NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 213

CARCINOGENESIS BIOASSAY OF BUTYL BENZYL PHTHALATE

(CAS NO. 85-68-7)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDY)

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

NATIONAL TOXICOLOGY PROGRAM

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

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In July 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP TECHNICAL REPORT
ON THE

CARCINOGENESIS BIOASSAY OF BUTYL BENZYL PHTHALATE (CAS NO. 85-68-7) IN F344/N RATS AND B6C3F$_1$ MICE (FEED STUDY)

NATIONAL TOXICOLOGY PROGRAM
Box 12233
Research Triangle Park
North Carolina 27709
and
Bethesda, Maryland 20205

August 1982

NTP-80-25
NIH Publication No. 82-1769

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

NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, NC 27709 (919-541-3991).

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

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 (702-487-4650).

Single copies of this carcinogenesis bioassay technical report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

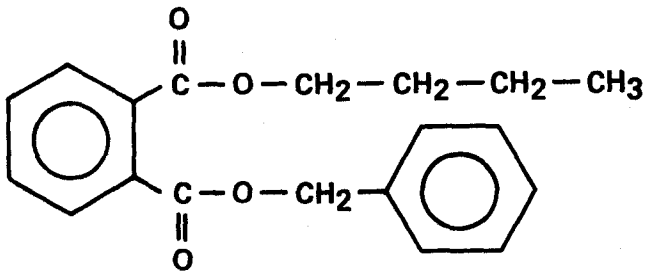
TABLE OF CONTENTS

TABLES

FIGURES

APPENDIXES

CARCINOGENESIS BIOASSAY OF BUTYL BENZYL PHTHALATE



BUTYL BENZYL PHTHALATE

CAS NO. 85-68-7
$C_{19}H_{20}O_4$ Mol. Wt. 312.37

ABSTRACT

A carcinogenesis bioassay of butyl benzyl phthalate, a plasticizer for vinyl chloride plastics, was accomplished by feeding diets containing 6,000 or 12,000 ppm of the phthalate to groups of 50 F344/N rats and 50 B6C3F1 mice of each sex for 28 to 103 weeks.

Mean body weights of dosed female rats and mice of each sex were lower than those of the control animals throughout most of the study.

After week 14, an increasing number of dosed male rats died as a result of an unexplained internal hemorrhaging, and all surviving male rats were killed at week 29 to 30. Because of compound-related mortality, butyl benzyl phthalate was not adequately tested for carcinogenicity in male F344/N rats.

Mononuclear cell leukemias occurred at a statistically significant (P=0.011) increased incidence in the high-dose group of female rats when compared with the control group and with a significantly (P=0.006) increasing trend (controls 7/49, 14%; low-dose 7/49, 14%; high-dose 18/50, 36%). The incidence in the high-dose group and the overall trend remained statistically significant (P=0.008 and P=0.019) when compared with the historical incidence for F344/N female rats with leukemia at this laboratory (77/399, 19%). Further, this leukoproliferation was generally characterized by splenomegaly and often by hepatomegaly.

Administration of butyl benzyl phthalate was not associated with increased incidences of any type of tumor among male or female mice.

Tumor rates were decreased in female rats for fibroadenomas of the mammary glands (20/49, 14/49, 9/50) and in male mice for lymphomas of the hematopoietic system (13/50, 11/49, 4/50) and for alveolar/bronchiolar adenomas or carcinomas (17/50, 11/49, 8/50).

Under the conditions of this bioassay, butyl benzyl phthalate was probably carcinogenic for female F344/N rats, causing an increased incidence of mononuclear cell leukemias. The male F344/N rat study was considered inadequate for evaluation due to compound-related toxicity and early mortality. Butyl benzyl phthalate was not carcinogenic for B6C3F1 mice of either sex.

CONTRIBUTORS

This bioassay of butyl benzyl phthalate was conducted at Mason Research Institute under a subcontract to Tracor Jitco, Inc., the prime contractor for the Carcinogenesis Testing Program. The chronic study was begun in April 1977 and completed in May 1979.

Principal Contributors at Mason Research Institute
57 Union Street
Worcester, Massachusetts 01608
(Conducted bioassay and evaluated tissues)

R. Fleischman, D.V.M.
Pathologist

A. Good, M.A.
Technical Coordinator

M. Hagopian, Ph.D.
Chemist

H. Lilja, Ph.D.
Principal Investigator

E. Massaro, Ph.D.
Principal Investigator

R. Monson, M.A.
Bioassay Coordinator

G. Wade, B.S.
Bioassay Coordinator

E. Zepp, M.A.
Operations Coordinator

Principal Contributors at Tracor Jitco
1776 East Jefferson Street
Rockville, Maryland 20852
(Prepared preliminary summary report)

E. Cremmins, M.A.
Technical Editor

C. Dean, B.S.
Production Editor

A. Jacobs, Ph.D.
Bioscience Writer

J. Joiner, Ph.D.
Statistician

L. Campbell, Ph.D.
Acting Director, Bioassay Program

S. Olin, Ph.D.
Program Associate Director

M. Stedham, D.V.M.
Pathologist

W. Theriault, Ph.D.
Manager, Technical Reports

Principal Contributors at the National Toxicology Program

National Institute of Environmental Health Sciences
Box 12233
Research Triangle Park
North Carolina 27709 and
Bethesda, Maryland 20205
(Evaluated the experiment, interpreted the results, and reported the findings)

J. Fielding Douglas, Ph.D.
Richard A. Griesemer, D.V.M., Ph.D.
Charles K. Grieshaber, Ph.D.
Larry Hart, Ph.D.
William V. Hartwell, Ph.D.
Joseph Haseman, Ph.D.
James E. Huff, Ph.D. (Chemical Manager)
William W. Kluwe, Ph.D.
Ernest E. McConnell, D.V.M.
John A. Moore, D.V.M.
Sherman F. Stinson, Ph.D.
Raymond Tennant, Ph.D.
Jerrold M. Ward, D.V.M., Ph.D.

The pathology report and selected slides were evaluated in February 1980 by the NTP Pathology Working Group (composed of Drs. J. Ward and S. Stinson) as described by Ward et al. (1978). The chemicals used in this bioassay of butyl benzyl phthalate were analyzed by the Midwest Research Institute, 425 Volker Blvd., Kansas City, Missouri 64110; analysis of the formulated diets and reanalysis of the bulk chemical were done by Mason Research Institute.

REVIEWERS

National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee

Margaret Hitchcock, Ph.D. (Chairperson)
John B. Pierce Foundation Laboratory
New Haven, Connecticut

Curtis Harper, Ph.D.
Associate Professor of Pharmacology
University of North Carolina
Chapel Hill, North Carolina

Thomas Shepard, M.D.
University of Washington
School of Medicine
Seattle, Washington

Alice Whittemore, Ph.D.
Stanford University School of Medicine
Palo Alto, California

Ad Hoc Subcommittee Panel of Experts

Frank Mirer, Ph.D.
(Principal Reviewer)
United Auto Workers
International Union
Detroit, Michigan

Sheldon Murphy, Ph.D.
(Principal Reviewer)
University of Texas Medical School
Houston, Texas

Norman Breslow, Ph.D.
University of Washington
Seattle, Washington

Joseph Highland, Ph.D.*
Environmental Defense Fund
Washington, D.C.

Charles Irving, Ph.D.
Veterans Administration Hospital
Cancer Research Laboratory
Memphis, Tennessee

Svend Nielsen, D.V.M., Ph.D.
Professor of Pathology
The University of Connecticut
Storrs, Connecticut

Bernard Schwetz, Ph.D.*
Toxicology Research Laboratory
Dow Chemical U.S.A.
Midland, Michigan

Roy Shore, Ph.D.
New York University Medical Center
New York, New York

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

Gary Williams, M.D.
Chief of Experimental Pathology
American Health Foundation
Valhalla, New York

*Unable to attend June 27, 1980 meeting

SUMMARY OF PEER REVIEW COMMENTS ON THE BIOASSAY OF BUTYL BENZYL PHTHALATE

On June 27, 1980, this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Room 1331, Switzer Building, 330 C Street, S.W., Washington, D.C.

Dr. Mirer, as a principal reviewer for the report on the bioassay of butyl benzyl phthalate, agreed with the conclusion in the report, that under the conditions of the bioassay, butyl benzyl phthalate was not clearly carcinogenic for female F344/N rats and because of compound-related toxicity and mortality, this chemical was not adequately tested for carcinogenicity in male F344/N rats. Butyl benzyl phthalate was not carcinogenic for B6C3F1 mice of either sex. Dr. Mirer stated that the incidence of mononuclear cell leukemia in high-dose female rats may have been related to the compound, but noted that the historical rate of leukemia in control F344/N rats was higher than in the controls for this bioassay.

As second principal reviewer, Dr. Murphy stated that the conclusions regarding the association of butyl benzyl phthalate exposure with increased leukemia were vague, but basically he agreed with the statement that in female F344/N rats leukemias of the hematopoietic system may have been related to administration of the test chemical. He commented that weight gain data for female rats made it questionable whether a maximum tolerated dose was used. Both reviewers were concerned with the lack of explanation for the internal hemorrhaging that resulted in high mortality and early termination of the study in male rats. Dr. Murphy also suggested that the discussion in the report could be expanded regarding the higher incidence of leukemia in female rats at the high dose.

Dr. Mirer moved that the report on the bioassay of butyl benzyl phthalate be accepted with the incorporation of suggested modifications, principally a clarifying discussion after a reexamination of the data surrounding the increased incidence of leukemia in female rats, and a statement in the summary on the lack of carcinogenic effects in mice. Dr. Murphy seconded the motion and the report was approved unanimously by the Peer Review Panel.

On 23 June 1981, this revised carcinogenesis bioassay report on butyl benzyl phthalate was reconsidered and the new conclusion was approved by the NTP Peer Review Panel. This open meeting took place in the auditorium, Building 101, National Institute of Environmental Health Sciences, Research Triangle Park, NC.

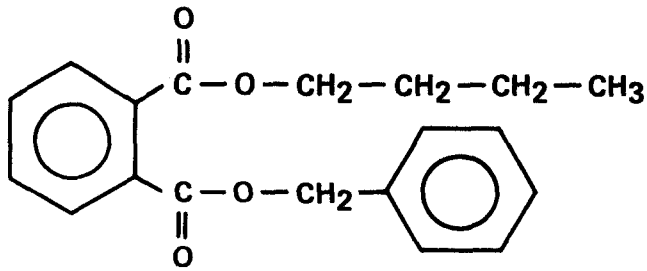
During the reevaluation of the relevant data (as suggested by the Panel), the experimental incidence rates for mononuclear cell leukemia were compared with the temporal historical rates in the test laboratory (since 1977) for "all leukemias": 77/399 (19%, range - 12 to 24%). According to this conservative approach, the incidence of mononuclear cell leukemia in the high-dose female F344/N rats remained significantly higher when compared with historical controls (P=0.008) and also when compared with concurrent controls (P=0.011); the dose-related trend statistic was significant for both comparisons (P=0.019 and P=0.006). Based on the reanalysis, the conclusion was revised to read that "butyl benzyl phthalate was probably carcinogenic for F344/N rats, causing an increased incidence of mononuclear cell leukemias." The data for male rats and B6C3F1 mice were not reexamined.

There was considerable discussion among the peer review panel members and NTP staff about historical control rates (especially with regard to the leukemias), about the definition of leukemias and how these lesions are diagnosed, and about what "probably carcinogenic" means. A consensus was reached that the revised conclusion was appropriate to the data.

Dr. Williams moved that the proposed conclusion for the report on the bioassay of butyl benzyl phthalate be accepted. Dr. Swenberg seconded the motion and the report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION

I. INTRODUCTION



BUTYL BENZYL PHTHALATE

CAS NO. 85-68-7
$C_{19}H_{20}O_4$ Mol. Wt. 312.37

Butyl benzyl phthalate (CAS No. 85-68-7; synonyms: BBP; benzyl butyl phthalate; phthalic acid; benzyl butyl ester; Santicizer 160) is a clear slightly viscous liquid added to polymers such as polyvinyl chloride to give flexibility and softness. Not chemically bound to the polymer, butyl benzyl phthalate is dispersed in the matrix of polymer chains (Autian, 1973) and thus under certain conditions may migrate from the polymer.

Butyl benzyl phthalate has been used as a component of car upholstery and air conditioner filters (Statsek, 1974; Singmaster and Crosby, 1976). Butyl benzyl phthalate is approved by the U.S. Food and Drug Administration for use in food contact articles, provided these contain less than 1% dibenzyl phthalate by weight (CFR, 1967; CFR, 1976). Although specific production figures for butyl benzyl phthalate are not available, annual production exceeds 5,000 pounds per year (USITC, 1979).

Butyl benzyl phthalate induces extra-medullary hematopoiesis in the liver and spleen and periportal hepatitis in Swiss-Webster mice administered 0.125 to 0.5 g/kg by the intraperitoneal route (Calley et al., 1966). The intraperitoneal LD_{50} for this strain of mouse was 3.16 g/kg. The LD_{50} values determined in this NTP/NCI bioassay are: 2.33 g/kg for F344/N rats, 6.16 g/kg for male B6C3F1 mice, and 4.17 g/kg for female mice.

Butyl benzyl phthalate was not mutagenic in *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, and TA 1537 with and without metabolic activation (NTP, 1980). Likewise a mutagenic response was not seen in *Escherichia coli* (wild or uvrA$^-$) and this phthalate gave negative results in differential killing assays using repair deficient strains of *E. coli* (uvrA$^-$, polA$^-$, recA$^-$) (Kuarata, 1975). Mammalian cytogenetic studies using Chinese hamster ovary cells to detect chromosome aberrations and sister chromatid exchanges were also negative (NTP unpublished results).

In the chick embryo system, butyl benzyl phthalate was shown to be lethal by Haberman et al. (1968) but not by Bower et al. (1970); abnormal development was not observed (Bower et al., 1970 and Haberman et al., 1968) and neonatal death rates at 21 days of age were not affected (Haberman et al., 1968).

Carcinogenesis studies have been completed by the NCI or are ongoing by the NTP on other phthlates and related compounds; diallyl phthalate (chronic testing phase); di(2-ethylhexyl)adipate caused liver tumors in mice (NTP, 1982a); di(2-ethylhexyl)phthalate induced liver tumors in male and female F344/N rats and in male and female B6C3F1 mice (NTP, 1982b); diethyl phthalate (prechronic testing completed); dimethyl terephthalate probably induced lung tumors in male B6C3F1 mice (NCI, 1979a); and phthalamide (NCI, 1979b) and phthalic anhydride (NCI, 1979c) did not cause any increase in tumor incidence in rats or mice.

Butyl benzyl phthalate did not induce a positive pulmonary adenoma response in strain A mice (Theiss et al., 1977). Three dose levels (160, 400, and 800 mg/kg), including a maximum tolerated dose, were administered to 3 groups of 20 mice for a total of 24 intraperitoneal injections (3,840; 9,600; and 19,200 mg/kg); all 60 mice survived the 24-week experiment.

Butyl benzyl phthalate was tested by the NCI Carcinogenesis Testing Program because consumer exposure to products containing this plasticizer may be widespread, because no long-term carcinogenesis studies were available or planned, and because butyl benzyl phthalate is part of a phthalate chemical class study.

II. MATERIALS AND METHODS

CHEMICAL ANALYSIS

DIETARY PREPARATION

ANIMALS

ANIMAL MAINTENANCE

RANGE-FINDING AND FOURTEEN-DAY STUDIES

SUBCHRONIC STUDIES

CHRONIC STUDIES

Clinical Examinations and Pathology

Data Recording and Statistical Analyses

CHEMICAL ANALYSIS

The butyl benzyl phthalate (CAS No. 85-68-7) used in this bioassay was manufactured by Monsanto (St. Louis, MO) and supplied by Missouri Solvents and Chemicals (Kansas City, MO) in two batches. Lot No. M2676 was used for the subchronic studies and the first 34 weeks of the chronic studies, and Lot No. M81977 was used for the final 70 weeks of the chronic studies. Both lots were stored in the dark in their original containers.

Elemental analysis, determination of boiling point, thin-layer and vapor-phase chromatography, and infrared, ultraviolet, and nuclear magnetic resonance spectrum analyses for both lots of butyl benzyl phthalate were consistent with those expected for the structure (Appendixes E and F). For Lot No. M81977, results of thin-layer chromatography by one solvent system indicated only one component, and those from a second system indicated the major component and one trace impurity. Results of vapor-phase chromatography by one solvent system indicated eight impurities — two had areas that were 0.22% and 0.59% of the major peak and the rest totaled less than 0.2% of the major peak area. Ten impurities were detected by vapor-phase chromatography by a second system — three with areas of 0.22%, 0.27%, and 1.19% of the major peak and the rest with a total area that was 0.4% of the major peak. A purity of 97.2% $\pm 1.1(\delta)\%$ was indicated by the results of (ester) hydrolysis titration. For Lot No. M2676, the results of thin-layer chromatography indicated the presence of only one component. Similar results were obtained from vapor-phase chromatography by one system, while those from a second system indicated an impurity whose area was 1.8% that of the major peak.

DIETARY PREPARATION

Test diets were prepared by mixing the chemical with an aliquot of powdered Wayne Lab Blox® animal feed (Allied Mills, Chicago, IL), which was combined with the rest of the feed by mixing in a Patterson-Kelly® intensifier bar V-blender for 15 minutes. Test diets were sealed in labeled plastic bags and stored at 4°C for no longer than 14 days.

The stability of butyl benzyl phthalate in feed was determined by assaying sample diet mixtures containing 100,000 ppm butyl benzyl phthalate which had been stored at -20°, 5°, 25°, or 45°C for 2 weeks. The compound was found to be stable in feed for 2 weeks at temperatures as high as 45°C (Appendix G).

The amounts of butyl benzyl phthalate present in feed were determined by the net ultraviolet absorbances at 275 nm of 50-ml methanol extracts of 2-g samples (Appendix H). At each dietary concentration, the mean of the analytical concentration was usually within ±10% of the theoretical value.

ANIMALS

Four-week-old F344/N rats and 4- to 5-week old B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center, Frederick, Maryland. The animals were observed for the presence of parasites and other diseases for 10 days and then assigned to control or dosed groups so that average cage weights were approximately equal for all animals of the same sex and species.

ANIMAL MAINTENANCE

Rats and mice were each housed five per cage in suspended polycarbonate cages (Lab Products, Inc., Garfield, NJ) covered with non-woven fiber filter sheet (Lab Products). Aspen-bed® hardwood chips (American Excelsior, Summerville, MA) were used as bedding. Clean bedding and cages were provided twice weekly and cage racks were changed every 2 weeks.

Water and Wayne Lab Blox® diet were available *ad libitum*, the former from an Edstrom automatic watering system (Waterford, WI) and the latter in stainless-steel, gang-style hoppers (Scientific Cages, Inc., Bryan, TX) that were changed once per week.

The temperature in the animal rooms was 18°-31°C, and the relative humidity was 10%-88%. During the course of this bioassay, room temperature recordings were in the range of 20° to 26°C (68°-78.8°F) eighty percent (436/545) of the time for rats and seventy-seven percent (235/305) for mice. The highest temperature listed for rats was 31°C (88°F) on four separate days between May and July, 1978 and for mice 30°C (86°F) on 5 days in May and July, 1978. Both studies were conducted from April 1977 to May 1979. Incoming air was prefiltered through Tri-Dek 15/40 denier Dacron filters followed by final filtration through a Cambridge "Aerosolve 95" filter. Air was changed 10 times per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice were housed by species in rooms in which chronic feed studies were also being conducted on the following chemicals:

(CAS 9000-30-0) Guar gum
(CAS 103-23-1) Di(2-ethylhexyl)adipate
(CAS 117-81-7) Di(2-ethylhexyl)phthalate.

RANGE-FINDING AND FOURTEEN-DAY STUDIES

Range-finding and fourteen-day feed studies were conducted using F344/N rats and B6C3F1 mice to determine the concentrations of butyl benzyl phthalate to be used in the subchronic studies.

In the range-finding test, groups of five males and five females of each species were administered a single dose of the test substance in corn oil by gavage. Rats were administered doses of 20, 10, 5, 2.5, 1.25, 0.63, 0.31, 0.16, or 0.08 g/kg body weight, and mice were administered doses of 20, 10, 5.0, 2.5, or 1.25 g/kg (Table 1). All surviving animals were killed after 14 days. The estimated LD_{50}'s were 2.33 g/kg for rats, 6.16 g/kg for male mice, and 4.17 g/kg for female mice.

In the fourteen-day studies, groups of five males and five females of each species were administered the test chemical in feed for 2 weeks at concentrations of 12,500 to 100,000 ppm for rats (Table 2) and 1,600 to 25,000 ppm for mice (Table 3). Groups of five males and five females of each species were maintained as untreated controls. Animals were fed control diets on day 15 and were killed on day 16.

No deaths occurred in rats. Weight gain was depressed in both male and female rats fed diets containing 25,000 ppm or more, and rats fed 100,000 ppm lost weight. Testicular atrophy was observed in all male rats fed 50,000 or 100,000 ppm. Thymic atrophy was observed in all rats fed 100,000 ppm. Two male mice died, one fed 3,100 ppm and one fed 6,300 ppm. Depressions in weight gain of mice were not dose related, and no compound-related effects were observed at necropsy.

TABLE 1. DOSAGE AND SURVIVAL OF RATS AND MICE ADMINISTERED A SINGLE DOSE OF BUTYL BENZYL PHTHALATE BY GAVAGE

	Dose (g/kg)	Survival *(a)*	
		Males	Females
Rats			
	0.08	5/5	5/5
	0.16	5/5	5/5
	0.31	5/5	5/5
	0.63	5/5	5/5
	1.25	5/5	5/5
	2.50	2/5	2/5
	5.00	0/5	0/5
	10.00	0/5	0/5
	20.00	0/5	0/5
Mice			
	1.25	5/5	5/5
	2.50	5/5	5/5
	5.00	2/5	1/5
	10.00	0/5	0/5
	20.00	2/5	1/5

(a) Number surviving/number per group

TABLE 2. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE FOR 14 DAYS

Dose (ppm)	Survival *(a)*	Mean Body Weights (grams)			Weight Change Relative to Controls *(b)* (Percent)
		Initial	Final	Change	
Males					
0	5/5	88.2	146.6	58.4	
12,500	5/5	88.4	145.2	56.8	- 3
25,000	5/5	88.4	135.4	47.0	- 20
50,000	5/5	88.4	105.8	17.4	- 70
100,000	5/5	88.2	74.2	-14.0	- 124
Females					
0	5/5	87.2	119.0	31.8	
12,500	5/5	87.2	121.0	33.8	+ 6
25,000	5/5	87.2	111.2	24.0	- 25
50,000	5/5	87.2	95.4	8.2	- 74
100,000	5/5	87.2	69.0	- 18.2	- 157

(a) Number surviving/number per group

(b) Weight change of the dosed group relative to that of the controls =

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

TABLE 3. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE FOR 14 DAYS

Dose (ppm)	Survival *(a)*	Mean Body Weights (grams)			Weight Change Relative to Controls *(b)* (Percent)
		Initial	Final	Change	
Males					
0	5/5	24.0	25.8	1.8	
1,600	5/5	24.0	27.6	3.6	+ 100
3,100	4/5	24.0	24.0	0	- 100
6,300	4/5	24.0	25.3	1.3	- 28
12,500	5/5	24.0	25.8	1.8	0
25,000	5/5	24.0	25.2	1.2	- 33
Females					
0	5/5	19.0	20.6	1.6	
1,600	5/5	19.0	19.8	0.8	- 50
3,100	5/5	19.0	20.2	1.2	- 25
6,300	5/5	19.0	20.2	1.2	- 25
12,500	5/5	19.0	19.8	0.8	- 50
25,000	5/5	19.0	19.8	0.8	- 50

(a) Number surviving/number per group

(b) Weight change of the dosed group relative to that of the controls =

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

SUBCHRONIC STUDIES

Subchronic studies were conducted to determine the concentrations to be used in the chronic studies. Diets containing 0, 1,600, 3,100, 6,300, 12,500, or 25,000 ppm butyl benzyl phthalate were fed for 13 weeks to groups of 10 rats and mice of each sex (Tables 4 and 5). Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day study, survivors were killed. Necropsies were performed on all animals, and tissues were taken for histopathologic analysis from animals in control and high-dose groups.

TABLE 4. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE FOR 13 WEEKS

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Change	
Males					
0	10/10	86.5	300	213.5	
1,600	10/10	86.5	332	245.5	+ 15.0
3,100	10/10	86.5	305	218.5	+ 2.3
6,300	9/10	86.5	298	211.5	- 0.9
12,500	9/10	86.5	308	221.5	+ 3.7
25,000	10/10	86.5	240	153.5	- 28.1
Females					
0	8/10	72.5	184	111.5	
1,600	10/10	72.5	186	113.5	+ 1.8
3,100	10/10	72.5	192	119.5	+ 7.2
6,300	10/10	72.5	182	109.5	- 1.8
12,500	10/10	72.5	184	111.5	0
25,000	10/10	72.5	183	110.5	- 0.9

(a) Number surviving/number per group

(b) Weight change of the dosed group relative to that of the controls =

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

TABLE 5. DOSAGE, SURVIVAL, AND MEAN BODY WEIGHTS OF MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE FOR 13 WEEKS

Dose (ppm)	Survival (a)	Mean Body Weights (grams)			Weight Change Relative to Controls (b) (Percent)
		Initial	Final	Change	
Males					
0	10/10	19.5	31.0	11.5	
1,600	10/10	19.5	29.4	9.9	- 13.9
3,100	10/10	19.5	28.5	9.0	- 21.7
6,300	10/10	19.5	28.4	8.9	- 22.6
12,500	10/10	19.5	28.1	8.6	- 25.2
25,000	10/10	19.5	27.0	7.5	- 34.8
Females					
0	10/10	16.4	24.2	7.8	
1,600	10/10	16.4	24.6	8.2	+ 5.1
3,100	10/10	16.4	24.8	8.4	+ 7.7
6,300	10/10	16.4	23.9	7.5	- 3.8
12,500	10/10	16.4	22.5	6.1	- 21.8
25,000	6/10 (c)	16.4	22.7	6.3	- 19.2

(a) Number surviving/number per group

(b) Weight change of the dosed group relative to that of the controls =

$$\frac{\text{Weight Change (Dosed Group)} - \text{Weight Change (Control Group)}}{\text{Weight Change (Control Group)}} \quad 100$$

(c) Deaths were accidental.

Rats: Four rats died — one male fed 6,300, one male fed 12,500, and two female controls. Depressed weight gain (28% when compared with controls) and testicular degeneration were observed in male rats fed 25,000 ppm. No compound-related effects were observed in female rats. Based on weight changes relative to controls, the doses selected for rats for the chronic study were 6,000 and 12,000 ppm butyl benzyl phthalate in the diet.

Mice: Accidental deaths occurred in four female mice fed 25,000 ppm. Weight gain was depressed by more than 10% in all groups of male mice and in female mice fed 12,500 or 25,000 ppm. No other compound-related effects were observed in mice. Based on weight changes relative to controls, the doses selected for mice on the chronic study were 6,000 and 12,000 ppm butyl benzyl phthalate in the diet.

CHRONIC STUDIES

The test groups, doses administered, and durations of the chronic studies are shown in Table 6.

Clinical Examinations and Pathology

Animals were inspected twice daily. Body weights were recorded every 4 weeks. Animals that were moribund and those that survived to the end of the study were anesthetized with carbon dioxide and then killed and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: abnormal lymph nodes, skin, mandibular lymph nodes, mammary gland, salivary gland, bone marrow, costochondral junction, thymus, larynx, trachea, lungs and bronchi, heart, thyroids, parathyroids, esophagus, stomach, duodenum, jejunum, ileum, colon, mesenteric lymph nodes, liver, gallbladder (mouse), pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles/prostate/testes, ovaries/uterus, brain, and pituitary.

Necropsies were performed on all animals found dead unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

TABLE 6. EXPERIMENTAL DESIGN OF CHRONIC FEEDING STUDIES WITH BUTYL BENZYL PHTHALATE IN RATS AND MICE

Test Group	Initial No. of Animals	Butyl Benzyl Phthalate (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male Rats				
Control	50	0	0	29 *(a)*
Low-Dose	50	6,000	28 *(a)*	1-2
High-Dose	50	12,000	28 *(a)*	1-2
Female Rats				
Control	50	0	0	106
Low-Dose	50	6,000	103	1-2
High-Dose	50	12,000	103	2
Male Mice				
Control	50	0	0	106
Low-Dose	50	6,000	103	1-2
High-Dose	50	12,000	103	2
Female Mice				
Control	50	0	0	106
Low-Dose	50	6,000	103	2-3
High-Dose	50	12,000	103	2

(a) All surviving male rats were killed at weeks 29-30 because of poor survival in the dosed groups.

Data Recording and Statistical Analyses

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each dose level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

Life table methods were used to analyze the weeks of death of animals with histologically observed tumors under the principles described by Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971).

III. RESULTS

RATS

Body Weights and Clinical Signs

Survival

Pathology

Statistical Analyses of Results

MICE

Body Weights and Clinical Signs

Survival

Pathology

Statistical Analyses of Results

RATS

Body Weights and Clinical Signs

Mean body weights of dosed rats of either sex were lower than those of the corresponding controls throughout most of the study (Figure 1). Feed consumption of dosed female rats was 70% to 80% that of the controls. No other compound-related clinical effects were observed.

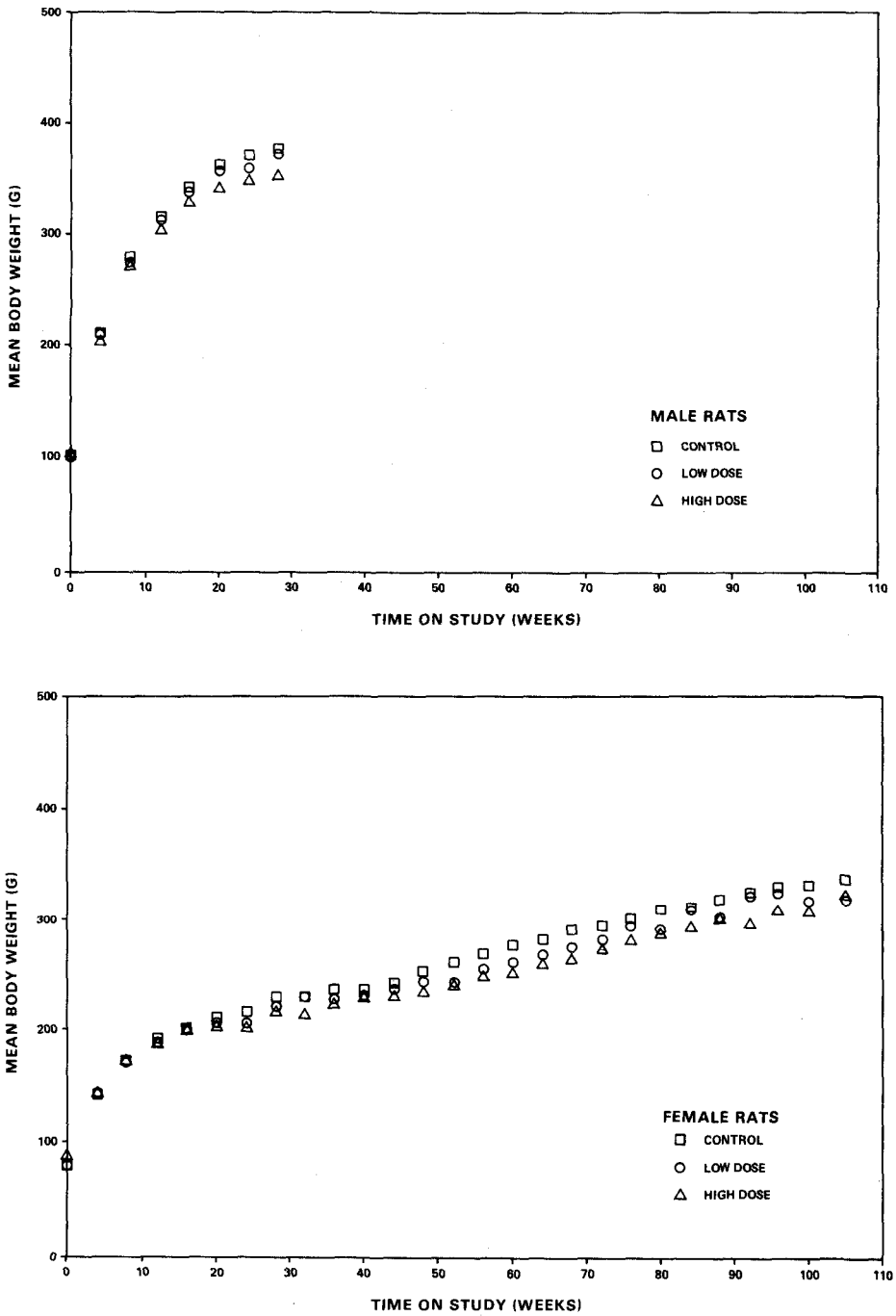


Figure 1. Growth Curves for Rats Fed Diets Containing Butyl Benzyl Phthalate

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing butyl benzyl phthalate at the doses of this bioassay, and those of the controls, are shown by the Kaplan and Meier curves in Figure 2. In male rats, the result of Tarone's test indicates a dose-related trend in mortality ($P<0.001$) due to shorter survival in the dosed groups than in the control group. The high-dose group survival was significantly shorter ($P<0.001$) than that in either of the other two groups. In females, the survival in all three groups was comparable.

Male dosed rats died prematurely. Internal hemorrhaging was suspected at gross necropsy, yet was not confirmed microscopically. At week 28, only 30% (15/50) of the high-dose group male rats were alive and deaths were occurring among low-dose males. All male rats were killed at weeks 29-30 when 49/50 (98%) of the control group, 40/50 (80%) of the low-dose group, and 15/50 (30%) of the high-dose group were alive. This toxic effect was not encountered in females, and 31/50 (62%) of the control group, 29/50 (58%) of the low-dose group, and 32/50 (64%) of the high-dose group lived to the end of the study at 105-106 weeks.

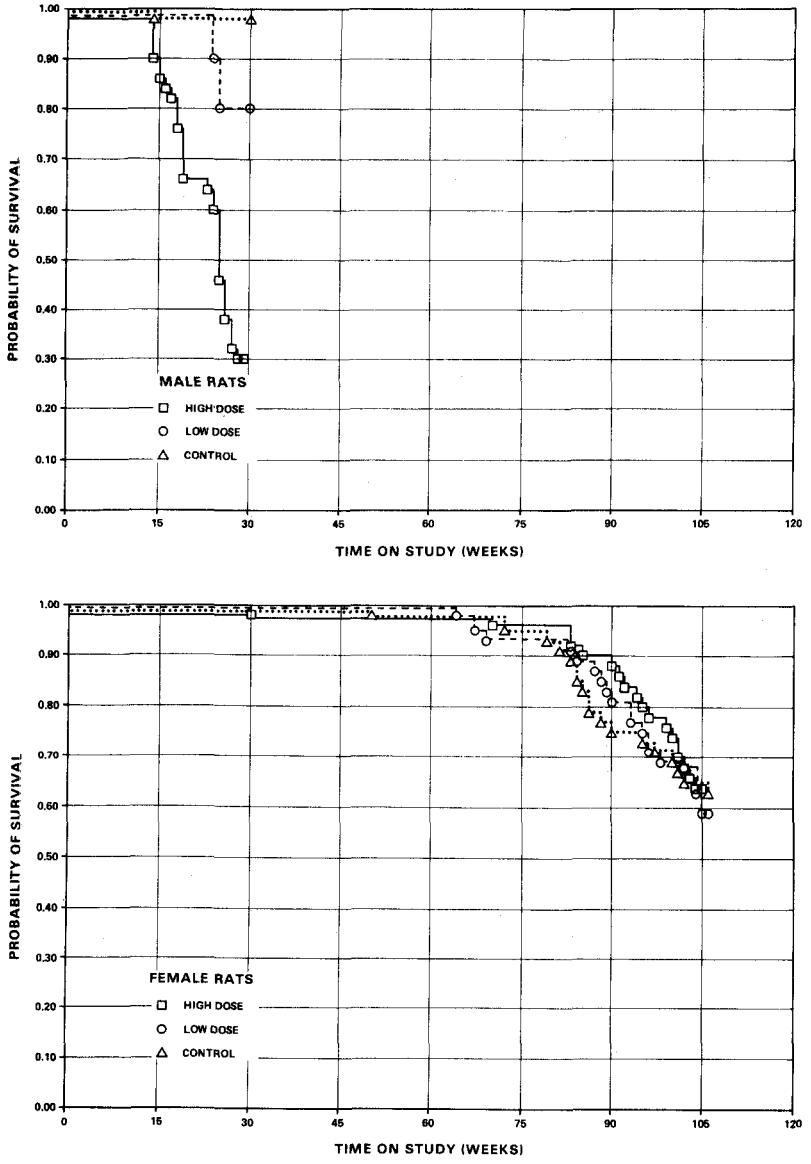


Figure 2. Survival Curves for Rats Fed Diets Containing Butyl Benzyl Phthalate

Pathology

The summary of histopathologic findings on tumor and non-tumor pathology in male rats is not given since all of the surviving males were killed early in the study after increasing numbers of animals died from hemorrhages. No histopathologic examinations were performed on these animals.

Histopathologic findings on neoplasms in female rats are summarized in Appendix A, Table A2; findings on nonneoplastic lesions in females are summarized in Appendix C, Table C2.

The following incidences of mononuclear cell leukemia affecting multiple organs of female rats appeared to be related to the administration of butyl benzyl phthalate:

	Control	Low Dose	High Dose
Number of animals examined	49	49	50
Mononuclear cell leukemia (total)	7 (14%)	7 (14%)	18 (36%)
Location:			
Multiple organs	6 (12%)	6 (12%)	17 (34%)
Spleen	0	1 (2%)	1 (2%)
Liver	1 (2%)	0	0

This leukoproliferative disorder was generally characterized by splenomegaly and often by hepatomegaly. The surfaces of the spleen and liver were often granular and roughened and the borders were frequently irregular. Microscopically, the red pulp of the spleen was congested and infiltrated by large numbers of poorly differentiated mononuclear cells with blastoid, donut-shaped, and reniform nuclei. The leukemic process spread to and infiltrated the capillary beds of numerous organs, including the lungs, spleen, and liver in all female rats, and to the kidneys, heart, pancreas, lymph nodes (mesenteric, mandibular, parotid, and lumbar), urinary bladder, ovaries, uterus, brain, thyroids, pituitary, adrenals, skin, bone marrow, and intercostal muscles in a few female rats. There were no apparent differences in tissue distribution or cytologic characteristics of the leukemia in dosed or control rats. The leukemia in dosed rats resembled that in the controls.

The remaining neoplastic, proliferative, degenerative, inflammatory, and developmental lesions observed in control and dosed female rats were considered to be unrelated to test compound administration and to be within the normal incidence limits in F344/N rats.

Statistical Analyses of Results

Histopathologic examinations were not performed in male rats due to early deaths. The statistical analyses were done on the tumor incidence of female rats only.

Table 7 contains the statistical analysis of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

Results of the Cochran-Armitage test indicate a significant dose-related trend (P=0.004), and a significantly higher (P=0.007) incidence of 18 females with leukemia and 1 with lymphoma in the high-dose group than in the control group was observed. All of the leukemias were diagnosed as mononuclear cell type. The historical incidence for studies in this laboratory since 1977 for female F344/N rats with "all leukemias" is 19% (77/399), ranging from 12% to 24%. In this bioassay, the rates equal 7/49 (14%) for controls, 7/49 (14%) for low-dose rats, and 18/50 (36%) for high-dose rats. Comparing the historical leukemia rates with the current incidence of mononuclear cell leukemia gives a trend statistic of P=0.019 and a control versus high-dose significance level of P=0.008.

There is a positive dose-related trend (P=0.050) in the incidence of adenoma in the pituitary of female rats, but the results of the Fisher exact test are not significant (20/45, 44%; 21/40, 53%; 26/41, 63%).

The Cochran-Armitage test indicates a departure from linear trend (P=0.013) in the incidence of female rats with islet-cell adenomas in the pancreas due to a higher incidence in the low-dose group than in the other two groups (0/47, 3/46, 0/46).

A negative trend (P=0.009) and a significantly lower (P=0.011) incidence of fibroadenomas in the mammary gland of the high-dose female rats than in the controls were observed (20/49, 14/49, 9/50).

The statistical analyses indicate that butyl benzyl phthalate was associated with an increased incidence of female F344/N rats with mononuclear cell leukemias. No statistical tests were done on the male rats.

TABLE 7. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN FEMALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE *(a)*

	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Sarcoma, NOS *(b)*	0/49(0)	3/49(6)	1/50(2)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		Infinite	Infinite
Lower Limit		0.602	0.053
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	84	96
Hematopoietic System:			
Mononuclear Cell Leukemia *(b)*	7/49(14)	7/49(14)	18/50(36)
P Values *(c),(d)*	P=0.006	N.S.	P=0.011
Relative Risk (Control) *(e)*		1.000	2.520
Lower Limit		0.324	1.115
Upper Limit		3.091	6.458
Weeks to First Observed Tumor	83	87	83
Hematopoietic System: Leukemia or Lymphoma *(b)*	7/49(14)	7/49(14)	19/50(38)
P Values *(c),(d)*	P=0.004	N.S.	P=0.007
Relative Risk (Control) *(e)*		1.000	2.660
Lower Limit		0.324	1.191
Upper Limit		3.091	6.758
Weeks to First Observed Tumor	83	87	83
Liver: Neoplastic Nodule *(b)*	1/49(2)	1/48(2)	3/50(6)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		1.021	2.940
Lower Limit		0.013	0.246
Upper Limit		78.494	151.180
Weeks to First Observed Tumor	106	106	100
Liver: Neoplastic Nodule or Hepatocellular Carcinoma *(b)*	1/49(2)	1/48(2)	4/50(8)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		1.021	3.920
Lower Limit		0.013	0.407
Upper Limit		78.494	188.989
Weeks to First Observed Tumor	106	106	100
Pituitary: Adenoma, NOS *(b)*	20/45(44)	21/40(53)	26/41(63)
P Values *(c),(d)*	P=0.050	N.S.	N.S.
Relative Risk (Control) *(e)*		1.181	1.427
Lower Limit		0.725	0.921
Upper Limit		1.904	2.179
Weeks to First Observed Tumor	84	87	83
Thyroid: C-Cell Adenoma or Carcinoma *(b)*	2/47(4)	3/46(7)	0/46(0)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		1.533	0.000
Lower Limit		0.184	0.000
Upper Limit		17.650	3.446
Weeks to First Observed Tumor	101	106	—

TABLE 7. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN FEMALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE *(a)* (Continued)

	Control	Low Dose	High Dose
Pancreatic Islets: Islet-Cell Adenoma *(b)*	0/47(0)	3/46(7)	0/46(0)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Departure from Linear Trend *(f)*	P=0.013		
Relative Risk (Control) *(e)*		Infinite	—
Lower Limit		0.616	—
Upper Limit		Infinite	—
Weeks to First Observed Tumor	—	106	—
Mammary Gland: Fibroadenoma *(b)*	20/49(41)	14/49(29)	9/50(18)
P Values *(c),(d)*	P=0.009(N)	N.S.	P=0.011(N)
Relative Risk (Control) *(e)*		0.700	0.441
Lower Limit		0.374	0.199
Upper Limit		1.279	0.904
Weeks to First Observed Tumor	86	90	94
Clitoral Gland: Carcinoma, NOS *(b)*	4/49(8)	1/49(2)	3/50(6)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.250	0.735
Lower Limit		0.005	0.113
Upper Limit		2.409	4.120
Weeks to First Observed Tumor	106	105	104
Clitoral Gland: Carcinoma, NOS or Adenoma, NOS *(b)*	4/49(8)	1/49(2)	4/50(8)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.250	0.980
Lower Limit		0.005	0.193
Upper Limit		2.409	4.980
Weeks to First Observed Tumor	106	105	104
Uterus: Endometrial Stromal Polyp *(b)*	12/49(24)	10/49(20)	11/49(22)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.833	0.917
Lower Limit		0.357	0.407
Upper Limit		1.901	2.045
Weeks to First Observed Tumor	50	90	83

(a) Dosed groups received doses of 6,000 or 12,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

MICE

Body Weights and Clinical Signs

A dose-related decrease in mean body weights of male and female mice was observed throughout the study (Figure 3). No other compound-related clinical signs were observed.

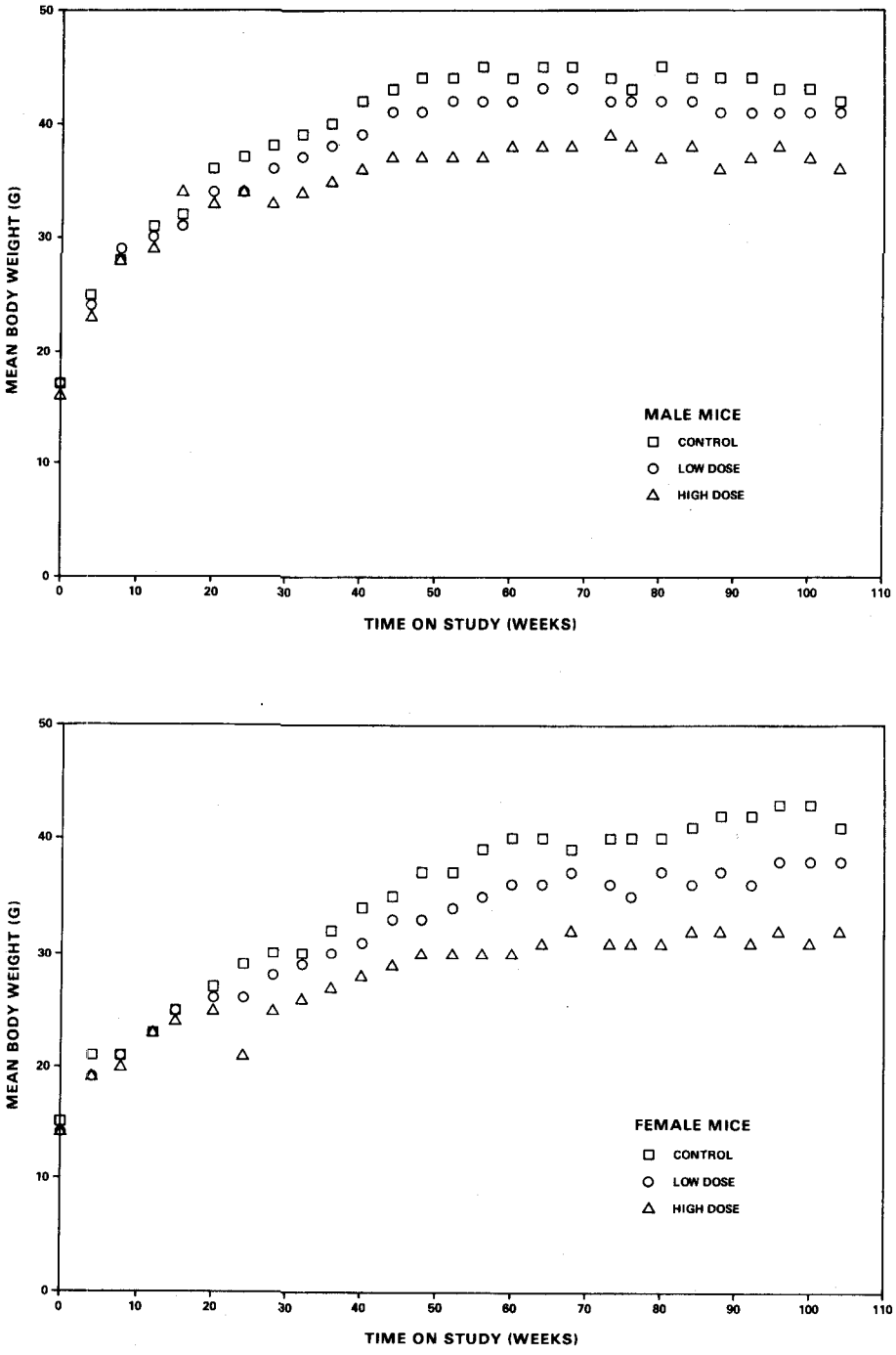


Figure 3. Growth Curves for Mice Fed Diets Containing Butyl Benzyl Phthalate

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing butyl benzyl phthalate at the doses of this bioassay, together with those for the controls, are shown by the Kaplan and Meier curves in Figure 4. The survival among all three groups of either sex was comparable.

In male mice, 44/50 (88%) of the control group, 43/49 (88%) of the low-dose group, and 42/50 (84%) of the high-dose group were alive at the end of the study of 105-106 weeks. In females, 35/50 (70%) of the control group and the low-dose group and 36/50 (72%) of the high-dose group lived to the end of the study at 105-106 weeks.

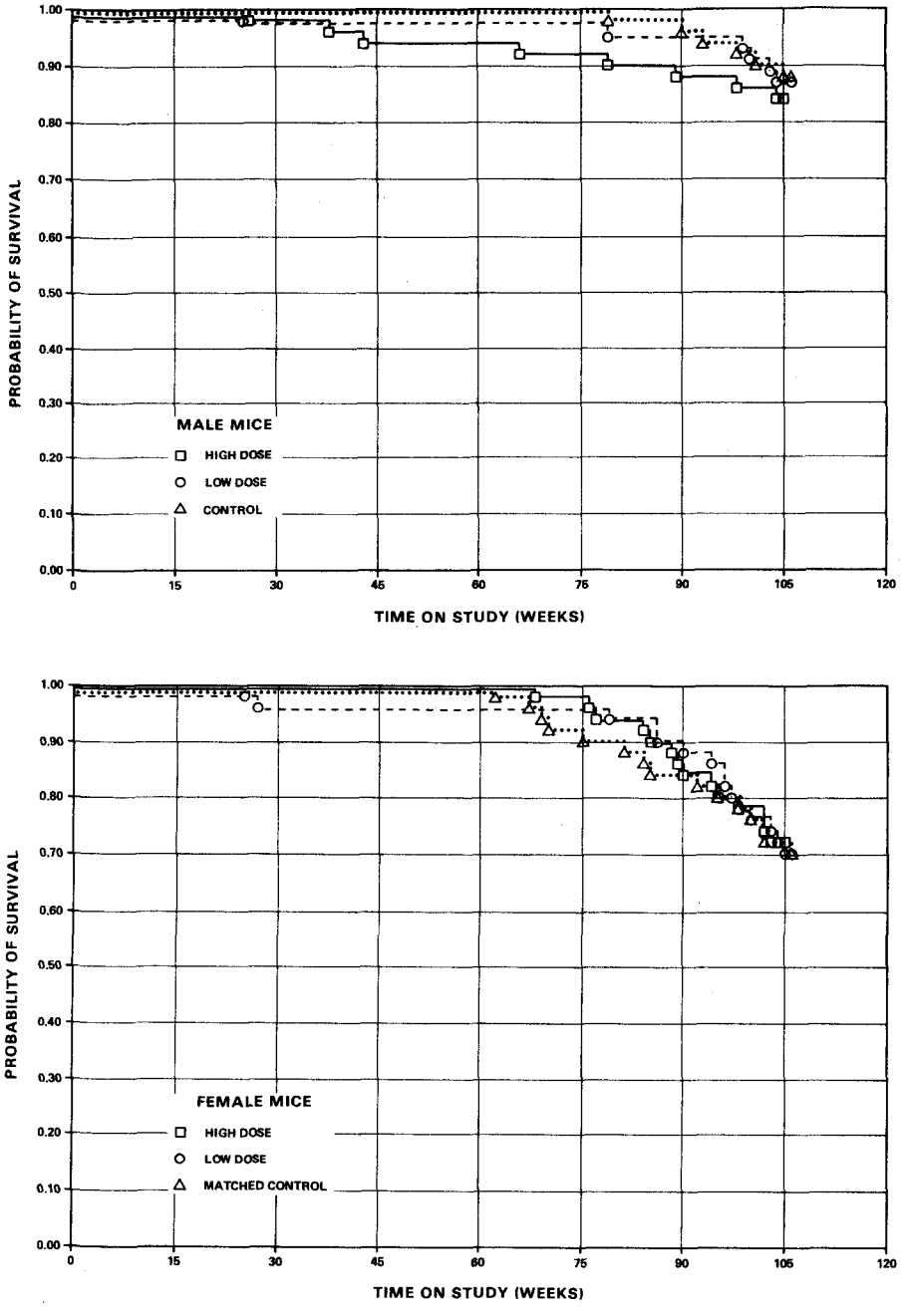


Figure 4. Survival Curves for Mice Fed Diets Containing Butyl Benzyl Phthalate

Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B, Tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2.

All neoplasms observed in mice were considered to be unrelated to the long-term administration of the test compound and to be within the normal incidence limits in B6C3F1 mice.

The nonneoplastic, proliferative, degenerative, inflammatory, and developmental lesions observed in male and female mice were also considered to be unrelated to test compound administration and to be within the normal incidence limits in B6C3F1 mice. No toxic lesions were seen.

The results of histopathologic examination indicated that butyl benzyl phthalate was not carcinogenic for B6C3F1 mice under the conditions of this bioassay.

Statistical Analyses of Results

Tables 8 and 9 contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, there was a significant trend (P=0.016) in the negative direction and significantly lower (P=0.007 and P=0.033) incidences in each of the dosed groups of animals with alveolar/bronchiolar adenomas in the lung. These negative results may have been a consequence of the higher incidence observed in the control group (13/50, 26%) as compared with the historical incidence of 280/3543 (8%) in B6C3F1 male mice. A departure from linear trend (P=0.049) was also indicated due to a sharp decline in the dosed groups' incidences relative to the control group.

Lymphomas or leukemia occurred with a negative trend (P=0.008) and a significantly lower (P=0.009) incidence in the high-dose male mice was observed when compared with controls.

In female mice, none of the statistical tests were significant.

In each of the 95% confidence intervals for relative risk shown in the tables, the value of less than one is included: this indicates the absence of significant positive results. Each of the intervals, except for the incidence of alveolar/bronchiolar adenomas in the lung of the low-dose group of males and for the incidence of lymphomas or leukemia in the high-dose group of males, has an upper limit greater than one indicating the theoretical possibility of tumor induction by butyl benzyl phthalate which could not be detected under the conditions of this test.

TABLE 8. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE *(a)*

	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma *(b)*	13/50(26)	3/49(6)	5/50(10)
P Values *(c),(d)*	P=0.016(N)	P=0.007(N)	P=0.033(N)
Departure from Linear Trend *(f)*	P=0.049		
Relative Risk (Control) *(e)*		0.235	0.385
Lower Limit		0.046	0.116
Upper Limit		0.792	1.054
Weeks to First Observed Tumor	106	105	89
Lung: Alveolar/Bronchiolar Carcinoma *(b)*	4/50(8)	8/49(16)	3/50(6)
P Values *(c),(d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		2.041	0.750
Lower Limit		0.588	0.115
Upper Limit		8.704	4.206
Weeks to First Observed Tumor	79	99	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma *(b)*	17/50(34)	11/49(22)	8/50(16)
P Values *(c),(d)*	P=0.024(N)	N.S.	P=0.032(N)
Relative Risk (Control) *(e)*		0.660	0.471
Lower Limit		0.314	0.195
Upper Limit		1.332	1.036
Weeks to First Observed Tumor	79	99	89
Hematopoietic System: Malignant Lymphoma, NOS *(b)*	12/50(24)	10/49(20)	4/50(8)
P Values *(c),(d)*	P=0.024(N)	N.S.	P=0.027(N)
Relative Risk (Control) *(e)*		0.850	0.333
Lower Limit		0.363	0.084
Upper Limit		1.941	1.014
Weeks to First Observed Tumor	98	99	105
Hematopoietic System: All Lymphomas *(b)*	13/50(26)	11/49(22)	4/50(8)
P Values *(c), (d)*	P=0.015(N)	N.S.	P=0.016(N)
Relative Risk (Control) *(e)*		0.863	0.308
Lower Limit		0.389	0.078
Upper Limit		1.878	0.917
Weeks to First Observed Tumor	98	99	105
Hematopoietic System: Lymphoma or Leukemia *(b)*	14/50(28)	11/49(22)	4/50(8)
P Values *(c), (d)*	P=0.008(N)	N.S.	P=0.009(N)
Relative Risk (Control) *(e)*		0.802	0.286
Lower Limit		0.367	0.073
Upper Limit		1.706	0.836
Weeks to First Observed Tumor	90	99	105

TABLE 8. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE *(a)* (Continued)

	Control	Low Dose	High Dose
Circulatory System:			
Angiosarcoma *(b)*	1/50(2)	4/49(8)	1/50(2)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		4.082	1.000
Lower Limit		0.423	0.013
Upper Limit		196.665	76.970
Weeks to First Observed Tumor	106	79	105
Liver: Hepatocellular Adenoma *(b)*	4/50(8)	7/48(15)	3/47(6)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		1.823	0.798
Lower Limit		0.497	0.123
Upper Limit		7.985	4.463
Weeks to First Observed Tumor	106	105	105
Liver: Hepatocellular Carcinoma *(b)*	9/50(18)	5/48(10)	11/47(23)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.579	1.300
Lower Limit		0.163	0.539
Upper Limit		1.775	3.220
Weeks to First Observed Tumor	101	105	79
Liver: Hepatocellular Adenoma or Carcinoma *(b)*	13/50(26)	12/48(25)	14/47(30)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.962	1.146
Lower Limit		0.447	0.560
Upper Limit		2.047	2.353
Weeks to First Observed Tumor	101	105	79

(a) Dosed groups received doses of 6,000 or 12,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

TABLE 9. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN FEMALE MICE FED DIETS *CONTAINING BUTYL BENZYL PHTHALATE (a)*

	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma *(b)*	5/50(10)	3/50(6)	1/50(2)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.600	0.200
Lower Limit		0.098	0.004
Upper Limit		2.910	1.699
Weeks to First Observed Tumor	106	106	105
Lung: Alveolar/Bronchiolar Carcinoma *(b)*	3/50(6)	0/50(0)	2/50(4)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.000	0.667
Lower Limit		0.000	0.058
Upper Limit		1.663	5.570
Weeks to First Observed Tumor	69	—	102
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma *(b)*	8/50(16)	3/50(6)	3/50(6)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.375	0.375
Lower Limit		0.067	0.067
Upper Limit		1.460	1.460
Weeks to First Observed Tumor	69	106	102
Hematopoietic System: Malignant Lymphoma, NOS *(b)*	15/50(30)	14/50(28)	15/50(30)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.933	1.000
Lower Limit		0.469	0.513
Upper Limit		1.845	1.948
Weeks to First Observed Tumor	84	97	90
Hematopoietic System: All Lymphomas *(b)*	17/50(34)	16/50(32)	17/50(34)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.941	1.000
Lower Limit		0.505	0.546
Upper Limit		1.746	1.831
Weeks to First Observed Tumor	84	97	77
Hematopoietic System: Lymphoma or Leukemia *(b)*	17/50(34)	16/50(32)	18/50(36)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		0.941	1.059
Lower Limit		0.505	0.587
Upper Limit		1.746	1.916
Weeks to First Observed Tumor	84	97	77
Liver: Hepatocellular Adenoma *(b)*	0/50(0)	3/50(6)	2/49(4)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		Infinite	Infinite
Lower Limit		0.601	0.302
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	—	106	105

TABLE 9. ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN FEMALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE *(a)* (Continued)

	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma *(b)*	2/50(4)	2/50(4)	4/49(8)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		1.000	2.041
Lower Limit		0.075	0.308
Upper Limit		13.326	21.737
Weeks to First Observed Tumor	106	106	103
Liver: Hepatocellular Adenoma or Carcinoma *(b)*	2/50(4)	5/50(10)	6/49(12)
P Values *(c), (d)*	N.S.	N.S.	N.S.
Relative Risk (Control) *(e)*		2.500	3.061
Lower Limit		0.432	0.581
Upper Limit		25.286	29.826
Weeks to First Observed Tumor	106	106	103

(a) Dosed groups received doses of 6,000 or 12,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in the control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. DISCUSSION AND CONCLUSIONS

After week 14 of the chronic study, an increasing number of deaths occurred among high-dose male rats and by week 24 increasing numbers of low-dose male rats were dying. Death in these animals was apparently the result of internal (and unexplained) hemorrhaging. Survival of the high-dose male rats had decreased to 30% by week 28 and the remaining male rats were killed at week 29 to 30 (49/50 controls, 40/50 low-dose, 15/50 high-dose). Compound-related hemorrhaging was not observed, however, in the subchronic study. Although 1/10 male rats receiving 6,300 ppm and 1/10 male rats receiving 12,500 ppm butyl benzyl phthalate died in the 13-week study, these deaths were not considered to be compound-related. Because of compound-related mortality, butyl benzyl phthalate was not adequately tested in male F344/N rats.

During most of the chronic study, mean body weights and feed consumption by dosed female rats were lower than those of the corresponding controls. No other compound-related clinical signs were observed for female rats, and survival of dosed and control females was comparable.

Mononuclear cell leukemia occurred with a dose-related trend (P=0.006) in female rats, and the incidences in the high-dose group were significantly higher than those in the controls (P=0.011). The historical rate for studies in this laboratory since 1977 for "all leukemias" is 77/399 (19%; 12% to 24%). The incidence of mononuclear cell leukemia in the high-dose female F344/N rats was significantly higher (P=0.008) when compared with historical controls and the dose-related trend was also significant (P=0.019).

The term mononuclear cell leukemia as used in this report refers to a neoplastic disease of F344/N rats which is characterized by infiltration of pleomorphic blast-like mononuclear cells in various organs, especially in the spleen, liver, and bone marrow. The tumor appears to originate in the spleen, and this organ is often markedly enlarged. The peripheral white blood cell count increases, and contains neoplastic cells, which resemble bone marrow stem cells. For these reasons, the term mononuclear cell leukemia is preferred, although the morphologic appearance of this lesion may be variable, depending on the degree and direction of differentiation of this pleuripotent stem cell. Other terms often used by pathologists within the Bioassay Program to describe this disease include leukemia, NOS; monocytic leukemia; and myelomonocytic cell leukemia (this last term is used in Appendix A, Table A2). Granulocytic leukemia is used occasionally if differentiation proceeds in this direction. The leukemias found in rats exposed to butyl benzyl phthalate were not different in cell morphology and tissue distribution from those in control rats.

Butyl benzyl phthalate was tested in the same room with three other chemicals — di(2-ethylhexyl)adipate, di(2-ethylhexyl)phthalate, and guar gum — undergoing carcinogenesis bioassays. Di(2-ethylhexyl)adipate (NTP 1982a) was carcinogenic for B6C3F1 mice, causing carcinomas of the liver in female mice and adenomas of the liver in male mice; di(2-ethylhexyl)phthalate (NTP 1982b) was carcinogenic for male and female F344/N rats, causing liver neoplastic nodules or carcinomas in males and hepatocellular carcinomas and neoplastic nodules of the liver in females, and for male and female B6C3F1 mice, causing hepatocellular carcinomas; guar gum (NTP 1982c) was not carcinogenic for male and female F344/N rats or B6C3F1 mice.

Although chemical cross-contamination among groups cannot be excluded completely, the responses in the separate testing experiments show that any adjacent chemical effect was absent, or minimal. The results of these other studies support the conclusion that butyl benzyl phthalate was probably carcinogenic for female F344/N rats, causing mononuclear cell leukemia. These data stand independently because the guar gum exposed groups did not show any compound related tumor development, because the di(2-ethylhexyl)adipate caused liver tumors in mice, because di(2-ethylhexyl)phthalate induced liver tumors in male and female rats and in male and female mice, and because none showed an increase in leukemia.

Carcinogenicity and other chronic effects of phthalic acid esters are summarized in the following paragraphs as documented in a recent NTP report (Kluwe, 1982).

Five lifetime feeding studies on di(2-ethylhexyl)phthalate have been reported: three with rats and two with mice (Table 10). At the 4,000-ppm dietary level, di(2-ethylhexyl)phthalate did not cause early deaths in male or female rats or increase the incidence of tumors (3 of 16 dosed rats and 5 of 16 controls had tumors when killed at the end of the study; Carpenter et al., 1953). Although the specific tumor sites were not reported, the authors concluded that di(2-ethylhexyl)phthalate was not carcinogenic to rats at

this dietary level. Di(2-ethylhexyl)phthalate also failed to increase tumor incidence in male or female rats when present at 5,000 ppm in the diet (Harris et al., 1956). This study also found no early deaths attributable to di(2-ethylhexyl)phthalate, but body weight gain was reduced by the 5,000-ppm di(2-ethylhexyl)phthalate dietary level, indicating that a near maximal-tolerated dose for this strain (Wistar) of rats was probably achieved. A third negative study was reported for mice supplied with 100 mg/kg (~670 ppm) di(2-ethylhexyl)phthalate in the diet for three generations. This dose would appear to be much lower than the maximum tolerated dose for mice. Renal cysts occurred in the F_1 and F_2 generations, suggesting an adverse effect of di(2-ethylhexyl)phthalate on fetal development (Omori, 1976).

Tests conducted by the NCI/NTP using the standard bioassay protocol (maximum tolerated dose and one-half maximum tolerated dose) identified di(2-ethylhexyl)phthalate as a hepatocarcinogen in male and female B6C3F1 mice and in male and female F344/N rats (Table 10). The effective dietary concentrations — 3,000 and 6,000 ppm for mice and 6,000 and 12,000 ppm for rats (NTP, 1982b) — were greater than those employed in the studies that did not find carcinogenic effects of di(2-ethylhexyl)phthalate. Thus, the discrepancies between the results of the various studies could be attributable simply to differences in the doses used or to strain differences. The former is suggested by the dose dependence of the tumorigenic effects in the NCI/NTP bioassay (NTP, 1982b).

Other reported chronic effects of di(2-ethylhexyl)phthalate on rats and mice maintained for 1 year on diets containing 500 or 1,000 ppm di(2-ethylhexyl)phthalate included dose-dependent interstitial nephritis, increased serum glutamic pyruvic transaminase activity (hepatotoxicity), and decreased serum concentration of glucose (Nagasaki et al., 1975). These effects appeared only in these Japanese studies, suggesting strain differences in response to di(2-ethylhexyl)phthalate. Nikoronow et al. (1973) reported that a diet containing 3,500 ppm di(2-ethylhexyl)phthalate produced a 30% mortality rate in Wistar rats within 1 year. The pathological basis for the mortality, not generally reported in other di(2-ethylhexyl)phthalate feeding studies, was not determined.

Guinea pigs survived a year of dietary exposure to di(2-ethylhexyl)phthalate (1,300 ppm) with no discernible adverse effects (Carpenter et al., 1953).

The only primate study located in the literature seems to suggest that rodents may be less susceptible to di(2-ethylhexyl)phthalate toxicity than are the higher species. Weekly intravenous infusions of small amounts of plasma-solubilized di(2-ethylhexyl)phthalate into rhesus monkeys (cumulative dose of 20-70 mg) produced focal parenchymal liver necrosis and inflammatory cell infiltration, as well as a subtle change in the kinetics of exogenously-administered sulfobromophthalein (Jacobson et al., 1977). The last effect was considered an indication of subtle hepatic dysfunction. As the doses used were equivalent to the amount of di(2-ethylhexyl) phthalate, or mono(2-ethylhexyl)phthalate, to which humans could be exposed via intravenous therapy or renal dialysis, a potential for di(2-ethylhexyl) phthalate-induced hepatotoxicity in humans may exist. Moreover, the effects of di(2-ethylhexyl)phthalate on the monkeys' livers did not disappear after the administration of di(2-ethylhexyl)phthalate was discontinued, suggesting prolonged or irreversible effects. Whether the differences between rodents and primates relate to differences in the sophistication of the methods chosen for toxicological analysis (sulfobromophthalein kinetics vs histopathology) or to fundamental species differences in response to phthalate esters is unknown.

A three-generation feeding study of di-n-butyl phthalate (100 mg/kg/day, 500-800 ppm) in mice produced renal cysts in the F_1 and F_2 generations, an effect similar to that of di(2-ethylhexyl)phthalate, but no increase in tumor incidence (Table 10; Omori, 1976). Since there was no indication that a maximum tolerated dose was reached, this study does not sufficiently indicate that di-n-butyl phthalate is non-carcinogenic.

Dietary di-n-butyl phthalate (1,250 or 2,500 ppm) produced no pathological abnormalities in rats exposed for 1 year, but 12,500 ppm in the diet was lethal within a week (Smith, 1953; Nikoronow et al., 1973). A single Russian report (English abstract) indicated that rats exposed "chronically" (length of time not specified) to 0.04 mg/liter di-n-butyl phthalate in air exhibited alterations in several hematological parameters (Voronin, 1974). The author cited the changes (increased leukocytes and erythrocytes, decreased lymphocytes and monocytes) as evidence of di-n-butyl phthalate hematotoxicity. Di-n-butyl phthalate did not produce similar effects in the feeding studies.

Butyl benzyl phthalate, under the conditions of the standard carcinogenesis bioassay reported here, was considered non-carcinogenic in mice, but was inadequately tested in male rats due to the high incidence of early deaths (Table 10). Female rats administered butyl benzyl phthalate exhibited a statistically significant increase in the incidence of mononuclear cell leukemias at the 12,000-ppm dietary level. Butyl benzyl phthalate was negative in the mouse lung adenoma assay (Table 10), a short-term *in vivo* assay that may signal long-term carcinogenic effects. Negative findings in this test, however, are of lesser significance than are positive findings. Butyl benzyl phthalate was judged to be non-carcinogenic to rats at a daily dose of 5 mg/kg (Effects of Santicizer 160, 1968; Table 10). This dose, however, would appear to be considerably lower than the maximum tolerated dose.

Aware of the current NCI/NTP bioassay results, the Interagency Testing Committee recommended to the Environmental Protection Agency that a more "thorough evaluation of the potential carcinogenicity of butyl benzyl phthalate be undertaken" (EPA, 1980; EPA, 1981; EPA, 1982).

Since completing and evaluating the results from this and other carcinogenesis bioassays on ortho phthalic acid esters, the National Toxicology Program has developed a continuing project to better characterize the toxic effects of select members in this chemical class. The detection of carcinogenic effects of di(2-ethylhexyl)phthalate (DEHP), di(2-ethylhexyl)adipate (DEHA), and butyl benzyl phthalate (BBP) in rodents has stimulated further interest in the chronic toxic potentials of phthalate esters and related chemicals, many of which are used in plastics or for other purposes that result in frequent human exposures. Recognizing the technical and economic importances of phthalates and the need for being able to better extrapolate rodent bioassay data to human health effects, the NTP will continue to study the deleterious effects of DEHP and to probe their mechanisms of action, as well as to evaluate the toxic potentials of several other phthalate esters both individually and comparatively. As an example: a six-month subchronic feeding study on butyl benzyl phthalate in Fischer 344 male rats will be conducted, with emphases on determining the cause of early deaths in the study reported in this technical report and on assessing reproductive function. The dose-dependent disposition and metabolism of BBP in rats will be studied to more appropriately correlate toxicity with pharmacokinetics and to ascertain the amounts of n-butanol and benzyl alcohol released as metabolites of butyl benzyl phthalate (benzyl alcohol is currently in chronic bioassay). A chronic carcinogenicity bioassay of butyl benzyl phthalate in male rats will be designed and initiated subsequent to a complete analysis of the subchronic study results.

Hodge, et al. (1953) reported that ethyl phthalyl ethyl glycolate in the diet at the 5% level (50,000 ppm) caused weight loss, compound-related deaths, and urinary tract caliculi (perhaps due to release of the glycol moiety) within 1 year. Adverse effects were not detected at the 0.5% level (5,000 ppm) when the rats were administered the chemical for 2 years (Table 10). Although the maximum tolerated dose may not have been achieved in the latter study, the premature deaths at 50,000 ppm suggest that 5,000 ppm in the diet was at least within an order of magnitude of the maximum tolerated dose.

Rats supplied with up to 20,000 ppm butyl phthalyl butyl glycolate in the diet for 2 years exhibited no increase in tumor incidence or other pathological effects (Table 10). Although this is a numerically high dietary concentration, neither early deaths nor a diminution of body weight gain was produced (Toxicity of BPhBG, 1950), indicating that the dose of butyl phthalyl butyl glycolate was probably below the maximum tolerated dose.

Neither phthalamide nor phthalic anhydride (the anhydrous form, phthalic anhydride, is mixed with specific alcohols to produce the phthalate esters) was carcinogenic under the conditions of the standard bioassay (NCI, 1979b; NCI, 1979c; Table 10). Thus, it would seem that carcinogenic effects of phthalate esters cannot be attributed to the phthalate moeity *per se* or to metabolism to phthalic acid.

Although judged not carcinogenic in rats under the standard bioassay conditions (NTP, 1982a), di(2-ethylhexyl)adipate did produce significant increases in the incidence of hepatocellular carcinomas in female mice and hepatocellular adenomas in male mice (Table 10).

The structural similarities between di(2-ethylhexyl)adipate and di(2-ethylhexyl)phthalate, and reports that both of these compounds produce liver tumors and dominant lethal mutations

in mice (Singh et al., 1974 and 1975) suggest similar, structurally-related mechanisms of action. Whether or not the monoester derivatives — mono(2-ethylhexyl)phthalate and mono(2-ethylhexyl) adipate — would also be carcinogenic and mutagenic under the same conditions as the diesters awaits further study.

In summary, two phthalate esters, di(2-ethylhexyl)phthalate and butyl benzyl phthalate, have been shown to induce carcinogenic responses in rodents. Some other phthalate ester compounds were not shown to be carcinogenic in lifetime feeding studies (Table 10), but the maximum tolerated doses were not used in most cases. Neither short-term, bacterial tests for mutagenic activity nor structure-activity relationships have been shown to be good indicators of the effects of the various phthalate esters in chronic studies. Therefore, the extensive *Salmonella* testing and the metabolism/disposition studies performed on many phthalate esters do not, at the present time, appear to adequately indicate the carcinogenic potential of individual phthalate esters (Kluwe, 1982).

Conclusions. Under the conditions of this bioassay, butyl benzyl phthalate was probably carcinogenic for female F344/N rats, causing an increased incidence of mononuclear cell leukemias. The male F344/N rat study was considered inadequate for evaluation due to compound-related toxicity and early mortality. Butyl benzyl phthalate was not carcinogenic for B6C3F1 mice of either sex.

TABLE 10. CARCINOGENICITY OF PHTHALATE ESTERS AND RELATED COMPOUNDS

Compound	Test Protocol	Results	Reference
Di(2-ethylhexyl) phthalate	Diet, 4,000 ppm, 2 yr., rat/Sherman, male and female	No increase in tumor incidence	Carpenter et al., 1953
	Diet, 5,000 ppm, 2 yr., rat/Wistar, male and female	No pathological effects	Harris et al., 1956
	Diet, 100 mg/kg (~670 ppm)*(a)*, 3 generations, mouse, male and female	No increase in tumor incidence reported	Omori, 1976
	Diet, 12,000 ppm, 2 yr., *(b)* rat/F344/N, male and female	Increased incidence of hepatocellular carcinomas in females and liver tumors in males	NTP, 1982b
	Diet, 3,000 or 6,000 ppm, *(b)* 2 yr., mouse/B6C3F1, male and female	Increased incidence of hepatocellular carcinomas	NTP, 1982b
Di-n-butyl phthalate	Diet, 100 mg/kg (~670 ppm), *(a)*, 3 generations, mouse, male and female	No increase in tumor incidence	Omori, 1976
Butyl benzyl phthalate	Intraperitoneal, 800 mg/kg, *(b)*, 3 times per week for 8 weeks, mouse/ Strain A, male	No lung adenomas *(c)*	Theiss, et al., 1977
	Diet, 5 mg/kg, 2 yr., rat/Wistar, male and female	No increase in tumor incidence	Effects of Santicizer 160, 1968
	Diet, 12,000 ppm, 2 yr., *(b)* rat/F344/N, female *(d)*	Increased incidence of mononuclear cell leukemia	Current study
	Diet, 12,000 ppm, 2 yr., *(b)* mouse/B6C3F1, male and female	No increase in tumor incidence	Current study
Ethyl phthalyl ethyl glycolate	Diet, 5,000 ppm, 2 yr., rat	No pathological effects	Hodge et al., 1953
Butyl phthalyl butyl glycolate	Diet, 200-20,000 ppm, 2 yr. rat/Sherman	No pathological effects	Toxicity of BPhBG (ca) 1950
Phthalic anhydride	Diet, 5,000 ppm, 2 yr., *(b)* rat/F344/N, male and female	No increase in tumor incidence	NCI, 1979c
	Diet, 12,500-50,000 ppm *(b)* 2 yr., mouse/B6C3F1, male and female	No increase in tumor incidence	NCI, 1979c
Phthalamide	Diet, 10,000 ppm (female) *(b)* 30,000 ppm (male), 2 yr., rat/F344/N, male and female	No increase in tumor incidence	NCI, 1979b
	Diet, 12,500 ppm (female) *(b)* 50,000 ppm (male), 2 yr., mouse/B6C3F1, male and female	No increase in tumor incidence	NCI, 1979b
Di(2-ethylhexyl) adipate	Diet, 25,000 ppm, 2 yr., *(b)* rat/F344/N, male and female	No increase in tumor incidence	NTP, 1982a
	Diet, 12,000 or 25,000 ppm *(b)* 2 yr., mouse/B6C3F1, male or female	Increased incidence of hepatocellular carcinomas in females (12,000 and 25,000 ppm) and hepatocellular adenomas in males (25,000 ppm)	NTP, 1982a

(a) Estimated concentration, based on a daily feed consumption of 15% of body weight.
(b) Maximum tolerated dose was used.
(c) Mouse lung adenoma assay.
(d) Carcinogenicity in males not adequately assessed due to high mortality rates.

V. REFERENCES

V. REFERENCES

Annual Book of ASTM Standards, American Society for Testing and Materials, Philadelphia, Part 29, Designation D1617-72, 1974, pp. 180-182.

Armitage, P., Statistical methods in medical research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

Autian, J. Toxicity and health threats of phthalate esters: review of the literature. Environ. Health Perspect. 4:3-25, 1973.

Berenblum, I., ed. Carcinogenicity testing. A report of the panel on carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Bower, R., Haberman, S., and Minton, P., Teratogenic effects in the chick embryo caused by esters of phthalic acid. J. Pharmacol. Exp. Ther. 171(2):314-324, 1970.

Calley, D., Autian, J., and Guess, W., Toxicology of a series of phthalate esters. J. Pharm. Sci. 55:158, 1966.

Carpenter, C., Weil, C., and Smyth, H., Chronic oral toxicity of di(2-ethylhexyl)phthalate for rats, guinea pigs, and dogs. Arch. Ind. Hyg. Occup. Med. 8:219-226, 1953.

CFR, U.S. Code of Federal Regulations 21: 121.2571, 1967.

CFR, U.S. Code of Federal Regulations 21: 121.2511, 1976.

Cox, D. R., Analysis of binary data, Methuen & Co., Ltd., London, 1970. pp. 48-52.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B34:187-220, 1972.

Effects of Santicizer 160, Effects after prolonged oral administration of Santicizer 160° (sic.) [butyl benzyl phthalate]. Submitted to the Environmental Protection Agency under Section 8(d) of the Toxic Substances Control Act of 1976, 8D HQ-1078-0280, 1968.

EPA, Environmental Protection Agency, Receipt of Seventh Report of the Interagency Testing Committee to the Administrator; Request for comments on priority list of chemicals, Fed. Register 45(229): 78432-78446 (25 November 1980).

EPA, Environmental Protection Agency, Alkyl phthalates and benzyl butyl phthalate; response to the Interagency Testing Committee, Fed. Register 46(210):53775-53777 (30 October 1981).

EPA, Environmental Protection Agency, Alkyl phthalates and benzyl butyl phthalate; follow-up response to the Interagency Testing Committee, Fed. Register 47(2):335-336 (5 January 1982).

Gart, J. J., The comparison of proportions: a review of significance tests, confidence limits and adjustments for stratification., Rev. Int. Stat. Inst. 39:148-169, 1971.

Gillio-Tos, M. and A. Vimercati, Kunststoffe, 56(6):409-412, 1966.

Haberman, S., Guess, W. L., Rowan, D. F., and Bower, R. K., Effects of plastics and their additives on human serum proteins, antibodies and developing chick embryos. Soc. Plastic Eng. J. 24:62-29, 1968.

Harris, R., Hodge, C., Maynard, E., and Blanchet, H., Chronic oral toxicity of 2-ethylhexyl phthalate in rats and dogs. Arch. Ind. Health 13:259-264, 1956.

Hodge, H., Maynard, E., Blanchet, H., Hyatt, R., Rowe, Z., and Spencer, H., Chronic oral toxicity of ethyl phthalyl ethyl glycolate in rats and dogs. Arch. Ind. Hyg. Occup. Med. 8:289-295, 1953.

Jacobson, M., Kevy, S., and Grand, R. Effects of a plasticizer leached from polyvinylchloride on the subhuman primate: A consequence of chronic transfusion therapy. J. Lab. Clin. Med. 89:1066-1079, 1977.

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. Statist. Assoc. 53:457-481, 1958.

Kluwe, W. M., Phthalic acid esters: Part 1, toxicological evaluation, Natl. Tox. Prog., Doc. No. 81-49, NTP, Research Triangle Park, North Carolina, 1982.

Kuarata, H., Studies on the mutagenic effects of phthalates. Report to Ministry of Health and Welfare (Japan), Scientific Research on Food Hygiene Program, 1975: as cited in Omori, Y. Recent progress in safety evaluation studies on plasticizers and plastics and their controlled use in Japan. Environ. Health Perspect. 17: 203-209, 1976.

Linhart, M. S., Cooper, J. A., Martin, R. L., Page, N. P., and Peters, J. A., Carcinogenesis bioassay data system. Comp. Biomed. Res. 7:230-248, 1974.

Nagasaki, H., Tomii, S., Mega, T., Hirao, K., Shinoshara, Y., and Ito, N., Chronic toxicity of dioctylphthalate (DOP) in male rats and mice, Chem. Abst. 54180r 83:117, 1975.

NCI, National Cancer Institute, NCI Technical report on the bioassay of dimethyl terephthalate, NCI TR 121, Department of Health, Education, and Welfare, Bethesda, Maryland, 1979a.

NCI, National Cancer Institute, NCI Technical Report on the bioassay of phthalamide, NCI TR 161, Department of Health, Education and Welfare, Bethesda, Maryland, 1979b.

NCI, National Cancer Institute, NCI Technical Report on the bioassay of phthalic anhydride, NCI TR 159, Department of Heath, Education and Welfare, Bethesda, Maryland, 1979c.

Nikoronow, M., Mazur, H., Pickacz, H., Effect of orally administered plasticizers in polyvinylchloride stabilizers in the rat. Toxicol. Appl. Pharmacol. 26:253-259, 1973.

NTP, National Toxicology Program, NTP Technical Bulletin 1(3):10, 1980.

NTP, National Toxicology Program, NTP Technical Report on the carcinogenesis bioassay of di(2-ethylhexyl)adipate, NTP TR 212, NIH Publication No. 81-1768, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982a.

NTP, National Toxicology Program, NTP Technical Report on the carcinogenesis bioassay of di(2-ethylhexyl)phthalate, NTP TR 217, NIH Publication No. 82-1773, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982b.

NTP, National Toxicology Program, NTP Technical Report on the carcinogenesis bioassay of guar gum, NTP TR 229, NIH Publication No. 82-1785, Department of Health and Human Services, Research Triangle Park, North Carolina, 1982c.

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

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administration of benzo (a) pyrene and ferric oxide. Cancer Res. 32:1073-1081, 1972.

Singh, A., Lawrence, W., and Autian, J., Mutagenic and antifertility sensitivities of mice to di-2-ethylhexylphthalate (DEHP) and dimethoxyethyl phthalate (DMEP). Toxicol. Appl. Pharmacol. 29:35-46, 1974.

Singh, A., Lawrence, W., and Autian, J., Dominant lethal mutations and antifertility effects of di-2-ethylhexyl adipate and diethyl adipate in male mice. Toxicol. Appl. Pharmacol. 32:566-576, 1975.

Singmaster, J. and Crosby, D., Plasticizers as interferences in pollutant analyses. Bull. Environ. Contam. Toxicol. 16(3):291-300, 1976.

Smith, C. C., Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate and methoxy oleate. Arch. Ind. Hyg. 7:310-318, 1953.

Statsek, N., Hygiene investigations of certain esters of phthalic acid and of polyvinylchloride materials plastificated thereby. Gig Sanit. 6:25-28, 1974.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62:679-682, 1975.

Theiss, J. C., Stoner, G. D., Shimkin, M. B., and Weisburger, E. K., Test for carcinogenicity of organic contaminants of United States drinking waters by pulmonary tumor response in strain A mice. Cancer Res., 37: 2717-2720, 1977.

Toxicity of BPhBG, a study of the toxicity of butyl phthalyl butyl glycollate (Santicizer B-16). Submitted to the Environmental Protection Agency under Section 8(d) of the Toxic Substances Control Act of 1976, 8D HQ-1078-0250, ca. 1950.

USITC, United States International Trade Commission, Synthetic Organic Chemicals -United States Production and Sales 1978, USITC Publication 1001, U.S. Government Printing Office, Washington, D.C., 1979.

Voronin, A., Toxicity and sanitizing characteristics of a dibutylphthalate plasticizer. Chem. Abstracts, 80:56220R, 1974.

Ward, J.M., Goodman, D. G., Griesemer, R. A., Hardisty, J. F., Schueler, R. L., Squire, R. A., and Strandberg, J. D., Quality assurance for pathology in rodent carcinogenesis tests. J. Environ. Path. Toxicol. 2:371-378, 1978.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	0	0	0
INTEGUMENTARY SYSTEM			
NONE			

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		1 (2%)
BASAL-CELL TUMOR			1 (2%)
BASAL-CELL CARCINOMA			1 (2%)
SARCOMA, NOS	1 (2%)		
*SUBCUT TISSUE	(49)	(49)	(50)
SARCOMA, NOS		3 (6%)	1 (2%)
FIBROMA	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	1 (2%)	1 (2%)
C-CELL CARCINOMA, METASTATIC	1 (2%)		
SARCOMA, NOS, METASTATIC		1 (2%)	
CARCINOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(50)
MYELOMONOCYTIC LEUKEMIA	6 (12%)	6 (12%)	17 (34%)
#SPLEEN	(47)	(47)	(50)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(45)	(43)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LUMBAR LYMPH NODE	(45)	(43)	(47)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(49)	(48)	(50)
MYELOMONOCYTIC LEUKEMIA	1 (2%)		
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(49)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(48)	(50)
NEOPLASTIC NODULE	1 (2%)	1 (2%)	3 (6%)
HEPATOCELLULAR CARCINOMA			1 (2%)
#SMALL INTESTINE	(48)	(47)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
#DUODENUM	(48)	(47)	(49)
LEIOMYOSARCOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(47)	(47)	(50)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(40)	(41)
ADENOMA, NOS	20 (44%)	21 (53%)	26 (63%)
#ADRENAL	(49)	(46)	(47)
CORTICAL ADENOMA			1 (2%)
CORTICAL CARCINOMA	1 (2%)		
PHEOCHROMOCYTOMA	2 (4%)	1 (2%)	
#THYROID	(47)	(46)	(46)
ADENOMA, NOS	1 (2%)		
FOLLICULAR-CELL ADENOMA		2 (4%)	
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA	2 (4%)	2 (4%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(47)	(46)	(46)
ISLET-CELL ADENOMA		3 (7%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
ADENOCARCINOMA, NOS	2 (4%)		
CARCINOSARCOMA		1 (2%)	
FIBROADENOMA	20 (41%)	14 (29%)	9 (18%)
*CLITORAL GLAND	(49)	(49)	(50)
CARCINOMA,NOS	4 (8%)	1 (2%)	3 (6%)
SQUAMOUS CELL CARCINOMA	2 (4%)		
ADENOMA, NOS			1 (2%)
ADENOCARCINOMA, NOS		1 (2%)	
#UTERUS	(49)	(49)	(49)
ADENOCARCINOMA, NOS			1 (2%)
LEIOMYOMA			1 (2%)
LEIOMYOSARCOMA			2 (4%)
ENDOMETRIAL STROMAL POLYP	12 (24%)	10 (20%)	11 (22%)
#CERVIX UTERI	(49)	(49)	(49)
SARCOMA, NOS			1 (2%)
#OVARY	(48)	(48)	(49)
GRANULOSA-CELL TUMOR			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(45)	(47)	(48)
ASTROCYTOMA			2 (4%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(49)	(50)
SARCOMA, NOS, METASTATIC			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHa	3	9	10
MORIBUND SACRIFICE	15	11	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	31	29	32
ANIMAL MISSING			
a INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	44	37	45
TOTAL PRIMARY TUMORS	78	74	87
TOTAL ANIMALS WITH BENIGN TUMORS	35	32	36
TOTAL BENIGN TUMORS	57	54	51
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	17	28
TOTAL MALIGNANT TUMORS	20	19	32
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	1
TOTAL SECONDARY TUMORS	1	4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	4
TOTAL UNCERTAIN TUMORS	1	1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
PAPILLOMA, NOS		1 (2%)	
FIBROSARCOMA			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(50)
FIBROMA	1 (2%)		
FIBROSARCOMA	1 (2%)	2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
CARCINOMA, NOS, METASTATIC	1 (2%)		
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	1 (2%)	4 (8%)
ALVEOLAR/BRONCHIOLAR ADENOMA	13 (26%)	3 (6%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (8%)	8 (16%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	9 (18%)	8 (16%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (2%)	
LEUKEMIA,NOS	1 (2%)		
#SPLEEN	(50)	(48)	(45)
MALIGNANT LYMPHOMA, NOS	2 (4%)		1 (2%)
#MESENTERIC L. NODE	(42)	(46)	(43)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
#PEYER'S PATCH	(48)	(47)	(46)
MALIGNANT LYMPHOMA, NOS	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(48)	(45)
ANGIOSARCOMA	1 (2%)	2 (4%)	
#LIVER	(50)	(48)	(47)
HEMANGIOSARCOMA			1 (2%)
ANGIOSARCOMA		2 (4%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(47)
HEPATOCELLULAR ADENOMA	4 (8%)	7 (15%)	3 (6%)
HEPATOCELLULAR CARCINOMA	9 (18%)	5 (10%)	11 (23%)
#STOMACH	(49)	(47)	(46)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOCARCINOMA, NOS		1 (2%)	
#ILEUM	(48)	(47)	(46)
ADENOCARCINOMA, NOS	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(45)	(43)	(45)
CORTICAL ADENOMA		1 (2%)	1 (2%)
#THYROID	(47)	(46)	(47)
FOLLICULAR-CELL ADENOMA	1 (2%)	1 (2%)	
FOLLICULAR-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(50)
CARCINOMA,NOS	1 (2%)		
PAPILLARY ADENOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(50)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	6	7
MORIBUND SACRIFICE	1		1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	44	43	42
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	38	31	26
TOTAL PRIMARY TUMORS	52	45	31
TOTAL ANIMALS WITH BENIGN TUMORS	17	12	9
TOTAL BENIGN TUMORS	20	13	9
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	22	18
TOTAL MALIGNANT TUMORS	32	32	22
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	4
TOTAL SECONDARY TUMORS	2	1	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
BASAL-CELL CARCINOMA	1 (2%)		
SEBACEOUS ADENOCARCINOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA	1 (2%)		1 (2%)
LEIOMYOSARCOMA, METASTATIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)		2 (4%)
LEIOMYOSARCOMA, METASTATIC		1 (2%)	
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	10 (20%)	11 (22%)	15 (30%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (4%)	2 (4%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
*HEMATOPOIETIC SYSTEM	(50)	(50)	(50)
NEOPLASM, NOS	1 (2%)	1 (2%)	
#SPLEEN	(50)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	4 (8%)	1 (2%)	
#MESENTERIC L. NODE	(39)	(42)	(39)
MALIGNANT LYMPHOMA, NOS	1 (3%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(50)	(49)
LEUKEMIA,NOS			1 (2%)
#PEYER'S PATCH	(45)	(47)	(45)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(39)	(42)	(39)
ANGIOSARCOMA			1 (3%)
#LIVER	(50)	(50)	(49)
ANGIOSARCOMA	1 (2%)	1 (2%)	
*MESENTERY	(50)	(50)	(50)
ANGIOSARCOMA		1 (2%)	
#UTERUS	(47)	(50)	(47)
ANGIOSARCOMA			1 (2%)
#OVARY/PAROVARIAN	(44)	(47)	(46)
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
HEPATOCELLULAR ADENOMA		3 (6%)	2 (4%)
HEPATOCELLULAR CARCINOMA	2 (4%)	2 (4%)	4 (8%)
#PANCREAS	(43)	(44)	(46)
ACINAR-CELL ADENOMA			1 (2%)
LEIOMYOSARCOMA, METASTATIC		1 (2%)	
#STOMACH	(47)	(45)	(44)
LEIOMYOSARCOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(39)	(41)
ADENOMA, NOS	1 (2%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL	(44)	(45)	(42)
CORTICAL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*VAGINA	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)		
#UTERUS	(47)	(50)	(47)
LEIOMYOMA	1 (2%)		
LEIOMYOSARCOMA			2 (4%)
ENDOMETRIAL STROMAL POLYP	1 (2%)		1 (2%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)		
#OVARY	(44)	(47)	(46)
CYSTADENOCARCINOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS			2 (4%)
PAPILLARY ADENOMA	1 (2%)		
PAPILLARY CYSTADENOMA, NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
*VERTEBRAL COLUMN	(50)	(50)	(50)
OSTEOSARCOMA	1 (2%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHə	15	15	13
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	35	36
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	33	27	31
TOTAL PRIMARY TUMORS	40	32	37
TOTAL ANIMALS WITH BENIGN TUMORS	10	9	7
TOTAL BENIGN TUMORS	10	9	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	26	19	25
TOTAL MALIGNANT TUMORS	29	22	30
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	
TOTAL UNCERTAIN TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

ə INCLUDES AUTOLYZED ANIMALS

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	0	0	0
INTEGUMENTARY SYSTEM			
NONE			

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
ACANTHOSIS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(50)
ATELECTASIS		1 (2%)	
CONGESTION, NOS	1 (2%)	1 (2%)	1 (2%)
EDEMA, NOS		1 (2%)	
HEMORRHAGE	1 (2%)	1 (2%)	
INFLAMMATION, FOCAL	2 (4%)	7 (15%)	3 (6%)
GRANULOMA, NOS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(42)	(46)	(40)
HYPOPLASIA, NOS		2 (4%)	
#SPLEEN	(47)	(47)	(50)
PIGMENTATION, NOS		1 (2%)	
HEMOSIDEROSIS	3 (6%)	2 (4%)	4 (8%)
#SPLENIC CAPSULE	(47)	(47)	(50)
RUPTURE			1 (2%)
#SPLENIC FOLLICLES	(47)	(47)	(50)
ATROPHY, NOS	2 (4%)	2 (4%)	4 (8%)
#SPLENIC RED PULP	(47)	(47)	(50)
ATROPHY, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(45)	(43)	(47)
PIGMENTATION, NOS			1 (2%)
#MEDIASTINAL L.NODE	(45)	(43)	(47)
HEMORRHAGE			1 (2%)
PIGMENTATION, NOS	1 (2%)	1 (2%)	2 (4%)
HEMOSIDEROSIS		1 (2%)	1 (2%)
#PANCREATIC L.NODE	(45)	(43)	(47)
HEMORRHAGE			1 (2%)
#MESENTERIC L. NODE	(45)	(43)	(47)
HEMORRHAGE			2 (4%)
ATROPHY, NOS		2 (5%)	1 (2%)
#PEYER'S PATCH	(48)	(47)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(29)	(24)	(28)
CYST, NOS		1 (4%)	4 (14%)
HEMORRHAGE		1 (4%)	1 (4%)
ATROPHY, NOS		1 (4%)	2 (7%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(50)
PERIVASCULITIS		1 (2%)	
#LUNG	(49)	(48)	(50)
PERIVASCULITIS	1 (2%)		
#HEART	(46)	(46)	(49)
DEGENERATION, NOS	1 (2%)		
#MYOCARDIUM	(46)	(46)	(49)
INFLAMMATION, INTERSTITIAL			1 (2%)
DEGENERATION, NOS	18 (39%)	19 (41%)	8 (16%)
#CARDIAC VALVE	(46)	(46)	(49)
INFLAMMATION, CHRONIC			1 (2%)
*AORTA	(49)	(49)	(50)
INFLAMMATION, NOS	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*PULMONARY ARTERY	(49)	(49)	(50)
MINERALIZATION	5 (10%)		1 (2%)
#OVARY	(48)	(48)	(49)
THROMBOSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(45)	(48)	(47)
INFLAMMATION, FOCAL		2 (4%)	
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#SUBMAXILLARY GLAND	(45)	(48)	(47)
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
#SUBMAXILLARY DUCT	(45)	(48)	(47)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#LIVER	(49)	(48)	(50)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	2 (4%)		2 (4%)
NECROSIS, DIFFUSE		1 (2%)	
METAMORPHOSIS FATTY	5 (10%)	9 (19%)	6 (12%)
BASOPHILIC CYTO CHANGE	43 (88%)	33 (69%)	35 (70%)
CLEAR-CELL CHANGE	1 (2%)		
#BILE DUCT	(49)	(48)	(50)
HYPERPLASIA, NOS		3 (6%)	6 (12%)
#PANCREAS	(47)	(46)	(46)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
ATROPHY, NOS		1 (2%)	1 (2%)
#PANCREATIC ACINUS	(47)	(46)	(46)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL	3 (6%)	1 (2%)	1 (2%)
#STOMACH	(46)	(49)	(49)
ULCER, FOCAL			1 (2%)
DEGENERATION, NOS	1 (2%)		
NECROSIS, FOCAL		1 (2%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, BASAL CELL		2 (4%)	
ACANTHOSIS		1 (2%)	
#GASTRIC MUCOSA	(46)	(49)	(49)
INFLAMMATION, ACUTE	1 (2%)		
#FORESTOMACH	(46)	(49)	(49)
ULCER, FOCAL	1 (2%)		
HYPERPLASIA, BASAL CELL		1 (2%)	
#COLON	(49)	(46)	(45)
NEMATODIASIS	5 (10%)	3 (7%)	4 (9%)
URINARY SYSTEM			
#KIDNEY	(47)	(47)	(50)
MINERALIZATION	1 (2%)		1 (2%)
HYDRONEPHROSIS		1 (2%)	
CYST, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL			1 (2%)
GLOMERULONEPHRITIS, MEMBRANOUS		1 (2%)	1 (2%)
NEPHROPATHY	32 (68%)	30 (64%)	20 (40%)
PIGMENTATION, NOS	2 (4%)	1 (2%)	4 (8%)
HEMOSIDEROSIS	1 (2%)	1 (2%)	5 (10%)
#KIDNEY/TUBULE	(47)	(47)	(50)
MINERALIZATION		1 (2%)	
PIGMENTATION, NOS	20 (43%)	28 (60%)	22 (44%)
HEMOSIDEROSIS		1 (2%)	
REGENERATION, NOS	1 (2%)	1 (2%)	
#KIDNEY/PELVIS	(47)	(47)	(50)
MINERALIZATION		1 (2%)	
HEMORRHAGE		1 (2%)	
#URINARY BLADDER	(49)	(43)	(48)
INFLAMMATION, ACUTE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(40)	(41)
CYST, NOS	10 (22%)	8 (20%)	3 (7%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEMORRHAGIC CYST		1 (3%)	
HEMOSIDEROSIS		1 (3%)	
HYPERPLASIA, FOCAL	1 (2%)	1 (3%)	
#ADRENAL	(49)	(46)	(47)
CYST, NOS			1 (2%)
CONGESTION, NOS	1 (2%)		
METAMORPHOSIS FATTY	1 (2%)	2 (4%)	
ANGIECTASIS	5 (10%)	4 (9%)	6 (13%)
#THYROID	(47)	(46)	(46)
HYPERPLASIA, C-CELL	3 (6%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(50)
GALACTOCELE	3 (6%)	5 (10%)	5 (10%)
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NOS	3 (6%)	1 (2%)	1 (2%)
*MAMMARY ACINUS	(49)	(49)	(50)
HYPERPLASIA, NOS	3 (6%)		
#UTERUS	(49)	(49)	(49)
MUCOCELE	1 (2%)		
HYDROMETRA	2 (4%)	3 (6%)	4 (8%)
HEMORRHAGE	1 (2%)		
ANGIECTASIS	1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(49)	(49)
HYPERPLASIA, FOCAL	1 (2%)		
HYPERPLASIA, CYSTIC	2 (4%)	1 (2%)	1 (2%)
#OVARY	(48)	(48)	(49)
CYST, NOS	3 (6%)	1 (2%)	2 (4%)
NECROSIS, FAT			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(45)	(47)	(48)
MALACIA			1 (2%)
INFARCT HEMORRHAGIC			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(49)	(50)
HYPERPLASIA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(49)	(49)	(50)
CYST, NOS	1 (2%)		
INFLAMMATION, NOS		1 (2%)	
*EPICARDIUM	(49)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
EPIDERMAL INCLUSION CYST	2 (4%)		
FIBROSIS		1 (2%)	1 (2%)
ALOPECIA		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
ABSCESS, CHRONIC	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(49)	(50)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
#LUNG	(50)	(49)	(50)
CONGESTION, NOS			2 (4%)
EDEMA, NOS			1 (2%)
HEMORRHAGE	1 (2%)		4 (8%)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL	1 (2%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(45)	(44)	(45)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#SPLEEN	(50)	(48)	(45)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#SPLENIC FOLLICLES	(50)	(48)	(45)
ATROPHY, NOS			2 (4%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MANDIBULAR L. NODE	(42)	(46)	(43)
PIGMENTATION, NOS			1 (2%)
#CERVICAL LYMPH NODE	(42)	(46)	(43)
HYPERPLASIA, NOS		1 (2%)	
#MEDIASTINAL L.NODE	(42)	(46)	(43)
ATROPHY, NOS		1 (2%)	
#PANCREATIC L.NODE	(42)	(46)	(43)
ATROPHY, NOS			1 (2%)
#MESENTERIC L. NODE	(42)	(46)	(43)
HEMORRHAGE		2 (4%)	6 (14%)
HEMORRHAGIC CYST	9 (21%)		
ATROPHY, NOS	2 (5%)		1 (2%)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#INGUINAL LYMPH NODE	(42)	(46)	(43)
PIGMENTATION, NOS	1 (2%)	4 (9%)	
HYPERPLASIA, NOS		1 (2%)	
#PEYER'S PATCH	(48)	(47)	(46)
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(23)	(17)	(20)
CYST, NOS	2 (9%)	1 (6%)	
NECROSIS, NOS			1 (5%)
ATROPHY, NOS			1 (5%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
PERIVASCULITIS	2 (4%)	1 (2%)	
#MESENTERIC L. NODE	(42)	(46)	(43)
LYMPHANGIECTASIS	1 (2%)	3 (7%)	
#MYOCARDIUM	(49)	(49)	(49)
DEGENERATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(47)
ABSCESS, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FOCAL	1 (2%)		
NECROSIS, HEMORRHAGIC		1 (2%)	
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	
#BILE DUCT	(50)	(48)	(47)
CYST, NOS	1 (2%)		
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#PANCREAS	(47)	(46)	(44)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	
ATROPHY, NOS		1 (2%)	1 (2%)
#PANCREATIC ACINUS	(47)	(46)	(44)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(49)	(47)	(46)
ECTOPIA	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, ACUTE FOCAL	1 (2%)		
NECROSIS, FOCAL	1 (2%)		
PIGMENTATION, NOS	1 (2%)		
#PEYER'S PATCH	(48)	(47)	(46)
HYPERPLASIA, NOS			1 (2%)
#JEJUNAL MUCOUS MEMBR	(48)	(47)	(46)
ULCER, FOCAL			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(48)
MINERALIZATION	1 (2%)		
GLOMERULONEPHRITIS, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	10 (21%)
NEPHROPATHY		1 (2%)	
GLOMERULOSCLEROSIS, NOS	1 (2%)		
INFARCT, HEALED			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
#KIDNEY/PELVIS	(50)	(49)	(48)
INFLAMMATION, ACUTE			1 (2%)
#URINARY BLADDER	(50)	(49)	(45)
INFLAMMATION, NECROTIZING		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
FIBROSIS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(43)	(41)
CYST, NOS	1 (3%)		
#THYROID	(47)	(46)	(47)
CYST, NOS	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(50)
CYST, NOS	2 (4%)		
EPIDERMAL INCLUSION CYST			1 (2%)
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#PROSTATE	(47)	(47)	(43)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, PAPILLARY			1 (2%)
*SEMINAL VESICLE	(50)	(49)	(50)
CYST, NOS			1 (2%)
#TESTIS	(49)	(48)	(47)
MINERALIZATION	3 (6%)	3 (6%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(45)	(47)	(49)
MINERALIZATION	2 (4%)		1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(50)
ABSCESS, NOS		1 (2%)	
*PERITONEUM	(50)	(49)	(50)
HEMOPERITONEUM		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
HEMORRHAGE		1	
NECROSIS, FOCAL	1		
NECROSIS, FAT		1	1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	8	9
ANIMAL MISSING/NO NECROPSY		1	

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING BUTYL BENZYL PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
FIBROSIS, FOCAL		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CONGESTION, NOS		2 (4%)	1 (2%)
HEMORRHAGE	2 (4%)		
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, FOCAL			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#BONE MARROW	(49)	(44)	(45)
MYELOFIBROSIS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC	3 (6%)	1 (2%)	1 (2%)
#SPLEEN	(50)	(48)	(48)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC	2 (4%)	1 (2%)	
#SPLENIC FOLLICLES	(50)	(48)	(48)
ATROPHY, NOS	1 (2%)	1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(39)	(42)	(39)
PIGMENTATION, NOS		1 (2%)	
#BRONCHIAL LYMPH NODE	(39)	(42)	(39)
INFLAMMATION ACUTE PUSTULAR	1 (3%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC L.NODE	(39)	(42)	(39)
HYPERPLASIA, NOS	1 (3%)		
#LUMBAR LYMPH NODE	(39)	(42)	(39)
HYPERPLASIA, NOS	1 (3%)		
#MESENTERIC L. NODE	(39)	(42)	(39)
HEMORRHAGIC CYST	1 (3%)		
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, RETICULUM CELL			1 (3%)
#LIVER	(50)	(50)	(49)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
#THYMUS	(20)	(22)	(19)
CYST, NOS		2 (9%)	
INFLAMMATION, ACUTE			1 (5%)
ATROPHY, NOS	1 (5%)		
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
PERIARTERITIS			1 (2%)
#HEART	(48)	(50)	(50)
ENDOCARDITIS, BACTERIAL	1 (2%)		
ABSCESS, NOS		1 (2%)	
#CARDIAC VALVE	(48)	(50)	(50)
NECROSIS, FIBRINOID	1 (2%)		
#AORTIC VALVE	(48)	(50)	(50)
NECROSIS, FOCAL			1 (2%)
#SUBMAXILLARY GLAND	(48)	(45)	(46)
ARTERIOLOSCLEROSIS		1 (2%)	
#OVARY	(44)	(47)	(46)
ARTERIOLOSCLEROSIS		1 (2%)	
DIGESTIVE SYSTEM			
#SUBMAXILLARY GLAND	(48)	(45)	(46)
NECROSIS, FOCAL		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(50)	(49)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (2%)		
INFARCT, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	
PIGMENTATION, NOS		1 (2%)	
#PANCREAS	(43)	(44)	(46)
DILATATION/DUCTS	1 (2%)		
CYSTIC DUCTS		1 (2%)	1 (2%)
INFLAMMATION, NOS		1 (2%)	
ABSCESS, NOS		1 (2%)	
ATROPHY, NOS		1 (2%)	
#PANCREATIC ACINUS	(43)	(44)	(46)
ATROPHY, NOS	2 (5%)		
ATROPHY, FOCAL			1 (2%)
#STOMACH	(47)	(45)	(44)
HYPERPLASIA, BASAL CELL			1 (2%)
ACANTHOSIS			1 (2%)
#ILEUM	(45)	(47)	(45)
ULCER, NOS			1 (2%)
ADHESION, NOS			1 (2%)
#ILEAL MUCOUS MEMBRAN	(45)	(47)	(45)
NECROSIS, NOS			1 (2%)
#COLON	(43)	(45)	(43)
NEMATODIASIS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(49)
MINERALIZATION			1 (2%)
HEMORRHAGE		1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	3 (6%)
ABSCESS, NOS		2 (4%)	
GLOMERULONEPHRITIS, CHRONIC	2 (4%)	1 (2%)	2 (4%)
NEPHROPATHY	1 (2%)		
#KIDNEY/TUBULE	(50)	(50)	(49)
PIGMENTATION, NOS			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REGENERATION, NOS		1 (2%)	
#KIDNEY/PELVIS	(50)	(50)	(49)
INFLAMMATION, CHRONIC	1 (2%)		
#URINARY BLADDER	(45)	(47)	(46)
INFLAMMATION, CHRONIC			1 (2%)
ADHESION, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(39)	(41)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL	(44)	(45)	(42)
METAMORPHOSIS FATTY			1 (2%)
#THYROID	(45)	(49)	(48)
CYSTIC FOLLICLES		1 (2%)	
HYPERPLASIA, EPITHELIAL	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(47)	(50)	(47)
HEMORRHAGE	1 (2%)		1 (2%)
INFLAMMATION, ACUTE			1 (2%)
ABSCESS, NOS	1 (2%)	1 (2%)	1 (2%)
#CERVIX UTERI	(47)	(50)	(47)
INFLAMMATION, CHRONIC	1 (2%)		
#UTERUS/ENDOMETRIUM	(47)	(50)	(47)
INFLAMMATION, ACUTE	3 (6%)	6 (12%)	4 (9%)
HYPERPLASIA, CYSTIC	31 (66%)	29 (58%)	36 (77%)
#ENDOMETRIAL GLAND	(47)	(50)	(47)
CYST, NOS	1 (2%)		
#OVARY	(44)	(47)	(46)
MINERALIZATION			2 (4%)
CYST, NOS	6 (14%)	5 (11%)	2 (4%)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
ABSCESS, NOS	4 (9%)	5 (11%)	3 (7%)
HYPERPLASIA, EPITHELIAL			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(46)	(43)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
#BRAIN	(50)	(46)	(43)
MINERALIZATION	1 (2%)	1 (2%)	3 (7%)
INFLAMMATION ACUTE PUSTULAR		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
ABSCESS, NOS	1 (2%)		
*INTERCOSTAL MUSCLE	(50)	(50)	(50)
INFLAMMATION ACUTE PUSTULAR			2 (4%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)	2 (4%)	1 (2%)
INFLAMMATION, ACUTE	2 (4%)	1 (2%)	2 (4%)
INFLAMMATION, CHRONIC		1 (2%)	
*MESENTERY	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
THORAX			
EMPYEMA		1	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
OMENTUM			
INFLAMMATION WITH FIBROSIS		1	
NECROSIS, FAT	1	1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	1	
AUTO/NECROPSY/HISTO PERF			1

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

APPENDIX E

ANALYSIS OF BUTYL BENZYL PHTHALATE
(Lot No. M2676)

MIDWEST RESEARCH INSTITUTE

APPENDIX E

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	73.06	6.45
Determined	73.12	6.44
	73.28	6.48

B. BOILING POINT

Determined	Literature Values
331° to 370°, at 742 mm Hg	370° at 760 mm Hg (Gillio-Tos and Vimercati, 1966

C. DENSITY

$d_4^{24.5}$ 1.1142 ± 0.0002 (δ)	d_{25}^{25} 1.115 (Gillio-Tos and Vimercati, 1966)

D. REFRACTIVE INDEX

n_D^{20} 1.5394	n_D^{25} = 1.535 (Gillio-Tos and Vimercati, 1966)

E. THIN-LAYER CHROMATOGRAPHY

Plates:

System 1: Silica gel G-25-UV254

System 2: Silica gel 60 F-254
Amount spotted: 100 and 300 μg
Ref. standard: Dimethyl terephthalate (12.6 mg/ml methanol)
Visualization: Ultraviolet, 254 nm. Sprayed with 20% resorcinol in ethanol containing 300 mg zinc chloride in 100 ml and then heated for 10 minutes at 150°C. The plate was then cooled and sprayed with 4N sulfuric acid and heated for 20 minutes at 120°C. Finally, the plate was sprayed with 40% aqueous potassium hydroxide.

System 1: Dichloromethane, 100%

R_f: 0.47
R_{st}: 1.19

System 2: Hexane: p-Dioxane (90:10)

R_f: 0.44
R_{st}: 0.76

F. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Tracor MT220

Detector: Flame ionization

Inlet temperature: 250°C

Detector temperature: 280°C

System 1

Column: 3% Dexsil 400 on 80/100 chromosorb W (AW), 1.8 m x 4 mm I.D., glass
Detector temperature: 280°C
Oven temperature program: 5 minutes at 150°C, then 150°C to 250°C at 10°C/minute
Sample injected: (6 μl) neat liquid, and 1.0% and 0.5% in methylene chloride to check for overloading and to quantitate the major peak
Results: Major peak and seven impurities

Peak	Retention Time (min)	Relative Retention Time	Relative Area (%)
1	4.7	0.57	0.1
2	4.8	0.58	0.2
3	5.8	0.70	0.06
4	7.6	0.91	0.4
5	8.0	0.96	0.2
6	8.3	1.0	100
7	9.8	1.2	0.4
8	11.0	1.3	0.4

System 2

Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm I.D., glass
Detector temperature: 276°C
Sample injected: (7 μl) neat liquid, and 1.0% and 0.5% in methylene chloride to check for overloading and to quantitate major peak
Oven Temperature program: 5 minutes at 150°C, then 150°C to 250°C at 10°C/minute
Results: Major peak and one impurity

Peak	Retention Time (min)	Relative Retention Time	Relative Area (%)
1	4.0	0.56	0.04
2	7.1	1.0	100

G. SPECTRAL DATA

(1) Infrared:

Instrument: Beckman IR-12
Cell: Neat sodium chloride plate
Results: See Figure 5

No literature spectrum found

(2) Ultraviolet/Visible:

Instrument: Cary 118

No literature values found. Experimental data are consistent with the structure.

λ max(nm)	$\varepsilon \times 10^{-3}$
281 s	1.17 ± 0.01 (δ)
275	1.30 ± 0.02 (δ)
269	1.20 ± 0.02 (δ)
264	1.16 ± 0.02 (δ)
257 s	1.31 ± 0.02 (δ)

(No absorbance between 350 and 800 nm (visible region)
Solvent: Methanol

(3) Nuclear magnetic resonance:
Instrument: Varian HA-100
Solvent: Chloroform-d with internal tetramethylsilane

No literature spectrum found. Experimental spectrum consistent with the structure.

Assignments (see Figure 6)

(a) t, (δ) = 0.88 ppm (J_{ab} = 7 Hz);
(b) m, (δ) 1.35 ppm;
(c) m, (δ) = 1.60 ppm;
(d) t, (δ) = 4.19 ppm, J_{cd} = 7 Hz;
(e) s, (δ) = 5.34 ppm;
(f) m, (δ) = 7.09 - 7.67 ppm;
(g) m, (δ) = 7.67 - 8.09 ppm;
(h) impurity, (δ) = 0.79 ppm

Integration Ratios:

(a + h) 3.01
(b + c) 4.01
(d) 2.15
(e) 2.01
(f) 6.81
(g) 2.01

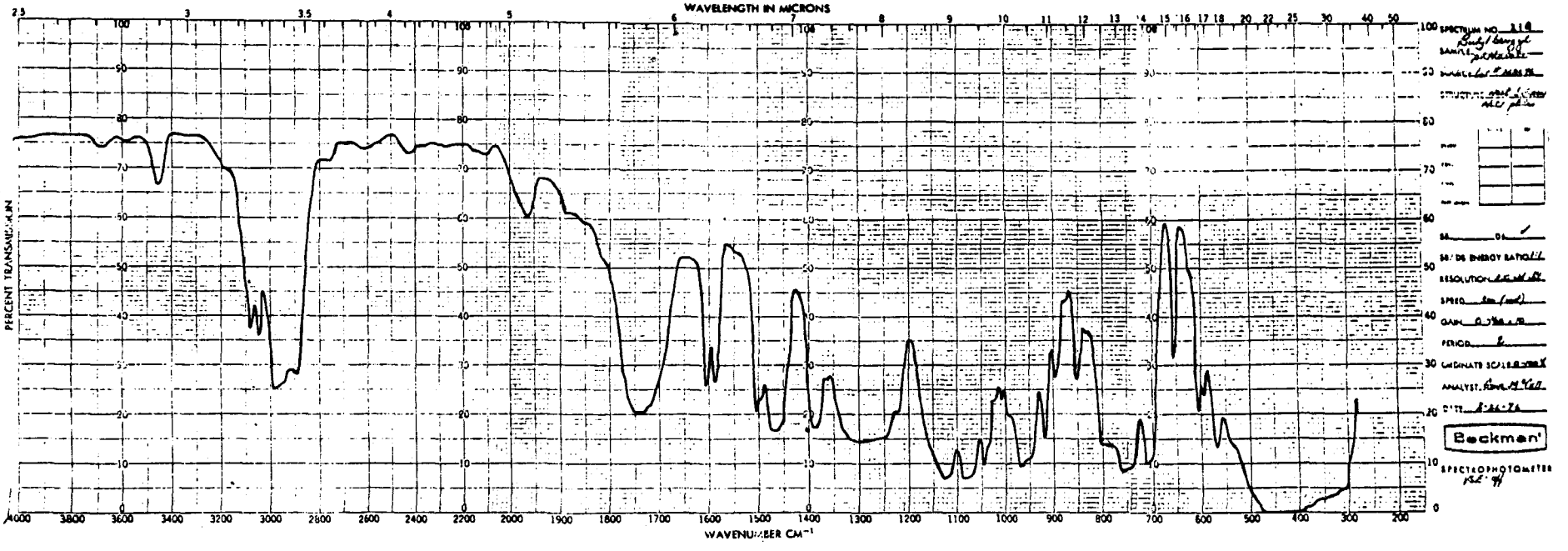


Figure 5. Infrared Absorption Spectrum of Butyl Benzyl Phthalate (Lot No. M2676)

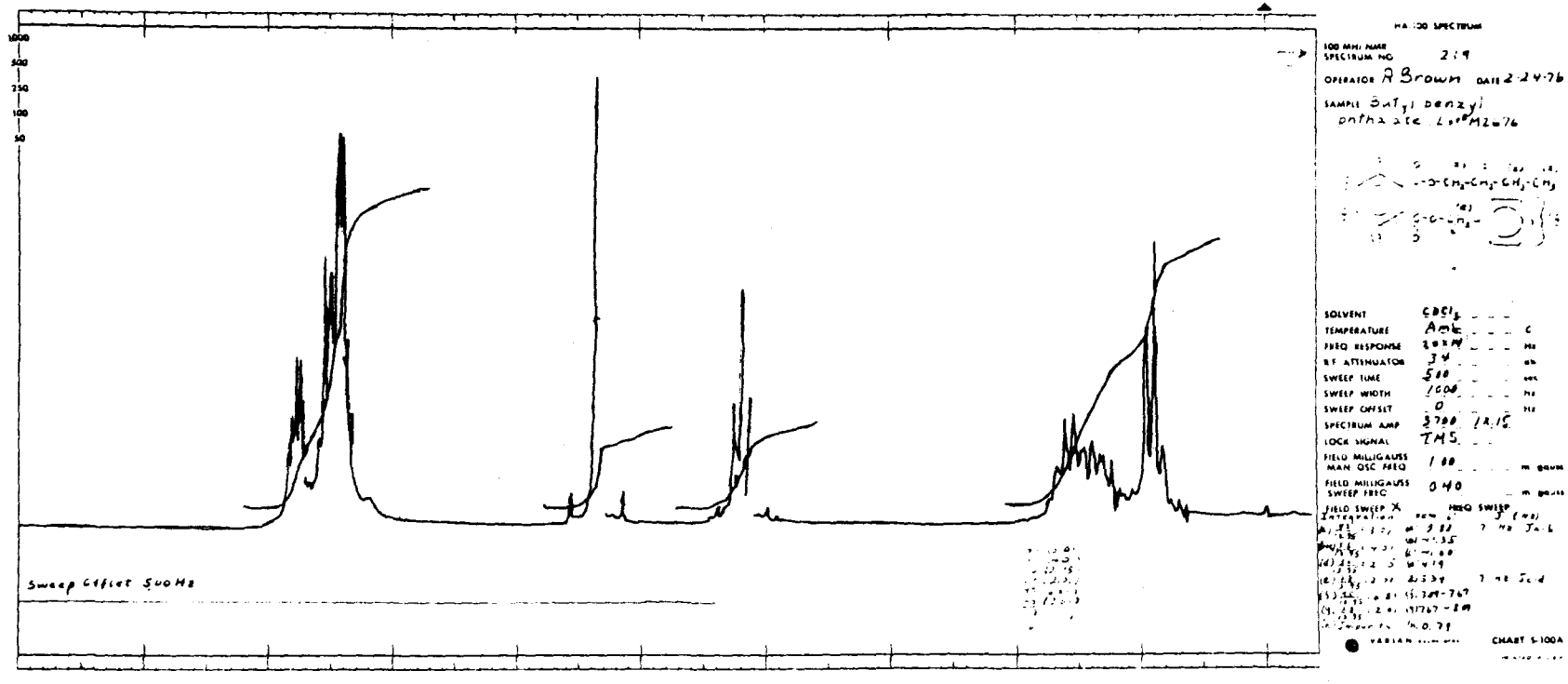


Figure 6. Nuclear Magnetic Resonance Spectrum of Butyl Benzyl Phthalate (Lot No. M2676)

APPENDIX F

ANALYSIS OF BUTYL BENZYL PHTHALATE
(Lot No. M81977)

MIDWEST RESEARCH INSTITUTE

APPENDIX F

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	73.06	6.45
Determined	73.73	6.43
	73.80	6.39

B. ESTER TITRATION

Free acid titration: No titratable free acidity

Ester titration: 97.20% ± 1.12% (Annual Book of ASTM Standards, 1974)

Samples of butyl benzyl phthalate were hydrolyzed for 2 to 16 hours at room temperature with potassium hydroxide. Test samples were titrated at 2-hr intervals to determine the time needed to complete hydrolysis. The 12-, 14-, and 16-hr samples indicated complete hydrolysis. The 14- and 16-hr samples were averaged to obtain the total ester value reported.

C. THIN-LAYER CHROMATOGRAPHY

Plates: Silica Gel G-25 UV_{254}

Amount Spotted: 100 and 300 g (10 mg/ml in methanol)

System 1:
Dichloromethane,100%
R_f: 0.394(major)
R_{st}: 1.25

System 2:
Hexane:p-dioxane(90:10)
R_f: 0.314(major), 0.215(trace)
R_{st}: 0.739,0.506(trace)

Ref. Standard: Dimethyl terephthalate (12.6 mg/ml methanol)

Visualization: Ultraviolet, 254 nm. Sprayed with 20% resorcinol in ethanol containing 300 mg zinc chloride in 100 ml and then heated for 10 min at 150°C. The plate was then cooled and sprayed with 4N sulfuric acid and heated for 20 min at 120°C. Finally, the plate was sprayed with 40% aqueous potassium hydroxide.

D. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Varian 3740
Detector: Flame ionization
Inlet temperature: 250°C
Carrier gas: Nitrogen
Carrier flow rate: 70 cc/min

System 1:

Column: 3% Dexsil 400 on 80/100 Chromosorb W(AW), 1.8 m x 4 mm I.D., glass
Detector temperature: 280°C
Oven temperature program: 5 min at 150°C, then 150° to 250°C at 10°C/min
Sample injected: (6 μl) neat liquid, and 1.0% and 0.5% in methylene chloride to check for overloading and quantitate the major peak.

Results: Major peak and 10 impurities. Three impurities had areas of 0.22%, 0.27%, and 1.19% of the major peak. The remaining seven impurities totaled less than 0.4% of the major peak.

Peak	Retention Time (min)	Retention Time (Relative to butyl benzyl phthalate)	Area (Percent) of butyl benzyl phthalate)
1	0.8	0.04	0.03
2	1.2	0.06	0.05
3	9.9	0.53	0.22
4	10.1	0.54	0.27
5	12.1	0.65	0.14
6	15.7	0.85	0.01
7	18.6	1.00	100
8	20.2	1.09	0.02
9	20.5	1.10	0.05
10	26.5	1.43	1.19
11	33.5	1.81	0.04

System 2

Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm I.D., glass
Detector temperature: 276°C
Oven temperature program: 5 min at 150°C, then 150° to 250°C at 10°C/min
Sample injected: (7 μl) neat liquid, and 1.0% and 0.5% in methylene chloride to check for overloading and quantitate major peak.
Results: Major peak and eight impurities. Two impurities had areas of 0.22% and 0.59% of the major peak area. The remaining six impurities had areas totaling less than 0.2% of the major peak area.

Peak	Retention Time (min)	Retention Time (Relative to butyl benzyl phthalate)	Area (Percent) of butyl benzyl phthalate)
1	2.8	0.11	0.01
2	4.4	0.17	0.02
3	8.0	0.31	0.01
4	14.7	0.57	0.22
5	17.0	0.66	0.07
6	24.3	0.94	0.01
7	25.8	1.00	100
8	31.4	1.22	0.03
9	48.8	1.89	0.59

E. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12
Cell: Neat liquid between silver chloride plates

Results: See Figure 7

No literature reference found. Spectrum is consistent with structure and IR previously done on Lot No. M2676, Batch No. 01, report dated 4/20/76.

(2) Ultraviolet/Visible

No absorbances between 800-350 nm
Instrument: Cary 118

λ max(nm)	ε x 10^{-3}
281 (shoulder)	1175.1 ± 4.2
274.5	1301.7 ± 6.3
268	1185.0 ± 5.6
263.8	1151.5 ± 5.6
257 (shoulder)	1309.7 ± 4.8
230 (shoulder)	8604.8 ± 18.4

Solvent: Methanol

No literature data found. Spectrum is consistent with UV/Vis previously done on Lot No. M2676, Batch No. 01, report dated 4/20/76.

(3) Nuclear Magnetic Resonance

Instrument: Varian HA-100
Solvent: Chloroform-d-with internal tetramethylsilane

No literature reference found. Spectrum consistent with structure and NMR previously done on Lot No. M2676, report dated 4/20/76.

Assignments: (See Figure 8)

(a) t, (δ) = 0.85 ppm (J_{a-b} = 7 Hz)
(b) m, (δ) = 1.30 ppm
(c) m, (δ) = 1.52 ppm (J_{cd} = 7 Hz)
(d) t, (δ) = 4.12 ppm
(e) s, (δ) = 5.26 ppm
(f) m, (δ) = 7.14-7.44 ppm
(g) m, (δ) = 7.54-7.72 ppm

Integration Ratios:

(a) 2.82
(b+c) 4.22
(d) 1.90
(e) 1.90
(f) 6.97
(g) 2.18

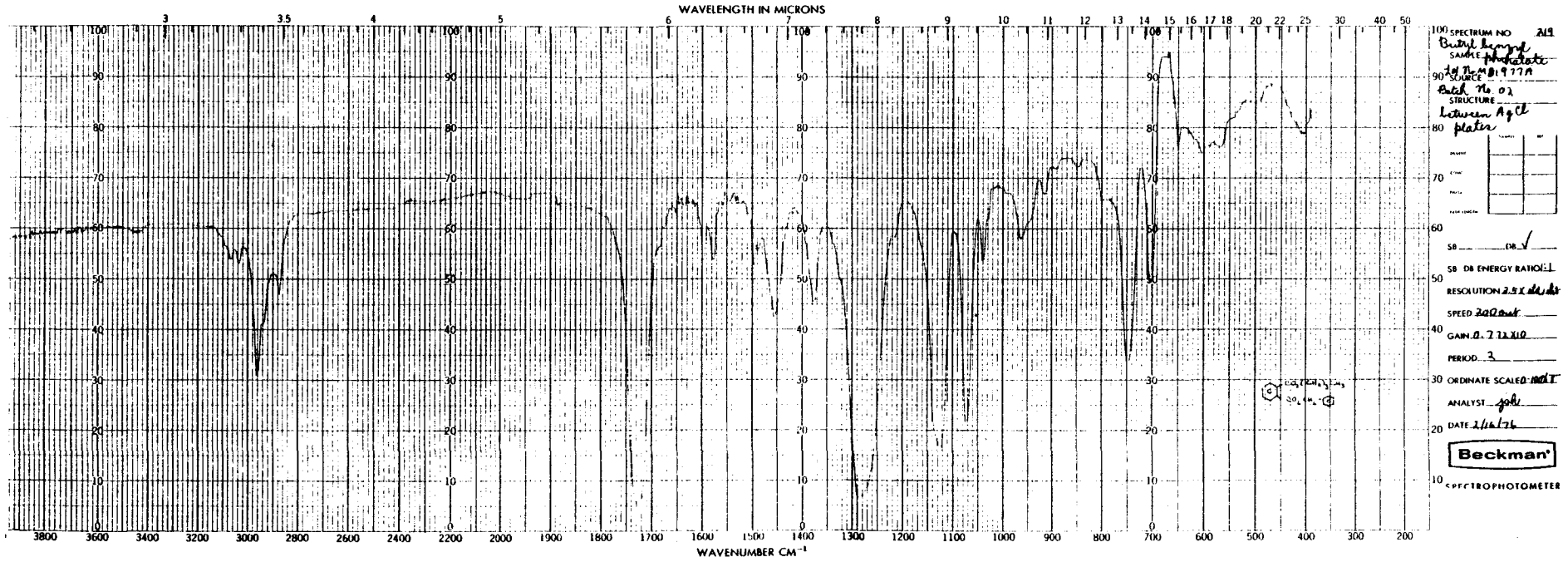


Figure 7. Infrared Absorption Spectrum of Butyl Benzyl Phthalate (Lot No. M81977)

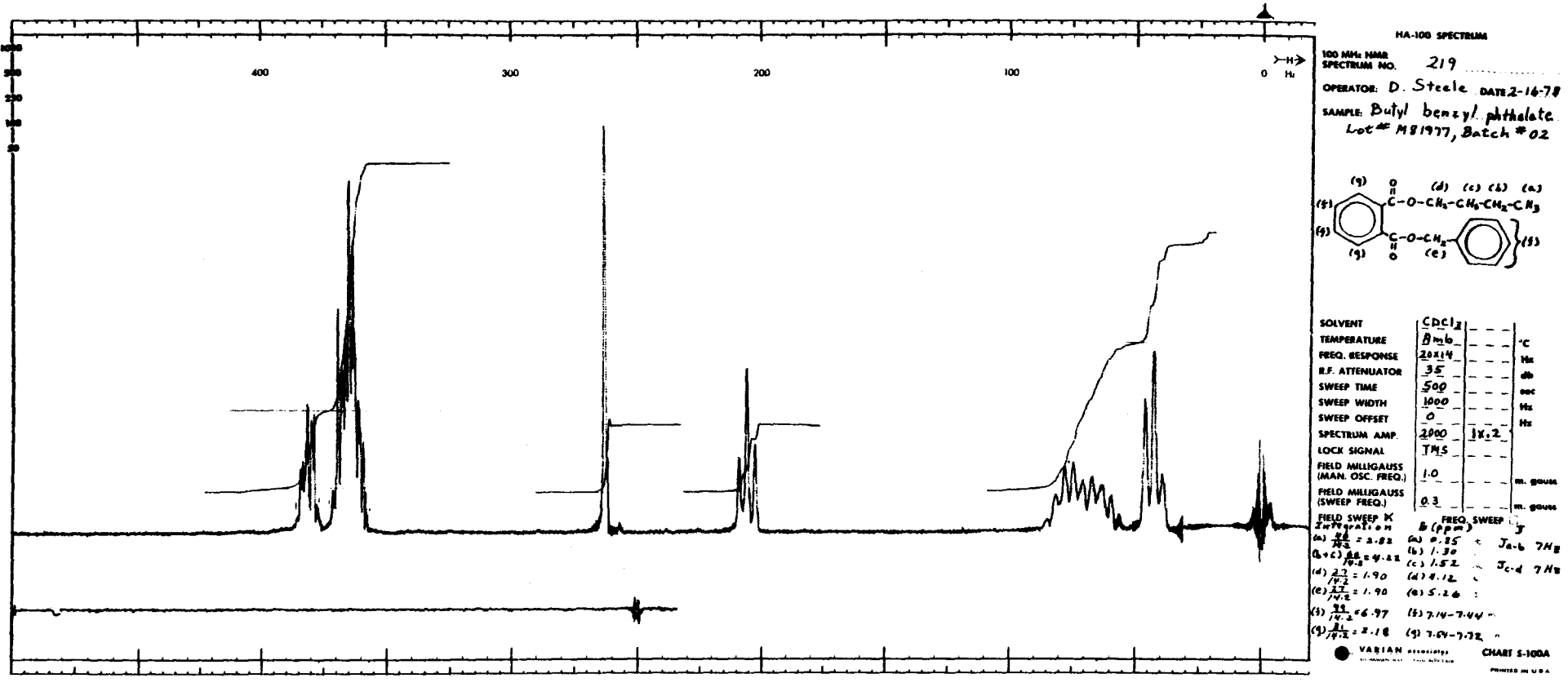


Figure 8. Nuclear Magnetic Resonance Spectrum of Butyl Benzyl Phthalate (Lot No. M81977)

APPENDIX G

STABILITY ANALYSIS OF BUTYL BENZYL PHTHALATE IN FORMULATED DIETS

(MIDWEST RESEARCH INSTITUTE)

1. Mixing and storage: Butyl benzyl phthalate (2.5010 g) and Wayne Lab-Blox® Rodent Feed (22.5011 g) were mixed in a mortar. Samples of the mixture were then removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively. These samples were analyzed by vapor-phase chromatography as described below.

2. Extraction and analysis: One-gram samples of each of the above stability mixtures were triturated twice with 50-ml portions of methanol. The supernatant solutions were combined and diluted to 100 ml with methanol in a volumetric flask, and this constituted the test solution.

Instrument: Bendix 2500
Column: 3% OV-17 on 80/100 Supelcoport glass, 1.8 m, 4 mm I.D.
Detection: Flame ionization
Oven temperature: 230°C, isothermal
Detector temperature: 255°C
Inlet temperature: 250°C

3. Results:

Sample (°C)	Average % Compound Recovered *(a)*
-20	10.0±.3
5	9.6±.3
25	9.8±.3
45	9.4±.3

(a) Corrected for a spike recovery value of 96.2%. Theoretical expected value, 10.0%.

There was no significant difference between the samples stored at the various temperatures.

4. Conclusion: Butyl benzyl phthalate mixed with feed is stable for 2 weeks at temperatures of up to 45°C.

APPENDIX H

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF BUTYL BENZYL PHTHALATE

Two-gram samples were extracted with 50 ml of methanol. The net ultraviolet absorbances of the supernatant extracts were determined at 275 nm and corrected for recovery with spiked controls.

Theoretical Concentration (ppm)	Number of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
6,000	13	5,681	4.99	5,200- 6,350
12,000	15	11,493	6.17	10,200-12,900

☆U.S. GOVERNMENT PRINTING OFFICE: 1982-361-132:671

NIH Publication No. 82-1769
August 1982