of atrophy of the seminiferous tubule in the testis and of hypospermia in the epididymis in 25,000 ppm males were significantly greater than those of the controls (Table 5). The degenerative changes of the testis and epididymis of the 25,000 ppm males were qualitatively and quantitatively similar to those observed in males in the 10-week modified mating study.

TABLE 5
Incidences of Selected Nonneoplastic Lesions in Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	300 ppm	900 ppm	2,800 ppm	8,300 ppm	25,000 ppm
Testis[a]	15	15	15	15	15	15
Seminiferous Tubule, Atrophy[b]	0	0	0	1 (1.0)[c]	0	15** (3.9)
Seminiferous Tubule, Giant Cells	0	0	0	0	0	5* (2.2)
Seminiferous Tubule, Hypospermia	0	0	0	0	0	15** (4.0)
Epididymis	15	15	15	15	15	15
Hypospermia	0	0	0	0	0	15** (4.0)
Tail, Detritus	0	0	0	1 (1.0)	0	13** (2.2)

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

Dose Selection Rationale: Based on lower mean body weights and increased incidences of nonneoplastic lesions in the 25,000 ppm rats, butyl benzyl phthalate exposure concentrations selected for male rats in the 2-year study were 0, 3,000, 6,000, or 12,000 ppm. In a previous 2-year NTP study, the only evidence of chronic toxicity or carcinogenicity in female rats was a marginal increase in the incidence of mononuclear cell leukemia. A higher exposure concentration of 24,000 ppm was added for females in this 2-year study to maximize the potential for observing adverse chemical effects. Therefore, exposure concentrations of 0, 6,000, 12,000, or 24,000 ppm were selected for females in the 2-year study.

2-Year Study

Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 6 and in the Kaplan-Meier survival curves (Figure 1). Survival of all exposed groups of males and females was similar to that of the controls.

Body Weights, Feed and Compound Consumption, and Clinical Findings

The mean body weight of the 12,000 ppm males was 4% to 10% less than that of the controls throughout most of the study, and the mean body weight of the 24,000 ppm females was 7% to 27% less than that of the controls throughout most of the study. Mean body weights of all 3,000 and 6,000 ppm males and of 6,000 and 12,000 ppm females were similar to those of the controls throughout the study (Tables 7 and 8, Figure 2). Feed consumption by the females exposed to 24,000 ppm was less than that by the controls at the beginning of the study, but was similar to that by the controls by week 6 (Tables H1 and H2). Feed consumption by all other exposed groups of males and females was similar to that by the controls. Dietary levels of 3,000, 6,000, or 12,000 ppm delivered average daily doses of approximately 120, 240, or 500 mg butyl benzyl phthalate/kg body weight to males. Dietary levels of 6,000, 12,000, or 24,000 ppm delivered average daily doses of approximately 300, 600, or 1,200 mg/kg to females. There were no clinical findings in exposed groups of rats related to butyl benzyl phthalate exposure.

Hematology and Hormone Assays

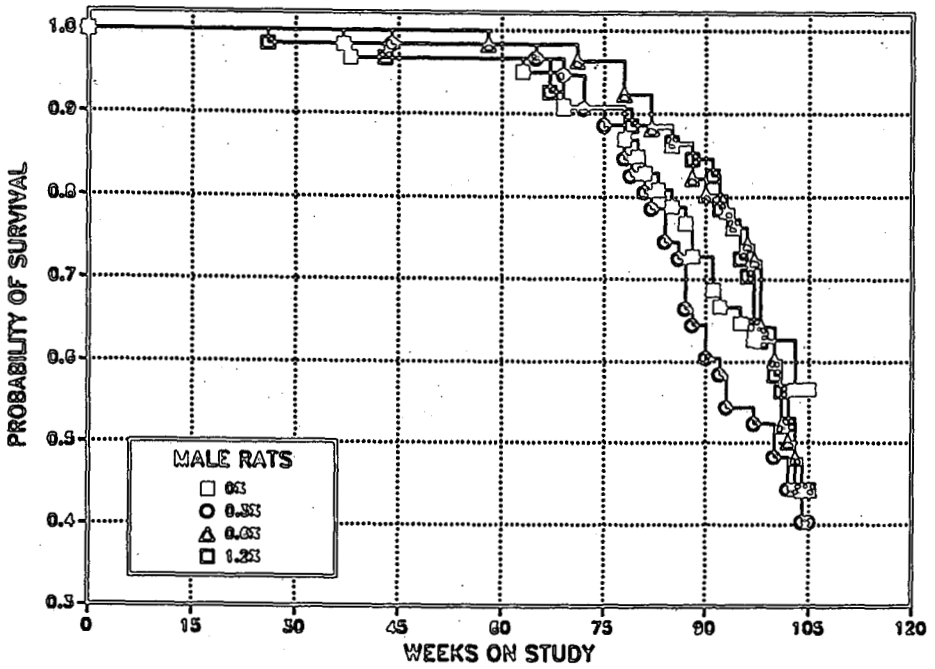
The hematology and hormone assay data for rats evaluated at 6, 8, and 15 months and at the end of the study are listed in Table E3. In general, hematology changes were sporadic and minor. At 6 months, a minimal decrease in erythrocyte count and an increase in mean cell hemoglobin, similar to that which occurred in the 26-week study, occurred in male rats in the 12,000 ppm group. In female rats, a decreased hematocrit value occurred at 15 months in the 24,000 ppm group. At this time point, however, the mean cell volume was decreased, indicating that smaller than normal red cells (microcytosis) were being released from the bone marrow. A microcytic anemia is usually associated with a reduced erythropoiesis and may be caused by a variety of conditions including decreased nutritional status. The marked mean body weight decrease of the 24,000 ppm female rats would be consistent with decreased nutrition. There was also a mild decrease in triiodothyronine concentrations in the 24,000 ppm females at 6 and 15 months and at study termination. No alterations in the thyroxine and thyroid-stimulating hormone concentrations occurred, suggesting that the decrease in triiodothyronine was related to a nonthyroidal disorder. In nonthyroidal disorders, decreases in circulating triiodothyronine concentrations have been attributed to the decreased conversion of thyroxine to triiodothyronine peripherally (e.g. liver) and can be related to numerous conditions including poor nutrition. It appears that thyroid hormones decrease in extrathyroidal tissues (except for the brain) as a response to situations in which preservation of energy is advantageous, and the triiodothyronine decrease observed in various nonthyroidal disorders is probably related to caloric deprivation or conservation.

TABLE 6
Survival of Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	10	10	10
Moribund	19	19	26	26
Natural deaths	3	11	2	2
Animals surviving to study termination	28	20	22	22
Percent probability of survival at end of study[b]	57	40	44	44
Mean survival (days)[c]	625	617	644	632
Survival analysis[d]	P=0.794	P=0.185	P=0.570	P=0.520

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Female				
Animals initially in study	60	60	60	60
15-Month interim evaluation[a]	10	10	10	10
Moribund	23	17	16	20
Natural deaths	2	4	5	1
Animals surviving to study termination	25	29	29	29
Percent probability of survival at end of study	50	59	58	59
Mean survival (days)	651	651	651	644
Survival analysis	P=0.770N	P=0.567N	P=0.596N	P=0.759N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations
[c] Mean of all deaths (uncensored, censored, and terminal sacrifice).
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the exposed columns. A negative trend or lower mortality in an exposure group is indicated by N.



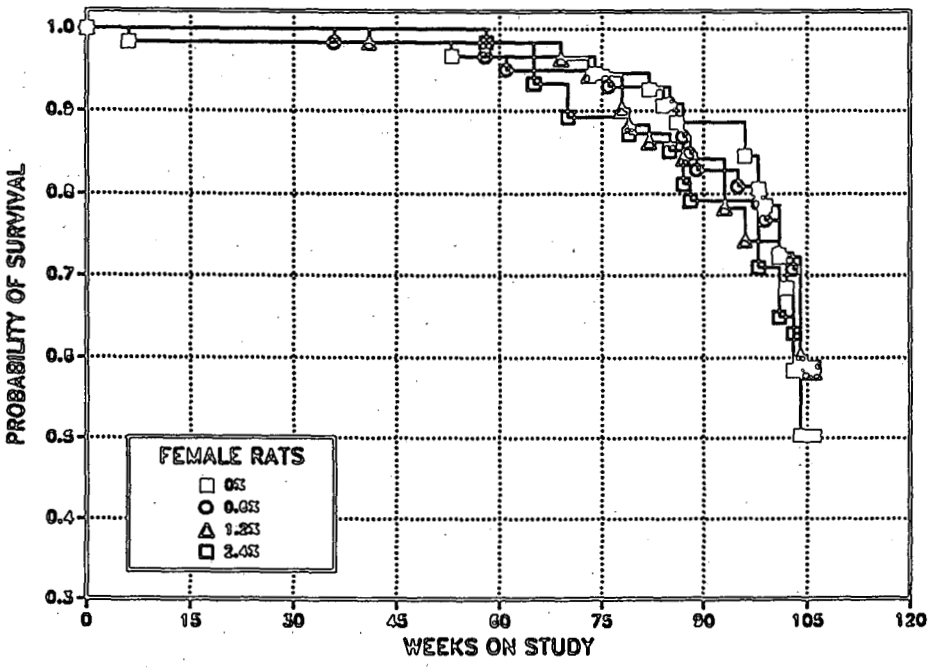


FIGURE 1
Kaplan-Meier Survival Curves for Rats Administered Butyl Benzyl Phthalate in Feed for 2 Years

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

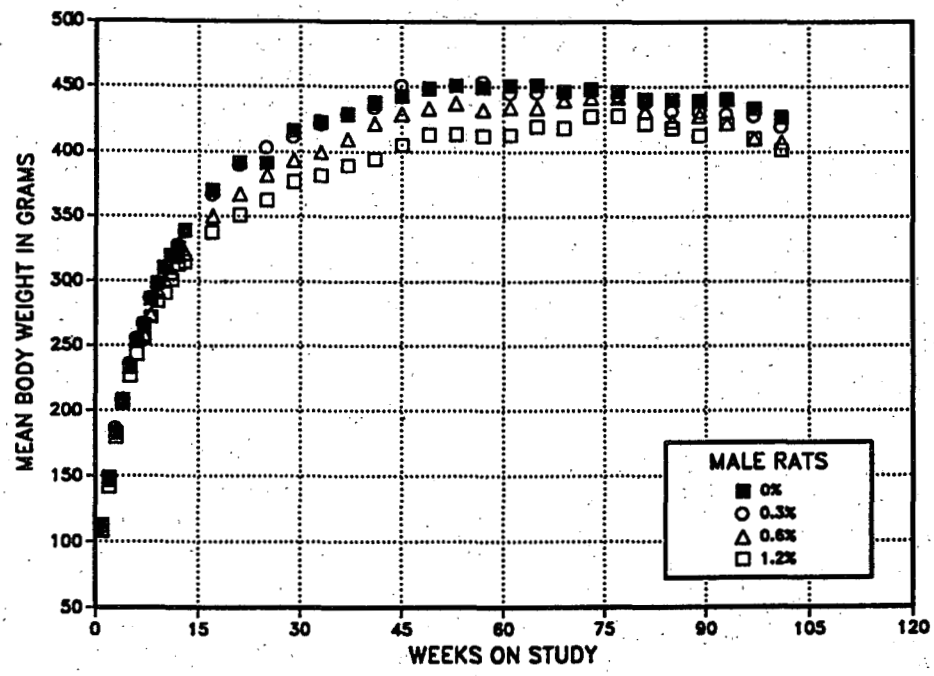
Weeks on Study	0 ppm		3,000 ppm			6,000 ppm			12,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	113	60	111	98	60	109	97	60	108	96	60
2	149	60	148	99	60	147	99	60	141	95	60
3	183	60	186	102	60	184	101	60	180	98	60
4	205	60	208	102	60	209	102	60	208	101	60
5	233	60	236	101	60	233	100	60	226	97	60
6	253	60	255	101	60	253	100	60	243	96	60
7	266	60	267	100	60	258	97	60	255	96	60
8	286	60	287	100	60	274	96	60	272	95	60
9	298	60	299	100	60	292	98	60	284	95	60
10	310	60	310	100	60	301	97	60	291	94	60
11	319	60	320	100	60	307	96	60	301	94	60
12	326	60	328	101	60	319	98	60	312	96	60
13	339	60	338	100	60	321	95	60	315	93	60
17	370	60	367	99	60	350	95	60	338	91	60
21	391	60	389	100	60	367	94	60	351	90	60
25	391	60	403	103	60	381	98	60	362	93	60
29	416	60	411	99	60	394	95	60	377	91	59
33	423	60	421	100	60	399	95	60	382	90	59
37	429	60	429	100	60	410	96	60	389	91	59
41	437	58	434	99	60	422	96	60	394	90	59
45	443	58	451	102	59	429	97	60	405	92	58
49	449	58	449	100	59	433	97	60	413	92	58
53	451	58	451	100	59	437	97	60	414	92	58
57	450	58	453	101	59	432	96	60	412	92	58
61	451	58	445	99	59	434	96	59	413	92	58
65	451	57	446	99	59	434	96	59	420	93	58
69[a]	446	46	445	100	47	440	99	49	419	94	46
73	448	45	447	100	45	441	99	48	427	95	45
77	446	45	443	99	44	441	99	48	428	96	45
81	440	42	437	99	41	431	98	46	422	96	44
85	440	40	431	98	37	424	96	44	418	95	44
89	439	36	430	98	32	428	98	41	413	94	42
93	440	33	429	97	28	422	96	40	422	96	39
97	433	32	428	99	27	410	95	37	411	95	35
101	426	31	419	98	24	407	96	29	402	94	29
Mean for weeks											
1-13	252		253	100		247	98		241	96	
14-52	417		417	100		398	95		379	91	
53-101	443		439	99		429	97		417	94	

[a] Interim evaluation occurred during week 66.

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Weeks on Study	0 ppm		6,000 ppm			12,000 ppm			24,000 ppm		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	99	60	98	99	60	96	97	60	92	93	60
2	121	60	121	101	60	122	101	60	114	94	60
3	135	60	135	100	60	134	99	60	127	94	60
4	142	60	145	102	60	142	100	60	137	97	60
5	153	60	151	99	60	149	97	60	142	93	60
6	159	59	162	102	60	157	99	60	151	95	60
7	167	59	167	100	60	163	98	60	157	94	60
8	171	59	171	100	60	167	97	60	160	94	60
9	175	59	176	100	60	171	98	60	166	95	60
10	178	59	179	101	60	175	98	60	171	96	60
11	180	59	183	102	60	176	98	60	170	95	60
12	186	59	187	100	60	181	97	60	175	94	60
13	185	59	187	101	60	179	97	60	176	95	60
17	198	59	197	100	60	193	98	60	183	93	60
21	204	59	204	100	60	196	97	60	187	92	60
25	213	59	213	100	60	206	97	60	193	91	60
29	218	59	219	100	60	211	97	60	197	91	60
33	223	59	225	101	60	216	97	60	199	89	60
37	231	59	231	100	59	224	97	60	203	88	60
41	238	59	239	100	59	230	97	60	206	87	60
45	240	59	244	102	59	239	99	59	207	86	60
49	258	59	261	101	59	248	96	59	212	82	60
53	269	59	270	100	59	258	96	59	214	80	60
57	276	58	275	100	59	266	96	59	214	78	60
61	281	58	280	99	58	270	96	59	216	77	59
65	286	58	287	100	57	277	97	59	220	77	59
69[a]	296	48	293	99	47	281	95	48	222	75	46
73	298	48	301	101	47	284	95	48	226	76	44
77	300	47	304	101	46	287	96	47	230	77	44
81	300	47	303	101	46	291	97	44	222	74	43
85	312	45	308	99	46	299	96	43	231	74	43
89	321	44	321	100	41	306	95	42	237	74	39
93	320	44	321	100	41	308	96	39	236	74	39
97	319	42	323	101	40	309	97	37	233	73	39
101	318	36	325	102	36	307	97	36	232	73	32
Mean for weeks											
1-13	158		159	101		155	98		149	94	
14-52	225		226	100		218	97		199	88	
53-101	300		301	100		288	96		226	75	

[a] Interim evaluation occurred during week 66.



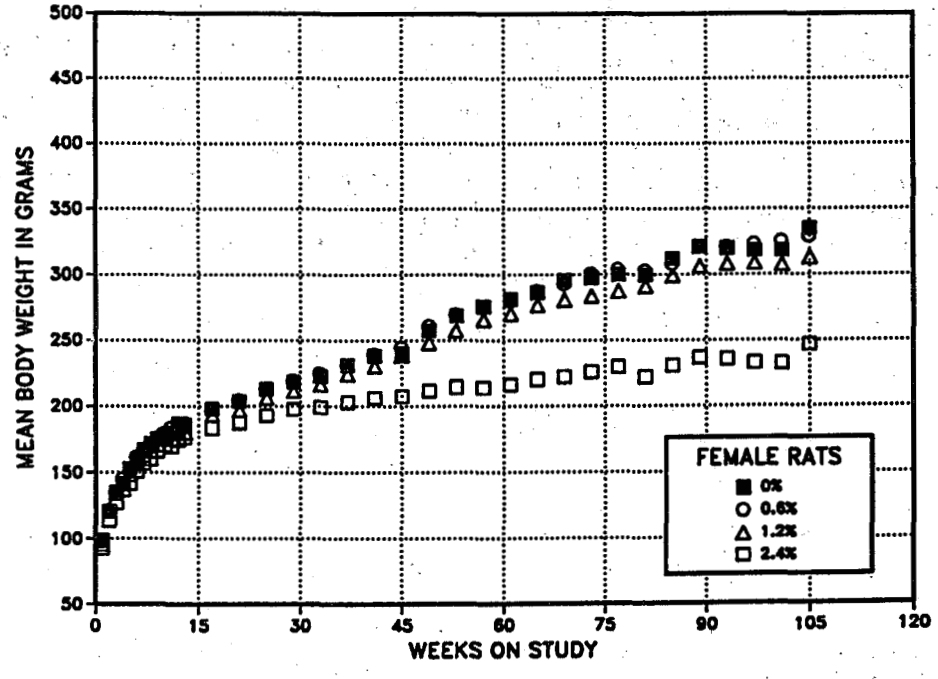


FIGURE 2
Growth Curves for Rats Administered Butyl Benzyl Phthalate in Feed for 2 Years

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the pancreas, urinary bladder, kidney, skin, preputial gland, mammary gland, pituitary gland, and other organs. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Pancreas: At 2 years, the incidences of pancreatic acinar cell adenoma and pancreatic acinar cell adenoma or carcinoma (combined) in 12,000 ppm males were significantly greater than those in the controls (Tables 9 and A3). The incidences of adenoma and of adenoma or carcinoma (combined) in this group exceeded the ranges of historical controls from NTP 2-year feed studies (Tables 9 and A4a). One carcinoma was observed in a 12,000 ppm male (Tables 9 and A1). This neoplasm has never been observed in the historical controls from NTP 2-year feed studies (Tables 9 and A4a). At 2 years, the incidence of focal hyperplasia of the pancreatic acinar cell in 12,000 ppm males was also significantly greater than that in the controls (Tables 9 and A5).

The foci of acinar cell hyperplasia were variably circumscribed and often minimally compressed the surrounding parenchyma. The glandular pattern within the hyperplasias was pronounced, and the cells were slightly enlarged compared to normal with mitotic figures occasionally observed. Generally, the adenomas were more discrete and were larger (greater than 3 mm in diameter) with greater compression of the surrounding parenchyma. Also, the acinar cell pattern was more heterogeneous with greater mitotic activity. The carcinoma was larger and more pleomorphic than the adenomas.

Two pancreatic acinar cell adenomas were observed in the 24,000 ppm females (Tables 9 and B1). While the incidence of this neoplasm (4%) was within the range of historical controls from NTP 2-year feed studies (Tables 9 and B4a), pancreatic neoplasms have only been observed in 3 of 1,194 females in the historical database. Hyperplasia, adenoma, and carcinoma of the pancreatic acinar cell represent a morphological and biological continuum with the morphological distinction between hyperplasias and adenomas not always clearly apparent. There were no increases in the incidences of hyperplasia or carcinoma in exposed females. However, because pancreatic neoplasms are rare in control groups, these lesions were classified as adenomas, and because a pancreatic tumorigenic effect occurred in the males, the pancreatic adenomas in the 24,000 ppm females were considered to be possibly related to exposure to butyl benzyl phthalate.

TABLE 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreas in Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Number Examined Microscopically	50	49	50	50
Acinus, Focal Hyperplasia[a]	4 (2.5)[b]	7 (2.1)	9 (2.3)	12 (2.3)
Acinus, Adenoma[c]				
Overall rate[d]	3/50 (6%)	2/49 (4%)	3/50 (6%)	10/50 (20%)
Adjusted rate[e]	10.7%	7.4%	13.6%	41.0%
Terminal rate[f]	3/28 (11%)	1/20 (5%)	3/22 (14%)	8/22 (36%)
First incidence (days)	729 (T)	569	729 (T)	709
Logistic regression test[g]	P=0.006	P=0.543N	P=0.548	P=0.016
Acinus, Carcinoma[h]				
Overall rate	0/50 (0%)	0/49 (0%)	0/50 (0%)	1/50 (2%)
Acinus, Adenoma or Carcinoma[i]				
Overall rate	3/50 (6%)	2/49 (4%)	3/50 (6%)	11/50 (22%)
Adjusted rate	10.7%	7.4%	13.6%	42.7%
Terminal rate	3/28 (11%)	1/20 (5%)	3/22 (14%)	8/22 (36%)
First incidence (days)	729 (T)	569	729 (T)	674
Logistic regression test	P=0.003	P=0.543N	P=0.548	P=0.014
	0 ppm	**6,000 ppm**	**12,000 ppm**	**24,000 ppm**
Female				
Number Examined Microscopically	50	50	50	50
Acinus, Focal Hyperplasia	1 (3.0)	4 (2.5)	2 (2.5)	0
Acinus, Adenoma[j]				
Overall rate	0/50 (0%)	0/50 (0%)	0/50 (0%)	2/50 (4%)

(T)Terminal sacrifice
* Significantly different (P≤0.05) from the control by the logistic regression test
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Historical incidence for 2-year NTP feed studies with untreated controls: 19/1,191 (1.6% ± 2.4%); range, 0%-10%
[d] Number of animals with neoplasm per number of animals with pancreas examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A lower incidence in an exposure group is indicated by N.
[h] Historical incidence: 0/1,919 (0.0%)
[i] Historical incidence: 19/1,191 (1.6% ± 2.4%); range, 0%-10%
[j] Historical incidence: 2/1,194 (0.2% ± 0.8%); range, 0%-4%

Urinary Bladder: At 2 years, transitional epithelial papillomas in the urinary bladder were observed in one control female and in two 24,000 ppm females (Tables 10 and B1). The incidence of this neoplasm exceeded the range of historical controls from NTP 2-year feed studies (Tables 10 and B4b). At 2 years, the incidence of transitional epithelial hyperplasia in 24,000 ppm females was significantly greater than in the controls (Tables 10 and B5).

The hyperplasias consisted of mild to moderate, focal to multifocal thickening of the transitional epithelium of the urinary bladder. The papillomas projected into the lumen of the urinary bladder and were characterized by papillary fronds that were covered with proliferative transitional epithelium and supported by a fibrous stalk. Hyperplasia and papillomas of the urinary bladder represent a morphological and biological continuum in the progression of proliferative lesions, and, although the two papillomas were small, they appeared to represent the next step in the progression of the observed hyperplasias. The occurrence of two papillomas and the supportive increase in the incidence of hyperplasia in the 24,000 ppm females suggest that these lesions may have been associated with exposure to butyl benzyl phthalate.

TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Urinary Bladder in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Number Examined Microscopically	50	50	50	50
Hyperplasia, Transitional Epithelium[a]	4 (1.5)[b]	0	1 (4.0)	10* (2.2)
Papilloma, Transitional Epithelium[c]	1	0	0	2

* Significantly different ($P \leq 0.05$) from the control by the logistic regression test
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Historical incidence for 2-year NTP feed studies with untreated controls: 4/1,182 (0.3% ± 0.8%); range, 0%-2%

Kidney: At the 15-month interim evaluation, the absolute right kidney weight of the 12,000 ppm females and the relative kidney weights of all exposed groups of males and the 24,000 ppm females were significantly greater than those of the controls (Table D3). The severities of renal tubule pigmentation in 12,000 ppm males and in 24,000 ppm females were greater than those in the controls at 15 months (Table 11). A marginal increase in severity was observed in 12,000 ppm males at 2 years. The pigment was yellowish-brown, coarsely granular, and present predominantly within the cytoplasm of tubule epithelial cells. Special stains performed on kidneys from four animals were positive for hemosiderin (Prussian Blue) and marginally positive for ceroid (AFB), suggesting an exacerbation of the pigment normally observed in aging rats. At 2 years, the incidences of mineralization of the kidney in 6,000 and 24,000 ppm females were significantly less than that in the controls (Tables 11 and B5), and the severity was decreased in exposed females at 15 months and 2 years. The incidences of nephropathy in 6,000, 12,000, and 24,000 ppm females and of transitional epithelial hyperplasia of the kidney in 12,000 ppm females were significantly greater than those in the controls (Tables 11 and B5).

Minimal to mild mineralization of the pars recta of the kidney is a common finding in female F344/N rats; the mineralization is often intraluminal, but may be intracellular. There were also slight increases in the incidences of nephropathy in exposed groups of

females. Nephropathy is extremely common in aging F344/N rats (particularly males) and was apparently marginally exacerbated in this study. Though these effects are possibly related to exposure to butyl benzyl phthalate, they are marginal changes of common background lesions and are of minimal biological significance. Also, there was an increase in the incidence of hyperplasia of the renal pelvic transitional epithelium in 12,000 ppm females. These hyperplasias were characterized by focal minimal thickening of the epithelium, which often contained a central focus of mineralization.

TABLE 11
Incidences of Nonneoplastic Lesions of the Kidney in Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm		3,000 ppm		6,000 ppm		12,000 ppm	
Male								
15-Month Interim Evaluation								
Number Examined Microscopically	10		10		10		10	
Nephropathy[a]	10	(1.4)[b]	10	(1.3)	10	(1.3)	9	(1.1)
Renal Tubule, Pigmentation	10	(1.0)	10	(1.0)	10	(1.2)	10	(2.0)
2-Year Study								
Number Examined Microscopically	50		50		50		50	
Mineralization	0		1	(1.0)	2	(1.5)	0	
Nephropathy	48	(2.1)	47	(2.2)	50	(2.3)	48	(2.0)
Renal Tubule, Pigmentation	49	(1.5)	48	(1.5)	50	(1.6)	50	(1.9)
Transitional Epithelium, Hyperplasia	6	(1.0)	10	(1.3)	6	(1.2)	1	(2.0)
	0 ppm		**6,000 ppm**		**12,000 ppm**		**24,000 ppm**	
Female								
15-Month Interim Evaluation								
Number Examined Microscopically	10		10		10		10	
Mineralization	10	(2.5)	9	(1.8)	9	(1.6)	8	(1.6)
Nephropathy	7	(1.0)	10	(1.0)	10	(1.0)	10	(1.0)
Renal Tubule, Pigmentation	10	(1.4)	10	(1.9)	10	(1.8)	10	(2.0)
2-Year Study								
Number Examined Microscopically	50		50		50		50	
Mineralization	43	(2.1)	34*	(1.7)	37	(1.4)	35*	(1.4)
Nephropathy	34	(1.4)	47**	(1.1)	43*	(1.1)	45**	(1.0)
Renal Tubule, Pigmentation	49	(1.4)	49	(1.5)	49	(1.4)	47	(1.6)
Transitional Epithelium, Hyperplasia	0		3	(1.0)	7**	(1.0)	4	(1.0)

* Significantly different ($P \leq 0.05$) from the control by the logistic regression test
** $P \leq 0.01$
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

Skin: At 2 years, the incidences of acanthosis and hyperkeratosis of the skin in 12,000 ppm males were significantly greater than in the controls (Tables 12 and A5). The identification of these lesions was based upon gross observations of crusty areas involving the skin of the tail. Microscopically, there was mild to moderate thickening (acanthosis) of the epidermis with excessive overlying keratin (keratinization). The pathogenesis of this minimal effect is uncertain.

TABLE 12
Incidences of Nonneoplastic Lesions of the Skin in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Number Necropsied	50	50	50	50
Acanthosis[a]	0	2 (3.0)[b]	2 (2.5)	10** (2.6)
Hyperkeratosis	0	2 (3.0)	3 (3.0)	13** (3.1)

** Significantly different ($P \leq 0.01$) from the control by the logistic regression test
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked

Preputial Gland: At 2 years, the incidences of preputial gland adenomas in males occurred with a negative trend (Tables 13 and A3). The incidence of adenoma or carcinoma (combined) of the preputial gland in 12,000 ppm males was significantly less than in the controls, and the incidences occurred with a negative trend (Tables 13 and A3). The incidence of preputial gland adenoma or carcinoma (combined) in 12,000 ppm males was less than the range of historical controls in NTP 2-year feed studies (Tables 13 and A4b). In no recent control group has the incidence been 0% as in the 12,000 ppm males in this study. The incidences of hyperplasia of the preputial gland in exposed groups were similar to that in the controls (Tables 13 and A5). A decrease in the incidence of clitoral gland neoplasms, which is the comparable tissue in females, was not observed (Table B1). Xenobiotics that affect one of these tissues often affect the other, but not always. It is not clear whether this marginal decrease in the incidence of preputial gland neoplasms was related to exposure to butyl benzyl phthalate.

TABLE 13
Incidences of Neoplasms and Nonneoplastic Lesions of the Preputial Gland in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	1 (3.0)[b]	2 (2.0)	2 (2.5)	1 (2.0)
Adenoma[c]				
Overall rate[d]	4/50 (8%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate[e]	12.8%	15.0%	4.5%	0.0%
Terminal rate[f]	2/28 (7%)	3/20 (15%)	1/22 (5%)	0/22 (0%)
First incidence (days)	644	729 (T)	729 (T)	—[h]
Logistic regression test[g]	P=0.022N	P=0.593N	P=0.165N	P=0.059N
Adenoma or Carcinoma[i]				
Overall rate	5/50 (10%)	8/50 (16%)	2/50 (4%)	0/50 (0%)
Adjusted rate	15.1%	33.9%	9.1%	0.0%
Terminal rate	2/28 (7%)	5/20 (25%)	2/22 (9%)	0/22 (0%)
First incidence (days)	614	626	729 (T)	—
Logistic regression test	P=0.006N	P=0.201	P=0.196N	P=0.032N

(T)Terminal sacrifice
[a] Number of animals with lesion
[b] Average severity of lesions in affected animals: 1 = minimal; 2 = mild; 3 = moderate; 4 = marked
[c] Historical incidence for 2-year NTP feed studies with untreated controls: 84/1,164 (7.2% ± 5.6%); range, 0%-24%
[d] Number of animals with neoplasm per number of animals with preputial gland examined microscopically
[e] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[f] Observed incidence in animals surviving until the end of the study
[g] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.
[h] Not applicable; no neoplasms in animal group
[i] Historical incidence: 124/1,164 (10.7% ± 7.5%); range, 2%-30%

Mammary Gland: At 2 years, the incidence of fibroadenoma of the mammary gland was significantly decreased in the 24,000 ppm females (Tables 14 and B3). The incidences of mammary gland fibroadenoma in the 6,000 and 12,000 ppm females only slightly exceeded the range of historical controls from NTP 2-year feed studies (Tables 14 and B4c). Fibroadenomas of the mammary gland are benign neoplasms that occur spontaneously at a high rate in female F344/N rats.

TABLE 14
Incidences of Neoplasms of the Mammary Gland in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Dose	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
15-Month Interim Evaluation				
Number Necropsied	10	10	10	10
Fibroadenoma[a]	0	1	1	0
2-Year Study				
Number Necropsied	50	50	50	50
Fibroadenoma[b]				
Overall rate[c]	28/50 (56%)	30/50 (60%)	31/50 (62%)	11/50 (22%)
Adjusted rate[d]	71.0%	72.8%	71.7%	28.9%
Terminal rate[e]	14/25 (56%)	18/29 (62%)	17/29 (59%)	5/29 (17%)
First incidence (days)	587	606	509	487
Logistic regression test[f]	P<0.001N	P=0.379	P=0.318	P<0.001N

[a] Number of animals with neoplasm
[b] Historical incidence for 2-year NTP feed studies with untreated controls: 465/1,202 (38.7% ± 12.7%); range, 8%-58%
[c] Number of animals with neoplasm per number of animals necropsied
[d] Kaplan-Meier estimated neoplasm incidence at the end of the study after adjustment for intercurrent mortality
[e] Observed incidence in animals surviving until the end of the study
[f] In the control column are the P values associated with the trend test. In the exposed group columns are the P values corresponding to the pairwise comparisons between the controls and that exposed group. The logistic regression test regards lesions in animals dying prior to terminal kill as nonfatal. A negative trend or lower incidence in an exposure group is indicated by N.

Pituitary Gland: At 2 years, the incidences of adenoma or carcinoma (combined) of the pars distalis of the pituitary gland in females occurred with a negative trend (0 ppm, 22/49; 6,000 ppm, 26/50; 12,000 ppm, 25/48; 24,000 ppm, 13/50; Table B3). Incidences of pituitary gland neoplasms in F344/N rats have been demonstrated to correlate with body weight (Seilkop, 1995). It is probable that the decrease in the incidences of these neoplasms in the 24,000 ppm females is associated with the significantly lower mean body weights of this group.

Other Organs: In addition to the effects mentioned above, there were other statistically significant increased or decreased incidences of nonneoplastic lesions. In males, there were increases in the incidences of granuloma in the liver (0 ppm, 0/50; 3,000 ppm, 0/50; 6,000 ppm, 0/50; 12,000 ppm, 7/50; Table A5) and of corpora amylacae (mineralization) in the prostate gland (17/50, 28/50, 32/50, 30/50); and a decrease in the incidence of bile duct hyperplasia (39/50, 41/50, 38/50, 27/50). In females, there were decreases in the incidences of focal hypertrophy of the adrenal cortex (0 ppm, 9/50; 6,000 ppm, 11/50; 12,000 ppm, 7/50; 24,000 ppm, 0/50; Table B5) and of cytoplasmic vacuolization of hepatocytes (7/50, 6/50, 2/50, 0/50). In general, these nonneoplastic lesions may occur spontaneously in high numbers and represent relatively insignificant changes within the individual organs and/or tissues. While the potential contribution of exposure to butyl benzyl phthalate and/or lower mean body weights as causal factors of these effects remains undetermined, the biological importance of the changes is considered minimal.

GENETIC TOXICOLOGY

Butyl benzyl phthalate was not mutagenic in a series of *in vitro* tests, but positive responses were obtained in two *in vivo* studies conducted with male mice.

Butyl benzyl phthalate was tested in two laboratories for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without Aroclor-induced S9 liver enzymes (Zeiger *et al.*, 1985; Table C1). Negative results were obtained in both laboratories in all strains using concentrations of butyl benzyl phthalate that were the highest permitted by experimental design. *In vitro* studies with mammalian cell systems also gave negative results with and without S9. No induction of trifluorothymidine resistance was obtained in L5178Y mouse lymphoma cells with concentrations of butyl benzyl phthalate that formed stable solutions (Myhr and Caspary, 1991; Table C2). Increases in mutant colonies were observed in the absence of S9 in cultures treated with concentrations that produced precipitation, but such responses were not considered valid by experimental quality control parameters. Therefore, the test was concluded to be negative. No induction of sister chromatid exchanges or chromosomal aberrations was observed in cultured Chinese hamster ovary cells treated with butyl benzyl phthalate (Galloway *et al.*, 1987; Tables C3 and C4). In the first sister chromatid exchange trial without S9, the response was considered to be equivocal due to a significant trend (P=0.004) in the absence of a significant increase in sister chromatid exchanges at any one dose level. However, results of the second trial were clearly negative, as was the single trial conducted with S9, and the test results were concluded to be negative overall.

No induction of sex-linked recessive lethal mutations was observed in germ dells of male *Drosophila melanogaster* administered 500 ppm butyl benzyl phthalate by injection or up to 50,000 ppm in dosed feed (Valencia *et al.*, 1985; Table C5).

In vivo studies with butyl benzyl phthalate in male mice gave positive results for induction of sister chromatid exchanges (Table C6) and chromosomal aberrations (Table C7) at standard sampling times over a dose range of 1,250 to 5,000 mg/kg. In the sister chromatid exchange test, a single trial with a sample time of 23 hours post-injection yielded a positive trend when the highest dose was excluded from the analysis (P=0.0067) because of a reduction in response at 5,000 mg/kg. The sister chromatid exchange test conducted with a 42-hour sample time also gave a weakly positive response by trend analysis. Neither trial was repeated. Positive trend analyses were obtained in each of two trials in the chromosomal aberrations test that used the standard harvest time of 17 hours post injection. The only significant dose in each trial was the highest dose (5,000 mg/kg). The single chromosomal aberrations trial that used a delayed harvest time of 36 hours showed no increases in chromosomal aberrations at any of the three dose levels tested.

In conclusion, butyl benzyl phthalate showed no evidence of mutagenicity in any of four *in vitro* tests performed with and without S9 activation enzymes. In addition, a test for induction of sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* gave negative results. Positive results were obtained in mouse bone marrow tests for sister chromatid exchanges and induction of chromosomal aberrations. Responses were weak, however, and the sister chromatid exchange test was not repeated.

DISCUSSION AND CONCLUSIONS

Butyl benzyl phthalate is widely used either directly as a plasticizer in a variety of commercial polymers or indirectly as a chemical intermediate. Consequently, there is human exposure in occupational settings and through a variety of consumer products including food, cosmetics, furniture and interior finishings, numerous household items, and product packaging. Butyl benzyl phthalate was originally nominated for chronic evaluation in rats and mice by the U.S. Environmental Protection Agency as part of a class study of phthalates. A previous study of butyl benzyl phthalate in rats and mice resulted in unexplained chemical-related mortality in male rats beginning at about week 14 of the study (NTP, 1982a). Therefore, the present studies were conducted only in rats.

Since male reproductive morphology and function have been affected by other phthalate esters, a 10-week modified mating study was conducted to assess the possibility that butyl benzyl phthalate could also adversely affect male reproduction. Following exposure to butyl benzyl phthalate in feed for 10 weeks, marked chemical-related effects on male reproductive parameters included decreased testis, epididymis, and seminiferous tubule weights accompanied by dramatically decreased sperm concentrations. It is likely that these effects were related to the marked testicular degeneration seen in the 10-week modified mating study and the 26-week feed study.

The 26-week study in males was designed to help characterize the chemical-related mortality observed in the previously conducted NTP studies (NTP, 1982a). In those studies, internal hemorrhaging was suspected at necropsy, but it was not confirmed microscopically. There were no chemical-related deaths in the present 26-week study and, in general, hematology changes were minimal. The most prominent change was a macrocytic responsive anemia in the 25,000 ppm male rats. A cause for the anemia was not readily evident, but chronic occult hemorrhaging could account for the responsive anemia. Evidence of an anemia was also present in the 10-week modified mating study, but it was not as prominent as that in the 26-week study.

In the 2-year study, there was evidence of an anemia in the 24,000 ppm female rats. However, it was a microcytic, rather than a macrocytic, anemia and was probably related to the markedly lower mean body weight gain (decreased nutritional status) of this group. The 24,000 ppm female rats also had lower triiodothyronine concentrations. This is probably also related to the lower mean body weight (hence, decreased nutrition).

The exposure concentrations used for males in the present 2-year study (3,000, 6,000, and 12,000 ppm) were based on results from the 10-week modified mating study and the 26-week study. In each of these studies, exposure concentrations up to 25,000 ppm did not cause any chemical-related deaths, but the 25,000 ppm concentration did result in 30% lower final mean body weights. However, the 8,300 ppm concentration had no adverse effects on mean body weights in the 26-week study, suggesting that the males could tolerate higher exposure concentrations. Consequently, 12,000 ppm was selected as the highest exposure concentration for males in the 2-year study. The exposure concentrations selected for females were based on the previous 2-year study conducted by the NTP (1982a) in which exposure concentrations of 0, 6,000, and 12,000 ppm did not affect survival of exposed groups of females, and mean body weights in exposed groups were slightly lower than those of the controls throughout the study. The only evidence of chronic toxicity or carcinogenicity in the previous 2-year study was a marginal increase in the incidence of mononuclear cell leukemia in the 12,000 ppm female rats. Consequently, these exposure concentrations were used for males in this study, and a higher exposure concentration of 24,000 ppm was added for females to maximize the potential for observing adverse chemical effects. Therefore, the exposure concentrations used for females in this study were 6,000, 12,000, and 24,000 ppm.

For male and female rats, the selected exposure concentrations had no effect on mortality or morbidity. Mean body weights of 3,000 and 6,000 ppm males and of 6,000 and 12,000 ppm females were similar to those of the controls. The exposure of males to 12,000 ppm butyl benzyl phthalate resulted in 4% to 10% lower mean body weights throughout most of the study, whereas the exposure of females to 24,000 ppm resulted in a more severe mean body weight depression of about 7% to 27% throughout the study. Although the survival of 24,000 ppm females was similar to that of the controls, the degree of mean body weight depression suggests that a lower exposure concentration could have been used. The selected exposure concentrations did allow adequate numbers of animals to be evaluated for chronic toxicity and carcinogenicity, and they also ranged from concentrations causing no effects to those which resulted in chemical-related adverse effects.

Chemical-related neoplasms and nonneoplastic lesions were observed in rats in the present 2-year study. Exposure to butyl benzyl phthalate resulted in marked increases in the incidences of pancreatic acinar cell hyperplasia and adenoma in 12,000 ppm male rats. In the F344/N rat, these lesions are generally characterized by a clear morphological continuum from focal acinar cell hyperplasia to adenoma to carcinoma. Ten adenomas and one carcinoma were observed in the 12,000 ppm males (11/50, 22%). This was statistically significant (P=0.014) by logistic regression analysis, which is appropriate for this typically nonfatal neoplasm. This incidence is well above the average incidence and well outside the range reported in the historical database from NTP 2-year feed studies. Although only one carcinoma was observed in a 12,000 ppm male, no carcinomas have ever been observed in NTP historical control rats from feed studies. With the exception of an increased incidence of pancreatic acinar cell hyperplasia in 12,000 ppm males, there were no other lesions at this site in male rats that could be attributed to the administration of butyl benzyl phthalate.

Even though the females were administered exposure concentrations that were twice as high as those administered to males, only two pancreatic acinar cell adenomas were observed in the 24,000 ppm group. These were probably related to exposure to butyl benzyl phthalate because of the rarity of this neoplasm. This difference in susceptibility between males and females has been reported for another peroxisome proliferator (hydrochlorofluorocarbon 123) (Malley *et al.*, 1995) and appears to be consistent with results of investigations showing that testosterone is stimulatory and estrogen is inhibitory for growth of acinar cell neoplasms in rat models (Lhoste *et al.*, 1987a,b; Sumi *et al.*, 1989; Longnecker and Sumi, 1990). Other peroxisome proliferators such as clofibrate and nafenopin have caused pancreatic acinar cell neoplasms, but these chemicals were only evaluated in male rats (Reddy and Qureshi, 1979; Svoboda and Azarnoff, 1979).

The biological significance of the marginal increase in the incidence of transitional epithelial papilloma in the urinary bladder of female rats is uncertain. While this neoplasm was observed in two 24,000 ppm females versus one in the control females, the appearance of this neoplasm in the exposed groups was associated with increases in the incidence and severity of transitional epithelial hyperplasia. This suggests that these lesions may have been associated with exposure to butyl benzyl phthalate.

Butyl benzyl phthalate did not cause liver neoplasms. Liver neoplasms would be expected because they have been observed with other peroxisome proliferators. In collaborative studies conducted concomitantly with the present 2-year study at the same laboratory, butyl benzyl phthalate was evaluated for its ability to induce hepatic peroxisomes in female F344/N rats as evidenced by the activities of two hepatic peroxisomal enzyme markers, palmitoyl CoA oxidase and carnitine acetyl transferase, when evaluated after 1 month and 1 year of exposure to butyl benzyl phthalate (Monsanto, 1994). It appears that butyl benzyl phthalate can cause a mild yet detectable increase in peroxisome proliferation, although at a lower concentration than that induced by only 3 weeks of exposure to the positive control, di(2-ethylhexyl)-phthalate (Table 15).

TABLE 15
Enzyme Activities in Female Rats Administered Butyl Benzyl Phthalate or Di(2-ethylhexyl)phthalate in Feed[a]

	Butyl Benzyl Phthalate				Di(2-ethylhexyl)phthalate[b]
Dose	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm	12,000 ppm
1 Month					
n	5	5	5	5	5
Palmitoyl CoA Oxidase (nmol/min/mg)	20.7 ± 5.6	22.2 ± 3.4	34.8 ± 10.6*	74.5 ± 18.2*	100.2 ± 38.4*
Carnitine Acetyl Transferase (nmol/min/mg)	5.0 ± 1.2	21.1 ± 3.9*	36.8 ± 13.6*	59.9 ± 17.2*	83.7 ± 28.5*
1 Year					
n	10	10	10	10	5
Palmitoyl CoA Oxidase (nmol/min/mg)	28.79 ± 5.56	33.11 ± 3.81	48.35 ± 10.57*	71.24 ± 11.94*	86.30 ± 12.24*
Carnitine Acetyl Transferase (nmol/min/mg)	17.13 ± 6.89	26.21 ± 4.18*	45.29 ± 6.82*	64.44 ± 14.06*	72.20 ± 10.46*

* Significantly different ($P \leq 0.05$) from the control group by the two-tailed Student's *t*-test
[a] Mean ± standard deviation. Study performed at Southern Research Institute. A detailed description of the protocol and these data are presented by Monsanto (1994).
[b] Di(2-ethylhexyl)phthalate was a positive control administered only for the last 3 weeks of the study.

These peroxisome proliferation results agree with work by other investigators. An association of hepatic peroxisome proliferation (induced by structurally dissimilar hypolipidemic drugs and certain phthalate ester plasticizers) with rodent liver tumorigenicity has been demonstrated by Reddy *et al.* (1980, 1986), who suggested that the hepatocarcinogenicity of these chemicals can be correlated with their ability to induce peroxisome proliferation and cyanide-insensitive palmitoyl CoA oxidase. In a ranking of seven phthalate esters and several common hypolipidemic agents by induction of normalized enzyme activity, the palmitoyl CoA oxidase value of butyl benzyl phthalate was two, compared to one for aspirin, 15 for di(2-ethylhexyl)phthalate, and 29 to 304 for hypolipidemic agents such as ciprofibrate and WY-14643 that are known peroxisome proliferators and liver carcinogens in rodents (Reddy and Lalwai, 1983; Barber *et al.*, 1987). Critical analysis of the literature on over 70 chemicals that induce peroxisome proliferation in rodent livers reveals a near perfect correlation between peroxisome proliferation and the eventual appearance of hepatocarcinogenicity (Ashby *et al.*, 1994). *In vivo* and *in vitro* investigations failed to provide evidence for significant peroxisome proliferation in the human liver by the chemicals studied. Furthermore, epidemiology studies have failed to demonstrate an increased incidence of tumors in humans exposed chronically to therapeutic hypolipidemic drugs known to cause peroxisome proliferation in rodents (reviewed by Ashby *et al.*, 1994).

Interestingly, there was no increase in the incidence of mononuclear cell leukemia in female rats as was observed previously, although the present study used exposure concentrations that were twice as high as those administered in the previous study (NTP, 1982a).

The decreased incidence of mammary gland neoplasms in female rats was observed previously (NTP, 1982a) and is attributable to lower mean body weights in the exposed groups. Using the NTP historical

control database, a logistic regression model for predicting mammary gland neoplasm incidence was developed based on survival and body weight at 1 year (Seilkop, 1995). Application of this model to the butyl benzyl phthalate data revealed that the predicted neoplasm incidence of 20.6% for the 24,000 ppm female exposure group agrees with the observed incidence (11/50, 22%). Thus, the chemical-related decrease in the incidence of this neoplasm appears to be due primarily to the lower mean body weights.

Butyl benzyl phthalate showed no evidence of mutagenicity in four standard *in vitro* tests (Zeiger *et al.*, 1985; Galloway *et al.*, 1987; Myhr and Caspary, 1991), and a germ cell mutation test in *Drosophila* gave negative results (Valencia *et al.*, 1985). Weak but statistically significant increases were, however, observed in mouse bone marrow tests for sister chromatid exchanges and chromosomal aberrations. The highest dose used in these tests was 5,000 mg/kg, which is approximately four times the highest dose used in these studies (1,200 mg/kg, 25,000 ppm). It was only at the highest dose (5,000 mg/kg) that increases in the chromosomal aberrations were observed *in vivo* in male mice.

CONCLUSIONS

Under the conditions of this 2-year feed study, there was *some evidence of carcinogenic activity** of butyl benzyl phthalate in male F344/N rats based on the increased incidences of pancreatic acinar cell adenoma and of acinar cell adenoma or carcinoma (combined). There was *equivocal evidence of carcinogenic activity* of butyl benzyl phthalate in female F344/N rats based on the marginally increased incidences of pancreatic acinar cell adenoma and of transitional epithelial papilloma of the urinary bladder.

Exposure of rats to butyl benzyl phthalate in feed for 2 years resulted in focal hyperplasia in the pancreas in male rats and in transitional epithelial hyperplasia in the urinary bladder of female rats.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 9. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 11.

REFERENCES

Agarwal, D.K., Maronpot, R.R., Lamb, J.C., IV, and Kluwe, W.M. (1985). Adverse effects of butyl benzyl phthalate on the reproductive and hematopoietic systems of male rats. *Toxicology* 35, 189-206.

Aldyreva, M.V., Klimova, T.S., Izyumova, A.S., and Timofievskaya, L.A. (1975). The influence of phthalate plasticizers of the generative function. *Gig. Tr. Prof. Zabol.* 19, 25-29.

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York.

Ashby, J., and Tennant, R.W. (1991). Definitive relationships among chemical structure, carcinogenicity, and mutagenicity for 301 chemicals tested by the U.S. NTP. *Mutat. Res.* 257, 229-306.

Ashby, J., Brady, A., Elcombe, C.R., Elliot, B.M., Ismael, J., Odum, J., Tugwood, J. D., Kettle, S., and Purchase, I.F.H. (1994). Mechanistically-based human hazard assessment of peroxisome proliferator-induced hepatocarcinogenesis. *Hum. Exp. Toxicol.* 13 (Suppl. 2), S1-S118.

Autian, J. (1973). Toxicity and health threats of phthalate esters: Review of the literature. *Environ. Health Perspect.* 4, 3-26.

Barber, E.D., Astill, B.D., Moran, E.J., Schneider, B.F., Gray, T.J.B., Lake, B.G., and Evans, J.G. (1987). Peroxisome induction studies on seven phthalate esters. *Toxicol. Ind. Health* 3, 7-24.

Bishop, J.B., Teaf, C.M., and Bhooshan., B. (1987). Assessment of fetal death rate among in utero progeny of B6C3F$_1$ and CD-1 mice after subcutaneous injections of males with butyl benzyl phthalate (BBP). *Environ. Mutagen.* 9, 15. (Abstr.)

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Bower, R.K., Haberman, S., and Minton, P.D. (1970). Teratogenic effects in the chick embryo caused by esters of phthalic acid. *J. Pharmacol. Exp. Ther.* 171, 314-324.

Calley, D., Autian, J., and Guess, W.L. (1966). Toxicology of a series of phthalate esters. *J. Pharm. Sci.* 55, 158-162.

Caspary, W.J., Lee, Y.J., Poulton, S., Myhr, B.C., Mitchell, A.D., and Rudd, C.J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality control guidelines and response categories. *Environ. Mol. Mutagen.* 12 (Suppl. 13), 19-36.

Castle, L., Mercer, A.J., Startin, J.R., and Gilbert, J. (1988). Migration from plasticized films into foods. 3. Migration of phthalate, sebacate, citrate and phosphate esters from films used for retail food packaging. *Food Addit. Contam.* 5, 9-20.

Castle, L., Mayo, A., and Gilbert, J. (1989). Migration of plasticizers from printing inks into foods. *Food Addit. Contam.* 6, 437-443.

Chemical Hazard Response Information System (CHRIS) (1992). Vol. 1. U.S. Coast Guard, Department of Transportation. Washington, DC.

Code of Federal Regulations (CFR) 21, Part 58.

Code of Federal Regulations (CFR) 21, § 170.3.

Code of Federal Regulations (CFR) **21**, §§ 175.105; 175.300; 175.320; 176.170; 176.180; 177.2420; 178.3740.

Code of Federal Regulations (CFR) **40**, § 261.33.

Code of Federal Regulations (CFR) **40**, Part 302.

Cosmetic Ingredient Review Expert Panel (CIRP) (1992). Final report on the safety assessment of butyl benzyl phthalate. *J. Am. Coll. Toxicol.* **11**, 1-23.

Cox, D.R. (1972). Regression models and life-tables. *J. R. Stat. Soc.* **B34**, 187-220.

Crawford, B.D. (1985). Perspectives on the somatic mutation model of carcinogenesis. In *Advances in Modern Environmental Toxicology. Mechanisms and Toxicity of Chemical Carcinogens and Mutagens* (M.A. Mehlman, W.G. Flamm, and R.J. Lorentzen, Eds.), pp. 13-59. Princeton Scientific Publishing Co. Inc., Princeton, NJ.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* **6**, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumour prevalence data. *Appl. Statist.* **32**, 236-248.

Dixon, W.J., and Massey, F.J., Jr. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, Inc., New York.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* **6**, 241-252.

Dunnett, C.W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* **50**, 1096-1121.

Eigenberg, D.A., Bozigian, H.P., Carter, D.E., and Sipes, I.G. (1986). Distribution, excretion, and metabolism of butylbenzyl phthalate in the rat. *J. Toxicol. Environ. Health* **17**, 445-456.

Ekwall, B., Nordensten, C., and Albanus, L. (1982). Toxicity of 29 plasticizers to HeLa cells in the MIT-24 system. *Toxicology* **24**, 199-210.

Elsisi, A.E., Carter, D.E., and Sipes, I.G. (1989). Dermal absorption of phthalate diesters in rats. *Fundam. Appl. Toxicol.* **12**, 70-77.

Ema, M., Murai, T., Itami, T., and Kawasaki, H. (1990). Evaluation of the teratogenic potential of the plasticizer butyl benzyl phthalate in rats. *J. Appl. Toxicol.* **10**, 339-343.

Ema, M., Itami, T., and Kawasaki, H. (1991). Evaluation of the embryolethality of butyl benzyl phthalate by conventional and pair-feeding studies in rats. *J. Appl. Toxicol.* **11**, 39-42.

Ema, M., Itami, T., and Kawasaki, H. (1992a). Embryolethality and teratogenicity of butyl benzyl phthalate in rats. *J. Appl. Toxicol.* **12**, 179-183.

Ema, M., Itami, T., and Kawasaki, H. (1992b). Effect of period of exposure on the developmental toxicity of butyl benzyl phthalate in rats. *J. Appl. Toxicol.* **12**, 57-61.

Ema, M., Itami, T., and Kawasaki, H. (1993). Teratogenic phase specificity of butyl benzyl phthalate in rats. *Toxicology* **79**, 11-19.

Erickson, N.G. (1965). The metabolism of diphenyl phthalate and butylbenzyl phthalate in the beagle dog. *Dis. Abstr.* **26**, 3014-3015.

European Chemical Industry Ecology and Toxicology Center (ECETOC) (1985). An assessment of the occurrence and effects of dialkyl-ortho-phthalates in the environment. *ECETOC Technical Report* **19**, 61.

Foster, P.M.D., Lake, B.G., Thomas, L.V., Cook, M.W., and Gangolli, S.D. (1981). Studies on the testicular effects and zinc excretion produced by various isomers of monobutyl-*o*-phthalate in the rat. *Chem.-Biol. Interact.* **34**, 233-238.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* 10 (Suppl. 10), 1-175.

Gangolli, S.D. (1982). Testicular effects of phthalate esters. *Environ. Health Perspect.* 45, 77-84.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *JNCI* 62, 957-974.

Gledhill, W.E., Kaley, R.G., Adams, W.J., Hicks, O., Michael, P.R., Saeger, V.W., and LeBlanc, G.A. (1980). An environmental safety assessment of butyl benzyl phthalate. *Environ. Sci. Technol.* 14, 301-305.

Gosselin, R.E., Smith, R.P., Hodge, H.C., and Braddock, J.E. (1984). *Clinical Toxicology of Commercial Products*, 5th ed., p. II-204. Williams & Wilkins, Baltimore, MD.

Gray, T.J.B., and Gangolli, S.D. (1986). Aspects of the testicular toxicity of phthalate esters. *Environ. Health Perspect.* 65, 229-235.

Haberman, S., Guess, W.L., Rowan, D.F., Bowman, R.O., and Bower, R.K. (1968). Effects of plastics and their additives on human serum proteins, antibodies and developing chick embryos. *Soc. Plast. Eng. J.* 24, 62-69.

Hammond, B.G. (1981). Toxicology of butyl benzyl phthalate. *Toxicologist* 1, 114. (Abstr.)

Hammond, B.G., Levinskas, G.J., Robinson, E.C., and Johannsen, F.R. (1987). A review of the subchronic toxicity of butyl benzyl phthalate. *Toxicol. Ind. Health* 3, 79-98.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* 58, 385-392.

Hawley's Condensed Chemical Dictionary (1987). 11th ed. (N.I. Sax and R.J. Lewis, Sr., Eds.), p. 184. Van Nostrand Reinhold, New York.

Hazardline (1983). Occupation Health Services, Inc., Secaucus, NJ. July 1, 1988.

Heindel, J.J., Gulati, D.K., Mounce, R.C., Russell, S.R., and Lamb, J.C., IV. (1989). Reproductive toxicity of three phthalic acid esters in a continuous breeding protocol. *Fundam. Appl. Toxicol.* 12, 508-518.

Hollander, M., and Wolfe, D.A. (1973). *Nonparametric Statistical Methods*, pp. 120-123. John Wiley and Sons, New York.

Howard, P.H., Banerjee, S., and Robillard, K.H. (1985). Measurement of water solubilities, octanol/water partition coefficients and vapor pressures of commercial phthalate esters. *Environ. Toxicol. Chem.* 4, 653-661.

International Agency for Research on Cancer (IARC) (1982). *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Industrial Chemicals and Dyestuffs*, Vol. 29, pp. 193-201. IARC, Lyon, France.

International Agency for Research on Cancer (IARC) (1987). *IARC Monographs on the Evaluation of the Carcinogenic Risk to Humans. Overall Evaluations of Carcinogenicity: An Updating of IARC Monographs Volumes 1 to 42*, (Suppl. 7). IARC, Lyon, France.

International Labor Office (1983). *Encyclopedia of Occupational Health and Safety,* Vol. I and II. International Labor Office, Geneva, Switzerland.

Jonckheere, A.R. (1954). A distribution-free *k*-sample test against ordered alternatives. *Biometrika* 41, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation from incomplete observations. *J. Am. Stat. Assoc.* 53, 457-481.

Kasuya, M. (1980). Toxicity of butylbenzyl phthalate (BBP) and other phthalate esters to nervous tissue in culture. *Toxicol. Lett.* 6, 373-378.

Keith, L.H., Garrison, A.W., Allen, F.R., Carter, M.H., Floyd, T.L., Pope, J.D., and Thruston, A.D., Jr. (1976). Identification of organic compounds in drinking water from thirteen U.S. cities. In *Identification and Analysis of Organic Pollutants in Water* (L.H. Keith, Ed.), pp. 329-373. Ann Arbor Science, Ann Arbor, MI.

Kluwe, W.M. (1982). Overview of phthalate ester pharmacokinetics in mammalian species. *Environ. Health Perspect.* **45**, 3-10.

Korhonen, A., Hemminki, K., and Vainio, H. (1983). Embryotoxic effects of phtalic acid derivatives, phosphates and aromatic oils used in the manufacturing of rubber on three day chicken embryos. *Drug Chem. Toxicol.* **6**, 191-207.

Kozumbo, W.J., Kroll, R., and Rubin, R.J. (1982). Assessment of the mutagenicity of phthalate esters. *Environ. Health Perspect.* **45**, 103-109.

Krauskopf, L.G. (1973). Studies on the toxicity of phthalates via ingestion. *Environ. Health Perspect.* **3**, 61-72.

Lamb, J.C., IV, Chapin, R.E., Teague, J., Lawton, A.D., and Reel, J.R. (1987). Reproductive effects of four phthalate acid esters in the mouse. *Toxicol. Appl. Pharmacol.* **88**, 255-269.

Lawrence, W.H. (1979). *Phthalate Esters: The Question of Safety. Toxicology Annual.* Vol 3, pp. 89-139, Marcel Dekker, Inc., New York.

Leyder, F., and Boulanger, P. (1983). Ultraviolet absorption, aqueous solubility, and octanol-water partition for several phthalates. *Bull. Environ. Contam. Toxicol.* **30**, 152-157.

Lhoste, E.F., Roebuck, B.D., Brinck-Johnsen, T., and Longnecker, D.S. (1987a). Effect of castration and hormone replacement on azaserine-induced pancreatic carcinogeneis in male and female Fischer rats. *Carcinogenesis* **8**, 699-703.

Lhoste, E.F., Roebuck, B.D., Stern, J.E., and Longnecker, D.S. (1987b). Effect of orchiectomy and testosterone on the early stages of azaserine-induced pancreatic carcinogenesis in the rat. *Pancreas* **2**, 38-43.

Longnecker, D.S., and Sumi, C. (1990). Effects of sex steroid hormones on pancreatic cancer in the rat. *Int. J. Pancreatol.* **4**, 159-165.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

McFall, J.A., Antoine, S.R., and DeLeon, I.R. (1985). Base-neutral extractable organic pollutants in biota and sediments from Lake Pontchartrain. *Chemosphere* **14**, 1561-1569.

McFee, A.F., Lowe, K.W., and San Sebastian, J.R. (1983). Improved sister-chromatid differentiation using paraffin-coated bromodeoxyuridine tablets in mice. *Mutat. Res.* **119**, 83-88.

McKnight, B., and Crowley, J. (1984). Tests for differences in tumor incidence based on animal carcinogenesis experiments. *J. Am. Stat. Assoc.* **79**, 639-648.

Malley, L.A., Carakostas, M., Hansen, J.F., Rusch, G.M., Kelly, D.P., and Trochimowicz, H.J. (1995). Two-year inhalation toxicity study in rats with hydrochlorofluorocarbon 123. *Fundam. Appl. Toxicol.* **25**, 101-114.

Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.

Margolin, B.H., Resnick, M.A., Rimpo, J.Y., Archer, P., Galloway, S.M., Bloom, A.D., and Zeiger, E. (1986). Statistical analyses for in vitro cytogenetic assays using Chinese hamster ovary cells. *Environ. Mutagen.* **8**, 183-204.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* 10, 71-80.

Milkov, L.E., Aldyreva, M.V., Popova, T.B., Lopukhova, K.A., Makarenko, Y.L., Malyar, L.M., and Shakhova, T.K. (1973). Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins. *Environ. Health Perspect.* 3, 175-178.

Miller, J.A., and Miller, E.C. (1977). Ultimate chemical carcinogens as reactive mutagenic electrophiles. In *Origins of Human Cancer* (H.H. Hiatt, J.D. Watson, and J.A. Winsten, Eds.), pp. 605-627. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Monsanto Company (1994). Immunotoxicology, peroxisomal proliferation, and cell proliferation studies of butyl benzyl phthalate (BBP) administered by dosed feed in female Fischer 344 rats. Prepared for Monsanto by Southern Research Institute. Monsanto Study No. XX-91-62/SRI Study No. A13-TXR-1.

Myhr, B.C., and Caspary, W.J. (1991). Chemical mutagenesis at the thymidine kinase locus in L5178Y mouse lymphoma cells: Results for 31 coded compounds in the National Toxicology Program. *Environ. Mol. Mutagen.* 18, 51-83.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH) (1976). National Occupational Health Survey (NOHS) (1972-1974) (database). U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Cincinnati, OH.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Exposure Survey (1981-1983), unpublished data as of July 1, 1990. NIOSH, Cincinnati, OH.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). Specification NIH-11-1335. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

National Toxicology Program (NTP) (1982a). Carcinogenesis Bioassay of Butyl Benzyl Phthalate (CAS No. 85-68-7) in F344/N Rats and B6C3F$_1$ Mice (Feed Study). Technical Report Series No. 213. NIH Publication No. 82-1769. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.

National Toxicology Program (NTP) (1982b). Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) in F344 Rats and B6C3F$_1$ Mice (Feed Study). Technical Report Series No. 217. NIH Publication No. 82-1773. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.

National Toxicology Program (NTP) (1982c). Carcinogenesis Bioassay of Di(2-ethylhexyl)adipate (CAS No. 103-23-1) in F344 Rats and B6C3F$_1$ Mice (Feed Study). Technical Report Series No. 212. NIH Publication No. 81-1768. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.

National Toxicology Program (NTP) (1983a). Carcinogenesis Bioassay of Diallyl Phthalate (CAS No. 131-17-9) in B6C3F$_1$ Mice (Gavage Study). Technical Report Series No. 242. NIH Publication No. 83-1798 U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC, and Bethesda, MD.

National Toxicology Program (NTP) (1983b). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations for Toxicity Testing for Rats and Mice, 10/31/82 version (updated October 1983). Research Triangle Park, NC.

National Toxicology Program (NTP) (1985). Toxicology and Carcinogenesis Studies of Diallylphthalate (CAS No. 131-17-9) in F344/N Rats (Gavage Studies). Technical Report Series No. 284. NIH Publication No. 85-2540. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

Omori, Y. (1976). Recent progress in safety evaluation studies on plasticizers and plastics and their controlled use in Japan. *Environ. Health Perspect.* **17**, 203-209.

Page, B.D., and Lacroix, G.M. (1992). Studies into the transfer and migration of phthalate esters from aluminum foil-paper laminates to butter and margarine. *Food Addit. Contam.* **9**, 197-212.

Patty's Industrial Hygiene and Toxicology (1967) (G.D. Clayton and F.E. Clayton, Eds.), Vol II, pp. 1904-1906. Interscience Publishers, New York.

Price, C.J., Field, E.A., Marr, M.C., Myers, C.B., Morrissey, R.E., and Schwetz, B.A. (1990). Developmental toxicity of butyl benzyl phthalate (BBP) in mice and rats. *Teratology* **41**, 586. (Abstr.)

Reddy, J.K., and Lalwai, N.D. (1983). Carcinogenesis by hepatic peroxisome proliferators: Evaluation of the risk of hypolipidemic drugs and industrial plasticizers to humans. *CRC Crit. Rev. Toxicol.* **12**, 1-58.

Reddy, J.K, and Qureshi, S.A. (1979). Tumorigenicity of the hypolipidaemic peroxisome proliferator ethyl-α-P-chlorophenoxyisobutyrate (clofibrate) in rats. *Br. J. Cancer* **40**, 476-482.

Reddy, J.K., Azarnoff, D.L., and Hignite, C.E. (1980). Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* **283**, 397-398.

Reddy, J.K., Reddy, M.K., Usman, M.I., Lalwani, N.D., and Rao, M.S. (1986). Comparison of hepatic peroxisome proliferative effect and its implication for hepatocarcinogenicity of phthalate esters, di(2-ethylhexyl) phthalate, and di(2-ethyhexyl) adipate with a hypolipidemic drug. *Environ. Health Perspect.* **65**, 317-327.

Robinson, E.C. (1991). Lack of neuropathological changes in rats after exposure to butyl benzyl phthalate. *J. Toxicol. Environ. Health* **32**, 345-347.

Rowland, I.R., Cottrell, R.C., and Phillips, J.C. (1977). Hydrolysis of phthalate esters by the gastrointestinal contents of the rat. *Food Cosmet. Toxicol.* **15**, 17-21.

Sadtler Standard Spectra. IR No. 57958. NMR No. 30796M. Sadtler Research Laboratories, Philadelphia.

Sax, N.I., and Lewis, R.J., Sr. (1989). *Dangerous Properties of Industrial Materials*, 7th ed., pp. 411-412. Van Nostrand Reinhold, New York.

Seilkop, S.K. (1995). The effect of body weight on tumor incidence and carcinogenicity testing in B6C3F$_1$ mice and F344 rats. *Fundam. Appl. Toxicol.* **24**, 247-259.

Shackelford, W.M., and Keith, L.H. (1976). Frequency of Organic Compounds Identified in Water (EPA-600/4-76-062), Athens, GA, U.S. Environmental Protection Agency, pp. 196-197.

Sheldon, L.S., and Hites, R.A. (1978). Organic compounds in the Delaware River. *Environ. Sci. Technol.* **12**, 1188-1194.

Sheldon, L.S., and Hites, R.A. (1979). Sources and movement of organic chemicals in the Delaware River. *Environ. Sci. Technol.* **13**, 574-579.

Shibko, S.I., and Blumenthal, H. (1973). Toxicology of phthalic esters used in food-packaging material. *Environ. Health Perspect.* **3**, 131-137.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* **28**, 519-531.

Wilson, J.G. (1973). *Environment and Birth Defects.* Academic Press, New York.

Yagi, Y., Nakamura, Y., Tomita, I., Tsuchikawa, K., and Shimoi, N. (1980). Teratogenic potential of di- and mono-(2-ethylhexyl)phthalate in mice. *J. Environ. Pathol. Toxicol.* **4**, 533-544.

Zeiger, E., Haworth, S., Mortelmans, K., and Speck, W. (1985). Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in *Salmonella. Environ. Mutagen.* **7**, 213-232.

Zeiger, E., Haseman, J.K., Shelby, M.D., Margolin, B.H., and Tennant, R.W. (1990). Evaluation of four in vitro genetic toxicity tests for predicting rodent carcinogenicity: Confirmation of earlier results with 41 additional chemicals. *Environ. Mol. Mutagen.* **16** (Suppl. 18), 1-14.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF BUTYL BENZYL PHTHALATE

TABLE A1	Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate	65
TABLE A2	Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate	70
TABLE A3	Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate	88
TABLE A4a	Historical Incidence of Pancreatic Neoplasms in Untreated Male F344/N Rats	94
TABLE A4b	Historical Incidence of Preputial Gland Neoplasms in Untreated Male F344/N Rats	94
TABLE A5	Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate	95

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	19	19	26	26
Natural deaths	3	11	2	2
Survivors				
Terminal sacrifice	28	20	22	22
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Endocrine System				
Adrenal medulla	(10)	(10)	(10)	(10)
Pheochromocytoma benign	1 (10%)		1 (10%)	
Pituitary gland	(9)	(10)	(10)	(10)
Pars distalis, adenoma	2 (22%)	2 (20%)		1 (10%)
Thyroid gland	(10)	(10)	(10)	(10)
C-cell, adenoma				1 (10%)
Genital System				
Testes	(10)	(10)	(10)	(10)
Bilateral, interstitial cell, adenoma	3 (30%)	3 (30%)	2 (20%)	5 (50%)
Interstitial cell, adenoma	4 (40%)	2 (20%)	7 (70%)	4 (40%)
Hematopoietic System				
Spleen	(10)	(10)	(10)	(10)
Histiocytic sarcoma	1 (10%)			
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Keratoacanthoma	1 (10%)			
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Histiocytic sarcoma	1 (10%)			
Leukemia mononuclear		1 (10%)	1 (10%)	
Mesothelioma malignant			3 (30%)	
Systems Examined With No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study				
Alimentary System				
Intestine large, colon	(44)	(48)	(49)	(48)
Adenocarcinoma			1 (2%)	
Carcinoid tumor malignant				1 (2%)
Intestine large, rectum	(49)	(50)	(50)	(50)
Leiomyosarcoma	1 (2%)			
Intestine large, cecum	(47)	(50)	(50)	(50)
Lipoma		1 (2%)		
Intestine small, duodenum	(50)	(50)	(50)	(50)
Intestine small, jejunum	(49)	(50)	(50)	(50)
Leiomyoma	1 (2%)			
Intestine small, ileum	(49)	(50)	(50)	(50)
Leiomyosarcoma		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Carcinoma, metastatic, pancreas				1 (2%)
Hepatocellular carcinoma			1 (2%)	
Hepatocellular adenoma	2 (4%)	1 (2%)		4 (8%)
Hepatocellular adenoma, multiple		1 (2%)		
Mesentery	(7)	(13)	(12)	(5)
Adenocarcinoma, metastatic, intestine large, colon			1 (8%)	
Oral mucosa		(1)	(2)	
Squamous cell papilloma		1 (100%)	2 (100%)	
Pancreas	(50)	(49)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Acinus, adenoma	3 (6%)	2 (4%)	3 (6%)	10 (20%)
Acinus, carcinoma				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Schwannoma malignant				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Carcinoma				1 (2%)
Tongue		(1)	(1)	(1)
Squamous cell papilloma			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Thymoma malignant, metastatic, thymus				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma malignant	2 (4%)		2 (4%)	2 (4%)
Pheochromocytoma benign	6 (12%)	8 (16%)	9 (18%)	8 (16%)
Pheochromocytoma benign, multiple	3 (6%)	2 (4%)		
Islets, pancreatic	(50)	(49)	(50)	(50)
Adenoma	5 (10%)	2 (4%)	4 (8%)	1 (2%)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, adenoma	9 (18%)	11 (22%)	11 (22%)	10 (20%)
Pars distalis, adenoma, multiple	1 (2%)			
Pars distalis, carcinoma		1 (2%)		
Pars intermedia, adenoma			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	5 (10%)	3 (6%)	3 (6%)	1 (2%)
Follicular cell, adenoma		1 (2%)		1 (2%)
Follicular cell, carcinoma				2 (4%)
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	1 (2%)	
Carcinoma	1 (2%)	6 (12%)	1 (2%)	
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Bilateral, interstitial cell, adenoma	37 (74%)	36 (72%)	40 (80%)	37 (74%)
Interstitial cell, adenoma	7 (14%)	10 (20%)	8 (16%)	8 (16%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Pheochromocytoma malignant, metastatic, adrenal medulla			1 (2%)	
Lymph node	(23)	(22)	(30)	(19)
Iliac, leiomyosarcoma, metastatic, intestine large, rectum	1 (4%)			
Lymph node, mandibular	(49)	(49)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Lymph node, mediastinal			(1)	
Adenocarcinoma, metastatic, intestine large, colon			1 (100%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Fibroma		1 (2%)		
Liposarcoma		1 (2%)		
Thymus	(49)	(48)	(50)	(48)
Thymoma benign		1 (2%)		1 (2%)
Thymoma malignant				1 (2%)
Integumentary System				
Mammary gland	(45)	(49)	(47)	(48)
Carcinoma		1 (2%)		
Fibroadenoma	2 (4%)	5 (10%)	2 (4%)	
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				2 (4%)
Basal cell carcinoma	1 (2%)		1 (2%)	1 (2%)
Keratoacanthoma	2 (4%)	2 (4%)	2 (4%)	1 (2%)
Squamous cell papilloma	1 (2%)		1 (2%)	1 (2%)
Sebaceous gland, adenoma			1 (2%)	
Subcutaneous tissue, fibroma	5 (10%)	2 (4%)	5 (10%)	5 (10%)
Subcutaneous tissue, lipoma				1 (2%)
Subcutaneous tissue, melanoma malignant	1 (2%)			
Subcutaneous tissue, schwannoma benign		1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Chordoma	1 (2%)			
Osteosarcoma				1 (2%)
Skeletal muscle	(1)		(1)	
Adenocarcinoma, metastatic, intestine large, colon			1 (100%)	
Hemangiosarcoma	1 (100%)			
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant		1 (2%)	1 (2%)	
Oligodendroglioma malignant	1 (2%)			
Spinal cord	(2)	(1)		(2)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		2 (4%)		
Alveolar/bronchiolar carcinoma		1 (2%)		1 (2%)
Chordoma, metastatic, bone	1 (2%)			
Pheochromocytoma malignant, metastatic, adrenal medulla			1 (2%)	
Thymoma malignant, metastatic, thymus				1 (2%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Special Senses System				
None				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Renal tubule, adenoma	1 (2%)	2 (4%)		
Renal tubule, carcinoma				1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, intestine large, colon			1 (2%)	
Systemic Lesions				
Multiple organs	(50)	(50)	(50)	(50)
Leukemia mononuclear	31 (62%)	28 (56%)	34 (68%)	30 (60%)
Mesothelioma malignant	1 (2%)	3 (6%)		1 (2%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	8	7	9	9
2-Year study	50	50	50	50
Total primary neoplasms				
15-Month interim evaluation	12	8	14	11
2-Year study	135	142	135	136
Total animals with benign neoplasms				
15-Month interim evaluation	8	7	9	9
2-Year study	46	49	49	47
Total benign neoplasms				
15-Month interim evaluation	11	7	10	11
2-Year study	94	98	94	91
Total animals with malignant neoplasms				
15-Month interim evaluation	1	1	4	
2-Year study	37	35	37	38
Total malignant neoplasms				
15-Month interim evaluation	1	1	4	
2-Year study	41	44	41	45
Total animals with metastatic neoplasms				
2-Year study	2		2	2
Total metastatic neoplasms				
2-Year study	2		12	3

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm

Number of Days on Study	255	261	438	477	479	541	544	557	562	576	593	607	611	614	631	631	644	662	677	711	718	718	729	729	729
Carcass ID Number	008	039	053	001	025	012	110	122	420	445	418	105	049	442	409	027	203	041	311	211	017	034	004	006	001
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma	X																								
Intestine large, cecum	+	A	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Intestine small, ileum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																		X							
Mesentery							+			+	+								+						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant														X											
Pheochromocytoma benign																	X								
Pheochromocytoma benign, multiple												X										X	X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X				X														X				
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma			X							X									X						
General Body System																									
Tissue NOS																	+								
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																	X			X					
Carcinoma														X											
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma				X					X	X	X	X	X	X		X	X	X	X	X		X	X	X	X
Interstitial cell, adenoma							X	X							X						X				

+: 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 Feed Study of Butyl Benzyl Phthalate: 0 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	730	730	730	730	730	731	
Carcass ID Number	014	015	019	020	024	026	029	033	036	037	038	043	044	046	002	007	013	016	023	028	030	032	035	047	048	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	44
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyosarcoma																										1
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leiomyoma	X																									1
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma						X																				2
Mesentery			+												+							+				7
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma			X							X											X					3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																	X									2
Pheochromocytoma benign																X	X	X	X	X						6
Pheochromocytoma benign, multiple																										3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma							X		X						X		X			X						5
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma	X			X				X		X		X					X									9
Pars distalis, adenoma, multiple																				X						1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma										X						X										5
General Body System																										
Tissue NOS																										1
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X								X															4
Carcinoma																										1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	37
Interstitial cell, adenoma	X			X																X						7

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm (continued)

Number of Days on Study	255	261	438	447	479	541	544	557	562	567	593	607	611	614	631	631	644	662	667	711	717	718	728	729	729	
Carcass ID Number	008	039	053	001	025	012	010	022	020	045	018	055	049	042	059	027	043	041	031	021	017	034	040	046	001	
Hematopoietic System																										
Blood																									+	
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node	+	+			+	+		+	+	+	+	+	+		+		+		+	+	+			+		
Iliac, leiomyosarcoma, metastatic, intestine large, rectum	X																									
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	
Fibroadenoma																									X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Basal cell carcinoma											X															
Keratoacanthoma																										
Squamous cell papilloma																										
Subcutaneous tissue, fibroma														X								X				
Subcutaneous tissue, melanoma malignant																										
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Chordoma														X												
Skeletal muscle			+																							
Hemangiosarcoma			X																							
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Oligodendroglioma malignant		X																								
Peripheral nerve														+		+										
Spinal cord																+										
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Chordoma, metastatic, bone														X												
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Special Senses System																										
Ear																										
Eye																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Renal tubule, adenoma																								X		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Leukemia mononuclear					X	X		X	X	X	X	X	X		X		X	X	X	X	X	X	X	X	X	
Mesothelioma malignant																			X							

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm (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	731	
Carcass ID Number	014	015	019	020	024	026	029	033	036	037	038	043	044	046	002	007	013	016	023	028	030	032	035	047	048	Total Tissues/ Tumors
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+		+	+	+	+	+															+		23
Iliac, leiomyosarcoma, metastatic, intestine large, rectum																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	45
Fibroadenoma																						X				2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma																										1
Keratoacanthoma								X																X		2
Squamous cell papilloma										X																1
Subcutaneous tissue, fibroma							X					X											X			5
Subcutaneous tissue, melanoma malignant																			X							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma																										1
Skeletal muscle																										1
Hemangiosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Oligodendroglioma malignant																										1
Peripheral nerve						+																				3
Spinal cord						+																				2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Chordoma, metastatic, bone																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																		+			+					2
Eye																		+								1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear		X		X	X	X	X				X			X	X	X					X	X	X	X		31
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 3,000 ppm

Number of Days on Study	3	4	4	4	4	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6
	0	5	7	9	9	1	4	4	5	6	6	8	8	0	0	0	0	1	2	2	4	4	4	7	9
	3	0	7	9	9	9	1	4	0	5	9	4	6	1	7	7	7	1	6	7	4	5	9	8	4
Carcass ID Number	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
	6	6	8	6	7	8	0	9	0	9	0	8	1	7	6	7	8	0	7	7	0	8	7	6	0
	3	8	5	4	1	9	6	1	7	3	0	6	0	7	9	0	3	3	2	8	4	4	4	1	2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lipoma																									
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma																									
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																								X	
Hepatocellular adenoma, multiple																									
Mesentery						+			+						+		+		+				+		
Oral mucosa																									
Squamous cell papilloma																									
Pancreas	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma											X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign								X						X											
Pheochromocytoma benign, multiple																									
Islets, pancreatic	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X								X								X	X			
Pars distalis, carcinoma	X																								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																			X						
Follicular cell, adenoma																									
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
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	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 3,000 ppm
(continued)

Number of Days on Study	698	708	708	723	723	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731		
Carcass ID Number	080	090	105	075	092	062	066	067	079	082	087	095	096	097	098	108	065	073	076	081	088	094	099	101	109	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lipoma					X																					1
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																					X					1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																										1
Hepatocellular adenoma, multiple																								X		1
Mesentery		+		+	+		+						+							+					+	13
Oral mucosa																						+				1
Squamous cell papilloma																						X				1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																						X				2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue														+												1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign		X						X						X	X			X	X							8
Pheochromocytoma benign, multiple	X																			X						2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma		X																								2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma								X			X					X	X		X					X	X	11
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma											X														X	3
Follicular cell, adenoma		X																								1
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma									X									X				X				3
Carcinoma				X	X				X						X									X		6
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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		36
Interstitial cell, adenoma																			X							10

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 3,000 ppm
(continued)

Number of Days on Study	3	4	4	4	4	5	5	5	5	5	5	5	5	6	6	6	6	6	6	6	6	6	6	6	6
	0	5	7	9	9	1	4	4	5	6	6	8	8	0	0	0	0	1	2	2	4	4	4	7	9
	3	0	7	9	9	9	1	4	0	5	9	4	6	1	7	7	7	1	6	7	4	5	9	8	4
Carcass ID Number	0	0	0	0	0	0	1	0	1	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1
	6	6	8	6	7	8	0	9	0	9	0	8	1	7	6	7	8	0	7	7	0	8	7	6	0
	3	8	5	4	1	9	6	1	7	3	0	6	0	7	9	0	3	3	2	8	4	4	4	1	2
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+						+	+		+	+		+	+	+	+		+	+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroma						X																			
Liposarcoma																									
Thymus	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma																						X		X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma																						X			
Subcutaneous tissue, schwannoma benign								X																	
Subcutaneous tissue, schwannoma malignant																						X			
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									
Peripheral nerve																									+
Spinal cord																									+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, adenoma															X										
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear				X		X	X		X	X		X	X	X	X	X				X	X			X	
Mesothelioma malignant																	X						X		

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 3,000 ppm (continued)

Number of Days on Study	698	708	708	723	723	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	
Carcass ID Number	080	090	105	075	092	626	666	667	679	682	687	695	696	697	698	108	065	073	076	081	088	094	099	101	109	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node		+	+	+				+		+		+	+	+						+		+				22
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroma																										1
Liposarcoma						X																				1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Thymoma benign													X													1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma										X																1
Fibroadenoma			X									X					X									5
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma						X									X											2
Subcutaneous tissue, fibroma																								X		2
Subcutaneous tissue, schwannoma benign																										1
Subcutaneous tissue, schwannoma malignant																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																								X		1
Peripheral nerve																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma						X													X							2
Alveolar/bronchiolar carcinoma																						X				1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																								X		2
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X	X	X	X				X			X	X		X		X		X	X	X		X		28
Mesothelioma malignant													X													3

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm

Number of Days on Study	400	494	542	542	569	574	592	611	614	628	651	655	670	674	681	681	683	684	697	697	701	702	702	703	708
Carcass ID Number	130	129	159	169	160	127	170	168	164	145	144	157	147	135	133	150	137	136	123	128	154	132	149	156	162
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Adenocarcinoma										X															
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Hepatocellular carcinoma																									
Mesentery	+		+			+				+	+			+	+							+		+	
Adenocarcinoma, metastatic, intestine large, colon										X															
Oral mucosa																									
Squamous cell papilloma																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																				+					
Squamous cell papilloma																				X					
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant															X								X		
Pheochromocytoma benign																		X				X			X
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																					X	X			
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X								X	X										X	
Pars intermedia, adenoma																				X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma														X		X									
General Body System																									
None																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	717	722	723	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	165	222	424	224	226	338	441	446	451	553	558	563	567	621	625	631	634	639	640	643	648	652	655	661	666	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Hepatocellular carcinoma		X																								1
Mesentery			+			+			+																	12
Adenocarcinoma, metastatic, intestine large, colon																										1
Oral mucosa														+											+	2
Squamous cell papilloma														X											X	2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Acinus, adenoma													X	X									X			3
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																										1
Squamous cell papilloma																										1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant																										2
Pheochromocytoma benign				X							X		X			X							X		X	9
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																							X	X		4
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma			X		X						X				X		X						X	X		11
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X																					3
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	400	494	542	542	569	574	592	611	614	628	651	655	670	674	681	681	683	684	697	697	701	702	702	703	708
Carcass ID Number	130	129	159	169	160	127	170	168	164	445	444	457	447	435	433	450	437	436	423	428	454	432	449	456	462
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																									
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Bilateral, interstitial cell, adenoma		X	X		X	X	X		X		X	X	X	X		X	X	X	X	X	X	X	X	X	
Interstitial cell, adenoma				X				X		X					X										X
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal medulla															X										
Lymph node	+		+	+		+		+	+	+	+	+	+	+	+	+	+	+	+			+		+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mediastinal										+															
Adenocarcinoma, metastatic, intestine large, colon										X															
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	I	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Keratoacanthoma													X												
Squamous cell papilloma												X													
Sebaceous gland, adenoma																									
Subcutaneous tissue, fibroma										X				X								X			X
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle										+															
Adenocarcinoma, metastatic, intestine large, colon										X															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	717	722	723	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	165	122	142	124	126	138	141	146	151	153	158	163	167	121	125	131	134	139	140	143	148	152	155	161	166	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																				X						1
Carcinoma												X														1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
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	40
Interstitial cell, adenoma			X	X												X										8
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Lymph node	+	+	+	+			+				+								+	+	+		+	+		30
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mediastinal																										1
Adenocarcinoma, metastatic, intestine large, colon																										1
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Fibroadenoma																			X	X						2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma				X																						1
Keratoacanthoma																								X		2
Squamous cell papilloma																										1
Sebaceous gland, adenoma																									X	1
Subcutaneous tissue, fibroma																								X		5
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Adenocarcinoma, metastatic, intestine large, colon																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant		X																								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	400	494	542	542	569	574	592	611	614	628	651	655	670	674	681	681	683	684	697	697	701	702	702	703	708
Carcass ID Number	130	129	159	169	160	127	170	168	164	145	144	157	147	135	133	150	137	136	123	128	154	132	149	156	162
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant, metastatic, adrenal medulla															X										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, intestine large, colon										X															
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X		X	X		X		X	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	717	722	723	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	165	122	142	124	126	138	141	146	151	153	158	163	167	121	125	131	134	139	140	143	148	152	155	161	166	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant, metastatic, adrenal medulla																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, intestine large, colon																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X	X			X	X		X	X		X						X	X	X	X	X			34

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm

Number of Days on Study	180	297	464	466	477	550	593	614	634	639	639	654	662	663	668	674	674	675	684	698	698	703	709	709	716
Carcass ID Number	219	186	187	201	224	207	216	225	184	185	228	193	188	206	202	189	204	215	190	181	194	191	217	227	205
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoid tumor malignant																									
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pancreas																X									
Hepatocellular adenoma			X																					X	
Mesentery									+				+				+							+	+
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																								X	X
Acinus, carcinoma																X									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Tongue																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma malignant, metastatic, thymus		X																							
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign											X				X		X		X	X					
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma																			X	X		X		X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																								X	
Follicular cell, adenoma																									
Follicular cell, carcinoma																				X					
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma							X		X	X	X		X	X	X	X	X	X	X	X	X	X	X		
Interstitial cell, adenoma			X	X		X		X				X													

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	716	716	718	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	211	212	209	183	195	198	200	210	218	220	222	226	230	182	192	196	197	199	203	208	213	214	221	223	229	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Carcinoid tumor malignant			X																							1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pancreas																										1
Hepatocellular adenoma																	X	X								4
Mesentery																										5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma				X							X	X				X		X		X		X			X	10
Acinus, carcinoma																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant			X																							1
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma													X													1
Tongue													+													1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma malignant, metastatic, thymus																										1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant					X									X												2
Pheochromocytoma benign											X													X	X	8
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma						X																				1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma									X	X						X	X			X						10
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																										1
Follicular cell, adenoma			X																							1
Follicular cell, carcinoma								X																		2
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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		37
Interstitial cell, adenoma												X			X										X	8

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	180	197	464	466	447	550	593	614	634	639	633	654	662	663	668	674	674	675	684	698	698	703	709	709	716
Carcass ID Number	219	186	187	201	224	207	216	225	184	185	228	193	188	206	202	189	204	215	190	181	194	191	217	227	205
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+				+	+	+	+			+	+	+	+	+		+	+			+			+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Thymoma benign																									
Thymoma malignant		X																							
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma											X														
Basal cell carcinoma																									
Keratoacanthoma																									
Squamous cell papilloma																									
Subcutaneous tissue, fibroma															X				X			X			
Subcutaneous tissue, lipoma																									
Subcutaneous tissue, schwannoma malignant																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma				X																					
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Peripheral nerve				+																					
Spinal cord				+																					+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar carcinoma																									
Thymoma malignant, metastatic, thymus		X																							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									+
Eye																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, carcinoma																									X
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X				X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	
Mesothelioma malignant									X																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	716	716	718	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	211	212	209	183	195	198	200	210	218	220	222	226	230	182	192	196	197	199	203	208	213	214	221	223	229	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+																+	+	+		+					19
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymoma benign							X																			1
Thymoma malignant																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma													X													2
Basal cell carcinoma																	X									1
Keratoacanthoma																		X								1
Squamous cell papilloma					X																					1
Subcutaneous tissue, fibroma																		X	X							5
Subcutaneous tissue, lipoma												X														1
Subcutaneous tissue, schwannoma malignant			X																							1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Peripheral nerve																										1
Spinal cord																										2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar carcinoma																X										1
Thymoma malignant, metastatic, thymus																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye						+																				1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X	X										X				X	X	X		X		X	X		30
Mesothelioma malignant																										1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	9/50 (18%)	10/50 (20%)	9/50 (18%)	8/50 (16%)
Adjusted rate[b]	28.2%	38.8%	34.5%	25.4%
Terminal rate[c]	6/28 (21%)	6/20 (30%)	6/22 (27%)	3/22 (14%)
First incidence (days)	607	544	684	639
Life table test[d]	P=0.473N	P=0.263	P=0.448	P=0.589N
Logistic regression test[d]	P=0.335N	P=0.410	P=0.562N	P=0.475N
Cochran-Armitage test[d]	P=0.402N			
Fisher exact test[d]		P=0.500	P=0.602N	P=0.500N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	10/50 (20%)	10/50 (20%)	11/50 (22%)	10/50 (20%)
Adjusted rate	30.1%	38.8%	38.6%	33.3%
Terminal rate	6/28 (21%)	6/20 (30%)	6/22 (27%)	5/22 (23%)
First incidence (days)	607	544	681	639
Life table test	P=0.469	P=0.348	P=0.370	P=0.496
Logistic regression test	P=0.487N	P=0.524	P=0.557	P=0.573N
Cochran-Armitage test	P=0.535			
Fisher exact test		P=0.598N	P=0.500	P=0.598N
Liver: Hepatocellular Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	6.5%	8.5%	0.0%	14.2%
Terminal rate	1/28 (4%)	1/20 (5%)	0/22 (0%)	2/22 (9%)
First incidence (days)	662	678	—[e]	464
Life table test	P=0.223	P=0.600	P=0.250N	P=0.291
Logistic regression test	P=0.240	P=0.661	P=0.227N	P=0.339
Cochran-Armitage test	P=0.232			
Fisher exact test		P=0.691N	P=0.247N	P=0.339
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/50 (4%)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted rate	6.5%	8.5%	4.2%	14.2%
Terminal rate	1/28 (4%)	1/20 (5%)	0/22 (0%)	2/22 (9%)
First incidence (days)	662	678	722	464
Life table test	P=0.212	P=0.600	P=0.529N	P=0.291
Logistic regression test	P=0.238	P=0.661	P=0.483N	P=0.339
Cochran-Armitage test	P=0.227			
Fisher exact test		P=0.691N	P=0.500N	P=0.339
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	0/50 (0%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	0.0%	15.0%	0.0%	4.5%
Terminal rate	0/28 (0%)	3/20 (15%)	0/22 (0%)	1/22 (5%)
First incidence (days)	—	729 (T)	—	729 (T)
Life table test	P=0.581	P=0.067	—	P=0.452
Logistic regression test	P=0.581	P=0.067	—	P=0.452
Cochran-Armitage test	P=0.634			
Fisher exact test		P=0.121	—	P=0.500

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.1%	19.8%	9.1%	0.0%
Terminal rate	2/28 (7%)	2/20 (10%)	2/22 (9%)	0/22 (0%)
First incidence (days)	729 (T)	645	729 (T)	—
Life table test	P=0.115N	P=0.128	P=0.607	P=0.292N
Logistic regression test	P=0.081N	P=0.165	P=0.607	P=0.292N
Cochran-Armitage test	P=0.092N			
Fisher exact test		P=0.218	P=0.691N	P=0.247N
Mammary Gland: Fibroadenoma or Carcinoma				
Overall rate	2/50 (4%)	6/50 (12%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.1%	24.3%	9.1%	0.0%
Terminal rate	2/28 (7%)	3/20 (15%)	2/22 (9%)	0/22 (0%)
First incidence (days)	729 (T)	645	729 (T)	—
Life table test	P=0.099N	P=0.066	P=0.607	P=0.292N
Logistic regression test	P=0.066N	P=0.090	P=0.607	P=0.292N
Cochran-Armitage test	P=0.077N			
Fisher exact test		P=0.134	P=0.691N	P=0.247N
Oral Cavity (Oral Mucosa or Tongue): Squamous Cell Papilloma				
Overall rate	0/50 (0%)	1/50 (2%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	5.0%	11.9%	0.0%
Terminal rate	0/28 (0%)	1/20 (5%)	2/22 (9%)	0/22 (0%)
First incidence (days)	—	729 (T)	697	—
Life table test	P=0.599	P=0.433	P=0.097	—
Logistic regression test	P=0.624N	P=0.433	P=0.121	—
Cochran-Armitage test	P=0.634			
Fisher exact test		P=0.500	P=0.121	—
Pancreas: Adenoma				
Overall rate	3/50 (6%)	2/49 (4%)	3/50 (6%)	10/50 (20%)
Adjusted rate	10.7%	7.4%	13.6%	41.0%
Terminal rate	3/28 (11%)	1/20 (5%)	3/22 (14%)	8/22 (36%)
First incidence (days)	729 (T)	569	729 (T)	709
Life table test	P=0.002	P=0.624N	P=0.548	P=0.011
Logistic regression test	P=0.006	P=0.543N	P=0.548	P=0.016
Cochran-Armitage test	P=0.005			
Fisher exact test		P=0.510N	P=0.661N	P=0.036
Pancreas: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/49 (4%)	3/50 (6%)	11/50 (22%)
Adjusted rate	10.7%	7.4%	13.6%	42.7%
Terminal rate	3/28 (11%)	1/20 (5%)	3/22 (14%)	8/22 (36%)
First incidence (days)	729 (T)	569	729 (T)	674
Life table test	P<0.001	P=0.624N	P=0.548	P=0.007
Logistic regression test	P=0.003	P=0.543N	P=0.548	P=0.014
Cochran-Armitage test	P=0.002			
Fisher exact test		P=0.510N	P=0.661N	P=0.020

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Pancreatic Islets: Adenoma				
Overall rate	5/50 (10%)	2/49 (4%)	4/50 (8%)	1/50 (2%)
Adjusted rate	17.9%	6.8%	15.2%	4.5%
Terminal rate	5/28 (18%)	0/20 (0%)	2/22 (9%)	1/22 (5%)
First incidence (days)	729 (T)	607	701	729 (T)
Life table test	P=0.150N	P=0.347N	P=0.623N	P=0.161N
Logistic regression test	P=0.097N	P=0.271N	P=0.518N	P=0.161N
Cochran-Armitage test	P=0.112N			
Fisher exact test		P=0.226N	P=0.500N	P=0.102N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	10/50 (20%)	11/50 (22%)	11/50 (22%)	10/49 (20%)
Adjusted rate	30.6%	42.2%	37.9%	35.1%
Terminal rate	7/28 (25%)	7/20 (35%)	6/22 (27%)	5/22 (23%)
First incidence (days)	438	499	569	684
Life table test	P=0.473	P=0.261	P=0.352	P=0.431
Logistic regression test	P=0.467N	P=0.460	P=0.545	P=0.575N
Cochran-Armitage test	P=0.546N			
Fisher exact test		P=0.500	P=0.500	P=0.579
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	10/50 (20%)	12/50 (24%)	11/50 (22%)	10/49 (20%)
Adjusted rate	30.6%	43.3%	37.9%	35.1%
Terminal rate	7/28 (25%)	7/20 (35%)	6/22 (27%)	5/22 (23%)
First incidence (days)	438	303	569	684
Life table test	P=0.506	P=0.197	P=0.352	P=0.431
Logistic regression test	P=0.456N	P=0.394	P=0.545	P=0.575N
Cochran-Armitage test	P=0.511N			
Fisher exact test		P=0.405	P=0.500	P=0.579
Preputial Gland: Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted rate	12.8%	15.0%	4.5%	0.0%
Terminal rate	2/28 (7%)	3/20 (15%)	1/22 (5%)	0/22 (0%)
First incidence (days)	644	729 (T)	729 (T)	—
Life table test	P=0.038N	P=0.644	P=0.231N	P=0.087N
Logistic regression test	P=0.022N	P=0.593N	P=0.165N	P=0.059N
Cochran-Armitage test	P=0.025N			
Fisher exact test		P=0.500N	P=0.181N	P=0.059N
Preputial Gland: Carcinoma				
Overall rate	1/50 (2%)	6/50 (12%)	1/50 (2%)	0/50 (0%)
Adjusted rate	2.7%	25.1%	4.5%	0.0%
Terminal rate	0/28 (0%)	3/20 (15%)	1/22 (5%)	0/22 (0%)
First incidence (days)	614	626	729 (T)	—
Life table test	P=0.135N	P=0.030	P=0.749	P=0.470N
Logistic regression test	P=0.099N	P=0.044	P=0.763N	P=0.504N
Cochran-Armitage test	P=0.111N			
Fisher exact test		P=0.056	P=0.753N	P=0.500N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Preputial Gland: Adenoma or Carcinoma				
Overall rate	5/50 (10%)	8/50 (16%)	2/50 (4%)	0/50 (0%)
Adjusted rate	15.1%	33.9%	9.1%	0.0%
Terminal rate	2/28 (7%)	5/20 (25%)	2/22 (9%)	0/22 (0%)
First incidence (days)	614	626	729 (T)	—
Life table test	P=0.014N	P=0.136	P=0.273N	P=0.045N
Logistic regression test	P=0.006N	P=0.201	P=0.196N	P=0.032N
Cochran-Armitage test	P=0.008N			
Fisher exact test		P=0.277	P=0.218N	P=0.028N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	3/50 (6%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	10.7%	10.0%	9.4%	9.1%
Terminal rate	3/28 (11%)	2/20 (10%)	1/22 (5%)	2/22 (9%)
First incidence (days)	729 (T)	729 (T)	655	729 (T)
Life table test	P=0.506N	P=0.654N	P=0.614	P=0.611N
Logistic regression test	P=0.426N	P=0.654N	P=0.641N	P=0.611N
Cochran-Armitage test	P=0.456N			
Fisher exact test		P=0.500N	P=0.661N	P=0.500N
Skin: Basal Cell Adenoma or Basal Cell Carcinoma				
Overall rate	1/50 (2%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	2.5%	0.0%	4.5%	11.3%
Terminal rate	0/28 (0%)	0/20 (0%)	1/22 (5%)	2/22 (9%)
First incidence (days)	593	—	729 (T)	639
Life table test	P=0.098	P=0.516N	P=0.742	P=0.289
Logistic regression test	P=0.101	P=0.502N	P=0.760	P=0.307
Cochran-Armitage test	P=0.097			
Fisher exact test		P=0.500N	P=0.753N	P=0.309
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Basal Cell Carcinoma				
Overall rate	4/50 (8%)	2/50 (4%)	4/50 (8%)	5/50 (10%)
Adjusted rate	12.9%	10.0%	13.8%	20.2%
Terminal rate	3/28 (11%)	2/20 (10%)	2/22 (9%)	4/22 (18%)
First incidence (days)	593	729 (T)	655	639
Life table test	P=0.272	P=0.478N	P=0.587	P=0.403
Logistic regression test	P=0.347	P=0.394N	P=0.618N	P=0.518
Cochran-Armitage test	P=0.309			
Fisher exact test		P=0.339N	P=0.643N	P=0.500
Skin (Subcutaneous Tissue): Fibroma				
Overall rate	5/50 (10%)	2/50 (4%)	5/50 (10%)	5/50 (10%)
Adjusted rate	16.0%	8.3%	15.9%	17.3%
Terminal rate	3/28 (11%)	1/20 (5%)	1/22 (5%)	2/22 (9%)
First incidence (days)	614	645	628	668
Life table test	P=0.407	P=0.343N	P=0.562	P=0.550
Logistic regression test	P=0.452	P=0.254N	P=0.601N	P=0.614N
Cochran-Armitage test	P=0.415			
Fisher exact test		P=0.218N	P=0.630N	P=0.630N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Testes: Adenoma				
Overall rate	44/50 (88%)	46/50 (92%)	48/50 (96%)	45/50 (90%)
Adjusted rate	97.8%	97.8%	98.0%	100.0%
Terminal rate	27/28 (96%)	19/20 (95%)	21/22 (95%)	22/22 (100%)
First incidence (days)	477	450	494	464
Life table test	P=0.354	P=0.049	P=0.122	P=0.205
Logistic regression test	P=0.568	P=0.371	P=0.383	P=0.619
Cochran-Armitage test	P=0.449			
Fisher exact test		P=0.370	P=0.134	P=0.500
Thyroid Gland (C-cell): Adenoma				
Overall rate	5/50 (10%)	3/50 (6%)	3/50 (6%)	1/50 (2%)
Adjusted rate	14.1%	12.8%	9.7%	3.6%
Terminal rate	2/28 (7%)	2/20 (10%)	1/22 (5%)	0/22 (0%)
First incidence (days)	438	626	674	709
Life table test	P=0.091N	P=0.475N	P=0.375N	P=0.130N
Logistic regression test	P=0.078N	P=0.356N	P=0.378N	P=0.105N
Cochran-Armitage test	P=0.079N			
Fisher exact test		P=0.357N	P=0.357N	P=0.102N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	0/50 (0%)	1/50 (2%)	0/50 (0%)	3/50 (6%)
Adjusted rate	0.0%	4.2%	0.0%	11.6%
Terminal rate	0/28 (0%)	0/20 (0%)	0/22 (0%)	1/22 (5%)
First incidence (days)	—	708	—	698
Life table test	P=0.042	P=0.449	—	P=0.096
Logistic regression test	P=0.050	P=0.473	—	P=0.121
Cochran-Armitage test	P=0.044			
Fisher exact test		P=0.500	—	P=0.121
All Organs: Malignant Mesothelioma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	1/50 (2%)
Adjusted rate	3.1%	10.9%	0.0%	2.4%
Terminal rate	0/28 (0%)	1/20 (5%)	0/22 (0%)	0/22 (0%)
First incidence (days)	677	607	—	634
Life table test	P=0.376N	P=0.245	P=0.477N	P=0.737N
Logistic regression test	P=0.411N	P=0.299	P=0.497N	P=0.762
Cochran-Armitage test	P=0.409N			
Fisher exact test		P=0.309	P=0.500N	P=0.753N
All Organs: Mononuclear Cell Leukemia				
Overall rate	31/50 (62%)	28/50 (56%)	34/50 (68%)	30/50 (60%)
Adjusted rate	71.8%	72.0%	74.1%	65.6%
Terminal rate	16/28 (57%)	10/20 (50%)	11/22 (50%)	7/22 (32%)
First incidence (days)	479	499	400	180
Life table test	P=0.469	P=0.364	P=0.268	P=0.478
Logistic regression test	P=0.466	P=0.233N	P=0.469	P=0.492N
Cochran-Armitage test	P=0.510			
Fisher exact test		P=0.342N	P=0.338	P=0.500N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
All Organs: Benign Neoplasms				
Overall rate	46/50 (92%)	49/50 (98%)	49/50 (98%)	47/50 (94%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	28/28 (100%)	20/20 (100%)	22/22 (100%)	22/22 (100%)
First incidence (days)	438	450	494	464
Life table test	P=0.374	P=0.033	P=0.144	P=0.199
Logistic regression test	P=0.590	P=0.204	P=0.583	P=0.601
Cochran-Armitage test	P=0.523			
Fisher exact test		P=0.181	P=0.181	P=0.500
All Organs: Malignant Neoplasms				
Overall rate	37/50 (74%)	35/50 (70%)	37/50 (74%)	38/50 (76%)
Adjusted rate	78.5%	80.8%	77.8%	77.5%
Terminal rate	18/28 (64%)	12/20 (60%)	12/22 (55%)	11/22 (50%)
First incidence (days)	255	303	400	180
Life table test	P=0.394	P=0.279	P=0.414	P=0.342
Logistic regression test	P=0.331	P=0.396N	P=0.526	P=0.469
Cochran-Armitage test	P=0.383			
Fisher exact test		P=0.412N	P=0.590N	P=0.500
All Organs: Benign or Malignant Neoplasms				
Overall rate	50/50 (100%)	50/50 (100%)	50/50 (100%)	50/50 (100%)
Adjusted rate	100.0%	100.0%	100.0%	100.0%
Terminal rate	28/28 (100%)	20/20 (100%)	22/22 (100%)	22/22 (100%)
First incidence (days)	255	303	400	180
Life table test	P=0.397	P=0.093	P=0.285	P=0.260
Logistic regression test	—[f]	—	—	—
Cochran-Armitage test	—			
Fisher exact test		P=1.000N	P=1.000N	P=1.000N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreas, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm 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 exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

TABLE A4a
Historical Incidence of Pancreatic Neoplasms in Untreated Male F344/N Rats[a]

	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence			
Total	19/1,191 (1.6%)	0/1,191 (0.0%)	19/1,191 (1.6%)
Standard deviation	2.4%		2.4%
Range	0%-10%		0%-10%

[a] Data as of 17 June 1994

TABLE A4b
Historical Incidence of Preputial Gland Neoplasms in Untreated Male F344/N Rats[a]

	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence			
Total	84/1,164 (7.2%)	41/1,164 (3.5%)	124/1,164 (10.7%)
Standard deviation	5.6%	3.4%	7.5%
Range	0%-24%	0%-14%	2%-30%

[a] Data as of 17 June 1994

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	19	19	26	26
Natural deaths	3	11	2	2
Survivors				
Terminal sacrifice	28	20	22	22
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Intestine large, colon	(10)	(10)	(10)	(10)
Parasite metazoan	1 (10%)		1 (10%)	
Intestine large, rectum	(10)	(10)	(10)	(9)
Parasite metazoan	2 (20%)		2 (20%)	1 (11%)
Intestine large, cecum	(10)	(10)	(10)	(10)
Edema	1 (10%)			
Parasite metazoan		1 (10%)		
Liver	(10)	(10)	(10)	(10)
Angiectasis	1 (10%)			
Basophilic focus	6 (60%)	4 (40%)	2 (20%)	1 (10%)
Clear cell focus	2 (20%)	2 (20%)	1 (10%)	1 (10%)
Eosinophilic focus	3 (30%)	1 (10%)	1 (10%)	2 (20%)
Granuloma		1 (10%)	1 (10%)	1 (10%)
Hemorrhage				1 (10%)
Hepatodiaphragmatic nodule	3 (30%)		1 (10%)	1 (10%)
Inflammation, subacute		1 (10%)	3 (30%)	1 (10%)
Mixed cell focus		2 (20%)	2 (20%)	
Bile duct, hyperplasia	5 (50%)	4 (40%)	4 (40%)	
Hepatocyte, vacuolization cytoplasmic	2 (20%)		1 (10%)	
Kupffer cell, pigmentation	1 (10%)		1 (10%)	
Lobules, necrosis				2 (20%)
Mesentery	(4)	(2)	(5)	(1)
Accessory spleen				1 (100%)
Fibrosis	1 (25%)		1 (20%)	
Hemorrhage			1 (20%)	
Fat, necrosis	4 (100%)	2 (100%)	2 (40%)	
Pancreas	(10)	(10)	(10)	(10)
Atrophy	3 (30%)	4 (40%)	5 (50%)	4 (40%)
Cytoplasmic alteration			1 (10%)	1 (10%)
Cardiovascular System				
Heart	(10)	(10)	(10)	(10)
Cardiomyopathy	8 (80%)	4 (40%)	6 (60%)	6 (60%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
15-Month Interim Evaluation (continued)				
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	4 (40%)	1 (10%)	1 (10%)	3 (30%)
Hyperplasia, focal	1 (10%)		1 (10%)	
Hypertrophy, focal				1 (10%)
Pituitary gland	(9)	(10)	(10)	(10)
Pars distalis, angiectasis		1 (10%)		
Pars distalis, cyst	1 (11%)		1 (10%)	1 (10%)
Pars distalis, hemorrhage				1 (10%)
Pars distalis, hyperplasia, focal	3 (33%)	1 (10%)	2 (20%)	2 (20%)
Thyroid gland	(10)	(10)	(10)	(10)
Ultimobranchial cyst			1 (10%)	2 (20%)
C-cell, hyperplasia			1 (10%)	
Follicle, cyst			1 (10%)	1 (10%)
Genital System				
Epididymis	(10)	(10)	(10)	(10)
Atypia cellular	1 (10%)	2 (20%)	1 (10%)	2 (20%)
Preputial gland	(10)	(10)	(10)	(10)
Inflammation, chronic	6 (60%)	6 (60%)	5 (50%)	6 (60%)
Inflammation, suppurative	2 (20%)		1 (10%)	1 (10%)
Prostate	(10)	(10)	(10)	(10)
Corpora amylacea	1 (10%)	2 (20%)	5 (50%)	7 (70%)
Inflammation, suppurative	5 (50%)	5 (50%)	6 (60%)	4 (40%)
Epithelium, hyperplasia	1 (10%)		1 (10%)	
Testes	(10)	(10)	(10)	(10)
Interstitial cell, hyperplasia	5 (50%)	6 (60%)	8 (80%)	4 (40%)
Hematopoietic System				
Bone marrow	(10)	(10)	(10)	(10)
Myelofibrosis		1 (10%)		
Lymph node	(2)		(2)	(1)
Deep cervical, hemorrhage	1 (50%)			
Deep cervical, pigmentation	1 (50%)			
Mediastinal, hemorrhage	2 (100%)		2 (100%)	1 (100%)
Mediastinal, pigmentation	2 (100%)		2 (100%)	1 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(10)
Ectasia		1 (10%)	1 (10%)	
Hemorrhage	2 (20%)		1 (10%)	
Hyperplasia, lymphoid	1 (10%)	1 (10%)		
Lymph node, mesenteric	(10)	(10)	(10)	(10)
Ectasia	1 (10%)			
Hemorrhage			2 (20%)	
Hyperplasia, lymphoid		1 (10%)		
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	2 (20%)			1 (10%)
Pigmentation, hemosiderin	10 (100%)	10 (100%)	10 (100%)	7 (70%)
Thymus	(10)	(10)	(10)	(9)
Cyst	1 (10%)			

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
15-Month Interim Evaluation (continued)				
Integumentary System				
Skin	(10)	(10)	(10)	(10)
Inflammation, chronic			1 (10%)	
Musculoskeletal System				
Bone	(10)	(10)	(10)	(10)
Femur, osteopetrosis	1 (10%)			
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Hemorrhage	1 (10%)			
Infiltration cellular, histiocyte	2 (20%)			2 (20%)
Alveolar epithelium, hyperplasia	1 (10%)		1 (10%)	1 (10%)
Nose	(10)	(10)	(10)	(10)
Exudate		2 (20%)	1 (10%)	
Foreign body		2 (20%)	1 (10%)	
Fungus		1 (10%)		
Mucosa, hyperplasia			1 (10%)	
Mucosa, metaplasia, squamous		1 (10%)		
Special Senses System				
Eye				(1)
Cataract				1 (100%)
Hemorrhage				1 (100%)
Retina, degeneration				1 (100%)
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Nephropathy	10 (100%)	10 (100%)	10 (100%)	9 (90%)
Renal tubule, pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Systems Examined With No Lesions Observed				
General Body System				
Nervous System				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study				
Alimentary System				
Intestine large, colon	(44)	(48)	(49)	(48)
Dilatation	1 (2%)			
Parasite metazoan	5 (11%)	3 (6%)	4 (8%)	4 (8%)
Intestine large, rectum	(49)	(50)	(50)	(50)
Parasite metazoan	10 (20%)	5 (10%)	1 (2%)	8 (16%)
Intestine large, cecum	(47)	(50)	(50)	(50)
Edema	3 (6%)	2 (4%)	4 (8%)	
Parasite metazoan		1 (2%)		1 (2%)
Ulcer	1 (2%)			1 (2%)
Intestine small, duodenum	(50)	(50)	(50)	(50)
Erosion		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis		2 (4%)	5 (10%)	2 (4%)
Basophilic focus	22 (44%)	20 (40%)	18 (36%)	14 (28%)
Clear cell focus	15 (30%)	8 (16%)	7 (14%)	8 (16%)
Cyst				2 (4%)
Degeneration, cystic	7 (14%)	7 (14%)	10 (20%)	6 (12%)
Eosinophilic focus	3 (6%)	8 (16%)	7 (14%)	4 (8%)
Granuloma				7 (14%)
Hematopoietic cell proliferation		2 (4%)		
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	1 (2%)	4 (8%)		7 (14%)
Inflammation, subacute				4 (8%)
Mixed cell focus	6 (12%)	3 (6%)	6 (12%)	7 (14%)
Bile duct, hyperplasia	39 (78%)	41 (82%)	38 (76%)	27 (54%)
Centrilobular, necrosis	1 (2%)	4 (8%)		2 (4%)
Hepatocyte, hyperplasia, focal		1 (2%)		
Hepatocyte, vacuolization cytoplasmic	6 (12%)	2 (4%)	2 (4%)	4 (8%)
Kupffer cell, pigmentation	1 (2%)	1 (2%)	5 (10%)	6 (12%)
Lobules, necrosis	2 (4%)		2 (4%)	1 (2%)
Mesentery	(7)	(13)	(12)	(5)
Accessory spleen		3 (23%)		1 (20%)
Angiectasis	1 (14%)			
Fat, necrosis	5 (71%)	8 (62%)	9 (75%)	3 (60%)
Pancreas	(50)	(49)	(50)	(50)
Atrophy	29 (58%)	33 (67%)	27 (54%)	29 (58%)
Edema			3 (6%)	
Acinus, cytoplasmic alteration	1 (2%)		1 (2%)	
Acinus, hyperplasia, focal	4 (8%)	7 (14%)	9 (18%)	12 (24%)
Salivary glands	(50)	(50)	(50)	(50)
Cytoplasmic alteration			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	1 (2%)		4 (8%)	3 (6%)
Ulcer	2 (4%)	1 (2%)	6 (12%)	3 (6%)
Mucosa, hyperplasia	2 (4%)		3 (6%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema	2 (4%)		3 (6%)	
Erosion	2 (4%)	5 (10%)	2 (4%)	1 (2%)
Ulcer	2 (4%)		1 (2%)	1 (2%)
Tongue		(1)	(1)	(1)
Epithelium, hyperplasia		1 (100%)		1 (100%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Cardiovascular System				
Blood vessel	(50)	(50)	(50)	(50)
Hypertrophy	2 (4%)		1 (2%)	
Inflammation, subacute	2 (4%)		1 (2%)	
Thrombosis			1 (2%)	
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	32 (64%)	32 (64%)	24 (48%)	34 (68%)
Thrombosis	2 (4%)	3 (6%)	1 (2%)	5 (10%)
Schwann cell, hyperplasia			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	11 (22%)	11 (22%)	9 (18%)	6 (12%)
Angiectasis		1 (2%)	3 (6%)	2 (4%)
Degeneration, fatty	3 (6%)	5 (10%)	6 (12%)	8 (16%)
Hemorrhage	1 (2%)			1 (2%)
Hyperplasia, focal	7 (14%)	6 (12%)	10 (20%)	4 (8%)
Hypertrophy		1 (2%)		
Hypertrophy, focal	2 (4%)	2 (4%)	5 (10%)	1 (2%)
Vacuolization cytoplasmic	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	6 (12%)	12 (24%)	17 (34%)	12 (24%)
Islets, pancreatic	(50)	(49)	(50)	(50)
Hyperplasia	3 (6%)	2 (4%)		2 (4%)
Metaplasia, hepatocyte		1 (2%)		
Parathyroid gland	(49)	(50)	(49)	(49)
Hyperplasia			1 (2%)	1 (2%)
Pituitary gland	(50)	(50)	(50)	(49)
Pars distalis, angiectasis	3 (6%)		3 (6%)	
Pars distalis, cyst	3 (6%)	5 (10%)	6 (12%)	2 (4%)
Pars distalis, hyperplasia	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Pars distalis, hyperplasia, focal	7 (14%)	11 (22%)	9 (18%)	5 (10%)
Pars distalis, necrosis				1 (2%)
Pars intermedia, angiectasis	3 (6%)		2 (4%)	
Pars intermedia, cyst	1 (2%)	1 (2%)	1 (2%)	
Pars intermedia, hyperplasia		1 (2%)	1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst	2 (4%)	2 (4%)	1 (2%)	2 (4%)
C-cell, hyperplasia	4 (8%)	10 (20%)	12 (24%)	7 (14%)
Follicle, cyst	2 (4%)	2 (4%)		4 (8%)
General Body System				
None				

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate
(continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Atypia cellular	28 (56%)	26 (52%)	29 (58%)	37 (74%)
Hypospermia	34 (68%)	37 (74%)	41 (82%)	43 (86%)
Preputial gland	(50)	(50)	(50)	(50)
Ectasia	11 (22%)	14 (28%)	20 (40%)	14 (28%)
Hyperplasia	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Inflammation, chronic	25 (50%)	17 (34%)	20 (40%)	17 (34%)
Inflammation, suppurative	9 (18%)	9 (18%)	14 (28%)	6 (12%)
Prostate	(50)	(50)	(50)	(50)
Corpora amylacea	17 (34%)	28 (56%)	32 (64%)	30 (60%)
Fibrosis				1 (2%)
Hemorrhage		1 (2%)		
Inflammation, suppurative	29 (58%)	29 (58%)	26 (52%)	19 (38%)
Epithelium, hyperplasia	4 (8%)	6 (12%)	5 (10%)	3 (6%)
Seminal vesicle	(50)	(50)	(50)	(50)
Dilatation	4 (8%)	2 (4%)		1 (2%)
Testes	(50)	(50)	(50)	(50)
Mineralization		1 (2%)		
Interstitial cell, hyperplasia	4 (8%)	7 (14%)	4 (8%)	6 (12%)
Seminiferous tubule, atrophy	2 (4%)	5 (10%)	6 (12%)	4 (8%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hypercellularity	1 (2%)	4 (8%)	1 (2%)	
Myelofibrosis	2 (4%)	3 (6%)	4 (8%)	5 (10%)
Lymph node	(23)	(22)	(30)	(19)
Iliac, hemorrhage	1 (4%)			
Iliac, hyperplasia, lymphoid	1 (4%)			
Iliac, pigmentation	1 (4%)			
Mediastinal, fibrosis			1 (3%)	
Mediastinal, hemorrhage	5 (22%)	5 (23%)		
Mediastinal, pigmentation	5 (22%)	7 (32%)	10 (33%)	5 (26%)
Pancreatic, ectasia			1 (3%)	
Pancreatic, hemorrhage		1 (5%)		1 (5%)
Pancreatic, hyperplasia, lymphoid			1 (3%)	
Pancreatic, pigmentation			4 (13%)	2 (11%)
Renal, hyperplasia, lymphoid	1 (4%)			
Renal, pigmentation	2 (9%)	2 (9%)	3 (10%)	2 (11%)
Lymph node, mandibular	(49)	(49)	(50)	(50)
Congestion	1 (2%)	2 (4%)		
Ectasia	6 (12%)	3 (6%)	4 (8%)	4 (8%)
Hemorrhage	4 (8%)	3 (6%)	2 (4%)	6 (12%)
Hyperplasia, lymphoid	3 (6%)	2 (4%)	1 (2%)	5 (10%)
Pigmentation	2 (4%)	5 (10%)	1 (2%)	
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	6 (12%)	4 (8%)	2 (4%)	1 (2%)
Hemorrhage	1 (2%)	3 (6%)		4 (8%)
Hyperplasia, lymphoid	2 (4%)	2 (4%)	1 (2%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Spleen	(50)	(50)	(50)	(50)
Developmental malformation		1 (2%)		
Fibrosis	5 (10%)	11 (22%)	14 (28%)	10 (20%)
Granuloma				1 (2%)
Hematopoietic cell proliferation	2 (4%)	8 (16%)	5 (10%)	7 (14%)
Hemorrhage		1 (2%)		
Metaplasia, osseous	1 (2%)			
Necrosis	1 (2%)		1 (2%)	
Pigmentation, hemosiderin	14 (28%)	1 (2%)	2 (4%)	6 (12%)
Lymphoid follicle, atrophy		1 (2%)		
Thymus	(49)	(48)	(50)	(48)
Cyst			1 (2%)	
Hemorrhage	1 (2%)	1 (2%)		
Integumentary System				
Mammary gland	(45)	(49)	(47)	(48)
Hyperplasia	10 (22%)	16 (33%)	10 (21%)	12 (25%)
Skin	(50)	(50)	(50)	(50)
Acanthosis		2 (4%)	2 (4%)	10 (20%)
Cyst epithelial inclusion		1 (2%)	1 (2%)	1 (2%)
Edema			1 (2%)	
Hemorrhage				1 (2%)
Hyperkeratosis		2 (4%)	3 (6%)	13 (26%)
Inflammation, chronic		1 (2%)		2 (4%)
Inflammation, chronic, focal	1 (2%)			
Metaplasia, osseous	1 (2%)			
Ulcer				3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, hyperostosis				1 (2%)
Cranium, osteopetrosis	1 (2%)	1 (2%)	1 (2%)	
Femur, osteopetrosis			3 (6%)	1 (2%)
Rib, osteopetrosis			1 (2%)	
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	4 (8%)	5 (10%)		2 (4%)
Gliosis				1 (2%)
Hemorrhage				2 (4%)
Hydrocephalus	2 (4%)	1 (2%)		1 (2%)
Mineralization	1 (2%)			1 (2%)
Necrosis			1 (2%)	2 (4%)
Cerebellum, necrosis				1 (2%)

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
2-Year Study (continued)				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion	1 (2%)	2 (4%)		1 (2%)
Foreign body		1 (2%)		
Hemorrhage		1 (2%)		1 (2%)
Infiltration cellular, histiocyte	13 (26%)	12 (24%)	14 (28%)	6 (12%)
Inflammation, subacute	1 (2%)	3 (6%)	1 (2%)	
Inflammation, suppurative	1 (2%)			
Metaplasia, osseous	1 (2%)	1 (2%)		
Alveolar epithelium, hyperplasia	7 (14%)	2 (4%)	6 (12%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Exudate	17 (34%)	10 (20%)	13 (26%)	17 (34%)
Foreign body	8 (16%)	4 (8%)	6 (12%)	5 (10%)
Fungus	12 (24%)	5 (10%)	8 (16%)	11 (22%)
Mucosa, hyperplasia	11 (22%)	8 (16%)	5 (10%)	12 (24%)
Mucosa, metaplasia, squamous	9 (18%)	5 (10%)	7 (14%)	2 (4%)
Special Senses System				
Eye	(1)			(1)
Atrophy	1 (100%)			
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		2 (4%)	3 (6%)	
Inflammation, suppurative		3 (6%)	9 (18%)	4 (8%)
Mineralization		1 (2%)	2 (4%)	
Nephropathy	48 (96%)	47 (94%)	50 (100%)	48 (96%)
Papilla, inflammation, suppurative		1 (2%)		
Papilla, necrosis		1 (2%)		
Renal tubule, atrophy		1 (2%)	1 (2%)	
Renal tubule, cytoplasmic alteration				1 (2%)
Renal tubule, dilatation		1 (2%)		1 (2%)
Renal tubule, hyperplasia			2 (4%)	
Renal tubule, pigmentation	49 (98%)	48 (96%)	50 (100%)	50 (100%)
Transitional epithelium, hyperplasia	6 (12%)	10 (20%)	6 (12%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Hemorrhage		1 (2%)		
Inflammation, suppurative		2 (4%)		
Transitional epithelium, hyperplasia				2 (4%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF BUTYL BENZYL PHTHALATE

TABLE B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 104

TABLE B2 Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 108

TABLE B3 Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 124

TABLE B4a Historical Incidence of Pancreatic Neoplasms in Untreated Female F344/N Rats 128

TABLE B4b Historical Incidence of Urinary Bladder Papilloma in Untreated Female F344/N Rats 128

TABLE B4c Historical Incidence of Mammary Gland Fibroadenoma in Untreated Female F344/N Rats 128

TABLE B5 Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 129

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	23	17	16	20
Natural deaths	2	4	5	1
Survivors				
Terminal sacrifice	25	29	29	29
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Liver	(10)	(10)	(10)	(10)
Hepatocellular adenoma			1 (10%)	
Endocrine System				
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, adenoma		1 (10%)	1 (10%)	
Thyroid gland	(10)	(10)	(10)	(10)
C-cell, adenoma		1 (10%)		
C-cell, adenoma, multiple	1 (10%)			
Follicular cell, adenoma	1 (10%)			
Genital System				
Uterus	(10)	(10)	(10)	(10)
Deciduoma benign	1 (10%)			
Polyp stromal	1 (10%)	1 (10%)		
Integumentary System				
Mammary gland	(10)	(10)	(10)	(10)
Fibroadenoma		1 (10%)	1 (10%)	
Nervous System				
Brain	(10)	(10)	(10)	(10)
Astrocytoma malignant			1 (10%)	
Systemic Lesions				
Multiple organs[b]	(10)	(10)	(10)	(10)
Leukemia mononuclear	1 (10%)			
Systems Examined With No Neoplasms Observed				
Cardiovascular System				
General Body System				
Hematopoietic System				
Musculoskeletal System				
Respiratory System				
Special Senses System				
Urinary System				

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study				
Alimentary System				
Intestine large, cecum	(50)	(50)	(49)	(49)
Intestine small, jejunum	(50)	(49)	(50)	(49)
Intestine small, ileum	(49)	(50)	(50)	(50)
Liver	(50)	(50)	(50)	(50)
Hepatocellular adenoma			1 (2%)	2 (4%)
Mesentery	(10)	(8)	(8)	(6)
Leiomyosarcoma, metastatic, uterus			1 (13%)	
Lipoma		1 (13%)		
Liposarcoma	1 (10%)			
Schwannoma malignant		1 (13%)		
Oral mucosa		(1)		(1)
Squamous cell carcinoma		1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma				2 (4%)
Salivary glands	(49)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(3)		(1)	
Squamous cell papilloma	2 (67%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Schwannoma malignant	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	5 (10%)	1 (2%)	
Carcinoma			1 (2%)	
Adrenal medulla	(49)	(49)	(50)	(50)
Ganglioneuroma		1 (2%)		
Pheochromocytoma benign	1 (2%)	2 (4%)		1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)		
Carcinoma			2 (4%)	
Pituitary gland	(49)	(50)	(48)	(50)
Pars distalis, adenoma	19 (39%)	26 (52%)	25 (52%)	13 (26%)
Pars distalis, adenoma, multiple	1 (2%)			
Pars distalis, carcinoma	2 (4%)			
Pars intermedia, adenoma				1 (2%)
Thyroid gland	(49)	(50)	(50)	(50)
C-cell, adenoma	4 (8%)	4 (8%)	3 (6%)	2 (4%)
C-cell, carcinoma	1 (2%)	2 (4%)		
C-cell, carcinoma, multiple				1 (2%)
Follicular cell, carcinoma		2 (4%)		
General Body System				
Tissue NOS	(1)			(1)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	4 (8%)	4 (8%)	4 (8%)
Carcinoma	4 (8%)	6 (12%)	5 (10%)	
Ovary	(50)	(50)	(50)	(50)
Arrhenoblastoma NOS			1 (2%)	
Granulosa cell tumor benign	2 (4%)			
Uterus	(50)	(50)	(50)	(50)
Deciduoma benign				1 (2%)
Leiomyoma	1 (2%)			
Leiomyosarcoma	1 (2%)		1 (2%)	
Polyp stromal	6 (12%)	9 (18%)	6 (12%)	7 (14%)
Sarcoma stromal		1 (2%)		
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(12)	(11)	(16)	(15)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Thymus	(49)	(50)	(50)	(48)
Carcinoma, metastatic, thyroid gland				1 (2%)
Thymoma benign			1 (2%)	
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Adenoma	2 (4%)			
Carcinoma	2 (4%)	1 (2%)	2 (4%)	
Fibroadenoma	28 (57%)	30 (60%)	31 (62%)	11 (22%)
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Basal cell carcinoma			1 (2%)	
Keratoacanthoma	1 (2%)	1 (2%)		
Squamous cell papilloma			1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)	1 (2%)	2 (4%)	
Subcutaneous tissue, lipoma			1 (2%)	
Musculoskeletal System				
None				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Carcinoma	1 (2%)			
Carcinoma, metastatic, pituitary gland	2 (4%)			

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)	1 (2%)	
Carcinoma, metastatic, mammary gland		1 (2%)		
Special Senses System				
None				
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Hemangioma				1 (2%)
Renal tubule, carcinoma	1 (2%)		1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Transitional epithelium, papilloma	1 (2%)			2 (4%)
Systemic Lesions				
Multiple organs	(50)	(50)	(50)	(50)
Leukemia mononuclear	21 (42%)	20 (40%)	21 (42%)	19 (38%)
Neoplasm Summary				
Total animals with primary neoplasms[c]				
15-Month interim evaluation	4	4	3	
2-Year study	49	48	45	42
Total primary neoplasms				
15-Month interim evaluation	5	4	4	
2-Year study	112	120	112	70
Total animals with benign neoplasms				
15-Month interim evaluation	3	4	2	
2-Year study	40	43	41	31
Total benign neoplasms				
15-Month interim evaluation	4	4	3	
2-Year study	77	86	77	48
Total animals with malignant neoplasms				
15-Month interim evaluation	1		1	
2-Year study	30	31	27	22
Total malignant neoplasms				
15-Month interim evaluation	1		1	
2-Year study	35	34	34	22
Total animals with metastatic neoplasms				
2-Year study	2	1	1	1
Total metastatic neoplasms				
2-Year study	2	1	1	1
Total animals with uncertain neoplasms - benign or malignant				
2-Year study			1	
Total uncertain neoplasms				
2-Year study			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm

Number of Days on Study	039	368	512	570	587	598	668	670	681	684	691	702	702	703	711	713	716	717	717	717	719	725	725	725	726
Carcass ID Number	309	308	316	348	344	303	324	337	349	343	341	311	313	320	302	328	304	305	314	322	345	307	310	323	339
Alimentary System																									
Esophagus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+					+									+	+	+								
Liposarcoma	X																								
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell papilloma								X																	
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																	+								
Squamous cell papilloma																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X																
Adrenal medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign			X																						
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma						X	X			X	X			X				X					X	X	X
Pars distalis, adenoma, multiple																									
Pars distalis, carcinoma																				X					
Thyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma												X	X	X											
C-cell, carcinoma																									
General Body System																									
Tissue NOS	+																								
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																							X		
Carcinoma													X												
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Granulosa cell tumor benign						X																			

+: 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 Feed Study of Butyl Benzyl Phthalate: 0 ppm (continued)

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	301	312	317	318	319	325	326	329	330	332	335	336	340	343	347	306	315	321	327	331	333	338	342	346	350	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery	+		+			+			+			+														10
Liposarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell papilloma																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue												+	+													3
Squamous cell papilloma												X	X													2
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant																					X					1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								1
Parathyroid gland	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X		X					X	X	X		X	X						X			X		X		19
Pars distalis, adenoma, multiple															X											1
Pars distalis, carcinoma																					X					2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma												X														4
C-cell, carcinoma																			X							1
General Body System																										
Tissue NOS																										1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																X									X	3
Carcinoma					X							X						X								4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Granulosa cell tumor benign						X																				2

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm (continued)

Number of Days on Study	039	368	512	570	587	598	668	670	681	684	691	702	702	703	711	713	716	717	711	717	719	725	725	725	726
Carcass ID Number	309	308	316	348	344	303	324	337	349	344	341	311	313	320	302	328	304	305	314	322	345	307	310	323	339
Genital System (continued)																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyoma																									
Leiomyosarcoma																									
Polyp stromal									X										X				X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+						+	+			+		+		+			+	+		+				
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																	X								
Fibroadenoma					X				X	X	X	X	X	X		X		X		X		X	X	X	X
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma															X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+																								
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Carcinoma, metastatic, pituitary gland																				X					
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																		X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye							+	+																	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, carcinoma								X																	
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma			X																						
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X			X		X		X		X		X	X	X	X	X		X			X	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 0 ppm (continued)

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	301	312	317	318	319	325	326	329	330	332	335	336	340	343	347	306	315	321	327	331	333	338	342	346	350	Total Tissues/ Tumors
Genital System (continued)																										
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyoma																X										1
Leiomyosarcoma											X															1
Polyp stromal												X			X				X							6
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node			+													+	+									12
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma								X				X														2
Carcinoma				X																						2
Fibroadenoma			X	X		X	X	X			X	X			X	X	X		X		X	X			X	28
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma										X																1
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																						X				1
Carcinoma, metastatic, pituitary gland																					X					2
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma											X															2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Special Senses System																										
Eye																										2
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, carcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																										1
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X		X							X	X				X			X			X			21

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm

Number of Days on Study	247	405	424	529	593	606	606	611	618	663	682	689	701	703	718	722	725	725	725	725	726	736	736	736	736
Carcass ID Number	401	390	415	362	368	379	382	402	361	388	392	372	394	397	389	364	363	367	376	400	399	365	366	370	373
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery	+						+								+										
Lipoma							X																		
Schwannoma malignant	X																								
Oral mucosa															+										
Squamous cell carcinoma															X										
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																			X	X				X	X
Adrenal medulla	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Ganglioneuroma																									
Pheochromocytoma benign														X		X									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X	X		X	X	X		X	X	X	X	X					X				X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																				X					
C-cell, carcinoma												X													
Follicular cell, carcinoma													X												
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X				X			
Carcinoma									X																
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal	X																X	X	X						
Sarcoma stromal																X									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	374	377	378	381	384	385	387	391	395	396	403	404	406	407	409	669	671	675	680	683	686	693	698	405	408	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery			+					+	+											+		+				8
Lipoma																										1
Schwannoma malignant																										1
Oral mucosa																										1
Squamous cell carcinoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma												X														5
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ganglioneuroma																					X					1
Pheochromocytoma benign																										2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																					X					1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma					X		X	X	X			X	X	X	X					X	X	X	X		X	26
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma														X							X		X			4
C-cell, carcinoma				X																						2
Follicular cell, carcinoma		X																								2
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma				X															X							4
Carcinoma		X					X					X						X			X					6
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal					X		X	X			X							X								9
Sarcoma stromal																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	2	4	4	5	5	6	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7
	4	0	2	2	9	0	0	1	1	6	8	8	0	0	1	2	2	2	2	2	2	3	3	3	3
	7	5	4	9	3	6	6	1	8	3	2	9	1	3	8	2	5	5	5	5	6	6	6	6	6
Carcass ID Number	4	3	4	3	3	3	3	4	3	3	3	3	3	3	3	3	3	3	3	4	3	3	3	3	3
	0	9	1	6	6	7	8	0	6	8	9	7	9	9	8	6	6	6	7	0	9	6	6	7	7
	1	0	5	2	8	9	2	2	1	8	2	2	4	7	9	4	3	7	6	0	9	5	6	0	3
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node				+	+	+				+		+				+								+	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma			X																						
Fibroadenoma						X		X		X	X	X		X	X	X	X	X	X	X		X	X		
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Subcutaneous tissue, fibroma													X												
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma												X													
Carcinoma, metastatic, mammary gland			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
None																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X	X		X	X	X	X	X						X		X	X				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 6,000 ppm
(continued)

Number of Days on Study	736	736	736	736	736	736	736	736	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	
Carcass ID Number	374	377	378	381	384	385	387	391	395	396	403	404	406	407	409	369	371	375	380	383	386	393	398	405	408	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node							+								+	+		+								11
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Fibroadenoma	X			X	X			X	X			X		X	X	X	X		X	X	X	X	X	X		30
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma							X																			1
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma, metastatic, mammary gland																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
None																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X					X	X						X	X				X				X	X	20

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm

Number of Days on Study	285	479	509	544	544	551	572	607	646	647	647	666	670	703	722	724	725	725	726	726	733	736	736	736	736
Carcass ID Number	467	461	427	423	426	466	450	440	449	430	431	432	455	424	436	460	443	447	444	457	468	421	422	428	429
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery								+	+		+		+		+				+			+			
Leiomyosarcoma, metastatic, uterus									X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue																									
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma							X																		
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma												X													
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X					X					X	X	X	X			X	X
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									
General Body System																									
None																									
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma									X													X			
Carcinoma							X									X							X		
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Arrhenoblastoma NOS																				X					
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leiomyosarcoma									X																
Polyp stromal															X	X				X				X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	737	737	738	738	738	738	738	738	
Carcass ID Number	434	435	438	439	442	445	448	425	433	437	441	446	451	452	453	454	462	465	469	470	456	458	459	463	464	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma														X												1
Mesentery			+																							8
Leiomyosarcoma, metastatic, uterus																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tongue																							+			1
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X																		1
Carcinoma																										1
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma	X																									2
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X	X	X		X	X	X					X	X	X	X	X	X	X			X	X	X		X	25
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma					X						X												X			3
General Body System																										
None																										
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma															X	X										4
Carcinoma											X												X			5
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Arrhenoblastoma NOS																										1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leiomyosarcoma																										1
Polyp stromal		X														X										6

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	2	4	5	5	5	5	5	6	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	8	7	0	4	4	5	7	0	4	4	4	6	7	0	2	2	2	2	2	2	3	3	3	3	3
	5	9	9	4	4	1	2	7	6	7	7	6	0	3	2	4	5	5	6	6	3	6	6	6	6
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
	6	6	2	2	2	6	5	4	4	3	3	3	5	2	3	6	4	4	4	5	6	2	2	2	2
	7	1	7	3	6	6	0	0	9	0	1	2	5	4	6	0	3	7	4	7	8	1	2	8	9
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node		+	+				+				+	+	+		+	+			+		+				
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									
Fibroadenoma			X	X	X			X	X	X			X	X		X	X	X	X	X	X	X	X	X	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Squamous cell papilloma																									
Subcutaneous tissue, fibroma																									
Subcutaneous tissue, lipoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear		+																							
Eye				+																					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Renal tubule, carcinoma																X									
Ureter									+																
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X			X					X	X			X	X	X	X	X	X	X	X			

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 12,000 ppm
(continued)

Number of Days on Study	736	736	736	736	736	736	736	737	737	737	737	737	737	737	737	737	737	737	737	737	738	738	738	738	738	
Carcass ID Number	434	435	438	439	442	445	448	425	433	437	441	446	451	452	453	454	462	465	469	470	456	458	459	463	464	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+						+								+	+								+	+	16
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymoma benign																					X					1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma												X			X											2
Fibroadenoma		X	X		X	X		X	X		X			X	X	X			X		X		X	X		31
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell carcinoma			X																							1
Squamous cell papilloma																X										1
Subcutaneous tissue, fibroma			X												X											2
Subcutaneous tissue, lipoma																					X					1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																								1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Eye																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, carcinoma																										1
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X				X	X		X			X			X	X									X	X	21

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 24,000 ppm

Number of Days on Study	404	452	452	452	487	487	551	592	607	607	611	681	684	684	684	703	703	703	717	722	725	737	737	737	737
Carcass ID Number	496	486	494	502	485	506	487	516	493	520	481	512	505	526	528	504	522	525	510	489	490	483	491	497	500
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular adenoma																									
Mesentery		+					+																+		+
Oral mucosa																									
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																			X				X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Schwannoma malignant																									
Endocrine System																									
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma								X				X		X		X				X	X		X	X	
Pars intermedia, adenoma					X																				
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																									X
C-cell, carcinoma, multiple			X																						
General Body System																									
Tissue NOS																		+							
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Deciduoma benign													X												
Polyp stromal					X																				

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 24,000 ppm
(continued)

Number of Days on Study	737	737	737	737	737	737	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	
Carcass ID Number	507	511	514	517	521	527	482	484	488	492	495	498	499	501	503	508	509	513	515	518	519	523	524	529	530	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma															X							X				2
Mesentery										+								+								6
Oral mucosa																		+								1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma																										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Blood vessel	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Schwannoma malignant													X													1
Endocrine System																										
Adrenal cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma benign																X										1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pars distalis, adenoma							X	X										X		X				X		13
Pars intermedia, adenoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma																									X	2
C-cell, carcinoma, multiple																										1
General Body System																										
Tissue NOS																										1
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma								X	X						X										X	4
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Deciduoma benign																										1
Polyp stromal			X						X							X			X				X		X	7

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate: 24,000 ppm
(continued)

Number of Days on Study	404	452	452	452	487	487	551	592	607	607	611	618	618	684	684	703	703	703	717	722	722	737	737	737	737
Carcass ID Number	496	486	494	502	485	506	487	516	493	520	481	512	505	526	528	504	522	525	510	489	490	483	491	497	500
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+					+			+	+			+	+		+		+						
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	I	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, thyroid gland			X																						
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibroadenoma					X	X					X	X	X					X							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell adenoma																									
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant	X																								
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Eye				+																+					
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma																									
Ureter							+																		
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Transitional epithelium, papilloma				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 Feed Study of Butyl Benzyl Phthalate: 24,000 ppm
(continued)

Number of Days on Study	737	737	737	737	737	737	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	738	
Carcass ID Number	507	511	514	517	521	527	482	484	488	492	495	498	499	501	503	508	509	513	515	518	519	523	524	529	530	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node								+			+	+				+				+				+		15
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Carcinoma, metastatic, thyroid gland																										1
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibroadenoma							X				X			X		X									X	11
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Basal cell adenoma								X																		1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle	+																									1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Astrocytoma malignant																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Eye	+																									3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma				X																						1
Ureter																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Transitional epithelium, papilloma																									X	2
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear			X						X			X				X		X		X			X		X	19

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Adrenal Cortex: Adenoma				
Overall rate[a]	1/50 (2%)	5/50 (10%)	1/50 (2%)	0/50 (0%)
Adjusted rate[b]	2.4%	15.6%	3.4%	0.0%
Terminal rate[c]	0/25 (0%)	3/29 (10%)	1/29 (3%)	0/29 (0%)
First incidence (days)	681	725	736 (T)	—[e]
Life table test[d]	P=0.123N	P=0.137	P=0.758N	P=0.515N
Logistic regression test[d]	P=0.146N	P=0.101	P=0.761	P=0.499N
Cochran-Armitage test[d]	P=0.134N			
Fisher exact test[d]		P=0.102	P=0.753N	P=0.500N
Clitoral Gland: Adenoma				
Overall rate	3/50 (6%)	4/50 (8%)	4/50 (8%)	4/50 (8%)
Adjusted rate	11.2%	13.0%	12.5%	13.8%
Terminal rate	2/25 (8%)	3/29 (10%)	3/29 (10%)	4/29 (14%)
First incidence (days)	725	725	646	736 (T)
Life table test	P=0.495	P=0.582	P=0.566	P=0.572
Logistic regression test	P=0.409	P=0.541	P=0.489	P=0.530
Cochran-Armitage test	P=0.446			
Fisher exact test		P=0.500	P=0.500	P=0.500
Clitoral Gland: Carcinoma				
Overall rate	4/50 (8%)	6/50 (12%)	5/50 (10%)	0/50 (0%)
Adjusted rate	14.3%	19.2%	14.9%	0.0%
Terminal rate	3/25 (12%)	5/29 (17%)	3/29 (10%)	0/29 (0%)
First incidence (days)	702	618	572	—
Life table test	P=0.037N	P=0.439	P=0.567	P=0.054N
Logistic regression test	P=0.052N	P=0.363	P=0.492	P=0.069N
Cochran-Armitage test	P=0.047N			
Fisher exact test		P=0.370	P=0.500	P=0.059N
Clitoral Gland: Adenoma or Carcinoma				
Overall rate	7/50 (14%)	10/50 (20%)	9/50 (18%)	4/50 (8%)
Adjusted rate	24.7%	31.4%	26.5%	13.8%
Terminal rate	5/25 (20%)	8/29 (28%)	6/29 (21%)	4/29 (14%)
First incidence (days)	702	618	572	736 (T)
Life table test	P=0.119N	P=0.402	P=0.491	P=0.198N
Logistic regression test	P=0.188N	P=0.291	P=0.377	P=0.265N
Cochran-Armitage test	P=0.158N			
Fisher exact test		P=0.298	P=0.393	P=0.262N
Mammary Gland: Fibroadenoma				
Overall rate	28/50 (56%)	30/50 (60%)	31/50 (62%)	11/50 (22%)
Adjusted rate	71.0%	72.8%	71.7%	28.9%
Terminal rate	14/25 (56%)	18/29 (62%)	17/29 (59%)	5/29 (17%)
First incidence (days)	587	606	509	487
Life table test	P<0.001N	P=0.494N	P=0.557	P=0.001N
Logistic regression test	P<0.001N	P=0.379	P=0.318	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.420	P=0.342	P<0.001N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Mammary Gland: Fibroadenoma or Adenoma				
Overall rate	28/50 (56%)	30/50 (60%)	31/50 (62%)	11/50 (22%)
Adjusted rate	71.0%	72.8%	71.7%	28.9%
Terminal rate	14/25 (56%)	18/29 (62%)	17/29 (59%)	5/29 (17%)
First incidence (days)	587	606	509	487
Life table test	P<0.001N	P=0.494N	P=0.557	P=0.001N
Logistic regression test	P<0.001N	P=0.379	P=0.318	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.420	P=0.342	P<0.001N
Mammary Gland: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	1/50 (2%)	2/50 (4%)	0/50 (0%)
Adjusted rate	14.6%	2.1%	6.9%	0.0%
Terminal rate	3/25 (12%)	0/29 (0%)	2/29 (7%)	0/29 (0%)
First incidence (days)	716	424	736 (T)	—
Life table test	P=0.044N	P=0.154N	P=0.277N	P=0.052N
Logistic regression test	P=0.052N	P=0.180N	P=0.304N	P=0.064N
Cochran-Armitage test	P=0.052N			
Fisher exact test		P=0.181N	P=0.339N	P=0.059N
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
Overall rate	29/50 (58%)	31/50 (62%)	32/50 (64%)	11/50 (22%)
Adjusted rate	71.8%	73.3%	74.0%	28.9%
Terminal rate	14/25 (56%)	18/29 (62%)	18/29 (62%)	5/29 (17%)
First incidence (days)	587	424	509	487
Life table test	P<0.001N	P=0.492N	P=0.560N	P<0.001N
Logistic regression test	P<0.001N	P=0.392	P=0.313	P<0.001N
Cochran-Armitage test	P<0.001N			
Fisher exact test		P=0.419	P=0.341	P<0.001N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	20/49 (41%)	26/50 (52%)	25/48 (52%)	13/50 (26%)
Adjusted rate	57.0%	63.4%	71.2%	36.1%
Terminal rate	11/25 (44%)	15/29 (52%)	19/29 (66%)	7/29 (24%)
First incidence (days)	598	529	607	592
Life table test	P=0.035N	P=0.316	P=0.396	P=0.086N
Logistic regression test	P=0.053N	P=0.168	P=0.127	P=0.121N
Cochran-Armitage test	P=0.036N			
Fisher exact test		P=0.180	P=0.182	P=0.088N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	22/49 (45%)	26/50 (52%)	25/48 (52%)	13/50 (26%)
Adjusted rate	61.3%	63.4%	71.2%	36.1%
Terminal rate	12/25 (48%)	15/29 (52%)	19/29 (66%)	7/29 (24%)
First incidence (days)	598	529	607	592
Life table test	P=0.018N	P=0.455	P=0.553	P=0.042N
Logistic regression test	P=0.025N	P=0.290	P=0.232	P=0.058N
Cochran-Armitage test	P=0.016N			
Fisher exact test		P=0.307	P=0.307	P=0.039N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Thyroid Gland (C-cell): Adenoma				
Overall rate	4/49 (8%)	4/50 (8%)	3/50 (6%)	2/50 (4%)
Adjusted rate	11.4%	13.0%	10.3%	6.9%
Terminal rate	1/25 (4%)	3/29 (10%)	3/29 (10%)	2/29 (7%)
First incidence (days)	702	725	736 (T)	736 (T)
Life table test	P=0.209N	P=0.596N	P=0.463N	P=0.326N
Logistic regression test	P=0.256N	P=0.644	P=0.507N	P=0.359N
Cochran-Armitage test	P=0.227N			
Fisher exact test		P=0.631N	P=0.489N	P=0.329N
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	5/49 (10%)	6/50 (12%)	3/50 (6%)	3/50 (6%)
Adjusted rate	15.1%	18.5%	10.3%	8.8%
Terminal rate	2/25 (8%)	4/29 (14%)	3/29 (10%)	2/29 (7%)
First incidence (days)	702	689	736 (T)	452
Life table test	P=0.186N	P=0.557	P=0.316N	P=0.337N
Logistic regression test	P=0.217N	P=0.499	P=0.363N	P=0.346N
Cochran-Armitage test	P=0.200N			
Fisher exact test		P=0.514	P=0.346N	P=0.346N
Uterus: Stromal Polyp				
Overall rate	6/50 (12%)	9/50 (18%)	6/50 (12%)	7/50 (14%)
Adjusted rate	19.6%	26.1%	18.0%	22.4%
Terminal rate	3/25 (12%)	5/29 (17%)	3/29 (10%)	6/29 (21%)
First incidence (days)	681	247	722	487
Life table test	P=0.498N	P=0.387	P=0.523N	P=0.571
Logistic regression test	P=0.535	P=0.287	P=0.611	P=0.470
Cochran-Armitage test	P=0.555			
Fisher exact test		P=0.288	P=0.620N	P=0.500
Uterus: Stromal Polyp or Stromal Sarcoma				
Overall rate	6/50 (12%)	10/50 (20%)	6/50 (12%)	7/50 (14%)
Adjusted rate	19.6%	28.2%	18.0%	22.4%
Terminal rate	3/25 (12%)	5/29 (17%)	3/29 (10%)	6/29 (21%)
First incidence (days)	681	247	722	487
Life table test	P=0.458N	P=0.304	P=0.523N	P=0.571
Logistic regression test	P=0.535N	P=0.206	P=0.611	P=0.470
Cochran-Armitage test	P=0.514N			
Fisher exact test		P=0.207	P=0.620N	P=0.500
All Organs: Mononuclear Cell Leukemia				
Overall rate	21/50 (42%)	20/50 (40%)	21/50 (42%)	19/50 (38%)
Adjusted rate	51.7%	46.6%	52.0%	46.1%
Terminal rate	7/25 (28%)	8/29 (28%)	10/29 (34%)	8/29 (28%)
First incidence (days)	368	405	509	452
Life table test	P=0.382N	P=0.402N	P=0.436N	P=0.398N
Logistic regression test	P=0.340N	P=0.500N	P=0.578	P=0.422N
Cochran-Armitage test	P=0.394N			
Fisher exact test		P=0.500N	P=0.580N	P=0.419N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
All Organs: Benign Neoplasms				
Overall rate	40/50 (80%)	43/50 (86%)	41/50 (82%)	31/50 (62%)
Adjusted rate	88.7%	91.4%	93.1%	74.9%
Terminal rate	20/25 (80%)	25/29 (86%)	26/29 (90%)	19/29 (66%)
First incidence (days)	512	247	509	452
Life table test	P=0.028N	P=0.500N	P=0.377N	P=0.060N
Logistic regression test	P=0.009N	P=0.287	P=0.457	P=0.047N
Cochran-Armitage test	P=0.007N			
Fisher exact test		P=0.298	P=0.500	P=0.038N
All Organs: Malignant Neoplasms				
Overall rate	30/50 (60%)	31/50 (62%)	27/50 (54%)	22/50 (44%)
Adjusted rate	70.1%	66.3%	63.8%	50.7%
Terminal rate	13/25 (52%)	14/29 (48%)	14/29 (48%)	9/29 (31%)
First incidence (days)	39	247	509	404
Life table test	P=0.068N	P=0.466N	P=0.240N	P=0.110N
Logistic regression test	P=0.032N	P=0.516	P=0.343N	P=0.067N
Cochran-Armitage test	P=0.038N			
Fisher exact test		P=0.500	P=0.343N	P=0.080N
All Organs: Benign or Malignant Neoplasms				
Overall rate	49/50 (98%)	48/50 (96%)	45/50 (90%)	42/50 (84%)
Adjusted rate	98.0%	96.0%	93.8%	84.0%
Terminal rate	24/25 (96%)	27/29 (93%)	26/29 (90%)	21/29 (72%)
First incidence (days)	39	247	509	404
Life table test	P=0.092N	P=0.252N	P=0.145N	P=0.123N
Logistic regression test	P=0.004N	P=0.552N	P=0.087N	P=0.027N
Cochran-Armitage test	P=0.004N			
Fisher exact test		P=0.500N	P=0.102N	P=0.015N

(T)Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pituitary gland, thyroid gland, and uterus; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated neoplasm 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 exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The life table test regards neoplasms in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in an exposure group is indicated by N.
[e] Not applicable; no neoplasms in animal group

TABLE B4a
Historical Incidence of Pancreatic Neoplasms in Untreated Female F344/N Rats[a]

	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Overall Historical Incidence			
Total	2/1,194 (0.2%)	1/1,194 (0.1%)	3/1,194 (0.3%)
Standard deviation	0.8%	0.4%	0.9%
Range	0%-4%	0%-2%	0%-4%

[a] Data as of 17 June 1994

TABLE B4b
Historical Incidence of Urinary Bladder Papilloma in Untreated Female F344/N Rats[a]

	Incidence in Controls
Overall Historical Incidence	
Total	4/1,182 (0.3%)
Standard deviation	0.8%
Range	0%-2%

[a] Data as of 17 June 1994

TABLE B4c
Historical Incidence of Mammary Gland Fibroadenoma in Untreated Female F344/N Rats[a]

	Incidence in Controls
Overall Historical Incidence	
Total	465/1,202 (38.7%)
Standard deviation	12.7%
Range	8%-58%

[a] Data as of 17 June 1994

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Disposition Summary				
Animals initially in study	60	60	60	60
15-Month interim evaluation	10	10	10	10
Early deaths				
Moribund	23	17	16	20
Natural deaths	2	4	5	1
Survivors				
Terminal sacrifice	25	29	29	29
Animals examined microscopically	60	60	60	60
15-Month Interim Evaluation				
Alimentary System				
Intestine large, colon	(10)	(10)	(10)	(10)
Parasite metazoan				1 (10%)
Intestine large, cecum	(9)	(10)	(10)	(10)
Parasite metazoan		1 (10%)		
Liver	(10)	(10)	(10)	(10)
Basophilic focus	10 (100%)	10 (100%)	8 (80%)	9 (90%)
Eosinophilic focus		1 (10%)	1 (10%)	
Granuloma	1 (10%)	3 (30%)	3 (30%)	
Hepatodiaphragmatic nodule	1 (10%)	1 (10%)	2 (20%)	
Bile duct, hyperplasia	1 (10%)			
Kupffer cell, pigmentation			1 (10%)	
Mesentery	(1)		(4)	
Accessory spleen			1 (25%)	
Fat, necrosis	1 (100%)		3 (75%)	
Pancreas	(10)	(10)	(10)	(10)
Atrophy	1 (10%)	2 (20%)		
Tongue				(1)
Epithelium, hyperplasia				1 (100%)
Cardiovascular System				
Heart	(10)	(10)	(10)	(10)
Cardiomyopathy	1 (10%)	1 (10%)	1 (10%)	
Endocrine System				
Adrenal cortex	(10)	(10)	(10)	(10)
Accessory adrenal cortical nodule	2 (20%)	1 (10%)	2 (20%)	4 (40%)
Angiectasis	5 (50%)	6 (60%)	7 (70%)	3 (30%)
Cyst				1 (10%)
Degeneration, fatty	1 (10%)	1 (10%)		
Hyperplasia, focal				1 (10%)
Hypertrophy, focal	1 (10%)	1 (10%)	2 (20%)	
Parathyroid gland	(9)	(10)	(10)	(10)
Cyst			1 (10%)	

[a] Number of animals examined microscopically at the site and the number of animals with lesion

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
15-Month Interim Evaluation (continued)				
Endocrine System (continued)				
Pituitary gland	(10)	(10)	(10)	(10)
Pars distalis, angiectasis	1 (10%)	2 (20%)	1 (10%)	
Pars distalis, cyst	6 (60%)	10 (100%)	8 (80%)	1 (10%)
Pars distalis, hemorrhage			1 (10%)	
Pars distalis, hyperplasia, focal	1 (10%)	2 (20%)		1 (10%)
Pars intermedia, cyst		1 (10%)		
Thyroid gland	(10)	(10)	(10)	(10)
Ultimobranchial cyst	1 (10%)	3 (30%)		
C-cell, hyperplasia		1 (10%)	1 (10%)	
Genital System				
Clitoral gland	(10)	(10)	(10)	(10)
Ectasia				1 (10%)
Inflammation, chronic	2 (20%)	2 (20%)	1 (10%)	
Ovary	(10)	(10)	(10)	(10)
Cyst				1 (10%)
Uterus	(10)	(10)	(10)	(10)
Hydrometra	2 (20%)	3 (30%)	2 (20%)	2 (20%)
Hyperplasia, cystic				1 (10%)
Inflammation, suppurative	1 (10%)			
Hematopoietic System				
Lymph node		(1)	(1)	(1)
Mediastinal, hemorrhage		1 (100%)	1 (100%)	1 (100%)
Mediastinal, pigmentation		1 (100%)	1 (100%)	1 (100%)
Lymph node, mandibular	(10)	(10)	(10)	(10)
Ectasia	1 (10%)	1 (10%)	2 (20%)	2 (20%)
Hemorrhage				1 (10%)
Hyperplasia, lymphoid	2 (20%)	1 (10%)	1 (10%)	
Pigmentation	3 (30%)	4 (40%)	4 (40%)	4 (40%)
Spleen	(10)	(10)	(10)	(10)
Hematopoietic cell proliferation	1 (10%)	3 (30%)	3 (30%)	3 (30%)
Pigmentation, hemosiderin	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Thymus	(9)	(9)	(10)	(10)
Hemorrhage		1 (11%)		
Integumentary System				
Mammary gland	(10)	(10)	(10)	(10)
Hyperplasia	3 (30%)	2 (20%)	3 (30%)	1 (10%)
Musculoskeletal System				
Bone	(10)	(10)	(10)	(10)
Cranium, hyperostosis		1 (10%)		
Cranium, osteopetrosis		1 (10%)	1 (10%)	1 (10%)
Femur, osteopetrosis		1 (10%)	1 (10%)	2 (20%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
15-Month Interim Evaluation (continued)				
Respiratory System				
Lung	(10)	(10)	(10)	(10)
Infiltration cellular, histiocyte	2 (20%)	1 (10%)	3 (30%)	2 (20%)
Nose	(10)	(10)	(10)	(10)
Exudate	1 (10%)	1 (10%)		
Foreign body		2 (20%)		
Fungus	1 (10%)			
Mucosa, metaplasia, squamous	1 (10%)			
Urinary System				
Kidney	(10)	(10)	(10)	(10)
Cyst	1 (10%)	2 (20%)		
Mineralization	10 (100%)	9 (90%)	9 (90%)	8 (80%)
Nephropathy	7 (70%)	10 (100%)	10 (100%)	10 (100%)
Renal tubule, atrophy	1 (10%)			
Renal tubule, pigmentation	10 (100%)	10 (100%)	10 (100%)	10 (100%)
Systems Examined With No Lesions Observed				
General Body System				
Nervous System				
Special Senses System				
2-Year Study				
Alimentary System				
Intestine large, colon	(50)	(48)	(49)	(50)
Parasite metazoan	3 (6%)	2 (4%)	3 (6%)	3 (6%)
Intestine large, rectum	(50)	(50)	(50)	(50)
Parasite metazoan	3 (6%)	5 (10%)	11 (22%)	4 (8%)
Intestine large, cecum	(50)	(50)	(49)	(49)
Edema	2 (4%)		2 (4%)	1 (2%)
Parasite metazoan	2 (4%)		1 (2%)	1 (2%)
Ulcer			1 (2%)	
Intestine small, ileum	(49)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		
Ulcer	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)	4 (8%)	
Basophilic focus	37 (74%)	38 (76%)	41 (82%)	39 (78%)
Clear cell focus	3 (6%)	3 (6%)	8 (16%)	9 (18%)
Cyst		1 (2%)	2 (4%)	2 (4%)
Developmental malformation	1 (2%)			
Eosinophilic focus	19 (38%)	15 (30%)	9 (18%)	20 (40%)
Granuloma	6 (12%)	11 (22%)	6 (12%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)			1 (2%)
Hepatodiaphragmatic nodule	9 (18%)	6 (12%)	6 (12%)	12 (24%)
Inflammation, subacute	3 (6%)			
Mixed cell focus	10 (20%)	4 (8%)	5 (10%)	6 (12%)
Thrombosis			1 (2%)	
Bile duct, hyperplasia	10 (20%)	12 (24%)	12 (24%)	9 (18%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Alimentary System (continued)				
Liver (continued)	(50)	(50)	(50)	(50)
Centrilobular, necrosis	1 (2%)		1 (2%)	
Hepatocyte, hyperplasia, focal		1 (2%)		
Hepatocyte, vacuolization cytoplasmic	7 (14%)	6 (12%)	2 (4%)	
Kupffer cell, hyperplasia	1 (2%)	1 (2%)		
Kupffer cell, pigmentation	4 (8%)	1 (2%)	5 (10%)	10 (20%)
Lobules, necrosis	6 (12%)	3 (6%)		6 (12%)
Mesentery	(10)	(8)	(8)	(6)
Accessory spleen	1 (10%)		1 (13%)	1 (17%)
Fat, necrosis	9 (90%)	7 (88%)	6 (75%)	6 (100%)
Oral mucosa		(1)		(1)
Hyperplasia				1 (100%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	18 (36%)	18 (36%)	16 (32%)	12 (24%)
Edema		1 (2%)		
Acinus, cytoplasmic alteration	1 (2%)	1 (2%)	1 (2%)	
Acinus, hyperplasia, focal	1 (2%)	4 (8%)	2 (4%)	
Salivary glands	(49)	(50)	(50)	(50)
Atrophy	1 (2%)	2 (4%)		
Hyperplasia	1 (2%)			
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	2 (4%)	1 (2%)	1 (2%)	
Ulcer		1 (2%)		1 (2%)
Mucosa, hyperplasia	1 (2%)	2 (4%)	2 (4%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema			1 (2%)	
Erosion	1 (2%)	2 (4%)		
Hyperplasia			1 (2%)	
Inflammation, chronic	1 (2%)			
Mineralization	1 (2%)			
Ulcer	3 (6%)	1 (2%)	1 (2%)	
Tongue	(3)		(1)	
Epithelium, hyperplasia	1 (33%)		1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	16 (32%)	21 (42%)	23 (46%)	13 (26%)
Mineralization				1 (2%)
Thrombosis	1 (2%)			1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	5 (10%)	6 (12%)	8 (16%)	6 (12%)
Angiectasis	22 (44%)	23 (46%)	26 (52%)	29 (58%)
Cyst				1 (2%)
Degeneration, fatty	10 (20%)	15 (30%)	11 (22%)	4 (8%)
Hemorrhage		1 (2%)		1 (2%)
Hyperplasia, focal	9 (18%)	4 (8%)	2 (4%)	2 (4%)
Hypertrophy, focal	9 (18%)	11 (22%)	7 (14%)	
Necrosis			1 (2%)	

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Endocrine System (continued)				
Adrenal medulla	(49)	(49)	(50)	(50)
Hyperplasia	1 (2%)	2 (4%)	4 (8%)	1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		1 (2%)		
Metaplasia, hepatocyte				1 (2%)
Parathyroid gland	(47)	(49)	(49)	(48)
Hyperplasia		1 (2%)		
Pituitary gland	(49)	(50)	(48)	(50)
Pars distalis, angiectasis	7 (14%)	7 (14%)	8 (17%)	7 (14%)
Pars distalis, cyst	24 (49%)	20 (40%)	16 (33%)	19 (38%)
Pars distalis, hyperplasia				1 (2%)
Pars distalis, hyperplasia, focal	12 (24%)	11 (22%)	9 (19%)	11 (22%)
Pars intermedia, angiectasis		3 (6%)	3 (6%)	
Pars intermedia, cyst		2 (4%)		
Pars intermedia, hyperplasia	1 (2%)	1 (2%)		
Thyroid gland	(49)	(50)	(50)	(50)
Ultimobranchial cyst	2 (4%)	1 (2%)	1 (2%)	
C-cell, hyperplasia	6 (12%)	6 (12%)	7 (14%)	3 (6%)
Follicle, cyst		1 (2%)	1 (2%)	
Follicular cell, hyperplasia				1 (2%)
General Body System				
Tissue NOS	(1)			(1)
Hemorrhage	1 (100%)			
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Ectasia	12 (24%)	21 (42%)	13 (26%)	10 (20%)
Hyperplasia	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Inflammation, chronic	4 (8%)	4 (8%)	3 (6%)	1 (2%)
Inflammation, suppurative	3 (6%)	5 (10%)	6 (12%)	3 (6%)
Ovary	(50)	(50)	(50)	(50)
Cyst	3 (6%)	5 (10%)	6 (12%)	5 (10%)
Inflammation, chronic	1 (2%)		1 (2%)	
Uterus	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Hydrometra	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Hyperplasia, cystic	4 (8%)	4 (8%)	1 (2%)	11 (22%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hypercellularity	1 (2%)	2 (4%)		1 (2%)
Myelofibrosis	4 (8%)	3 (6%)	6 (12%)	6 (12%)
Lymph node	(12)	(11)	(16)	(15)
Iliac, hyperplasia, lymphoid		1 (9%)		
Iliac, pigmentation		1 (9%)		
Mediastinal, hemorrhage	1 (8%)	1 (9%)	1 (6%)	1 (7%)
Mediastinal, hyperplasia, lymphoid		1 (9%)		
Mediastinal, pigmentation	2 (17%)	5 (45%)	9 (56%)	9 (60%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Hematopoietic System (continued)				
Lymph node (continued)	(12)	(11)	(16)	(15)
Pancreatic, hemorrhage			1 (6%)	
Pancreatic, pigmentation	1 (8%)	2 (18%)	3 (19%)	3 (20%)
Renal, hemorrhage		2 (18%)	2 (13%)	
Renal, hyperplasia, lymphoid		1 (9%)		
Renal, pigmentation	1 (8%)	5 (45%)	3 (19%)	4 (27%)
Lymph node, mandibular	(49)	(50)	(50)	(50)
Ectasia	1 (2%)	4 (8%)	2 (4%)	3 (6%)
Hemorrhage	5 (10%)	9 (18%)	3 (6%)	5 (10%)
Hyperplasia, lymphoid	6 (12%)	6 (12%)	3 (6%)	
Pigmentation	12 (24%)	23 (46%)	13 (26%)	16 (32%)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Ectasia	1 (2%)			
Hemorrhage	3 (6%)	3 (6%)	3 (6%)	2 (4%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	
Spleen	(50)	(50)	(50)	(50)
Developmental malformation				1 (2%)
Fibrosis	1 (2%)	2 (4%)	3 (6%)	1 (2%)
Hematopoietic cell proliferation	10 (20%)	12 (24%)	8 (16%)	14 (28%)
Hemorrhage		1 (2%)		
Necrosis	1 (2%)			
Pigmentation, hemosiderin	19 (38%)	20 (40%)	21 (42%)	29 (58%)
Thymus	(49)	(50)	(50)	(48)
Cyst		1 (2%)		
Hemorrhage	1 (2%)		1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(49)	(50)	(50)	(50)
Ectasia	12 (24%)	9 (18%)	20 (40%)	11 (22%)
Hyperplasia	30 (61%)	39 (78%)	22 (44%)	17 (34%)
Inflammation, chronic active			1 (2%)	
Skin	(50)	(50)	(50)	(50)
Acanthosis	1 (2%)	2 (4%)		1 (2%)
Cyst epithelial inclusion	1 (2%)		1 (2%)	
Developmental malformation			1 (2%)	
Hemorrhage			2 (4%)	
Hyperkeratosis	3 (6%)	2 (4%)	3 (6%)	
Inflammation, chronic	1 (2%)			
Ulcer	2 (4%)	4 (8%)		2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis	7 (14%)	10 (20%)	9 (18%)	6 (12%)
Femur, osteopetrosis	5 (10%)	6 (12%)	6 (12%)	3 (6%)
Femur, osteosclerosis			1 (2%)	
Rib, osteopetrosis				1 (2%)
Skeletal muscle	(1)			(1)
Hemorrhage	1 (100%)			1 (100%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
2-Year Study (continued)				
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	9 (18%)	6 (12%)	9 (18%)	8 (16%)
Gliosis	1 (2%)			
Hemorrhage		1 (2%)	1 (2%)	
Hydrocephalus	2 (4%)	3 (6%)		1 (2%)
Necrosis		1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion			1 (2%)	1 (2%)
Hemorrhage		2 (4%)		1 (2%)
Infiltration cellular, histiocyte	21 (42%)	22 (44%)	18 (36%)	19 (38%)
Inflammation, subacute	1 (2%)		2 (4%)	1 (2%)
Alveolar epithelium, hyperplasia	2 (4%)		4 (8%)	2 (4%)
Nose	(50)	(50)	(50)	(50)
Exudate	6 (12%)	3 (6%)	2 (4%)	4 (8%)
Foreign body	5 (10%)	2 (4%)	1 (2%)	
Fungus	3 (6%)	1 (2%)	1 (2%)	
Mucosa, hyperplasia	3 (6%)	2 (4%)	2 (4%)	1 (2%)
Mucosa, metaplasia, squamous	3 (6%)		1 (2%)	1 (2%)
Special Senses System				
Eye	(2)		(1)	(3)
Atrophy	1 (50%)			
Cataract				2 (67%)
Hemorrhage	1 (50%)			1 (33%)
Retina, degeneration				2 (67%)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Calculus, microscopic observation only				1 (2%)
Cyst			2 (4%)	2 (4%)
Hydronephrosis			1 (2%)	1 (2%)
Mineralization	43 (86%)	34 (68%)	37 (74%)	35 (70%)
Nephropathy	34 (68%)	47 (94%)	43 (86%)	45 (90%)
Renal tubule, atrophy		1 (2%)	3 (6%)	2 (4%)
Renal tubule, cytoplasmic alteration	1 (2%)			
Renal tubule, dilatation	1 (2%)		2 (4%)	1 (2%)
Renal tubule, necrosis	1 (2%)			1 (2%)
Renal tubule, pigmentation	49 (98%)	49 (98%)	49 (98%)	47 (94%)
Transitional epithelium, hyperplasia		3 (6%)	7 (14%)	4 (8%)
Ureter			(1)	(1)
Dilatation				1 (100%)
Transitional epithelium, hyperplasia				1 (100%)
Urinary bladder	(50)	(50)	(50)	(50)
Edema	1 (2%)		1 (2%)	
Hemorrhage			1 (2%)	
Transitional epithelium, hyperplasia	4 (8%)		1 (2%)	10 (20%)

APPENDIX C
GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL 138
MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL 138
CHINESE HAMSTER OVARY CELL CYTOGENETICS PROTOCOLS 139
DROSOPHILA MELANOGASTER TEST PROTOCOL 140
MOUSE BONE MARROW SISTER CHROMATID EXCHANGE TEST PROTOCOLS 141
MOUSE BONE MARROW CHROMOSOMAL ABERRATIONS TEST PROTOCOLS 141
RESULTS 142
TABLE C1 Mutagenicity of Butyl Benzyl Phthalate in *Salmonella typhimurium* 143
TABLE C2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate 145
TABLE C3 Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Butyl Benzyl Phthalate 151
TABLE C4 Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Butyl Benzyl Phthalate 152
TABLE C5 Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Butyl Benzyl Phthalate 152
TABLE C6 Induction of Sister Chromatid Exchanges in Mouse Bone Marrow Cells by Butyl Benzyl Phthalate 153
TABLE C7 Induction of Chromosomal Aberrations in Mouse Bone Marrow Cells by Butyl Benzyl Phthalate 154

GENETIC TOXICOLOGY

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1985). Butyl benzyl phthalate was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, and 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. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and at least five doses of butyl benzyl phthalate. In the absence of toxicity, 10,000 or 11,500 μg/plate was selected as the high dose. All trials were repeated.

In this assay, a positive response 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 that is not dose-related, is not reproducible, or is of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr and Caspary (1991). Butyl benzyl phthalate was supplied as a coded aliquot by Radian Corporation. The high dose of butyl benzyl phthalate was determined by toxicity. Mouse lymphoma L5178Y cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to medium containing THG (thymidine, hypoxanthine, and glycine) for 1 day, and to normal medium for 3 to 5 days. For cloning, the horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL medium. This volume included the S9 fraction in those experiments performed with metabolic activation. Incubation with butyl benzyl phthalate continued for 4 hours, at which time the medium plus butyl benzyl phthalate was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, 3×10^6 cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant ($TK^{-/-}$) cells; 600 cells were plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days. The test was initially performed without S9. If a clearly positive response was not obtained, the test was repeated using freshly prepared S9 from the livers of Aroclor 1254-induced male Fischer 344/N rats.

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for butyl benzyl phthalate to be

considered positive, i.e., capable of inducing TFT resistance. A single significant response led to a "questionable" conclusion, and the absence of both a trend and peak response resulted in a "negative" call.

Chinese Hamster Ovary Cell Cytogenetics Protocols

Testing was performed as reported by Galloway *et al.* (1987). Butyl benzyl phthalate was sent to the laboratory as a coded aliquot by Radian Corporation. It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) 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 butyl benzyl phthalate; the high dose was limited by toxicity to 1,250 μg/mL. A single flask per dose was used, and tests yielding equivocal or positive results were repeated.

Sister Chromatid Exchange Test: In the SCE test without S9, CHO cells were incubated for 26 hours with butyl benzyl phthalate in supplemented McCoy's 5A medium. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing butyl benzyl phthalate was removed and replaced with fresh medium plus 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 butyl benzyl phthalate, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing serum and BrdU and no butyl benzyl phthalate, 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. All slides were scored blind, and those from a single test were read by the same person. Fifty second-division metaphase cells were scored for frequency of SCEs/cell from each dose level.

Statistical analyses were conducted on the slopes of the dose-response curves and the individual dose points (Galloway *et al.*, 1987). 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. An increase of 20% or greater at any single dose was considered weak evidence of activity; increases at two or more doses resulted in a determination that the trial was positive. A statistically significant trend ($P \leq 0.05$) in the absence of any responses reaching 20% above background led to a call of equivocal.

Chromosomal Aberrations Test: In the Abs test without S9, cells were incubated in McCoy's 5A medium with butyl benzyl phthalate for 12 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 butyl benzyl phthalate and S9 for 2 hours, after which the treatment medium was removed and the cells were incubated for 12 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the same manner as for the treatment without S9. The harvest time for the Abs test was based on the cell cycle information obtained in the SCE test.

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. One hundred first-division metaphase cells were scored at each dose level. 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).

Chromosomal aberration data are presented as percentage of cells with aberrations. To arrive at a statistical call for a trial, analyses were conducted on both the dose response curve and individual dose points. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) were considered weak evidence for a positive response; significant differences for two or more doses indicated the trial was positive. A positive trend test in the absence of a statistically significant increase at any one dose resulted in an equivocal call (Galloway *et al.*, 1987). Ultimately, the trial calls were based on a consideration of the statistical analyses as well as the biological information available to the reviewers.

DROSOPHILA MELANOGASTER TEST PROTOCOL

The assays for induction of sex-linked recessive lethal (SLRL) mutations were performed with adult flies as described by Valencia *et al.* (1985). Butyl benzyl phthalate was supplied as a coded aliquot by Radian Corporation. It was assayed in the 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. Because no response was obtained, butyl benzyl phthalate was retested by injection into adult males.

To administer butyl benzyl phthalate 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 anesthetized with ether and immobilized on a strip of tape. Injection into the thorax, under the wing, was performed with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of butyl benzyl phthalate at a level that would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. Oral exposure was achieved by allowing Canton-S males to feed for 72 hours on a solution of butyl benzyl phthalate in 5% sucrose. In the injection experiments, 24- to 72-hour old Canton-S males were treated with a solution of butyl benzyl phthalate dissolved in 9% ethanol:1% Tween-80 and allowed to recover for 24 hours. A concurrent solvent control group was also included. In the adult exposures, treated males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days (in each case, sample sperm from successive matings were treated at successively earlier post-meiotic stages). F_1 heterozygous females were mated with their siblings and 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 vials containing fewer than 5% of the expected number of wild-type males after 17 days; these were retested to confirm the response.

SLRL data were analyzed by simultaneous comparison with the concurrent and historical controls, using a normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered positive if $P \leq 0.01$ and the mutation frequency in the tested group was greater than 0.10%, or if $P \leq 0.05$ 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 0.05 and 0.01 but the frequency in the treatment group was between 0.10% and 0.15% or (b) the P value was between 0.10 and 0.05 but the frequency in the treatment group was greater than 0.10%. A test was considered negative if $P \geq 0.10$ or if the frequency in the treatment group was less than 0.10%.

Mouse Bone Marrow Sister Chromatid Exchange Test Protocols

Doses were selected based on available LD_{50} information, and the high dose was limited by experimental design to 5,000 mg/kg. Butyl benzyl phthalate was tested for the induction of SCEs in mouse bone marrow using two protocols. Male $B6C3F_1$ mice (five animals per dose group) were injected intraperitoneally with butyl benzyl phthalate dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene.

The first protocol had a standard harvest time of 23 hours, and the second protocol had a delayed harvest time of 42 hours. The mice were implanted subcutaneously with a BrdU tablet (McFee *et al.*, 1983) 24 hours before harvest (1 hour before butyl benzyl phthalate treatment in the case of the standard protocol). The use of BrdU allowed selection of the appropriate cell population (cells in the second metaphase following butyl benzyl phthalate treatment) for scoring. Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed 23 or 42 hours after treatment (24 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). The cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained using fluorescence-plus-Giemsa and scored.

Twenty-five second-division metaphase cells were scored from each of four animals per treatment group. Responses were evaluated as SCEs/cell, and the data were analyzed by a trend test (Margolin *et al.*, 1986).

Mouse Bone Marrow Chromosomal Aberrations Test Protocols

Doses were selected based on available LD_{50} information, and the high dose was limited by experimental design to 5,000 mg/kg. Butyl benzyl phthalate was tested for induction of Abs in mouse bone marrow using two different protocols. The first protocol used a standard harvest time of 17 hours, and the second protocol used a delayed harvest time of 36 hours.

Male $B6C3F_1$ mice (10 animals per dose group) were injected intraperitoneally with butyl benzyl phthalate dissolved in corn oil (injection volume = 0.4 mL). Solvent control mice received equivalent injections of corn oil only. The positive control was dimethylbenzanthracene (100 mg/kg). The mice were subcutaneously implanted with a BrdU tablet (McFee *et al.*, 1983) 18 hours before the scheduled harvest. (For the standard protocol, this required BrdU implantation to precede injection with butyl benzyl phthalate by 1 hour.) The use of BrdU allowed selection of the appropriate cell population for scoring. (Abs induced by chemical administration are present in maximum number at the first metaphase following treatment; they decline in number during subsequent nuclear divisions due to cell death.) Two hours before sacrifice, the mice received an intraperitoneal injection of colchicine in saline. The animals were killed 17 or 36 hours after butyl benzyl phthalate injection (18 hours after BrdU dosing). One or both femurs were removed, and the marrow was flushed out with phosphate-buffered saline (pH 7.0). Cells were treated with a hypotonic salt solution, fixed, and dropped onto chilled slides. After a 24-hour drying period, the slides were stained and scored.

Fifty first-division metaphase cells were scored from each of eight animals per treatment group. The types of aberrations observed (gaps, breaks, rearrangements, and chromatid versus chromosome) were recorded separately for each animal. The mean total number of aberrations and the mean percentage of cells with aberrations (excluding gaps) were determined for each treatment group. The values for percent cells with aberrations were analyzed by a one-tailed trend test (Margolin *et al.*, 1986), and significance was set at $P=0.025$.

RESULTS

Butyl benzyl phthalate was not mutagenic in a series of *in vitro* tests, but positive responses were obtained in two *in vivo* studies conducted with male mice.

Butyl benzyl phthalate was tested by two laboratories for mutagenicity in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 with and without Aroclor-induced S9 liver enzymes (Table C1; Zeiger *et al.*, 1985). Negative results were obtained at both laboratories in all strains using concentrations of butyl benzyl phthalate that were the highest permitted by experimental design. *In vitro* studies with mammalian cell systems also gave negative results with and without S9. No induction of trifluorothymidine resistance was obtained in L5178Y mouse lymphoma cells with concentrations of butyl benzyl phthalate that formed stable solutions (Table C2; Myhr and Caspary, 1991). Increases in mutant colonies were observed in the absence of S9 in cultures treated with concentrations that produced precipitation, but such responses were not considered valid by experimental quality control parameters. Therefore, the test was concluded to be negative. No induction of SCEs or Abs was observed in CHO cells treated with butyl benzyl phthalate (Tables C3 and C4; Galloway *et al.*, 1987). In the first SCE trial without S9, the response was considered to be equivocal due to a significant trend (P=0.004) in the absence of a significant increase in SCEs at any one dose level. However, results of the second trial were clearly negative, as was the single trial conducted with S9, and the test results were concluded to be negative overall.

No induction of sex-linked recessive lethal mutations was observed in germ cells of male *Drosophila melanogaster* administered 500 ppm butyl benzyl phthalate by injection or up to 50,000 ppm in feed (Table C5; Valencia *et al.*, 1985).

In vivo studies with butyl benzyl phthalate in male mice gave positive results for induction of SCEs (Table C6) and Abs (Table C7) at standard sampling times over a dose range of 1,250 to 5,000 mg/kg. In the SCE test, a single trial with sample time of 23 hours post-injection yielded a positive trend when the highest dose was excluded from the analysis (P=0.0067) because of a reduction in response at the 5,000 mg/kg level. The SCE test conducted with a 42-hour sample time also gave a weakly positive response by trend analysis. Neither trial was repeated. Positive trend analyses were obtained in each of two trials in the Abs test that used the standard harvest time of 17 hours post injection. The only significant dose in each trial was the highest dose (5,000 mg/kg). The single Abs trial that used a delayed harvest time of 36 hours showed no increases in Abs at any of the three dose levels tested.

In conclusion, butyl benzyl phthalate showed no evidence of mutagenicity in any of four *in vitro* tests performed with and without S9 activation enzymes. A test for induction of SLRL mutations in germ cells of male *Drosophila melanogaster* also gave negative results. Positive results were obtained in mouse bone marrow tests for induction of SCEs and Abs. Responses were rather weak, however, and the SCE test was not repeated.

TABLE C1
Mutagenicity of Butyl Benzyl Phthalate 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
Study performed at EG&G Mason Research, Inc.							
TA100	0	129 ± 2.7	130 ± 4.4	113 ± 6.2	142 ± 4.4	111 ± 10.0	141 ± 4.6
	100	140 ± 6.2	133 ± 8.4	118 ± 2.3	123 ± 5.5	120 ± 1.2	150 ± 13.7
	333	134 ± 7.5	142 ± 2.7	110 ± 8.9	130 ± 4.9	118 ± 11.2	140 ± 7.2
	1,000	135 ± 4.2	146 ± 5.1	107 ± 5.0	118 ± 9.8	117 ± 5.0	125 ± 9.2
	3,333	138 ± 4.7	137 ± 12.5	128 ± 3.3	127 ± 2.6	109 ± 7.8	122 ± 8.7
	10,000	140 ± 3.0	136 ± 7.8	121 ± 13.5	119 ± 15.7	102 ± 0.6	124 ± 4.1
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[c]		1,539 ± 54.0	1,321 ± 82.6	1,767 ± 89.4	2,031 ± 117.4	929 ± 12.3	1,191 ± 36.6
TA1535	0	24 ± 1.5	16 ± 0.9	14 ± 1.0	10 ± 2.1	9 ± 2.0	12 ± 0.9
	100	19 ± 2.1	15 ± 2.1	10 ± 3.7	9 ± 2.4	8 ± 0.9	11 ± 1.0
	333	16 ± 0.9	16 ± 2.0	8 ± 1.5	16 ± 1.2	9 ± 1.7	12 ± 2.3
	1,000	19 ± 1.8	17 ± 2.2	10 ± 0.6	8 ± 2.4	6 ± 1.0	16 ± 1.3
	3,333	16 ± 1.8	13 ± 2.0	9 ± 1.5	12 ± 4.5	4 ± 0.3	7 ± 0.7
	10,000	16 ± 0.3	14 ± 1.3	6 ± 1.8	7 ± 0.7	7 ± 0.6	9 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,041 ± 90.4	1,270 ± 48.2	146 ± 7.6	147 ± 7.8	47 ± 4.9	57 ± 3.4
TA1537	0	4 ± 0.0	7 ± 1.5	8 ± 0.9	8 ± 2.2	12 ± 1.0	7 ± 1.5
	100	5 ± 1.5	9 ± 2.6	9 ± 0.6	10 ± 3.8	9 ± 0.7	11 ± 0.6
	333	5 ± 0.7	6 ± 0.3	7 ± 0.6	8 ± 3.3	10 ± 1.5	6 ± 2.5
	1,000	5 ± 1.0	11 ± 1.3	9 ± 1.0	7 ± 0.3	11 ± 3.3	11 ± 2.2
	3,333	7 ± 1.2	7 ± 0.7	8 ± 2.4	4 ± 0.6	11 ± 2.1	8 ± 1.5
	10,000	8 ± 1.7	7 ± 0.9	7 ± 0.6	5 ± 1.2	3 ± 0.3	6 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		265 ± 30.6	466 ± 30.3	140 ± 5.0	222 ± 24.1	58 ± 4.4	112 ± 8.7
TA98	0	23 ± 1.0	22 ± 0.9	32 ± 0.9	32 ± 3.7	32 ± 1.5	27 ± 3.8
	100	19 ± 2.1	26 ± 4.3	30 ± 2.3	30 ± 2.0	27 ± 0.6	36 ± 0.9
	333	21 ± 1.9	27 ± 3.4	23 ± 5.7	27 ± 1.5	28 ± 4.2	30 ± 4.0
	1,000	15 ± 2.7	16 ± 0.9	26 ± 1.5	27 ± 1.2	31 ± 3.1	28 ± 4.3
	3,333	19 ± 2.2	21 ± 5.4	27 ± 1.8	23 ± 2.3	20 ± 4.9	25 ± 2.5
	10,000	22 ± 2.9	21 ± 4.0	19 ± 2.9	17 ± 2.4	20 ± 1.8	20 ± 1.2
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		1,624 ± 52.5	1,459 ± 51.8	1,569 ± 74.6	1,928 ± 50.6	762 ± 37.1	1,056 ± 30.3

TABLE C1
Mutagenicity of Butyl Benzyl Phthalate in *Salmonella typhimurium* (continued)

Strain	Dose (μg/plate)	Revertants/plate -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
Study performed at Case Western Reserve University							
TA100	0	99 ± 1.2	108 ± 9.8	160 ± 8.6	169 ± 8.0	164 ± 15.7	142 ± 11.7
	333	104 ± 2.9	117 ± 1.8	180 ± 14.5	176 ± 10.2	162 ± 2.2	143 ± 3.0
	1,000	109 ± 0.9	117 ± 4.2	162 ± 3.5	169 ± 2.2	138 ± 11.4	145 ± 13.3
	3,333	123 ± 11.2	125 ± 5.5	156 ± 2.9	149 ± 8.8	157 ± 3.0	159 ± 12.3
	10,000	125 ± 2.2	121 ± 9.9	222 ± 4.6	180 ± 17.0	171 ± 13.5	170 ± 12.2
	11,550	155 ± 2.9	132 ± 19.3	196 ± 10.0	178 ± 18.4	186 ± 4.0	163 ± 1.2
Trial summary		Equivocal	Negative	Negative	Negative	Negative	Negative
Positive control		733 ± 35.0	722 ± 23.9	2,057 ± 90.3	1,023 ± 28.3	2,440 ± 73.1	1,359 ± 165.2
TA1535	0	5 ± 1.0	8 ± 0.3	6 ± 1.2	13 ± 2.0	8 ± 1.8	13 ± 0.9
	333	3 ± 0.3	6 ± 0.6	8 ± 0.7	12 ± 4.4	5 ± 0.7	8 ± 0.3
	1,000	2 ± 0.9	4 ± 0.9	5 ± 0.7	9 ± 1.0	7 ± 1.5	9 ± 0.9
	3,333	4 ± 0.0	7 ± 0.7	5 ± 1.8	10 ± 0.3	8 ± 0.9	10 ± 2.1
	10,000	3 ± 0.3	7 ± 1.7	5 ± 1.0	8 ± 1.5	4 ± 1.0	9 ± 2.1
	11,550	6 ± 0.9	9 ± 1.5	6 ± 1.0	10 ± 1.8	5 ± 1.8	8 ± 1.5
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		435 ± 50.6	957 ± 173.9	93 ± 9.3	117 ± 4.6	160 ± 13.5	182 ± 15.7
TA1537	0	2 ± 0.6	5 ± 2.3	8 ± 0.6	10 ± 2.3	7 ± 1.5	9 ± 1.2
	333	3 ± 1.2	5 ± 1.7	7 ± 0.3	9 ± 0.3	5 ± 0.3	8 ± 2.3
	1,000	5 ± 1.0	6 ± 3.2	5 ± 0.3	8 ± 1.2	7 ± 0.3	7 ± 2.3
	3,333	3 ± 0.7	7 ± 0.6	5 ± 0.0	10 ± 2.0	6 ± 0.9	9 ± 1.9
	10,000	3 ± 0.9	5 ± 1.2	4 ± 0.0	7 ± 2.2	8 ± 1.5	7 ± 2.6
	11,550	2 ± 0.3	6 ± 1.3	8 ± 1.5	10 ± 2.0	7 ± 0.3	8 ± 0.9
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		787 ± 185.8	507 ± 94.8	122 ± 5.7	71 ± 3.8	117 ± 30.4	122 ± 19.9
TA98	0	12 ± 2.3	22 ± 6.2	19 ± 1.8	25 ± 3.6	21 ± 5.7	22 ± 1.5
	333	8 ± 0.7	13 ± 0.7	19 ± 1.0	28 ± 4.3	23 ± 1.9	21 ± 2.1
	1,000	11 ± 0.7	13 ± 3.8	21 ± 2.4	27 ± 2.3	23 ± 1.7	24 ± 2.6
	3,333	11 ± 0.6	14 ± 3.1	18 ± 4.4	26 ± 2.2	25 ± 2.0	23 ± 1.8
	10,000	13 ± 0.9	17 ± 3.7	14 ± 1.0	21 ± 1.5	21 ± 2.5	26 ± 3.8
	11,550	11 ± 2.1	17 ± 2.0	25 ± 3.0	28 ± 4.0	29 ± 2.3	29 ± 0.6
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		195 ± 27.9	283 ± 19.2	1,275 ± 368.8	542 ± 28.1	1,454 ± 115.4	622 ± 110.8

[a] The detailed protocol and these data are presented in Zeiger *et al.* (1985).
[b] Revertants are presented as mean ± standard error from three plates.
[c] The positive controls in the absence of metabolic activation were sodium azide (TA1535 and TA100), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for metabolic activation with all strains was 2-aminoanthracene.

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate[a]

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[b]	Average Mutant Fraction[c]
-S9						
Trial 1						
Ethanol		77	72	85	37	
		94	122	108	38	
		74	92	67	30	
		114	114	96	28	33
Methyl methanesulfonate	5[d]	62	37	606	325	
		33	18	491	501	
		57	33	556	327	384[e]
Butyl benzyl phthalate	5	74	61	87	39	
		69	82	94	45	
		72	77	74	34	40
	10	92	72	98	36	
		78	68	101	43	
		73	76	92	42	40
	20	79	40	95	40	
		71	34	90	42	
		90	38	152	56	46
	30	63	14	120	63	
		52	23	141	91	77[e]
	40	54	18	173	107	
		71	28	132	62	
		50	23	126	84	85[e]
	60[f]	59	5	176	99	
		70	10	217	104	
		55	7	191	116	106[e]

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate
(continued)

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 2						
Ethanol		84	130	80	32	
		93	130	105	38	
		82	40	170	69	46
		Toxic				
Methyl methanesulfonate	5[d]	37	12	730	658	
		95	101	724	255	456[e]
Butyl benzyl phthalate	20	57	16	167	98	
		58	40	63	36	
		57	48	80	47	60
	30	75	56	110	49	
		66	45	106	53	
		91	34	128	47	50
	40	69	25	99	48	
		77	28	153	67	
		71	29	125	59	58
	50	67	31	95	47	
		65	26	99	51	49
		Toxic				
	60[f]	49	20	115	79	
		54	23	97	60	69
		Toxic				
	80	67	18	132	66	
		39	15	103	88	
		73	27	183	84	79[e]

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate (continued)

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
−S9 (continued)						
Trial 3						
Ethanol		67	79	82	41	
		83	110	66	27	
		88	117	70	26	
		92	94	80	29	31
Methyl methanesulfonate	5[d]	58	26	432	248	
		49	30	567	384	
		54	32	638	398	343[e]
Butyl benzyl phthalate	10	61	69	60	33	
		79	72	90	38	35
	20	79	61	78	33	
		94	39	110	39	36
	30	86	39	127	49	
		90	27	114	42	
		67	36	86	43	45
	40	54	24	79	49	
		65	17	68	35	
		95	43	110	39	41
	60[f]	101	32	135	45	
		95	33	115	40	
		99	30	102	34	40
	80	60	11	94	52	
		68	14	106	52	52[e]

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate
(continued)

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 4						
Ethanol		105	109	62	20	
		89	80	68	26	
		95	99	70	24	
		99	113	82	28	24
Methyl methanesulfonate	5[d]	76	73	617	271	
		88	93	780	295	
		83	69	791	318	295[e]
Butyl benzyl phthalate	10	73	48	35	16	
		89	66	46	17	
		79	72	49	21	18
	20	72	53	41	19	
		87	64	54	21	
		76	50	53	23	21
	30	95	22	49	17	
		82	20	61	25	
		92	20	57	21	21
	40	Toxic				
		Toxic				

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate (continued)

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9						
Trial 1						
Ethanol		104	103	195	62	
		82	76	222	91	
		80	69	138	57	
		104	153	164	52	66
Methyl cholanthrene	2.5[d]	49	27	768	521	
		41	19	683	562	
		41	10	693	568	550[e]
Butyl benzyl phthalate	30	80	76	150	63	
		77	63	203	88	
		80	86	158	66	72
	40	78	73	116	50	
		78	59	149	64	
		80	113	104	44	52
	50	83	82	128	52	
		75	50	123	55	
		79	65	101	42	50
	60[f]	96	52	206	71	
		81	67	198	81	
		74	58	165	75	76
	80	78	51	195	84	
		76	55	169	74	
		80	62	184	77	78
	100	79	58	190	80	
		73	53	147	67	
		79	47	186	78	75

TABLE C2
Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Butyl Benzyl Phthalate
(continued)

Compound	Concentration (nL/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
+S9 (continued)						
Trial 2						
Ethanol		75	106	112	50	
		86	104	73	28	
		90	101	107	40	
		78	89	104	45	41
Methyl cholanthrene	2.5[d]	69	50	602	291	
		76	58	631	277	
		64	48	687	359	309[e]
Butyl benzyl phthalate	30	64	65	95	50	
		60	59	76	42	
		74	64	69	31	41
	40	94	81	94	33	
		86	57	137	53	
		83	66	77	31	39
	50	93	59	103	37	
		96	67	122	42	
		85	77	94	37	39
	60[f]	82	54	92	38	
		93	73	111	40	
		71	68	89	42	40
	80	99	61	121	41	
		83	69	90	36	
		92	80	109	39	39
	100	92	42	99	36	
		102	60	98	32	
		88	45	96	36	35

[a] Study performed at Litton Bionetics, Inc. The detailed protocol and these data are presented in Myhr and Caspary (1991).
[b] Mutant fraction (MF) (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/10^6 cells treated).
[c] Mean from three replicate plates of approximately 10^6 cells each
[d] Chemical was used as a positive control, and the dose is presented as μg/mL.
[e] Significant positive response ($P \leq 0.05$)
[f] Precipitate formed at this dose level.

TABLE C3
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by Butyl Benzyl Phthalate[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/ Chromosome	SCEs/ Cell	Hrs in BrdU	Relative Change of SCEs/ Chromosome[b] (%)
-S9								
Trial 1								
Summary: Equivocal								
Dimethylsulfoxide		50	1,045	480	0.45	9.6	26.0	
Triethylenemelamine	0.015	50	1,045	1,596	1.52	31.9	26.0	232.50
Butyl benzyl phthalate	0.40	50	1,047	549	0.52	11.0	26.0	14.16
	1.25	50	1,046	568	0.54	11.4	26.0	18.22
	4.00	50	1,046	566	0.54	11.3	26.0	17.80
					P=0.004[c]			
Trial 2								
Summary: Negative								
Dimethylsulfoxide		50	1,043	460	0.44	9.2	26.0	
Triethylenemelamine	0.015	50	1,048	1,482	1.41	29.6	26.0	220.64
Butyl benzyl phthalate	0.40	50	1,046	481	0.45	9.6	26.0	4.27
	1.25	50	1,052	469	0.44	9.4	26.0	1.08
	4.00	50	1,048	405	0.38	8.1	26.0	-12.38
	12.50	25	523	219	0.41	8.8	26.0	-5.06
	12.50	25	519	230	0.44	9.2	28.0	0.48
					P=0.934			
+S9								
Summary: Negative								
Dimethylsulfoxide		50	1,042	420	0.40	8.4	26.0	
Cyclophosphamide	1	50	1,036	1,711	1.65	34.2	26.0	309.74
Butyl benzyl phthalate	125	50	1,034	466	0.45	9.3	26.0	11.81
	400	50	1,040	466	0.44	9.3	26.0	11.16
	1,250	50	1,039	410	0.39	8.2	26.0	-2.10
					P=0.618			

[a] Study performed at Columbia University. The detailed protocol and these data are presented in Galloway *et al.* (1987). SCE=sister chromatid exchange; BrdU=bromodeoxyuridine.
[b] SCEs/chromosome in treated cells versus SCEs/chromosome in solvent control cells
[c] Significance of SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE C4
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by Butyl Benzyl Phthalate[a]

-S9					+S9				
Dose (μg/mL) Scored	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)	Dose (μg/mL) Scored	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Harvest time: 14.0 hours Summary: Negative					Harvest time: 14.0 hours Summary: Negative				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	1	0.01	1.0
Triethylenemelamine					Cyclophosphamide				
0.15	100	31	0.31	25.0	15	100	54	0.54	35.0
Butyl benzyl phthalate					Butyl benzyl phthalate				
125	100	1	0.01	1.0	125	100	2	0.02	2.0
400	100	2	0.02	2.0	400	100	3	0.03	3.0
1,250	100	1	0.01	1.0	1,250	100	1	0.01	1.0
				P=0.419[b]					P=0.431

[a] Study performed at Columbia University. The detailed protocol and these data are presented in Galloway *et al.* (1987). Abs=aberrations.
[b] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

TABLE C5
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by Butyl Benzyl Phthalate[a]

Route of Exposure	Dose (ppm)	Incidence of Death (%)	No. of Lethals/No. of X Chromosomes Tested			Total[b]
			Mating 1	Mating 2	Mating 3	
Feed	0		0/1,148	0/1,126	0/1,019	0/3,293 (0.00%)[c]
	10,000	40	1/1,122	1/1,090	3/1,107	5/3,319 (0.15%)
Feed	0		3/1,058	1/1,038	2/992	6/3,088 (0.19%)[c]
	50,000	10	0/828	0/835	1/692	1/2,355 (0.04%)
Injection	0		2/2,071	3/1,897	1/1,581	6/5,549 (0.11%)
	500	10	1/2,007	2/1,930	2/1,933	5/5,870 (0.09%)

[a] Study performed at University of Wisconsin, Madison. The detailed protocol and these data are presented in Valencia *et al.* (1985). After 17 days, presumptive lethal mutations were identified as vials containing no wild-type males; the parental flies were retested. Results were not significant at the 5% level (Margolin *et al.*, 1983).
[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials
[c] P=0.419 for pooled data from both feed studies.

TABLE C6
Induction of Sister Chromatid Exchanges in Mouse Bone Marrow Cells by Butyl Benzyl Phthalate[a]

Compound	Dose (mg/kg)	Mean SCEs/Cell
Trial 1		
Harvest time: 23 hours		
Corn oil[b]		5.98 ± 0.44
Dimethylbenzanthracene[c]	2.5	9.38 ± 0.32
Butyl benzyl phthalate	1,250	6.60 ± 0.85
	2,500	9.22 ± 0.86
	5,000	6.99 ± 0.74
		P=0.197[d]
Trial 2		
Harvest time: 42 hours		
Corn oil		4.97 ± 0.21
Dimethylbenzanthracene	2.5	12.69 ± 0.54
Butyl benzyl phthalate	1,250	5.75 ± 0.20
	2,500	4.56 ± 0.26
	5,000	5.99 ± 0.21
		P=0.025

[a] Study performed at Brookhaven National Laboratories. SCE=sister chromatid exchange. Twenty-five first-division metaphase cells were scored from each of four animals per treatment group.
[b] Solvent control
[c] Positive control
[d] One-tailed trend analysis (Margolin *et al.*, 1986); significant at P=0.025. For the 23-hour sample time, exclusion of the 5,000 mg/kg dose and recalculation of the trend resulted in a P value of 0.0067, which is significant.

TABLE C7
Induction of Chromosomal Aberrations in Mouse Bone Marrow Cells by Butyl Benzyl Phthalate[a]

Dose (mg/kg)	Total Cells	No. of Abs	Abs/ Cell	Cells with Abs (%)
Trial 1 - Harvest time: 17 hours				
Corn oil[b]				
	400	2.3	0.01	0.75
Dimethylbenzanthracene[c]				
100	400	11.6	0.13	11.50
Butyl benzyl phthalate				
1,250	400	2.4	0.02	1.50
2,500	400	0.8	0.01	0.75
5,000	400	3.4	0.04	3.25*
				P=0.003[d]
Trial 2 - Harvest time: 17 hours				
Corn oil				
	400	1.6	0.01	1.00
Dimethylbenzanthracene				
100	400	16.4	0.18	14.00
Butyl benzyl phthalate				
2,500	400	4.0	0.02	2.25
3,750	400	2.3	0.02	2.00
5,000	400	6.6	0.05	4.25**
				P=0.003
Trial 3 - Harvest time: 36 hours				
Corn oil				
	400	0.5	0.0	0.25
Dimethylbenzanthracene				
100	400	56.1	0.89	26.75
Butyl benzyl phthalate				
1,250	400	1.0	0.01	1.50
2,500	400	0.4	0.00	0.25
5,000	400	0.5	0.01	0.50
				P=0.464

* Positive (P=0.006)
** P=0.002
[a] Study performed at Brookhaven National Laboratories. Abs=aberrations. Fifty first-division metaphase cells were scored from each of eight animals per treatment group.
[b] Solvent control
[c] Positive control
[d] One-tailed trend analysis (Margolin *et al.*, 1986); significant at P=0.025

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

TABLE D1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate 156
TABLE D2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate 157
TABLE D3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of Butyl Benzyl Phthalate 158

TABLE D1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	2,800 ppm	25,000 ppm
n	15	15	15	15
Necropsy body wt	317 ± 4	315 ± 4	310 ± 4	225 ± 5**
Brain				
Absolute	1.924 ± 0.017	1.925 ± 0.017	1.903 ± 0.013	1.880 ± 0.019
Relative	6.08 ± 0.07	6.12 ± 0.08	6.15 ± 0.08	8.38 ± 0.13**
Heart				
Absolute	1.067 ± 0.032	1.032 ± 0.026	1.057 ± 0.026	0.845 ± 0.027**
Relative	3.37 ± 0.09	3.27 ± 0.07	3.41 ± 0.09	3.75 ± 0.11**
R. Kidney				
Absolute	1.231 ± 0.028	1.206 ± 0.026	1.269 ± 0.029	0.926 ± 0.023**
Relative	3.89 ± 0.07	3.84 ± 0.11	4.09 ± 0.08	4.11 ± 0.07
Liver				
Absolute	13.207 ± 0.264	13.394 ± 0.230	13.170 ± 0.230	9.987 ± 0.276**
Relative	41.67 ± 0.51	42.51 ± 0.63	42.44 ± 0.44	44.28 ± 0.62**
Lung				
Absolute	1.407 ± 0.036	1.473 ± 0.040	1.423 ± 0.040	1.141 ± 0.036**
Relative	4.45 ± 0.12	4.68 ± 0.14	4.60 ± 0.15	5.07 ± 0.15**
Prostate Gland				
Absolute	0.609 ± 0.058	0.614 ± 0.061	0.604 ± 0.050	0.276 ± 0.034**
Relative	1.93 ± 0.19	1.95 ± 0.20	1.94 ± 0.15	1.23 ± 0.15*
Seminal Vesicle				
Absolute	1.517 ± 0.081	1.290 ± 0.081	1.333 ± 0.078	1.035 ± 0.059**
Relative	4.80 ± 0.27	4.09 ± 0.25	4.29 ± 0.24	4.58 ± 0.22
R. Testis				
Absolute	1.497 ± 0.017	1.378 ± 0.081	1.502 ± 0.019	0.442 ± 0.014**
Relative	4.73 ± 0.06	4.36 ± 0.25	4.85 ± 0.06	1.97 ± 0.06**
Thymus				
Absolute	0.277 ± 0.012	0.293 ± 0.017	0.271 ± 0.009	0.239 ± 0.010
Relative	0.87 ± 0.04	0.92 ± 0.05	0.87 ± 0.03	1.07 ± 0.05**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	900 ppm	2,800 ppm	8,300 ppm	25,000 ppm
n	12	14	14	14	15	11
Necropsy body wt	367 ± 16	386 ± 5	400 ± 6	369 ± 8	376 ± 7	249 ± 13**
Brain						
Absolute	1.966 ± 0.031	2.068 ± 0.018*	2.081 ± 0.012**	2.030 ± 0.023	2.033 ± 0.018	1.916 ± 0.040
Relative	5.46 ± 0.22	5.37 ± 0.08	5.22 ± 0.07	5.53 ± 0.09	5.43 ± 0.11	7.98 ± 0.52**
Heart						
Absolute	1.182 ± 0.066	1.179 ± 0.029	1.226 ± 0.039	1.139 ± 0.034	1.140 ± 0.036	0.796 ± 0.035**
Relative	3.24 ± 0.14	3.06 ± 0.09	3.07 ± 0.10	3.10 ± 0.09	3.03 ± 0.08	3.24 ± 0.09
R. Kidney						
Absolute	1.277 ± 0.114	1.281 ± 0.039	1.365 ± 0.031	1.249 ± 0.037	1.422 ± 0.045	1.021 ± 0.058*
Relative	3.50 ± 0.27	3.32 ± 0.09	3.42 ± 0.06	3.41 ± 0.12	3.77 ± 0.09	4.13 ± 0.12**
Liver						
Absolute	12.181 ± 0.531	13.017 ± 0.199	13.759 ± 0.382	12.599 ± 0.333	14.261 ± 0.349**	11.782 ± 0.757
Relative	33.24 ± 0.46	33.76 ± 0.36	34.40 ± 0.66	34.16 ± 0.55	37.92 ± 0.71**	47.23 ± 1.23**
Lung						
Absolute	1.570 ± 0.059	1.726 ± 0.053	1.745 ± 0.046	1.648 ± 0.036	1.638 ± 0.042	1.147 ± 0.045**
Relative	4.30 ± 0.09	4.47 ± 0.10	4.37 ± 0.09	4.48 ± 0.09	4.36 ± 0.10	4.69 ± 0.15
Prostate Gland						
Absolute	0.768 ± 0.096	0.812 ± 0.105	0.579 ± 0.043	0.799 ± 0.084	0.731 ± 0.091	0.504 ± 0.099
Relative	2.05 ± 0.21	2.10 ± 0.27	1.44 ± 0.09	2.19 ± 0.24	1.93 ± 0.23	1.96 ± 0.37
Seminal Vesicle						
Absolute	1.387 ± 0.105	1.654 ± 0.103	1.798 ± 0.048*	1.489 ± 0.114	1.538 ± 0.089	0.979 ± 0.104*
Relative	3.78 ± 0.25	4.31 ± 0.29	4.51 ± 0.13	4.00 ± 0.26	4.11 ± 0.25	3.85 ± 0.29
R. Testis						
Absolute	1.517 ± 0.043	1.581 ± 0.016	1.619 ± 0.029	1.559 ± 0.027	1.594 ± 0.024	0.457 ± 0.026**
Relative	4.18 ± 0.12	4.11 ± 0.06	4.06 ± 0.08	4.24 ± 0.09	4.25 ± 0.08	1.86 ± 0.09**
Thymus						
Absolute	0.251 ± 0.023	0.263 ± 0.013	0.381 ± 0.115	0.259 ± 0.015	0.281 ± 0.017	0.182 ± 0.015
Relative	0.67 ± 0.04	0.68 ± 0.03	0.98 ± 0.32	0.71 ± 0.04	0.75 ± 0.05	0.74 ± 0.05

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

TABLE D3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluation in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
n	10	10	10	10
Necropsy body wt	458 ± 10	460 ± 7	430 ± 9*	416 ± 8**
R. Epididymis				
Absolute	0.428 ± 0.016	0.457 ± 0.023	0.473 ± 0.021	0.433 ± 0.009
Relative	0.93 ± 0.02	0.99 ± 0.05	1.10 ± 0.05*	1.04 ± 0.02*
R. Kidney				
Absolute	1.608 ± 0.045	1.767 ± 0.054	1.667 ± 0.038	1.706 ± 0.048
Relative	3.52 ± 0.10	3.83 ± 0.08*	3.88 ± 0.07**	4.10 ± 0.08**
Liver				
Absolute	15.463 ± 0.524	16.120 ± 0.482	15.479 ± 0.382	15.761 ± 0.483
Relative	33.75 ± 0.92	35.00 ± 0.83	36.00 ± 0.55	37.82 ± 0.78**
R. Testis				
Absolute	1.638 ± 0.122	1.820 ± 0.180	1.580 ± 0.106	1.771 ± 0.165
Relative	3.62 ± 0.36	3.97 ± 0.41	3.69 ± 0.27	4.27 ± 0.42
	0 ppm	**6,000 ppm**	**12,000 ppm**	**24,000 ppm**
Female				
n	10	10	10	10
Necropsy body wt	279 ± 7	279 ± 6	282 ± 5	215 ± 4**
R. Kidney				
Absolute	0.931 ± 0.022	1.004 ± 0.025	1.013 ± 0.022*	0.868 ± 0.019
Relative	3.34 ± 0.07	3.61 ± 0.12	3.59 ± 0.05	4.04 ± 0.12**
Liver				
Absolute	8.629 ± 0.189	8.785 ± 0.230	9.301 ± 0.305	8.377 ± 0.153
Relative	31.01 ± 0.81	31.58 ± 0.90	33.00 ± 1.01	38.96 ± 0.57**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights(absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX E
HEMATOLOGY AND HORMONE ASSAY RESULTS

TABLE E1 Hematology Data for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate 160
TABLE E2 Hematology Data for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate 161
TABLE E3 Hematology and Hormone Assay Data for Rats at 6, 8, and 15 Months and at Study Termination in the 2-Year Feed Study of Butyl Benzyl Phthalate 163

TABLE E1
Hematology Data for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	2,800 ppm	25,000 ppm
n	14	15	15	15
Hematocrit (%)	39.9 ± 0.5	39.8 ± 0.6	40.7 ± 0.5	38.2 ± 0.6
Hemoglobin (g/dL)	16.1 ± 0.2	16.0 ± 0.1	16.3 ± 0.2	15.5 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.20 ± 0.09	8.22 ± 0.10	8.41 ± 0.09	7.25 ± 0.11**
Reticulocytes ($10^6/\mu L$)	0.12 ± 0.01[b]	0.12 ± 0.02[c]	0.15 ± 0.01[c]	0.18 ± 0.02[d]
Mean cell volume (fL)	48.7 ± 0.1	48.4 ± 0.1	48.3 ± 0.1	52.7 ± 0.2**
Mean cell hemoglobin (pg)	19.7 ± 0.1	19.5 ± 0.1	19.4 ± 0.2	21.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)	40.4 ± 0.3	40.3 ± 0.3	40.1 ± 0.4	40.7 ± 0.3
Platelets ($10^3/\mu L$)	755.1 ± 10.8	738.5 ± 15.2	786.3 ± 14.5	963.5 ± 24.2**
Leukocytes ($10^3/\mu L$)	7.36 ± 0.23	7.50 ± 0.26[e]	7.58 ± 0.23	6.51 ± 0.21
Segmented neutrophils ($10^3/\mu L$)	1.33 ± 0.12[b]	1.14 ± 0.08[f]	1.31 ± 0.11[g]	1.12 ± 0.05[d]
Lymphocytes ($10^3/\mu L$)	6.29 ± 0.22[b]	6.83 ± 0.19[f]	6.47 ± 0.29[g]	5.38 ± 0.24[d]
Monocytes ($10^3/\mu L$)	0.08 ± 0.02[b]	0.01 ± 0.01*[f]	0.04 ± 0.03[g]	0.05 ± 0.02[d]
Eosinophils ($10^3/\mu L$)	0.04 ± 0.02[b]	0.04 ± 0.04[f]	0.05 ± 0.02[g]	0.04 ± 0.02[d]

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=11
[d] n=12
[e] n=14
[f] n=7
[g] n=10

TABLE E2
Hematology Data for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	900 ppm	2,800 ppm	8,300 ppm	25,000 ppm
n	13	14	15	14	15	11
Hematocrit (%)						
Day 30	43.3 ± 1.0[b]	42.7 ± 0.8[b]	45.0 ± 0.7	43.8 ± 1.1[b]	42.3 ± 0.8	40.0 ± 1.1[b]
Day 60	37.9 ± 0.7[c]	38.1 ± 0.6	38.1 ± 0.5	37.9 ± 0.3[b]	36.8 ± 0.4	36.4 ± 0.5*[c]
Day 90	35.5 ± 0.6	37.1 ± 1.2	35.6 ± 1.1	36.2 ± 0.8	34.6 ± 0.8	31.7 ± 1.3*
Day 120	39.8 ± 1.1	40.9 ± 0.4	40.7 ± 0.7	40.2 ± 0.4	39.0 ± 0.5	—[d]
Day 150	39.5 ± 1.1	40.6 ± 0.7	39.7 ± 0.5[c]	38.3 ± 0.9	38.5 ± 0.6[e]	—
Day 180	37.8 ± 0.9[f]	38.6 ± 0.9	39.5 ± 0.8[c]	38.4 ± 1.0	37.6 ± 0.9[c]	34.5 ± 0.7
Hemoglobin (g/dL)						
Day 30	16.2 ± 0.4[b]	15.7 ± 0.3[b]	16.8 ± 0.3	16.2 ± 0.3[b]	16.2 ± 0.3	15.9 ± 0.4[b]
Day 60	15.3 ± 0.2[c]	15.5 ± 0.2	15.5 ± 0.2	15.6 ± 0.1[b]	15.3 ± 0.1	15.6 ± 0.2[c]
Day 90	14.2 ± 0.2	14.6 ± 0.4	14.2 ± 0.5	14.4 ± 0.2	14.1 ± 0.3	13.0 ± 0.6
Day 120	15.5 ± 0.4	15.9 ± 0.2	15.9 ± 0.3	15.8 ± 0.2	15.5 ± 0.2	—
Day 150	15.4 ± 0.4	15.8 ± 0.2	15.4 ± 0.2[c]	15.0 ± 0.3	15.3 ± 0.2[c]	—
Day 180	14.5 ± 0.3[f]	14.8 ± 0.3	15.1 ± 0.3[c]	14.7 ± 0.4	14.7 ± 0.3	13.7 ± 0.3
Erythrocytes ($10^6/\mu L$)						
Day 30	8.02 ± 0.20[b]	7.95 ± 0.16[b]	8.33 ± 0.13	7.99 ± 0.18[b]	7.83 ± 0.14	7.22 ± 0.20**[b]
Day 60	7.50 ± 0.11[c]	7.61 ± 0.14	7.57 ± 0.09	7.54 ± 0.07[b]	7.23 ± 0.05*	6.71 ± 0.09**[c]
Day 90	7.20 ± 0.14	7.39 ± 0.20	7.15 ± 0.24	7.22 ± 0.09	6.89 ± 0.17	6.07 ± 0.27**
Day 120	8.05 ± 0.21	8.28 ± 0.08	8.17 ± 0.12	8.13 ± 0.09	7.81 ± 0.09*	—
Day 150	7.97 ± 0.22	8.20 ± 0.13	8.02 ± 0.09[c]	7.74 ± 0.17*	7.74 ± 0.11[c]	—
Day 180	7.71 ± 0.17[f]	7.90 ± 0.19	8.06 ± 0.16[c]	7.81 ± 0.22	7.67 ± 0.19[c]	6.69 ± 0.13**
Reticulocytes ($10^6/\mu L$)						
Day 30	0.09 ± 0.01[b]	0.10 ± 0.01[b]	0.06 ± 0.01	0.09 ± 0.01[b]	0.11 ± 0.01	0.10 ± 0.01[b]
Day 60	0.12 ± 0.02[c]	0.17 ± 0.03	0.13 ± 0.02	0.15 ± 0.01[b]	0.13 ± 0.02	0.20 ± 0.02*[c]
Day 90	0.15 ± 0.01	0.20 ± 0.02	0.17 ± 0.02	0.14 ± 0.01	0.14 ± 0.02	0.26 ± 0.03*
Day 120	0.10 ± 0.02	0.10 ± 0.01	0.09 ± 0.01[c]	0.10 ± 0.02[e]	0.11 ± 0.01	—
Day 150	0.15 ± 0.02	0.15 ± 0.01	0.13 ± 0.02[c]	0.15 ± 0.01	0.14 ± 0.01[c]	—
Day 180	0.15 ± 0.01[f]	0.15 ± 0.01	0.14 ± 0.02[c]	0.12 ± 0.02	0.14 ± 0.02[c]	0.17 ± 0.03
Mean cell volume (fL)						
Day 30	54.0 ± 0.3[b]	53.7 ± 0.2[b]	53.9 ± 0.3	54.7 ± 0.3[b]	53.9 ± 0.2	55.3 ± 0.3**[b]
Day 60	50.5 ± 0.5[c]	50.1 ± 0.4	50.3 ± 0.4	50.2 ± 0.2[b]	50.9 ± 0.5	54.2 ± 0.3**[c]
Day 90	49.3 ± 0.3	50.1 ± 0.5	49.8 ± 0.4	50.1 ± 0.6	50.2 ± 0.2*	52.4 ± 0.3**
Day 120	49.4 ± 0.2	49.4 ± 0.2	49.7 ± 0.3	49.5 ± 0.2	49.9 ± 0.2	—
Day 150	49.6 ± 0.2	49.5 ± 0.3	49.5 ± 0.2[c]	49.6 ± 0.2	49.7 ± 0.1[e]	—
Day 180	49.0 ± 0.4[f]	48.8 ± 0.2	49.0 ± 0.2[c]	49.2 ± 0.2	49.0 ± 0.1	51.6 ± 0.1**
Mean cell hemoglobin (pg)						
Day 30	20.3 ± 0.1[b]	19.8 ± 0.1[b]	20.2 ± 0.1	20.3 ± 0.1[b]	20.7 ± 0.2	22.0 ± 0.2**[b]
Day 60	20.5 ± 0.1[c]	20.3 ± 0.2	20.5 ± 0.1	20.7 ± 0.1[b]	21.1 ± 0.2**	23.3 ± 0.2**[c]
Day 90	19.8 ± 0.1	19.8 ± 0.1	20.0 ± 0.2	20.0 ± 0.1	20.4 ± 0.1**	21.4 ± 0.1**
Day 120	19.2 ± 0.1	19.2 ± 0.0	19.5 ± 0.1*	19.4 ± 0.1*	19.9 ± 0.1**	—
Day 150	19.4 ± 0.1	19.3 ± 0.1	19.3 ± 0.1[c]	19.5 ± 0.1	19.8 ± 0.1**[c]	—
Day 180	18.8 ± 0.1[f]	18.7 ± 0.1	18.7 ± 0.1[c]	18.8 ± 0.1	19.1 ± 0.1*[c]	20.5 ± 0.2**
Mean cell hemoglobin concentration (g/dL)						
Day 30	37.5 ± 0.3[b]	36.9 ± 0.3[b]	37.4 ± 0.3	37.2 ± 0.3[b]	38.4 ± 0.4	39.8 ± 0.3**[b]
Day 60	40.5 ± 0.3[c]	40.6 ± 0.2	40.6 ± 0.2	41.3 ± 0.2*[b]	41.5 ± 0.3*	43.0 ± 0.4**[c]
Day 90	40.1 ± 0.2	39.5 ± 0.5	40.1 ± 0.2	40.0 ± 0.6	40.7 ± 0.1	40.9 ± 0.2**
Day 120	38.9 ± 0.1	38.8 ± 0.1	39.2 ± 0.1	39.2 ± 0.2	39.9 ± 0.2**	—
Day 150	39.1 ± 0.2	39.1 ± 0.2	38.9 ± 0.2[c]	39.3 ± 0.2	39.6 ± 0.1*[e]	—
Day 180	38.4 ± 0.2[f]	38.4 ± 0.2	38.2 ± 0.2[c]	38.4 ± 0.2	39.0 ± 0.2*[c]	39.8 ± 0.3**

TABLE E2
Hematology Data for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	300 ppm	900 ppm	2,800 ppm	8,300 ppm	25,000 ppm
n	13	14	15	14	15	11
Platelets ($10^3/\mu L$)						
Day 30	914.9 ± 27.4[b]	892.3 ± 17.3[b]	881.8 ± 33.5	926.9 ± 26.5[b]	954.6 ± 19.7	869.9 ± 16.2[b]
Day 60	743.1 ± 26.9[c]	748.8 ± 20.1	735.5 ± 22.7	757.3 ± 16.4[b]	731.8 ± 9.5	714.9 ± 30.0[c]
Day 90	767.5 ± 12.6	740.9 ± 18.4	751.7 ± 21.9	707.8 ± 11.1*	743.1 ± 11.8[c]	734.6 ± 28.6
Day 120	676.4 ± 17.4	718.1 ± 21.3	713.9 ± 21.4	719.7 ± 15.0	709.7 ± 23.8	—
Day 150	694.2 ± 25.9	679.2 ± 21.8	699.6 ± 16.5[c]	695.9 ± 14.8	704.9 ± 16.5	—
Day 180	636.8 ± 15.5[f]	659.4 ± 13.0	656.6 ± 13.5[c]	650.7 ± 14.2	676.1 ± 9.7	685.0 ± 12.7
Leukocytes ($10^3/\mu L$)						
Day 30	7.67 ± 0.39[b]	7.74 ± 0.36[b]	7.78 ± 0.40	7.98 ± 0.36[b]	7.88 ± 0.34[c]	8.96 ± 0.34[b]
Day 60	9.24 ± 0.25[c]	8.42 ± 0.17	9.16 ± 0.31	8.57 ± 0.32[b]	8.43 ± 0.26	9.57 ± 0.37[c]
Day 90	8.24 ± 0.38	8.34 ± 0.25	8.60 ± 0.33	7.81 ± 0.25	8.07 ± 0.18	8.44 ± 0.36
Day 120	8.12 ± 0.36	8.72 ± 0.23	8.33 ± 0.27	8.12 ± 0.22	8.28 ± 0.29	—
Day 150	9.39 ± 0.41[f]	9.07 ± 0.31	9.30 ± 0.52[c]	8.84 ± 0.23	9.37 ± 0.27	—
Day 180	8.58 ± 0.19[f]	8.88 ± 0.21	8.83 ± 0.42[c]	9.45 ± 0.48	9.25 ± 0.33	9.25 ± 0.28
Segmented neutrophils ($10^3/\mu L$)						
Day 30	0.94 ± 0.13[b]	0.89 ± 0.09[b]	0.91 ± 0.08	1.00 ± 0.09[b]	0.92 ± 0.10[c]	0.87 ± 0.11[b]
Day 60	1.60 ± 0.17[c]	1.33 ± 0.12	1.40 ± 0.11	1.41 ± 0.08[b]	1.19 ± 0.08	0.99 ± 0.10**[c]
Day 90	1.50 ± 0.12	1.50 ± 0.13	1.48 ± 0.16	1.36 ± 0.18	1.58 ± 0.13	1.34 ± 0.08
Day 120	1.47 ± 0.13	1.75 ± 0.12	1.37 ± 0.10	1.49 ± 0.15	1.58 ± 0.11	—
Day 150	1.75 ± 0.16[f]	1.40 ± 0.12	1.71 ± 0.11[c]	1.60 ± 0.14	1.82 ± 0.17	—
Day 180	1.53 ± 0.19[f]	1.80 ± 0.11	1.47 ± 0.12[c]	2.04 ± 0.18	2.01 ± 0.12	1.50 ± 0.09
Lymphocytes ($10^3/\mu L$)						
Day 30	6.60 ± 0.40[b]	6.72 ± 0.34[b]	6.72 ± 0.38	6.83 ± 0.37[b]	6.83 ± 0.29[c]	7.96 ± 0.29**[b]
Day 60	7.32 ± 0.16[c]	6.86 ± 0.15	7.50 ± 0.26	6.99 ± 0.30[b]	7.07 ± 0.23	8.39 ± 0.29[c]
Day 90	6.39 ± 0.32	6.52 ± 0.20	6.78 ± 0.28	6.21 ± 0.25	6.20 ± 0.12	6.77 ± 0.36
Day 120	6.51 ± 0.35	6.76 ± 0.13	6.76 ± 0.25	6.47 ± 0.19	6.57 ± 0.27	—
Day 150	7.45 ± 0.32[f]	7.51 ± 0.25	7.38 ± 0.49[c]	6.95 ± 0.23	7.43 ± 0.25	—
Day 180	6.84 ± 0.24[f]	6.90 ± 0.19	7.19 ± 0.36[c]	7.23 ± 0.40	7.06 ± 0.32	7.47 ± 0.25
Monocytes ($10^3/\mu L$)						
Day 30	0.09 ± 0.02[b]	0.07 ± 0.02[b]	0.07 ± 0.01	0.07 ± 0.02[b]	0.07 ± 0.02[c]	0.09 ± 0.02[b]
Day 60	0.11 ± 0.03[c]	0.06 ± 0.01	0.12 ± 0.03	0.08 ± 0.02[b]	0.09 ± 0.03	0.10 ± 0.03[c]
Day 90	0.17 ± 0.04	0.12 ± 0.02	0.15 ± 0.03	0.10 ± 0.02	0.10 ± 0.02	0.16 ± 0.03
Day 120	0.02 ± 0.01	0.06 ± 0.03	0.04 ± 0.01	0.04 ± 0.02	0.05 ± 0.02	—
Day 150	0.09 ± 0.02[f]	0.04 ± 0.02	0.04 ± 0.02[c]	0.11 ± 0.02	0.02 ± 0.01	—
Day 180	0.08 ± 0.03[f]	0.06 ± 0.02	0.04 ± 0.01[c]	0.06 ± 0.02	0.05 ± 0.02	0.15 ± 0.07
Eosinophils ($10^3/\mu L$)						
Day 30	0.03 ± 0.01[b]	0.06 ± 0.01[b]	0.07 ± 0.02	0.06 ± 0.02[b]	0.07 ± 0.03[c]	0.04 ± 0.01[b]
Day 60	0.10 ± 0.02[c]	0.10 ± 0.03	0.07 ± 0.02	0.05 ± 0.02*[b]	0.05 ± 0.02*	0.05 ± 0.02[c]
Day 90	0.14 ± 0.03	0.11 ± 0.03	0.12 ± 0.02	0.12 ± 0.03	0.12 ± 0.04	0.12 ± 0.03
Day 120	0.10 ± 0.03	0.11 ± 0.03	0.15 ± 0.03	0.09 ± 0.02	0.09 ± 0.02	—
Day 150	0.08 ± 0.03[f]	0.11 ± 0.03	0.14 ± 0.03[c]	0.16 ± 0.04	0.09 ± 0.02	—
Day 180	0.11 ± 0.03[f]	0.11 ± 0.03	0.09 ± 0.02[c]	0.10 ± 0.02	0.12 ± 0.04	0.11 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=15
[c] n=14
[d] Not measured at this time point
[e] n=13
[f] n=12

TABLE E3
Hematology and Hormone Assay Data for Rats at 6, 8, and 15 Months and at Study Termination in the 2-Year Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male				
Hematology				
6 Months				
n	10	10	10	10
Hematocrit (%)	47.1 ± 0.4	47.7 ± 0.5	46.7 ± 0.5	46.7 ± 0.4
Hemoglobin (g/dL)	15.2 ± 0.1	15.3 ± 0.1	15.0 ± 0.1	15.1 ± 0.1
Erythrocytes ($10^6/\mu L$)	9.28 ± 0.08	9.31 ± 0.07	9.13 ± 0.08	9.08 ± 0.06*
Reticulocytes ($10^6/\mu L$)	0.11 ± 0.01	0.09 ± 0.01	0.11 ± 0.02	0.09 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.08 ± 0.03	0.03 ± 0.02	0.07 ± 0.03	0.06 ± 0.02
Mean cell volume (fL)	50.8 ± 0.3	51.3 ± 0.3	51.2 ± 0.2	51.5 ± 0.4
Mean cell hemoglobin (pg)	16.4 ± 0.1	16.4 ± 0.1	16.5 ± 0.1	16.7 ± 0.0**
Mean cell hemoglobin concentration (g/dL)	32.3 ± 0.2	32.1 ± 0.1	32.1 ± 0.1	32.5 ± 0.2
Platelets ($10^3/\mu L$)	594.8 ± 12.5	586.3 ± 10.9	595.9 ± 22.2	616.2 ± 21.6
Leukocytes ($10^3/\mu L$)	10.58 ± 0.38	9.99 ± 0.35	10.38 ± 0.57	10.63 ± 0.55
Segmented neutrophils ($10^3/\mu L$)	1.85 ± 0.24	1.77 ± 0.15	2.04 ± 0.19	2.34 ± 0.40
Lymphocytes ($10^3/\mu L$)	8.15 ± 0.24	7.92 ± 0.36	7.80 ± 0.48	7.74 ± 0.29
Atypical lymphocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)	0.32 ± 0.06	0.22 ± 0.06	0.44 ± 0.10	0.42 ± 0.07
Eosinophils ($10^3/\mu L$)	0.26 ± 0.05	0.09 ± 0.04*	0.09 ± 0.02	0.12 ± 0.04
15 Months				
n	9	10	10	10
Hematocrit (%)	42.8 ± 0.7	45.1 ± 0.8	44.0 ± 0.4	43.7 ± 0.6
Hemoglobin (g/dL)	14.3 ± 0.2	15.0 ± 0.3	14.6 ± 0.1	14.6 ± 0.1
Erythrocytes ($10^6/\mu L$)	8.40 ± 0.13	8.75 ± 0.15	8.58 ± 0.08	8.45 ± 0.09
Reticulocytes ($10^6/\mu L$)	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01	0.15 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.20 ± 0.10	0.04 ± 0.03	0.04 ± 0.02	0.03 ± 0.02
Mean cell volume (fL)	51.0 ± 0.4	51.5 ± 0.3	51.3 ± 0.3	51.6 ± 0.4
Mean cell hemoglobin (pg)	17.0 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.3	33.3 ± 0.2	33.2 ± 0.1	33.4 ± 0.2
Platelets ($10^3/\mu L$)	731.6 ± 36.9	715.4 ± 18.2	723.4 ± 24.9	734.4 ± 14.5
Leukocytes ($10^3/\mu L$)	8.29 ± 0.55	8.59 ± 0.74	8.05 ± 0.59	8.43 ± 0.63
Segmented neutrophils ($10^3/\mu L$)	3.26 ± 0.39	3.23 ± 0.27	3.47 ± 0.37	3.42 ± 0.31
Lymphocytes ($10^3/\mu L$)	4.64 ± 0.33	4.80 ± 0.49	4.12 ± 0.36	4.60 ± 0.46
Atypical lymphocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)	0.27 ± 0.03	0.41 ± 0.09	0.32 ± 0.07	0.34 ± 0.06
Eosinophils ($10^3/\mu L$)	0.11 ± 0.04	0.14 ± 0.05	0.13 ± 0.05	0.08 ± 0.03

TABLE E3
Hematology and Hormone Assay Data for Rats at 6, 8, and 15 Months and at Study Termination in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	3,000 ppm	6,000 ppm	12,000 ppm
Male (continued)				
Hormone Assays				
n	10	10	10	10
Thyroid-stimulating hormone (ng/mL)				
6 Months	3 ± 1[b]	8 ± 6	6 ± 2[c]	4 ± 1
8 Months	1 ± 0	3 ± 0**	2 ± 0	2 ± 0
15 Months	1 ± 0	1 ± 0	2 ± 0*	1 ± 0
Study termination	2 ± 0[b]	1 ± 0	1 ± 0	2 ± 0
Triiodothyronine (ng/dL)				
6 Months	80 ± 9[c]	100 ± 7[b]	82 ± 7[d]	77 ± 12[d]
15 Months	115 ± 7[c]	114 ± 4[b]	123 ± 4[b]	116 ± 2[c]
Study termination	117 ± 10	80 ± 11	121 ± 17[c]	117 ± 11[c]
Thyroxine (μg/dL)				
6 Months	4 ± 0[e]	4 ± 0[c]	4 ± 0[c]	4 ± 0[c]
15 Months	4 ± 1	4 ± 0	5 ± 0	3 ± 0[b]
Study termination	4 ± 0	3 ± 0	4 ± 0	4 ± 0
Adrenocorticotropic hormone (pg/mL)				
6 Months	3,102 ± 542	3,420 ± 636	3,014 ± 462	1,450 ± 365*
15 Months	1,263 ± 310[b]	1,599 ± 452[b]	2,299 ± 494	2,754 ± 757
Study termination	741 ± 122	641 ± 91	704 ± 130	835 ± 122
Testosterone (ng/mL)				
6 Months	1 ± 0[f]	1 ± 0[g]	1[h]	2 ± 0*[f]
8 Months	1 ± 0	1 ± 0	2 ± 0	2 ± 0[b]
15 Months	2 ± 0[d]	2 ± 1[i]	2 ± 1[b]	2 ± 0[e]
Study termination	5 ± 5	1 ± 0	1 ± 0	1 ± 0*

TABLE E3
Hematology and Hormone Assay Data for Rats at 6, 8, and 15 Months and at Study Termination in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Female				
Hematology				
6 Months				
n	9	10	10	10
Hematocrit (%)	45.4 ± 0.4	46.0 ± 0.4	43.7 ± 0.9	45.1 ± 0.5
Hemoglobin (g/dL)	14.9 ± 0.1	15.1 ± 0.1	14.5 ± 0.3	15.0 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.31 ± 0.08	8.42 ± 0.07	8.11 ± 0.18	8.32 ± 0.10
Reticulocytes ($10^6/\mu L$)	0.12 ± 0.02	0.09 ± 0.01	0.13 ± 0.02	0.15 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.07 ± 0.02	0.07 ± 0.03	0.07 ± 0.02	0.04 ± 0.02
Mean cell volume (fL)	54.6 ± 0.2	54.6 ± 0.3	53.9 ± 0.3	54.2 ± 0.3
Mean cell hemoglobin (pg)	17.9 ± 0.1	17.9 ± 0.1	17.9 ± 0.1	18.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.8 ± 0.1	32.8 ± 0.2	33.2 ± 0.2	33.3 ± 0.1
Platelets ($10^3/\mu L$)	645.8 ± 45.0	608.2 ± 17.5	573.0 ± 14.7	566.1 ± 17.7
Leukocytes ($10^3/\mu L$)	7.46 ± 0.82	7.59 ± 0.58	7.43 ± 0.37	8.17 ± 0.58
Segmented neutrophils ($10^3/\mu L$)	1.58 ± 0.45	1.35 ± 0.18	1.50 ± 0.14	1.04 ± 0.12
Lymphocytes ($10^3/\mu L$)	5.41 ± 0.35	5.85 ± 0.38	5.55 ± 0.35	6.62 ± 0.46
Atypical lymphocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monocytes ($10^3/\mu L$)	0.33 ± 0.07	0.30 ± 0.07	0.30 ± 0.05	0.41 ± 0.06
Eosinophils ($10^3/\mu L$)	0.15 ± 0.06	0.09 ± 0.02	0.08 ± 0.02	0.09 ± 0.03
15 Months				
n	9	10	10	10
Hematocrit (%)	44.9 ± 0.6	45.3 ± 0.4	44.7 ± 0.8	42.8 ± 0.6*
Hemoglobin (g/dL)	15.1 ± 0.3	15.5 ± 0.1	15.2 ± 0.2	14.7 ± 0.2
Erythrocytes ($10^6/\mu L$)	7.91 ± 0.15	8.12 ± 0.08	7.96 ± 0.15	7.79 ± 0.11
Reticulocytes ($10^6/\mu L$)	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.01	0.13 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.28 ± 0.06	0.16 ± 0.04	0.13 ± 0.02	0.47 ± 0.10
Mean cell volume (fL)	56.8 ± 0.5	55.8 ± 0.3	56.2 ± 0.2	55.0 ± 0.2**
Mean cell hemoglobin (pg)	19.1 ± 0.1	19.1 ± 0.1	19.1 ± 0.1	18.8 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.7 ± 0.2	34.2 ± 0.1	33.9 ± 0.1	34.3 ± 0.1*
Platelets ($10^3/\mu L$)	628.9 ± 50.8	696.5 ± 24.0	664.4 ± 23.7	691.9 ± 21.5
Leukocytes ($10^3/\mu L$)	4.20 ± 0.26	5.20 ± 0.69	4.97 ± 0.26	6.34 ± 0.66**
Segmented neutrophils ($10^3/\mu L$)	1.36 ± 0.19	1.57 ± 0.28	1.22 ± 0.11	1.28 ± 0.15
Lymphocytes ($10^3/\mu L$)	2.75 ± 0.15	3.48 ± 0.61	3.62 ± 0.23*	4.87 ± 0.59**
Atypical lymphocytes ($10^3/\mu L$)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.02 ± 0.02
Monocytes ($10^3/\mu L$)	0.04 ± 0.01	0.08 ± 0.03	0.06 ± 0.02	0.12 ± 0.02
Eosinophils ($10^3/\mu L$)	0.05 ± 0.01	0.08 ± 0.02	0.07 ± 0.02	0.06 ± 0.02

TABLE E3
Hematology and Hormone Assay Data for Rats at 6, 8, and 15 Months and at Study Termination in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

	0 ppm	6,000 ppm	12,000 ppm	24,000 ppm
Female (continued)				
Hormone Assays				
n	10	10	10	10
Thyroid-stimulating hormone (ng/mL)				
6 Months	2 ± 1	1 ± 0[b]	2 ± 2[b]	0 ± 0
15 Months	1 ± 0	1 ± 0	2 ± 0	2 ± 0
Study termination	1 ± 0	1 ± 0[b]	1 ± 0	1 ± 0
Triiodothyronine (ng/dL)				
6 Months	86 ± 8[i]	99 ± 7[e]	82 ± 5[c]	60 ± 4*
15 Months	126 ± 5	125 ± 3	117 ± 4	98 ± 5**
Study termination	130 ± 20[e]	101 ± 6[g]	97 ± 6[g]	83 ± 5*[d]
Thyroxine (μg/dL)				
6 Months	4 ± 0	4 ± 0[b]	4 ± 0[b]	3 ± 0
15 Months	3 ± 0	3 ± 0	3 ± 0	2 ± 0**
Study termination	3 ± 0	3 ± 0	3 ± 0	3 ± 0
Adrenocorticotropic hormone (pg/mL)				
6 Months	1,946 ± 328	1,912 ± 413	1,838 ± 255	1,966 ± 311
15 Months	1,565 ± 368	816 ± 88	1,609 ± 303	731 ± 106[b]
Study termination	666 ± 76	496 ± 68	634 ± 78	334 ± 35**
Prolactin (ng/mL)				
15 Months	26 ± 3	42 ± 11	37 ± 11	28 ± 6
Study termination	86 ± 15[f]	131 ± 25[e]	172 ± 37[g]	80 ± 42[e]

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n=9
[c] n=8
[d] n=5
[e] n=6
[f] n=2
[g] n=4
[h] n=1; no standard error calculated
[i] n=7

APPENDIX F
REPRODUCTIVE TISSUE EVALUATIONS AND MODIFIED MATING STUDY RESULTS

TABLE F1 Summary of Reproductive Tissue Evaluations for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate 168
TABLE F2 Fertility, Maternal Body Weight, and Developmental Toxicity Data for Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate 169
TABLE F3 Summary of Reproductive Tissue Evaluations for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate 170

TABLE F1
Summary of Reproductive Tissue Evaluations for Male Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	2,800 ppm	25,000 ppm
n	15	14	15	15
Weights (g)				
Necropsy body wt	317 ± 4	315 ± 4[b]	310 ± 4	225 ± 5**
R. cauda	0.221 ± 0.040	0.196 ± 0.023	0.179 ± 0.005	0.068 ± 0.005**[c]
R. epididymis	0.541 ± 0.042	0.505 ± 0.026[b]	0.486 ± 0.013	0.230 ± 0.009**
R. testis	1.497 ± 0.017	1.378 ± 0.081[b]	1.502 ± 0.019	0.442 ± 0.014**
Epididymal spermatozoal parameters				
Motility (%)	79.21 ± 3.38	73.24 ± 6.14[b]	76.49 ± 3.29	—[d]
Concentration (10^6/g cauda epididymal tissue)	373.94 ± 39.52	324.14 ± 49.56	261.47 ± 39.50*	0.567 ± 0.39**
Abnormal (%)	0.987 ± 0.106	0.857 ± 0.064	0.960 ± 0.105	—

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test (organ weights and body weights) or by Dunn's or Shirley's test (epididymal spermatozoal parameters)
** $P \leq 0.01$
[a] Data are presented as mean ± standard error.
[b] n=15
[c] n=14
[d] Not measured at this exposure level due to there being too few sperm to assess

TABLE F2
Fertility, Maternal Body Weight, and Developmental Toxicity Data for Rats in the 10-Week Modified Mating Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	2,800 ppm	25,000 ppm
Fertility				
Male fertility index[b]	23/30 (77%)	25/30 (83%)	28/30 (93%)	10/30 (33%)**
Female fertility index[c]	20/23 (87%)	23/25 (92%)	23/28 (82%)	0/0 (0%)**
Days of mating[d]	4.47 ± 0.38	4.30 ± 0.41	4.83 ± 0.28	6.23 ± 0.21**
Maternal body wt (g)				
n	19	21	23	30[e]
Mating day 0	191 ± 3	195 ± 2	188 ± 2	192 ± 2
Gestation day 0	200 ± 3	202 ± 3	198 ± 2	
Gestation day 6	208 ± 3	211 ± 2	207 ± 2	
Gestation day 9	215 ± 3	218 ± 3	213 ± 2	
Gestation day 13	225 ± 3	228 ± 3	224 ± 2	
Developmental Toxicity				
n	20	23	23	
Litters with dead fetuses	0 (0%)	2 (9%)	1 (4%)	
Live fetuses/total fetuses	203/203	189/192	232/233	
Live fetuses/litter	10.15 ± 0.83	8.22 ± 0.94	10.09 ± 0.60	
Corpora lutea/litter	12.65 ± 0.36	12.13 ± 0.59	12.22 ± 0.38	
Implantations/litter	10.60 ± 0.83	8.78 ± 0.92	10.83 ± 0.64	
Litters with resorptions	6 (30%)	7 (30%)	13 (57%)	
Resorptions/litter	0.50 ± 0.22	0.44 ± 0.15	0.70 ± 0.16	

** Significantly different ($P \leq 0.01$) from the control group by the Chi-square test or Dunn's or Shirley's test
[a] Data for days of mating, maternal body weights, and live fetuses, corpora lutea, implantations, and resorptions per litter are presented as mean ± standard error. Maternal body weight differences from the control group are not significant by Dunnett's or Williams' test. Differences from the control group for developmental toxicity data are not significant by the Chi-square test or Dunn's or Shirley's test.
[b] Females determined to be sperm positive at the end of the mating period/females housed with males
[c] Females pregnant at necropsy/females initially determined to be sperm positive
[d] Days until females were determined to be sperm positive (measured for 30 females per exposure group)
[e] No litters at this exposure level

TABLE F3
Summary of Reproductive Tissue Evaluations for Male Rats in the 26-Week Feed Study of Butyl Benzyl Phthalate[a]

	0 ppm	300 ppm	8,300 ppm	25,000 ppm
n	12	14	15	11
Weights (g)				
Necropsy body wt	367 ± 16	386 ± 5	369 ± 8[b]	249 ± 13**
R. cauda	0.227 ± 0.020	0.197 ± 0.007	0.212 ± 0.007	0.109 ± 0.009**
R. epididymis	0.531 ± 0.019	0.549 ± 0.018	0.559 ± 0.014	0.282 ± 0.025**
R. testis	1.517 ± 0.043	1.581 ± 0.016	1.559 ± 0.027[b]	0.457 ± 0.026**
Epididymal spermatozoal parameters				
Motility (%)	74.15 ± 9.62	75.49 ± 8.42	75.94 ± 6.28	—[c]
Concentration (10^6/g cauda epididymal tissue)	284.52 ± 32.23	285.75 ± 40.18	376.79 ± 22.68	2.06 ± 2.06**
Abnormal (%)	1.017 ± 0.097	0.879 ± 0.115	0.840 ± 0.094	—

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test (organ weights and body weights) or by Dunn's or Shirley's test (epididymal spermatozoal parameters)
[a] Data are presented as mean ± standard error.
[b] n=14
[c] Not measured at this exposure level due to there being too few sperm to assess

APPENDIX G
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF BUTYL BENZYL PHTHALATE 172
PREPARATION AND ANALYSIS OF DOSE FORMULATIONS 173
FIGURE G1 Infrared Absorption Spectrum of Butyl Benzyl Phthalate 175
FIGURE G2 Nuclear Magnetic Resonance Spectrum of Butyl Benzyl Phthalate 176
TABLE G1 Preparation and Storage of Dose Formulations in the Feed Studies of Butyl Benzyl Phthalate 177
TABLE G2 Results of Analysis of Dose Formulations Administered to Rats in the 10-Week Modified Mating Study and in the 26-Week Feed Study of Butyl Benzyl Phthalate 178
TABLE G3 Results of Analysis of Dose Formulations Administered to Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 179
TABLE G4 Results of Referee Analysis of Dose Formulations Administered to Rats in the 10-Week Modified Mating Study and the 26-Week and 2-Year Feed Studies of Butyl Benzyl Phthalate 182

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF BUTYL BENZYL PHTHALATE

Butyl benzyl phthalate was obtained from Chem Central (Kansas City, MO) in two lots (C090882 and L-121 1-87). Lot C090882 was used during the 10-week modified mating study and the 26-week study, and lot L-121 1-87 was used during the 2-year study. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). Reports on analyses performed in support of the butyl benzyl phthalate studies are on file at the National Institute of Environmental Health Sciences.

Both lots of the chemical, a clear, colorless, viscous liquid, were identified as butyl benzyl phthalate by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy. All spectra were consistent with the structure of butyl benzyl phthalate, and the infrared and nuclear magnetic resonance spectra were consistent with the literature spectra (*Sadtler Standard Spectra*) of butyl benzyl phthalate (Figures G1 and G2).

The purity of both lots was determined by elemental analyses, Karl Fischer water analysis, functional group titrations (free acid titration and ester hydrolysis), thin-layer chromatography (TLC), and gas chromatography. For one type of functional group titration, samples of butyl benzyl phthalate were diluted with methanol and titrated with 0.01 N sodium hydroxide. The titration was monitored potentiometrically with a combination mV/pH electrode filled with aqueous 3 M potassium chloride. For the second type of functional group titration, samples of butyl benzyl phthalate were dissolved in excess aqueous 1 N potassium hydroxide and 2-propanol. After gentle agitation, the excess potassium hydroxide was neutralized with 0.5 N hydrochloric acid. The titration was monitored visually with phenolphthalein indicator or potentiometrically with a combination pH/mV electrode filled with aqueous 3 M potassium chloride. TLC was performed on Silica Gel 60 F-254 plates with two solvent systems: 1) *n*-hexane: *p*-dioxane (90:10) and 2) methylene chloride. Plates were examined under visible light and/or short wavelength (254 nm) ultraviolet light and with a spray of 20% antimony (V) chloride in carbon tetrachloride or chloroform. For lot C090882, the plates were heated at 110° C for 20 minutes. Dimethyl terephthalate was used as a reference standard. Gas chromatography was performed using a flame ionization detector. For each lot, two systems were used:

A) 3% SP-2100 on 100/120 Supelcoport glass column, with a nitrogen carrier gas at a flow rate of 70 mL/minute and an oven temperature program of 50° C for 5 minutes, then 50° to 270° C at 10° C per minute, and

B) 3% SP-2401 on 100/120 Supelcoport glass column, with a nitrogen carrier gas at a flow rate of 70 mL/minute and an oven temperature program of 50° C for 5 minutes, then 50° to 250° C at 10° C per minute.

For lot L-121 1-87, an additional system was also used:

C) DB-5 Megabore fused silica column, with a helium carrier gas at a flow rate of 10 mL/minute, a nitrogen make-up gas at a flow rate of 20 mL/minute, and an oven temperature program of 150° to 300° C at 10° C per minute with a 17-minute final hold.

For lot C090882, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for butyl benzyl phthalate. Karl Fischer water analysis indicated 0.23% ± 0.01% water. Functional group

titrations indicated free acid values of 0.011% ± 0.001% and 0.00158 ± 0.0004 mEq acid/g sample and purities of 99.5% ± 0.2% and 100.2% ± 0.4%. TLC indicated a major spot and a slight trace impurity by system 1 and only a major spot by system 2. Gas chromatography using system A indicated one major peak and four impurities with a cumulative area of 3.0% relative to the major peak. The largest impurity had an area estimated to be 2.3% relative to the major peak. Gas chromatography using system B indicated one major peak and four impurities with a cumulative area of 2.8% relative to the major peak. Another analysis of lot C090882 by gas chromatography using system B indicated one major peak and three impurities with a cumulative area of 2.27% relative to the major peak. The largest impurity had an area estimated to be 1.76% relative to the major peak. The largest impurity was identified as benzyl phthalate by gas chromatography using system A, and the sample contained 1.43% ± 0.03% benzyl phthalate. Major peak comparisons of lot C090882 with lot M2676 with system A but with an isothermal oven temperature of 250° C indicated a purity of 100.3% ± 0.8% for lot C090882 relative to lot M2676. The overall purity of lot C090882 was determined to be greater than 97%.

For lot L-121 1-87, elemental analyses for carbon and hydrogen were in agreement with the theoretical values for butyl benzyl phthalate. Karl Fischer water analysis indicated 0.11% ± 0.01% water. Functional group titrations indicated 0.00112 ± 0.00003 mEq of acid/g of sample and a purity of 99.1 ± 0.4%. TLC indicated a major spot and two slight trace impurities by system 1 and a major spot and a trace impurity by system 2. Gas chromatography using system B with a final oven temperature of 270° C with a 20-minute final hold indicated one major peak and five impurities with a combined area of 1.20% relative to the major peak. Gas chromatography using system C indicated one major peak and three impurities with a combined area of 1.04% relative to the major peak. Major peak comparisons of lot L-121 1-87 with lot M2676 with system A but with an isothermal oven temperature of 250° C and *n*-pentacosane as an internal standard indicated a purity of 99.3% ± 0.4% for lot L-121 1-87 relative to lot M2676. The overall purity of lot L-121 1-87 was determined to be greater than or equal to 99%.

Stability studies of the bulk chemical were performed by the analytical chemistry laboratory. Gas chromatography was performed using system A except with an isothermal oven temperature of 250° C and *n*-pentacosane as an internal standard. These studies indicated that butyl benzyl phthalate was stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. To ensure stability, the bulk chemical was stored in the dark at 4° C in tightly sealed 1-gallon amber-glass bottles during the 10-week modified mating study and the 26-week study and in the dark at approximately 22° C in 1-gallon amber-glass bottles with Teflon®-lined lids during the 2-year study. Stability was monitored using gas chromatography during the 10-week modified mating study, the 26-week study, and the 2-year study. No significant degradation of the bulk chemical was detected.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared weekly by mixing butyl benzyl phthalate with feed (Table G1). A butyl benzyl phthalate/feed premix was prepared by hand and then blended with feed in a Patterson-Kelly twin-shell blender for 25 minutes using an intensifier bar for the initial 15 minutes for the 10-week modified mating study and for the 26-week study and for 15 minutes using an intensifier bar for the initial 5 minutes for the 2-year study. Formulations were stored in plastic bags inside containers at 5° C for up to 4 weeks.

Homogeneity studies of the 300, 6,250, and 25,000 ppm dose formulations were performed by the study laboratory and the analytical chemistry laboratory. In addition, homogeneity studies of the 3,000 and 24,000 ppm dose formulations were performed by the study laboratory. The samples were taken in triplicate from the three blender ports. For the 300, 3,000, 24,000 and 25,000 ppm formulations, the samples were extracted with hexane and clarified by centrifugation. Aliquots of the extracts were shaken with 0.1 N sodium hydroxide and were centrifuged to separate the two phases. For each sample, an aliquot

from the hexane layer was pipetted into a vial and evaporated under a stream of nitrogen. An internal standard solution of octaphenone in acetonitrile:water (80:20) was added, and the solutions were purified and then analyzed using high-performance liquid chromatography (HPLC). HPLC was performed with a Brownlee MPLC RP-18 column using ultraviolet detection (280 nm) and a mobile phase of water:acetonitrile (15:85, 25:75, 30:70, or 40:60) at a flow rate of 1.0 or 1.4 mL/minute. For the 6,250 ppm formulation, the samples were extracted with acetonitrile and clarified by centrifugation. Aliquots of the extracts were mixed with water and an internal standard solution of octaphenone in acetonitrile and were further diluted with acetonitrile. The solutions were filtered and analyzed using the same HPLC system used for the 300, 3,000, 24,000 and 25,000 ppm formulations but with a mobile phase ratio of 30:70 and a flow rate of 1.4 mL/minute. Stability studies of the 300 and 6,250 ppm dose formulations were also performed using the same HPLC methods described for the homogeneity analyses of the 300 and 6,250 ppm formulations, respectively. Homogeneity was confirmed and the stability of the dose formulations was confirmed for at least 4 weeks at temperatures up to 5° C when stored protected from light.

Periodic analyses of the dose formulations of butyl benzyl phthalate were conducted at the study laboratories and analytical chemistry laboratory using HPLC. For the 10-week modified mating study, the 26-week study, and the 2-year study, the formulations were analyzed every 8 to 9 weeks (Tables G2 and G3). All of the dose formulations analyzed were within 10% of the target concentration. Results of periodic referee analyses performed by the analytical chemistry laboratory agreed with the results obtained by the study laboratory (Table G4).

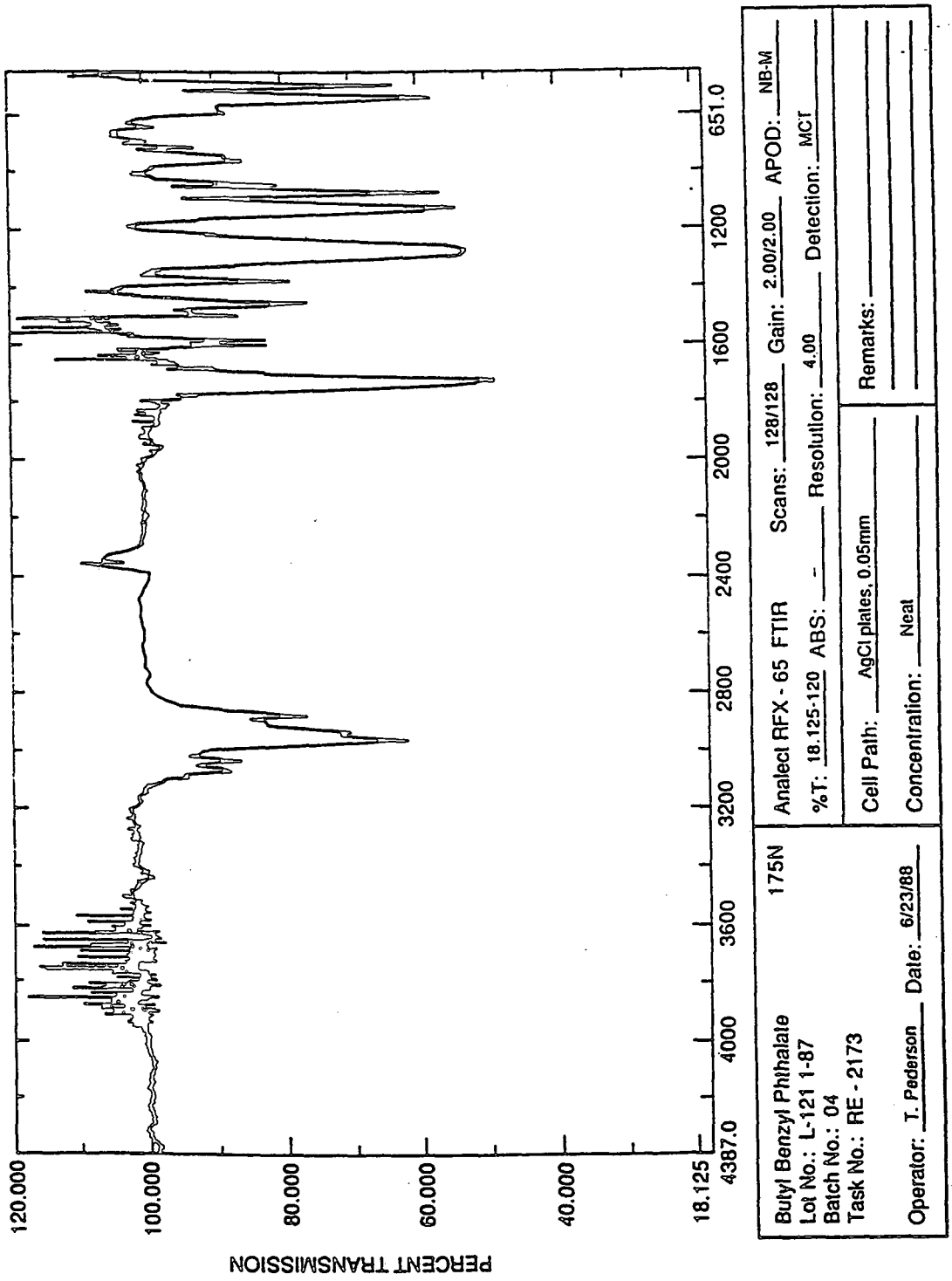


FIGURE G1
Infrared Absorption Spectrum of Butyl Benzyl Phthalate

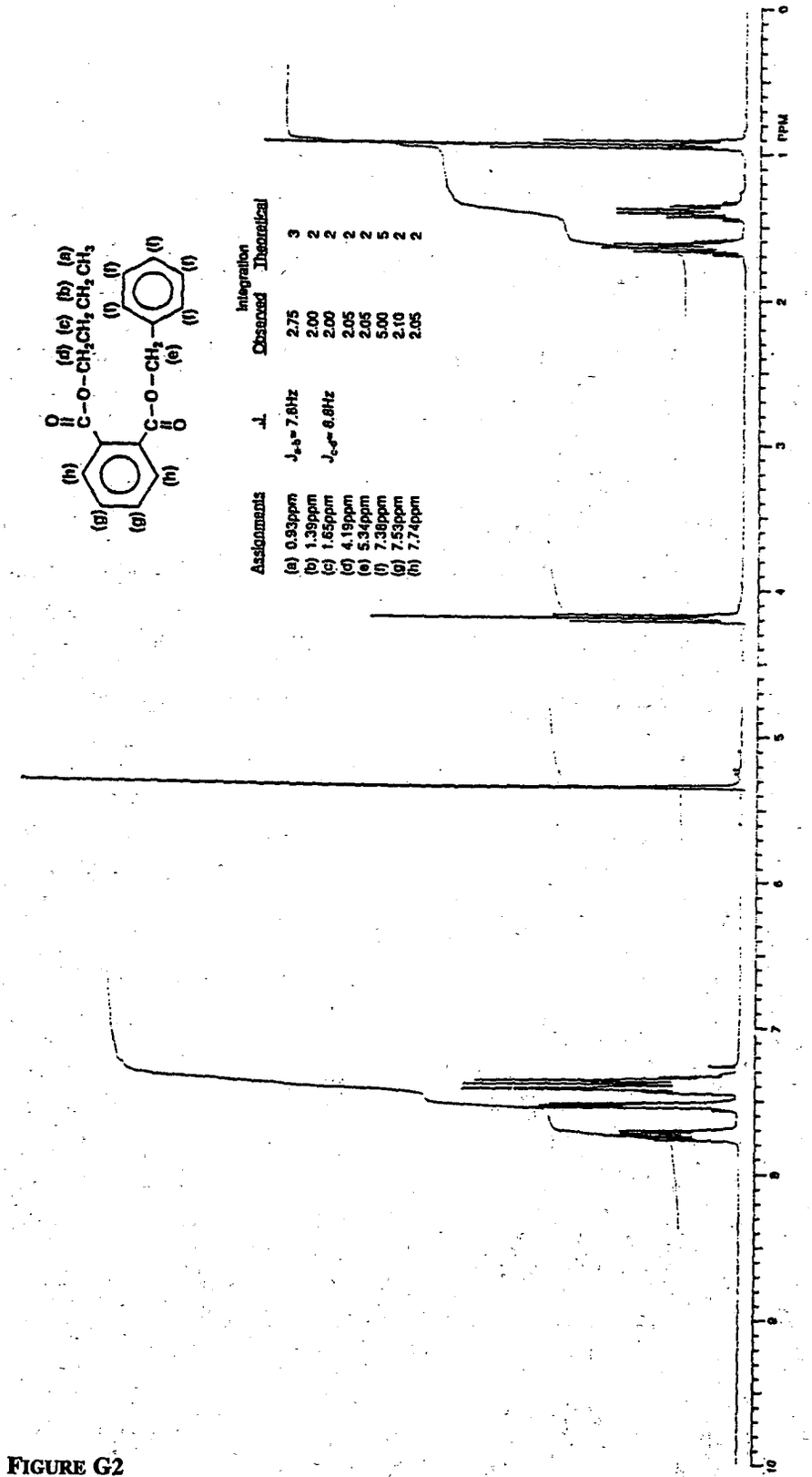


FIGURE G2
Nuclear Magnetic Resonance Spectrum of Butyl Benzyl Phthalate

TABLE G1
Preparation and Storage of Dose Formulations in the Feed Studies of Butyl Benzyl Phthalate

10-Week Modified Mating Study	26-Week Study	2-Year Study
Preparation		
A premix of feed and butyl benzyl phthalate was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 15 minutes and off for 10 minutes. Doses were prepared weekly.	A premix of feed and butyl benzyl phthalate was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 15 minutes and off for 10 minutes. Doses were prepared weekly.	A premix of feed and butyl benzyl phthalate was prepared, then layered into the remaining feed and blended in a Patterson-Kelly twin-shell blender with the intensifier bar on for 5 minutes and off for 10 minutes. Doses were prepared weekly.
Chemical Lot Number		
C090882	C090882	L-121 1-87
Maximum Storage Time		
4 weeks	4 weeks	4 weeks
Storage Conditions		
Stored in plastic bags placed in rigid containers at 5° C	Stored in plastic bags placed in rigid containers at 5° C	Stored in double-thickness plastic bags placed in rigid plastic containers at -20° C or 5° C
Study Laboratory		
Hazleton Laboratories America, Inc. (Rockville, MD)	Hazleton Laboratories America, Inc. (Rockville, MD)	Southern Research Institute (Birmingham, AL)
Referee Laboratory		
Midwest Research Institute (Kansas City, MO)	Midwest Research Institute (Kansas City, MO)	Midwest Research Institute (Kansas City, MO)

TABLE G2
Results of Analysis of Dose Formulations Administered to Rats in the 10-Week Modified Mating Study and in the 26-Week Feed Study of Butyl Benzyl Phthalate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
19 September 1984	22 September 1984	300	310[b]	+3
		300	310[c]	+3
		300	310[d]	+3
		300	308[b]	+3
		300	308[c]	+3
		300	310[d]	+3
		900	850	-6
		900	852	-5
		2,800	2,790	0
		2,800	2,790	0
		8,300	7,680	-7
		25,000	24,400[b]	-2
		25,000	24,400[c]	-2
		25,000	24,400[d]	-2
13 November 1984	14 November 1984	300	321	+7
		900	973	+8
		2,800	2,800	0
		8,300	8,450	+2
		25,000	23,900	-4
8 January 1985	9 January 1985	300	285	-5
		900	850	-6
		2,800	2,690	-4
		8,300	7,830	-6
		25,000	23,600	-6
12 March 1985	13 March 1985	300	299	0
		900	891	-1
		2,800	2,630	-6
		8,300	7,830	-6
		25,000	23,900	-4

[a] Results of duplicate analyses
[b] Sample selection from top left of twin-shell blender
[c] Sample selection from top right of twin-shell blender
[d] Sample selection from bottom of twin-shell blender

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
19 June 1989	20 June 1989	3,000	2,860	-5
		6,000	5,700	-5
		6,000	5,500	-8
		12,000	11,400	-5
		12,000	11,600	-3
		24,000	23,200	-3
21 August 1989	22-23 August 1989	3,000	3,000	0
		6,000	5,960	-1
		6,000	5,980	0
		12,000	12,300	+3
		12,000	12,000	0
		12,000	12,200	+2
		24,000	24,100	0
		24,000	24,800	+3
23 October 1989	24-25 October 1989	3,000	3,020	+1
		6,000	5,960	-1
		6,000	5,980	0
		12,000	12,200	+2
		12,000	12,000	0
		12,000	12,200	+2
		24,000	24,100	0
		24,000	23,700	-1
18 December 1989	18-19 December 1989	3,000	2,940	-2
		6,000	6,040	+1
		6,000	6,000	0
		12,000	11,900	-1
		12,000	12,000	0
		12,000	12,000	0
		24,000	24,600	+3
		24,000	24,200	+1
12 February 1990	13 February 1990	3,000	2,960	-1
		6,000	5,940	-1
		6,000	5,940	-1
		12,000	12,000	0
		12,000	12,000	0
		12,000	11,900	-1
		24,000	24,300	+1
		24,000	24,200	+1
26 March 1990	27-28 March 1990	3,000	2,820	-6
		6,000	5,920	-1
		6,000	5,870	-2
		12,000	12,000	0
		12,000	11,600	-3
		12,000	12,300	+3
		24,000	23,800	-1
		24,000	23,000	-4

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
21 May 1990	21-22 May 1990	3,000	2,870	-4
		6,000	5,960	-1
		6,000	5,990	0
		12,000	11,740	-2
		12,000	11,990	0
		12,000	12,170	+1
		24,000	23,910	0
		24,000	23,630	-2
23 July 1990	24-27 July 1990	3,000	3,110	+4
		6,000	6,540	+9
		6,000	6,370	+6
		12,000	12,450	+4
		12,000	12,620	+5
		12,000	12,590	+5
		24,000	24,370	+2
		24,000	24,510	+2
24 September 1990	25 September 1990-2 October 1990	3,000	2,870	-4
		6,000	5,910	-2
		6,000	5,890	-2
		12,000	11,800	-2
		12,000	11,800	-2
		12,000	11,700	-3
		24,000	23,400	-3
		24,000	23,400	-3
26 November 1990	27 November 1990	3,000	2,970	-1
		6,000	5,980	0
		6,000	6,020	0
		12,000	12,000	0
		12,000	12,000	0
		12,000	12,000	0
		24,000	23,800	-1
		24,000	23,900	0
21 January 1991	22 January 1991	3,000	3,120	+4
		6,000	5,600	-7
		6,000	5,770	-4
		12,000	12,000	0
		12,000	11,630	-3
		12,000	12,080	+1
		24,000	23,920	0
		24,000	23,820	-1

TABLE G3
Results of Analysis of Dose Formulations Administered to Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
18 March 1991	18-19 March 1991	3,000	2,990	0
		6,000	5,900	-2
		6,000	5,730	-5
		6,000	5,950	-1
		12,000	11,600	-3
		12,000	11,800	-2
		12,000	11,900	-1
		24,000	23,700	-1
		24,000	23,600	-2
		24,000	23,500	-2
13 May 1991	13-15 May 1991	3,000	3,020	+1
		6,000	5,910	-2
		6,000	5,930	-1
		6,000	5,970	-1
		12,000	11,900	-1
		12,000	11,900	-1
		12,000	12,000	0
		24,000	24,100	0
		24,000	24,000	0
		24,000	24,000	0
8 July 1991	9-10 July 1991	6,000	5,980	0
		12,000	12,000	0
		24,000	24,100	0
9 September 1991	9-10 September 1991	6,000	5,820	-3
		12,000	12,000	0
		24,000	23,800	-1
4 November 1991	4-5 November 1991	6,000	6,110	+2
		12,000	11,500	-4
		24,000	24,200	+1
6 January 1992	6 January 1992	6,000	5,970	-1
		12,000	11,700	-3
		24,000	23,600	-2
9 March 1992	9-10 March 1992	6,000	5,940	-1
		12,000	11,900	-1
		24,000	24,000	0

[a] Results of duplicate analyses

TABLE G4
Results of Referee Analysis of Dose Formulations Administered to Rats in the 10-Week Modified Mating Study and the 26-Week and 2-Year Feed Studies of Butyl Benzyl Phthalate

Date Prepared	Target Concentration (ppm)	Determined Concentration (ppm) Study Laboratory[a]	Determined Concentration (ppm) Referee Laboratory[b]
10-Week Modified Mating Study and 26-Week Study (Hazleton Laboratories America, Inc.)			
19 September 1984	300	308	305 ± 3
8 January 1985	8,300	7,830	7,960 ± 120
2-Year Study (Southern Research Institute)			
19 June 1989	12,000	11,400	11,800 ± 500

[a] Results of duplicate analyses
[b] Results of triplicate analyses (mean ± standard error)

APPENDIX H
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDY OF BUTYL BENZYL PHTHALATE

TABLE H1 Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 184
TABLE H2 Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate 185

TABLE H1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

	0 ppm		3,000 ppm			6,000 ppm			12,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	14.6	149	15.1	148	308	14.8	147	606	14.1	141	1,198
3	15.9	183									
4	15.6	205									
5	17.8	233	19.3	236	245	17.8	233	456	19.7	226	1,043
6	17.5	253	17.9	255	210	17.1	253	404	18.4	243	905
7	16.5	266									
8	17.4	286									
9	16.5	298	15.6	299	156	15.2	292	313	15.6	284	660
10	16.2	310	16.8	310	163	17.0	301	339	16.6	291	686
11	15.3	319									
12	16.4	326									
13	16.4	339	16.6	338	147	17.2	321	321	16.8	315	642
17	17.3	370	16.3	367	134	12.4	350	212	12.3	338	438
21	15.9	391	17.8	389	137	18.6	367	305	17.5	351	599
25	20.5	391	16.4	403	122	17.0	381	268	15.5	362	512
29	14.7	416	15.6	411	114	15.1	394	231	14.4	377	458
33	14.7	423	16.2	421	115	14.3	399	214	15.6	382	490
37	15.0	429	15.6	429	109	16.1	410	236	15.2	389	469
41	17.1	437	16.4	434	114	15.8	422	225	16.5	394	502
45	17.5	443				16.3	429	228	17.0	405	503
49	16.1	449	16.0	449	107	16.0	433	221	15.9	413	463
53	16.3	451	17.1	451	114	15.6	437	215	16.7	414	484
57	15.3	450	15.2	453	101	16.0	432	222	16.2	412	470
61	15.7	451	17.4	445	117	16.0	434	221	16.1	413	468
65	15.4	451	16.0	446	107	15.7	434	217	15.1	420	431
69	14.2	446	15.5	445	104	14.5	440	198	15.1	419	434
73	15.1	448	14.5	447	97	14.6	441	199	15.4	427	433
77	14.1	446	15.3	443	104	13.8	441	188	14.4	428	404
81	13.5	440	13.2	437	91	11.9	431	166	13.3	422	378
85	13.6	440	14.2	431	99	13.8	424	195	14.3	418	409
89	13.9	439	14.0	430	97	14.2	428	199	13.6	413	396
93						13.1	422	186	13.0	422	370
97	14.5	433	13.5	428	95	12.4	410	182	12.7	411	372
101	12.4	426	12.8	419	92	13.9	407	204	11.6	402	347
Mean for weeks											
1-13	16.3	264	16.9	264	205	16.5	258	407	16.9	250	856
14-52	16.5	417	16.3	413	119	15.7	398	238	15.5	379	493
53-101	14.5	443	14.9	440	101	14.3	429	199	14.4	417	415

[a] Grams of feed consumed per animal per day
[b] Milligrams of butyl benzyl phthalate consumed per kilogram body weight per day

TABLE H2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of Butyl Benzyl Phthalate

	0 ppm		6,000 ppm			12,000 ppm			24,000 ppm		
Week	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day[b] (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg)
2	10.3	121	11.1	121	551	10.9	122	1,076	7.5	114	1,582
3	11.0	135									
4	11.3	142									
5	11.1	153	12.6	151	499	11.8	149	951			
6	10.6	159	11.6	162	431	11.0	157	838	11.3	151	1,791
7	10.9	167									
8	10.9	171									
9	11.3	175									
10	10.9	178	10.8	179	362	10.9	175	749	10.5	171	1,478
11	10.8	180									
12	11.0	186									
13	10.3	185	10.5	187	337	10.1	179	677	9.8	176	1,338
17	10.2	198	10.4	197	317	10.2	193	634	9.7	183	1,274
21	10.3	204	10.0	204	292	10.2	196	623	9.9	187	1,269
25			10.4	213	293	9.9	206	575	10.1	193	1,254
29	9.3	218	9.8	219	269	9.6	211	547	9.0	197	1,090
33	9.7	223	10.2	225	272	9.4	216	520	9.3	199	1,127
37	10.7	231	10.9	231	282	10.7	224	575	9.6	203	1,133
41	10.7	238				11.2	230	581			
45	11.4	240	11.4	244	281	11.5	239	579	9.7	207	1,119
49	12.5	258	11.4	261	262	11.8	248	570	10.4	212	1,177
53	11.6	269	12.1	270	270	11.9	258	553	10.1	214	1,130
57	11.1	276	11.9	275	260	11.8	266	534	10.6	214	1,184
61	11.4	281	11.6	280	249	11.4	270	506	9.7	216	1,073
65	11.7	286	11.6	287	243	11.6	277	502	10.1	220	1,104
69	11.1	296	11.3	293	233	11.5	281	491	9.9	222	1,068
73	11.1	298	10.9	301	218	11.2	284	473	9.8	226	1,046
77	11.3	300	11.2	304	221	11.4	287	475	9.8	230	1,028
81	11.7	300	11.4	303	227	11.2	291	462	10.0	222	1,084
85	11.8	312	11.7	308	228	12.0	299	480	10.8	231	1,126
89	12.1	321	12.4	321	231	11.7	306	460	10.6	237	1,074
93	10.6	320	11.8	321	221	11.1	308	432	10.5	236	1,069
97	11.4	319	11.8	323	219	12.1	309	469	10.4	233	1,070
101	10.2	318	11.5	325	211	11.7	307	457	10.1	232	1,049
Mean for weeks											
1-13	10.9	163	11.3	160	436	10.9	156	858	9.8	153	1,547
14-52	10.6	226	10.6	224	284	10.5	218	578	9.7	198	1,180
53-101	11.3	300	11.6	301	233	11.6	288	484	10.2	226	1,085

[a] Grams of feed consumed per animal per day
[b] Milligrams of butyl benzyl phthalate consumed per kilogram body weight per day

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

TABLE I1 Ingredients of NIH-07 Rat and Mouse Ration 188
TABLE I2 Vitamins and Minerals in NIH-07 Rat and Mouse Ration 188
TABLE I3 Nutrient Composition of NIH-07 Rat and Mouse Ration 189
TABLE I4 Contaminant Levels in NIH-07 Rat and Mouse Ration 190

TABLE I1
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 were ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE I2
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 I3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation[b]	Range	Number of Samples
Protein (% by weight)	23.60 ± 0.71	21.30 — 25.20	32
Crude fat (% by weight)	5.45 ± 0.32	4.80 — 5.90	32
Crude fiber (% by weight)	3.49 ± 0.31	3.00 — 4.10	32
Ash (% by weight)	6.82 ± 0.33	6.01 — 7.27	32
Amino Acids (% of total diet)			
Arginine	1.287 ± 0.084	1.100 — 1.390	10
Cystine	0.306 ± 0.075	0.181 — 0.400	10
Glycine	1.160 ± 0.050	1.060 — 1.220	10
Histidine	0.580 ± 0.024	0.531 — 0.608	10
Isoleucine	0.917 ± 0.034	0.867 — 0.965	10
Leucine	1.972 ± 0.052	1.850 — 2.040	10
Lysine	1.273 ± 0.051	1.200 — 1.370	10
Methionine	0.437 ± 0.115	0.306 — 0.699	10
Phenylalanine	0.994 ± 0.125	0.665 — 1.110	10
Threonine	0.896 ± 0.055	0.824 — 0.985	10
Tryptophan	0.223 ± 0.160	0.107 — 0.671	10
Tyrosine	0.677 ± 0.105	0.564 — 0.794	10
Valine	1.089 ± 0.057	0.962 — 1.170	10
Essential Fatty Acids (% of total diet)			
Linoleic	2.389 ± 0.233	1.830 — 2.570	9
Linolenic	0.277 ± 0.036	0.210 — 0.320	9
Vitamins			
Vitamin A (IU/kg)	6,966 ± 1,512	4,290 — 12,540	32
Vitamin D (IU/kg)	4,450 ± 1,382	3,000 — 6,300	4
α-Tocopherol (ppm)	36.92 ± 9.32	22.5 — 48.9	9
Thiamine (ppm)	18.94 ± 2.06	15.0 — 25.0	32
Riboflavin (ppm)	7.92 ± 0.93	6.10 — 9.00	10
Niacin (ppm)	100.95 ± 25.92	65.0 — 150.0	9
Pantothenic acid (ppm)	30.30 ± 3.60	23.0 — 34.6	10
Pyridoxine (ppm)	9.25 ± 2.62	5.60 — 14.0	10
Folic acid (ppm)	2.51 ± 0.64	1.80 — 3.70	10
Biotin (ppm)	0.267 ± 0.049	0.19 — 0.35	10
Vitamin B_{12} (ppb)	40.14 ± 20.04	10.6 — 65.0	10
Choline (ppm)	3,068 ± 314	2,400 — 3,430	9
Minerals			
Calcium (%)	1.19 ± 0.09	1.02 — 1.37	32
Phosphorus (%)	0.94 ± 0.05	0.80 — 1.03	32
Potassium (%)	0.887 ± 0.067	0.772 — 0.971	8
Chloride (%)	0.526 ± 0.092	0.380 — 0.635	8
Sodium (%)	0.315 ± 0.034	0.258 — 0.370	10
Magnesium (%)	0.168 ± 0.008	0.151 — 0.180	10
Sulfur (%)	0.274 ± 0.063	0.208 — 0.420	10
Iron (ppm)	356.2 ± 90.0	255.0 — 523.0	10
Manganese (ppm)	92.24 ± 5.35	81.70 — 99.40	10
Zinc (ppm)	58.14 ± 9.91	46.10 — 81.60	10
Copper (ppm)	11.50 ± 2.40	8.090 — 15.39	10
Iodine (ppm)	3.70 ± 1.14	1.52 — 5.83	10
Chromium (ppm)	1.71 ± 0.45	0.85 — 2.09	9
Cobalt (ppm)	0.797 ± 0.23	0.490 — 1.150	6

TABLE I4
Contaminant Levels in NIH-07 Rat and Mouse Ration[a]

Nutrient	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.40 ± 0.18	0.10 — 0.80	32
Cadmium (ppm)	0.11 ± 0.06	0.05 — 0.20	32
Lead (ppm)	0.33 ± 0.38	0.10 — 2.10	32
Mercury (ppm)	0.03 ± 0.01	0.02 — 0.05	32
Selenium (ppm)[c]	0.35 ± 0.10	0.20 — 0.55	31
Aflatoxins (ppb)[d]	<5.0		31
Nitrate nitrogen (ppm)[e]	11.60 ± 5.15	1.80 — 20.0	32
Nitrite nitrogen (ppm)[e]	0.24 ± 0.20	0.10 — 1.00	32
BHA (ppm)[f]	1.42 ± 0.85	1.00 — 4.0	31
BHT (ppm)[f]	1.29 ± 1.10	1.00 — 7.00	31
Aerobic plate count (CFU/g)[f]	102,209 ± 92,146	4,700 — 380,000	32
Coliform (MPN/g)[g]	20.50 ± 22.30	3.00 — 93.00	32
Escherichia coli (MPN/g)	3.25 ± 1.08	3.00 — 9.0	32
Total nitrosoamines (ppb)[g]	7.05 ± 2.60	2.00 — 13.70	32
N-Nitrosodimethylamine (ppb)[g]	5.12 ± 1.60	1.00 — 9.40	32
N-Nitrosopyrrolidine (ppb)[g]	1.68 ± 1.25	0.00 — 4.70	32
Pesticides (ppm)			
α-BHC	<0.01		31
β-BHC	<0.02		31
γ-BHC	<0.01		31
δ-BHC	<0.01		31
Heptachlor	<0.01		31
Aldrin	<0.01		31
Heptachlor epoxide	<0.01		31
DDE	<0.01		31
DDD	<0.01		31
DDT	<0.01		31
HCB	<0.01		31
Mirex	<0.01		31
Methoxychlor	<0.05		31
Dieldrin	<0.01		31
Endrin	<0.01		31
Telodrin	<0.01		31
Chlordane	<0.05		31
Toxaphene	<0.1		31
Estimated PCBs	<0.2		31
Ronnel	<0.01		31
Ethion	<0.02		31
Trithion	<0.05		31
Diazinon	<0.1		31
Methyl parathion	<0.02		31
Ethyl parathion	<0.02		31
Malathion	0.28 ± 0.26	<0.05 — 1.00	34
Endosulfan I	<0.01		31
Endosulfan II	<0.01		31
Endosulfan sulfate	<0.03		31

[a] CFU=colony-forming units, MPN= most probable number, BHC=hexachlorocyclohexane or benzene hexachloride
[b] For values less than the limit of detection, the detection limit is given as the mean.
[c] No selenium measurement was recorded for the lot milled 4 May 1990.
[d] No aflatoxin measurement was recorded for the lot milled 2 October 1989.
[e] Sources of contamination: alfalfa, grains, and fish meal
[f] Sources of contamination: soy oil and fish meal. No BHA or BHT measurements were recorded for the lot milled 1 November 1989.
[g] All values were corrected for percent recovery.

APPENDIX J
SENTINEL ANIMAL PROGRAM

METHODS 192
RESULTS 193

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 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.

Serum samples were collected from randomly selected rats during the 10-week modified mating study, the 26-week study, and the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to Microbiological Associates, Inc. (Bethesda, MD), for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

Method and Test	Time of Analysis
10-Week Modified Mating Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM (pneumonia virus of mice)	Study termination
RCV/SDA (rat coronavirus/ sialodacryoadenitis virus)	Study termination
Sendai	Study termination
Hemagglutination Inhibition	
H-1 (Toolan's H-1 virus)	Study termination
KRV (Kilham rat virus)	Study termination
26-Week Study	
ELISA	
M. arthritidis	13 weeks, study termination
M. pulmonis	13 weeks, study termination
PVM	13 weeks, study termination
RCV/SDA	13 weeks, study termination
Sendai	13 weeks, study termination
Hemagglutination Inhibition	
H-1	13 weeks, study termination
KRV	13 weeks, study termination

Method and Test	Time of Analysis
2-Year Study	
ELISA	
M. arthritidis	Study termination
M. pulmonis	Study termination
PVM	6, 12, 14, and 17 months, study termination
RCV/SDA	6, 12, 14, and 17 months, study termination
Sendai	6, 12, 14, and 17 months, study termination
Immunofluorescence Assay	
RCV/SDA	Study termination
Hemagglutination Inhibition	
H-1	6, 12, 14, and 17 months, study termination
KRV	6, 12, 14, and 17 months, study termination

RESULTS

One rat had a positive titer to *M. arthritidis* at the end of the 2-year study. Further evaluation of the sample positive for *M. arthritidis* by immunoblot and Western blot procedures indicated that the positive titer may have been due to a cross reaction with antibodies of nonpathogenic *Mycoplasma* or other agents. There were no clinical findings or histopathologic changes of *M. arthritidis* infection in the rat with the positive titer. Accordingly, the *M. arthritidis*-positive titer was considered to be a false positive.

DEPARTMENT OF
HEALTH & HUMAN SERVICES

Public Health Service
National Toxicology Program
Central Data Management
P.O. Box 12233, MD E1-02
Research Triangle Park, NC 27709

SPECIAL FOURTH-CLASS RATE
POSTAGE AND FEES PAID
DHHS/NIH
Permit No. G-811

Official Business
Penalty for Private Use - $300

NIH Publication No. 97-3374
September 1997