National Toxicology Program
Toxicity Report Series
Number 30

NTP Technical Report
on Toxicity Studies of

Dibutyl Phthalate

(CAS No. 84-74-2)

Administered in Feed
to F344/N Rats and B6C3F$_1$ Mice

Daniel S. Marsman, D.V.M., Ph.D., Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 95-3353
March 1995

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund (Superfund) by an interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

Note to the Reader

The National Toxicology Program (NTP) is made up of four charter agencies of the United States Department of Health and Human Services (DHHS):

- the National Cancer Institute (NCI) of the National Institutes of Health;
- the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health;
- the National Center for Toxicological Research (NCTR) of the Food and Drug Administration; and
- the National Institute for Occupational Safety and Health (NIOSH) of the Centers for Disease Control.

In July 1981, the Carcinogenesis Bioassay Testing Program was transferred from NCI to NIEHS. NTP coordinates the relevant Public Health Service programs, staff, and resources that are concerned with basic and applied research and with biological assay development and validation.

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

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

The studies described in this toxicity study report were performed under the direction of NIEHS and were conducted in compliance with NTP laboratory health and safety requirements. These studies met or exceeded all applicable federal, state, and local health and safety regulations. Animal care and use were in accord and compliance with the Public Health Service Policy on Humane Care and Use of Animals.

Single copies of this report are available without charge, while supplies last, from the NTP Central Data Management (telephone number 919/541-1371).

NTP Central Data Management
NIEHS
Post Office Box 12233
Research Triangle Park, NC 27709

National Toxicology Program
Toxicity Report Series
Number 30

NTP Technical Report
on Toxicity Studies of

Dibutyl Phthalate

(CAS No. 84-74-2)

Administered in Feed
to F344/N Rats and B6C3F$_1$ Mice

Daniel S. Marsman, D.V.M., Ph.D., Study Scientist
National Toxicology Program
Post Office Box 12233
Research Triangle Park, NC 27709

NIH Publication 95-3353
March 1995

These studies were supported in part by funds from the Comprehensive Environmental Response, Compensation, and Liability Act trust fund (Superfund) by an interagency agreement with the Agency for Toxic Substances and Disease Registry, U.S. Public Health Service.

United States Department of Health and Human Services
Public Health Service
National Institutes of Health

CONTRIBUTORS

This NTP report on the toxicity studies of dibutyl phthalate is based primarily on studies that took place from October 1987 through November 1989.

National Toxicology Program
Evaluated experiment, interpreted results, and reported findings

Daniel S. Marsman, D.V.M., Ph.D., Study Scientist
John R. Bucher, Ph.D.
Robert E. Chapin, Ph.D.
Michael R. Elwell, D.V.M., Ph.D.
Joel Mahler, D.V.M.
H. B. Matthews, Ph.D.
Joseph H. Roycroft, Ph.D.
Michael D. Shelby, Ph.D.
Gregory S. Travlos, D.V.M.
Kristine L. Witt, M.S.
Oak Ridge Associated Universities

Battelle Columbus Laboratories
Principal contributors

Perry J. Kurtz, Ph.D., Principal Investigator
Arthur C. Peters, D.V.M., Principal Investigator
Joanne M. Killinger, Ph.D.
Lawrence E. Mezza, D.V.M.
Ronald L. Persing, D.V.M.
Michael J. Ryan, D.V.M., Ph.D.
John D. Toft II, D.V.M., M.S.

NTP Pathology Working Group
Evaluated slides and prepared pathology report

Paul K. Hildebrandt, D.V.M., Chair
PATHCO, Inc.
Michael R. Elwell, D.V.M., Ph.D.
National Toxicology Program
Jeffrey Everitt, D.V.M.
Chemical Industry Institute of Toxicology
William F. MacKenzie, D.V.M., M.S.
Experimental Pathology Laboratories, Inc.
Joel Mahler, D.V.M.
National Toxicology Program
Cynthia C. Shackelford, D.V.M., M.S., Ph.D.
National Toxicology Program

Experimental Pathology Laboratories, Inc.
Provided pathology quality assessment

John Peckham, D.V.M., M.S., Ph.D.
Gary Riley, M.V.Sc., Ph.D.

Environmental Health Research and Testing, Inc.
Provided sperm morphology, vaginal cytology evaluation, and reproductive assessment by continuous breeding (rats)

Leta Hommel Barnes
Teresa Cocanougher, B.A.
Dushyant K. Gulati, Ph.D.
Susan Russell, B.A.

Research Triangle Institute
Provided reproductive assessment by continuous breeding (mice)

James C. Lamb IV, Ph.D.
A. Davis Lawton, M.S.
Jerry R. Reel, Ph.D.

Analytical Sciences, Inc.
Provided statistical analyses

Sarah R. Kennedy, M.S.
Steven Seilkop, M.S.
Janet L. Teague, M.S.

Biotechnical Services, Inc.
Provided toxicity report preparation

C. Michael Bailey, B.S. Pharm., Principal Investigator
Janet L. Elledge, B.A.
Daphne D. Lambright, Ph.D.
Waynette D. Sharp, B.A., B.S.

PEER REVIEW

The draft report on the toxicity studies of dibutyl phthalate was evaluated in July 1994 by the reviewers listed below. These reviewers serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, reviewers determine if the design and conditions of these NTP studies are appropriate and ensure that the toxicity study report presents the experimental results and conclusions fully and clearly.

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

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

TABLE OF CONTENTS

ABSTRACT 5

INTRODUCTION 13
Physical and Chemical Properties, Production, Use, and Exposure 13
Metabolism and Disposition 14
Toxicity 15
Study Rationale and Design 19

MATERIALS AND METHODS 23
Procurement and Characterization of Dibutyl Phthalate 23
Dose Formulations 23
Toxicity Study Designs 24
Genetic Toxicity Studies 34
Statistical Methods 36
Quality Assurance 37

RESULTS 39
Maximum Perinatal Exposure Determination Feed Study in F344/N Rats 39
13-Week Feed Study with Perinatal Exposure in F344/N Rats 45
13-Week Feed Study in F344/N Rats 62
Continuous Breeding Study in Sprague-Dawley Rats 71
Perinatal Peroxisome Determination Studies in F344/N Rats 73
Maximum Perinatal Exposure Determination Feed Study in B6C3F_1 Mice 75
13-Week Feed Study in B6C3F_1 Mice 81
Continuous Breeding Study in Swiss (CD-1®) Mice 86
Genetic Toxicity 86

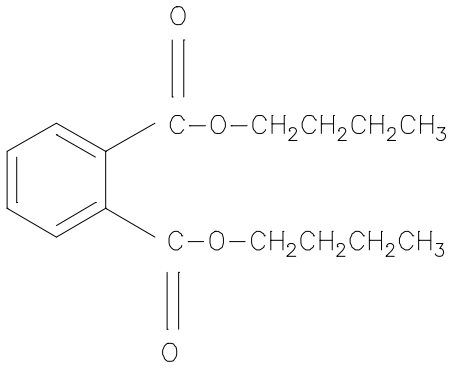
DISCUSSION 87

REFERENCES 93

APPENDIXES
Appendix A Summary of Nonneoplastic Lesions in Rats and Mice A-1
Appendix B Organ Weights and Organ-Weight-to-Body-Weight Ratios B-1
Appendix C Hematology and Clinical Chemistry Results C-1
Appendix D Reproductive Tissue Evaluations and Estrous Cycle Characterization D-1
Appendix E Continuous Breeding Studies E-1
Appendix F Perinatal Peroxisome Determination Studies F-1
Appendix G Genetic Toxicology G-1

ABSTRACT

Dibutyl Phthalate



CAS Number	84-74-2
Molecular Formula	$C_{16}H_{22}O_4$
Molecular Weight	278.35

Synonyms: 1,2-Benzenedicarboxylic acid dibutyl ester; benzene-*o*-dicarboxylic acid di-n-butyl ester; *o*-benzenedicarboxylic acid dibutyl ester; butyl phthalate; n-butyl phthalate; DBP; dibutyl 1,2-benzene dicarboxylate; dibutylphthalate; di-n-butylphthalate; di(*n*-butyl) phthalate; dibutyl-*o*-phthalate; phthalic acid dibutyl ester

Trade Names: Celluflex DBP; Elaol; Ergoplast FDB; Ersoplast FDA; Genoplast B; Hexaplas M/B; Palatinol C; Polycizer DBP; PX 104; RC Plasticizer DBP; Staflex DBP; Uniflex DBP; Unimoll DB; Witcizer 300; Witicizer 300

Dibutyl phthalate is a phthalate ester with extensive use in industry in such products as plastic (PVC) piping, various varnishes and lacquers, safety glass, nail polishes, paper coatings, dental materials, pharmaceuticals, and plastic food wrap. Concomitant with this extensive worldwide use is the high potential for human exposure to dibutyl phthalate in the workplace and the home environment through direct sources as well as indirectly, through contamination of water, air, and foodstuffs. Because existing toxicity information was considered inadequate, the effects of exposure to dibutyl phthalate were examined in male and female F344/N rats and B6C3F$_1$ mice in 13-week feed studies. Furthermore, due to concern over the potential for pervasive exposure of humans to dibutyl phthalate, additional perinatal studies examined rats and mice exposed as pups *in utero*, for the 4 weeks of lactation, and for an additional 4 weeks postweaning. Additional studies examined the effects on rats of combining perinatal and adult subchronic exposure. Due to the recognized biologic activity of this and other phthalates, hepatic peroxisome proliferation during the *in utero* and lactational phases and testicular toxicity

during the perinatal period were also examined. Finally, reproductive assessment by continuous breeding (including crossover mating trials and offspring assessment) and genetic toxicity studies were also conducted.

In the maximum perinatal exposure (MPE) determination study in rats, dibutyl phthalate was administered in the diet to dams during gestation and lactation, and to the pups postweaning for four additional weeks, at concentrations of 0, 1,250, 2,500, 5,000, 7,500, 10,000, and 20,000 ppm. Decreased weight gains were noted in dams exposed to 20,000 ppm during gestation and to dams exposed to 10,000 ppm during lactation. The gestation index (number of live pups per breeding female) was significantly lower in the 20,000 ppm group than in the controls, and pup mortality in this group was marked (100% by Day 1 of lactation); however, survival was 89% or greater in all other treatment groups. The mean body weight of pups in the 10,000 ppm group at Day 28 of lactation was approximately 90% of the mean weight of control pups. Pups were weaned onto diets containing dibutyl phthalate at the same concentrations fed to dams. After an additional 4 weeks of dietary administration, final mean body weights of pups in the 10,000 ppm groups were 92% of the control value for males and 95% of the control value for females. Hepatomegaly (increased relative liver weight) was observed in males in all exposed groups and in females receiving 2,500 ppm or greater. No gross lesions were observed at necropsy. Moderate hypospermia of the epididymis was diagnosed in all male rats in the 7,500 and 10,000 ppm groups; mild hypospermia of the epididymis was diagnosed in 2 of 10 males in the 5,000 ppm group. No degeneration of the germinal epithelium was detected in the testis of these rats. Thus, although toxicologically important, the epididymal hypospermia was not considered to be life threatening, and 10,000 ppm was recommended as the MPE concentration for male and female rats.

In the subsequent subchronic toxicity study of dibutyl phthalate with perinatal exposure, dams were administered diet containing 0 or the MPE concentration (10,000 ppm) during gestation and lactation, and weaned pups were administered the same diets as their dams received for an additional 4 weeks, until the beginning of the 13-week exposure phase. Male and female rats then received diets containing dibutyl phthalate at concentrations of 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm for 13 weeks. No mortality or toxicity was observed in dams during the perinatal phase of the study; however, before pups were culled at 4 days postpartum, the percentage of live pups per litter was 86% to 93% that of the controls. Through weaning, litter

weights of exposed pups ranged from 89% to 92% of the control values. Ten control and ten exposed pups per sex were examined at the time of weaning; hepatomegaly and markedly increased peroxisomal enzyme activities (approximately 19-fold greater than the control values) were observed in exposed pups. Body weights of the perinatally exposed pups remained lower than those of the controls throughout the 4-week period before the 13-week adult exposures began.

During the 13-week adult exposure phase, the final mean body weight of males in the MPE:0 ppm control group (MPE rats, returned to the base diet for 13 weeks), was 95% that of the controls. The body weight gain of females in the MPE:0 ppm group was greater than that of the unexposed controls, and the final body weights of these two groups were similar. Body weight gains of rats treated with dibutyl phthalate as adults decreased with increasing exposure concentration; for rats that received the MPE concentration followed by 40,000 ppm for 13 weeks, final body weights were 51% of the control value for males and 74% of the control value for females. Hepatomegaly apparently regressed in rats in the MPE:0 ppm groups but was observed in male rats receiving 5,000 ppm or greater and in females receiving 2,500 ppm or greater. In males that received 20,000 ppm as adults, testis and epididymal weights were less than in the controls; males in the 40,000 ppm group also had a lower testis weight than the controls. Results of hematologic analyses conducted at the end of the 13-week exposure period suggested a mild anemia in male rats administered 10,000 ppm or greater as adults and female rats administered 40,000 ppm as adults. Hypocholesterolemia and hypotriglyceridemia were observed in male and female rats at the higher exposure concentrations. Hypotriglyceridemia was detected in females receiving 20,000 or 40,000 ppm and in males receiving 10,000 ppm or greater. Elevations in alkaline phosphatase activities and bile acid concentrations in male and female rats receiving 20,000 or 40,000 ppm as adults were indicative of cholestasis. Microscopic examination revealed hepatocellular cytoplasmic alteration, consistent with glycogen depletion, in male and female rats receiving a concentration of 10,000 ppm or greater. In the liver of rats receiving 40,000 ppm, small, fine, eosinophilic granules were also observed in the cytoplasm of hepatocytes. Ultrastructural examination suggested the presence of increased numbers of peroxisomes. Lipofuscin accumulation was detected in rats that received 10,000 ppm or greater. Consistent with the regression of the hepatomegaly in rats in the MPE:0 and MPE:2,500 ppm groups, peroxisomal enzyme activity was not elevated in these groups. Marked elevations of peroxisomal enzyme activity were

detected, however, in males receiving 5,000 ppm or greater and in females receiving 10,000 ppm or greater; at the 40,000 ppm concentration, the highest concentration tested, enzyme activities were approximately 20-fold greater than the control values. Histopathologic examination of the testes revealed degeneration of the germinal epithelium, a mild to moderate focal lesion in rats in the 10,000 and 20,000 ppm groups and a marked, diffuse lesion in all males receiving 40,000 ppm; at 40,000 ppm, an almost complete loss of the germinal epithelium resulted. Testicular zinc concentrations were lower in the 40,000 ppm group than in the controls, a finding consistent with the marked loss of germinal epithelium at this exposure concentration. Spermatogenesis was evaluated in rats in the 0, 2,500, 10,000, and 20,000 ppm groups; rats administered 20,000 ppm had fewer spermatid heads per testis than the unexposed controls, and epididymal spermatozoal concentration was less than that in the MPE:0 ppm group.

For comparison with the perinatal subchronic study, a standard 13-week evaluation of the toxicity of dibutyl phthalate in male and female rats was also conducted. In this study, rats received dibutyl phthalate at the same dietary concentrations used in the 13-week exposure phase of the study with perinatal exposure: 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm. No deaths occurred in the standard study. Markedly reduced final mean body weights were observed in males and females in the 40,000 ppm groups (45% and 73% of control body weights, respectively); final mean body weights of males receiving 10,000 ppm or greater and females receiving 20,000 ppm or greater were lower than those of the controls. Hepatomegaly was observed in males that received 5,000 ppm or greater and in females that received 10,000 ppm or greater. Testis and epididymal weights of males in the 20,000 and 40,000 ppm groups were lower than those of the controls. A minimal anemia was detected in male rats receiving 5,000 ppm or greater. Hypocholesterolemia was observed in male and female rats receiving 20,000 or 40,000 ppm, and hypotriglyceridemia was detected in males in all exposed groups and in females receiving 10,000 ppm or greater. Elevations in alkaline phosphatase activity and bile acid concentration in male and female rats were considered indicative of cholestasis. Morphologic evaluation again confirmed the toxicity of dibutyl phthalate to the liver and testes of rats. Microscopic examination of the liver revealed hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in male and female rats receiving 10,000 ppm or greater. In the liver of rats in the 40,000 ppm groups, small, fine, eosinophilic granules were also observed in the cytoplasm of hepatocytes. Ultrastructural examination suggested the presence of increased numbers of peroxisomes, and peroxisomal enzyme activity was elevated in the

livers of male and female rats administered 5,000 ppm or greater; the enzyme activities in the 40,000 ppm groups were approximately 13-fold greater than the control value for males and 32-fold greater than the control value for females. Lipofuscin accumulation was detected in rats receiving 10,000 ppm or greater. Histopathologic examination of the testes revealed degeneration of the germinal epithelium, a mild to marked focal lesion in the 10,000 and 20,000 ppm groups and a marked, diffuse lesion in all males in the 40,000 ppm group; at 40,000 ppm, an almost complete loss of the germinal epithelium resulted. Testicular zinc concentrations were lower in the 20,000 and 40,000 ppm groups than in the controls. Serum testosterone values were also lower at these concentrations than in the controls. Spermatogenesis was evaluated in males in the 0, 2,500, 10,000, and 20,000 ppm groups; at 20,000 ppm, spermatid heads per testis and per gram testis, epididymal spermatozoal motility, and the number of epididymal spermatozoa per gram epididymis were lower than in the controls. All of these findings are consistent with the marked loss of germinal epithelium at these exposure concentrations.

In the continuous breeding study, Sprague-Dawley rats received 0, 1,000, 5,000, or 10,000 ppm dibutyl phthalate in feed. Mean body weights of exposed dams at delivery and during lactation generally decreased with increasing exposure concentration. The mean pup weight at birth in the 10,000 ppm group was significantly lower than the control pup weight. The average number of live pups per litter in all exposed groups was lower than in the controls. Crossover mating trials in the F_0 generation revealed no effects on the fertility of male or female rats receiving 10,000 ppm. In contrast to the F_0 rats, mating, pregnancy, and fertility indices of F_1 rats were lower in the 10,000 ppm group than in the controls. Germinal epithelial degeneration of the testes and absence or underdevelopment of the epididymides were noted in F_1 males in the 10,000 ppm group. Interstitial cell hyperplasia was noted in 7 of 10 males in the 10,000 ppm group. These effects document the male and female reproductive toxicity of dibutyl phthalate in F_1 rats receiving 10,000 ppm and do not exclude the possibility of developmental toxicity to F_2 offspring.

In the MPE determination study in mice, dams received 0, 1,250, 2,500, 5,000, 7,500, 10,000, or 20,000 ppm dibutyl phthalate in feed during gestation and lactation; pups were weaned onto the same diets as the dams received and were exposed for an additional 4 weeks. The gestation period was longer in dams that received 2,500 ppm or greater than in the controls, and

gestational body weight gain depressions were noted in dams receiving 7,500 ppm or greater. Only 5 of 20 females in the 10,000 ppm group delivered live pups, and none of the 20 females receiving 20,000 ppm delivered live pups. Only one pup in the 10,000 ppm group survived past Lactation Day 1; the number of live pups per litter in the 7,500 ppm group also remained low throughout lactation. No deaths of either male or female pups occurred after weaning. Initial (postweaning) and final body weights of male pups receiving 2,500 ppm or greater were significantly less than those of the control group. The mean body weights of exposed female pups were similar to the control body weight at weaning and remained similar throughout the 4 weeks postweaning. Hepatomegaly was present in male mice in all exposed groups, and the absolute liver weight of males administered 7,500 ppm was greater than that of the controls; although a similar change was apparent in females, no statistical differences between the liver weights of exposed and control females were detected. No treatment-related gross lesions were identified at necropsy, and no histopathologic lesions definitively associated with treatment were observed in male or female mice in the 7,500 ppm groups. The one surviving male pup in the 10,000 ppm group had cytoplasmic alteration in the liver, consistent with peroxisome proliferation. Developmental toxicity and fetal and pup mortality were suggested at concentrations as low as 7,500 ppm. No subchronic toxicity study with prior MPE exposure was conducted with mice, although an MPE concentration of 5,000 ppm was suggested by the data.

In a standard 13-week toxicity study, mice received 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm dibutyl phthalate in feed. No deaths occurred during this study. Mean body weights and weight gains of male and female mice decreased with increasing exposure concentration, and the decreases were significant for males and females that received 5,000 ppm or greater. Relative liver weights were greater in males and females receiving 5,000 ppm or greater than in the controls. A minimal anemia was suggested in female mice in the 20,000 ppm group. Although no gross lesions were observed at necropsy, microscopic examination revealed hepatocellular cytoplasmic alterations, consistent with glycogen depletion, in male mice receiving 10,000 or 20,000 ppm and female mice receiving 20,000 ppm. Small, fine, eosinophilic granules, consistent with peroxisome proliferation, were also observed in the cytoplasm of hepatocytes in males and females in the 20,000 ppm groups. Lipofuscin accumulation in the liver was detected in mice receiving 10,000 ppm or greater.

In a continuous breeding study using Swiss (CD-1®) mice, animals received 0, 300, 3,000, or 10,000 ppm dibutyl phthalate in feed. The fertility index, average number of litters per breeding pair, and average number of live pups per litter in the 10,000 ppm group were lower than in the controls. Crossover mating trials of mice receiving 10,000 ppm revealed effects on dams in the F_0 generation, with a lower fertility index, number of live pups per litter, and pup weight than in the controls. Liver weights were greater in males and females, and the uterine weight was less in exposed dams than in the controls. No other changes were observed at necropsy or on histopathologic examination. These data document the female reproductive toxicity of dibutyl phthalate in F_0 mice.

Dibutyl phthalate was not mutagenic in *Salmonella typhimurium* strain TA98, TA100, TA1535, or TA1537 with or without exogenous metabolic activation but did induce mutations in L5178Y mouse lymphoma cells treated without metabolic activation. In peripheral blood samples obtained from male and female mice at the end of the 13-week study, frequencies of micronucleated normochromatic erythrocytes were similar between exposed and control mice.

Together, the studies in rodents suggest that young rodents (*in utero* and perinatal) respond in a manner qualitatively similar to that of adult rats and mice. Dibutyl phthalate induced toxic effects in rodents as pups *in utero* and during the lactational phases of development and also affected young adults, as evidenced by fetotoxicity and lethality, body weight gain decrements, increased liver weights, hepatic peroxisome proliferation, testicular toxicity, and female reproductive toxicity. Dibutyl phthalate was lethal to rat fetuses and rat and mouse neonates at dietary concentrations that were not toxic to dams. Otherwise, there was no teratogenic or morphologic evidence that rodent young were uniquely sensitive to the effects of short-term dibutyl phthalate treatment.

INTRODUCTION

Physical and Chemical Properties, Production, Use, and Exposure

Dibutyl phthalate, a phthalate ester with extensive world-wide use in industry, is a colorless, oily liquid with a strong, bitter taste and a slight, aromatic odor (USEPA, 1985; Grant, 1986). Dibutyl phthalate has a specific gravity of 1.0459 at 20° C and a boiling point of 340° C (*Merck Index*, 1983). It is very soluble in acetone, benzene, alcohol, and ether (*Merck Index*, 1983), but only slightly soluble in water (13 mg/L at 25° C, or 11.2 mg/L at 20° C) (USEPA/ECAO, 1980; Howard, 1985).

Dibutyl phthalate is used extensively in the manufacture of plastics (such as PVC piping and carpet backing), various paints, varnishes and lacquers, medical supplies (such as transfusion and dental materials), safety glass for automobiles, food packaging, cosmetics (such as nail polishes and perfume oils), textiles (as a lubricant and as an insect repellant for impregnation in clothing), and paper coatings (RTECS, 1994). Although used as an insect repellant, dibutyl phthalate is not considered to be as effective as dimethyl phthalate (Martin and Worthing, 1974). Because of this worldwide use, there is a high potential for human exposure through direct sources in the workplace and the home environment. In addition, numerous indirect sources exist in water, air, and foodstuffs due to the pervasiveness of dibutyl phthalate in the environment and by contamination through leachable products. The potential for long-term human exposure to environmental chemicals such as pesticides and plasticizers, particularly *in utero* and in neonates, has raised additional concerns (NRC, 1993).

Phthalate esters are known to leach from finished plastics into blood and into milk and other foodstuffs and thereby enter the human body (Guess *et al.*, 1967). It has been estimated that 150 mg of dibutyl phthalate will migrate into 1 kg of cheese with a 15% fat content (Overcash, 1982). Dibutyl phthalate has been identified in plasma and tissues of patients undergoing hemodialysis or receiving blood transfusions (Ching *et al.*, 1981).

Production of dibutyl phthalate in the United States in 1977 and 1982 was 7,720,000 kg (RTECS, 1994). Additionally, 747 kg dibutyl phthalate was imported in the U.S. in 1977 and 303 kg was imported in 1982 (RTECS, 1994). Dibutyl phthalate is produced by 36 companies at 49 sites in the U.S. (RTECS, 1994). The most recent provisional data from the NIOSH National Occupational Exposure Survey (NOES) estimates that 512,633 workers, including 198,247 females, are potentially exposed to dibutyl phthalate (NIOSH, 1994). In 1974, the total number of potentially exposed

workers was 258,823, according to the National Occupational Hazard Survey; this number rose to 512,631 in 1983 (NIOSH, 1994).

Metabolism and Disposition

METABOLISM AND DISPOSITION IN HUMANS

As noted above, phthalate esters have been detected in the blood of individuals ingesting food that had been in contact with flexible plastics (Guess *et al.*, 1967; Overcash, 1982). The concentration of dibutyl phthalate in the blood was much higher following ingestion of such food products (up to 0.35 ppm) than the average pretest value of 0.02 ppm (Tomita *et al.*, 1977). Concentrations detected in human adipose tissue have varied from 0.10 to 0.79 ppm (Mes *et al.*, 1974; Mes and Campbell, 1976).

METABOLISM AND DISPOSITION IN ANIMALS

In a comparison of several phthalate diesters, 157 µmol/kg doses were applied to the skin of male F344 rats, and urine and feces were collected every 24 hours for 7 days after application (Elsisi *et al.*, 1989). The cumulative percentage of the dose excreted was greatest for dibutyl phthalate (about 60% of the applied dose) and diethyl phthalate (about 50% of the applied dose). In *in vitro* experiments comparing human and rat skin, rat skin was consistently more permeable to phthalate esters than human skin (Scott *et al.*, 1987). Elsisi *et al.* (1989) suggested that the extent of dermal absorption of phthalate diesters decreases as the length and size of the alkyl side chain increase.

After oral administration to rats and mice, dibutyl phthalate is thought to be metabolized by nonspecific esterases in the gastrointestinal tract to monobutyl phthalate and butanol prior to absorption into the bloodstream (Cater *et al.*, 1977). Metabolism, absorption, and excretion in urine and feces are considered rapid; for rats, 31% to 44% of the administered dose of dibutyl phthalate was excreted in the urine and 20% to 22% was excreted in the feces 24 hours after administration. The maximum concentration of the metabolites in plasma and various organs was reached approximately 20 to 30 minutes after administration. Only traces of the parent compound, dibutyl phthalate, were found in the excreta. Monobutyl phthalate was the major metabolite (70% to 80% of the total dose) and was excreted mainly in urine, as were two products of omega-oxidation (2% to 3%) and two products of omega-1 oxidation, mono-(3-hydroxy-butyl) phthalate and mono-(4-hydroxy butyl) phthalate (3% to 6%) (Albro and Moore, 1974; Williams and Blanchfield, 1975; Bedford, 1977). Further hydrolysis of monobutyl phthalate to phthalic acid is thought to occur slowly (Plunkett, 1976). Studies of dibutyl phthalate, dimethylphthalate, and di(2-ethylhexyl) phthalate with everted gut sac preparations from the small intestine of rats suggested that esterases

of the mucosal epithelium hydrolyzed the diesters to monoesters during absorption. When the esterases were inhibited by an organophosphate, the absorption of dibutyl phthalate was significantly reduced (Rowland *et al.*, 1977; White *et al.*, 1980).

No specific organ affinity was observed in rats that received a single intravenous dose of [^{14}C]-dibutyl phthalate. One hour after injection, 6% of the administered ^{14}C was in the liver, whereas 76% of the ^{14}C administered with di(2-ethylhexyl) phthalate was found in the liver an hour after injection (Tanaka, 1978).

Toxicity

TOXIC EFFECTS IN HUMANS

In one clinical case, a chemical worker accidentally swallowed approximately 10 g dibutyl phthalate. Subacute signs included severe, bilateral keratitis with loss of corneal epithelium and transitory nephritis with hematuria and crystalluria (Grant, 1986). No other information was found in the literature.

TOXIC EFFECTS IN ANIMALS

Dibutyl phthalate is of low to moderate acute toxicity in mammals. Median lethal inhalation (LC_{50}) concentrations and parenteral oral or dermal (LD_{50}) values are given in Table 1. Dibutyl phthalate is a known peroxisome proliferator; this class of chemicals induces pleiotropic effects in rats and mice, including hypolipidemia (specifically hypotriglyceridemia), body weight gain depressions, and often a marked hepatomegaly associated with hepatocellular replication, peroxisome proliferation, and the induction or modulation of a variety of enzymes or enzymatic pathways. Dibutyl phthalate in particular has been shown to cause growth retardation in rats when administered in the diet at a concentration of 2,500 ppm for 1 year (Smith, 1953); increased liver weights when administered to rats by gavage for 3 months (Nikonorow *et al.*, 1973); and increased or decreased liver cytochrome P_{450} activity (isotypes unspecified) in rats (Walseth *et al.*, 1982; Walseth and Nilsen, 1986). The pentobarbital sleeping times of male rats fed 1% dibutyl phthalate for 26 days were decreased, presumably by induction of the cytochrome P_{450} system, compared with the sleeping times of rats fed only the basal diet (Shibata *et al.*, 1984). Consistent with its status as a peroxisome-proliferating chemical, dibutyl phthalate uncouples oxidative phosphorylation in rats (Gosselin *et al.*, 1984; Melnick and Schiller, 1985). The liver weight of a dog treated with 2.0 mL dibutyl phthalate per kilogram body weight per day for 2 months was increased (USEPA, 1954).

TABLE 1 Summary of Selected Animal Toxicity Data for Dibutyl Phthalate

Species	Route of Exposure	LC_{50} or LD_{50}	Reference
Rat	Inhalation	25 g/m^3	ACGIH, 1986
Rat	Inhalation	4.25 g/m^3	Antonyuk and Aldyreva, 1973
Rat	Intraperitoneal	3.05 mL/kg	Singh *et al.*, 1972
Rat	Intraperitoneal	4 g/kg	Calley *et al.*, 1966
Rat	Intramuscular	8 g/kg	Autian, 1973
Rat	Intramuscular	8 g/kg	Sandmeyer and Kirwin, 1981
Rat	Oral	>20 g/kg	Lehman, 1955
Rat	Oral	12 g/kg	Sax, 1984
Rat	Oral	8 to 10 g/kg	Krauskopf, 1973
Mouse	Inhalation	25 g/m^3	Izmerov *et al.*, 1982
Mouse	Intravenous	720 mg/kg	Miyahara *et al.*, 1973
Mouse	Intraperitoneal	4.0 g/kg	Autian, 1973
Mouse	Intraperitoneal	3.57 g/kg	Goldemberg and Safrin, 1977
Mouse	Oral	9 g/kg	Konarova, 1979
Mouse	Oral	5.3 g/kg	Antonyuk and Aldyreva, 1973
Mouse	Subcutaneous	20.8 g/kg	SRRI, 1989
Rabbit	Dermal	20 mL/kg	Autian, 1973
Rabbit	Dermal	>20 g/kg	Union Carbide, 1971
Guinea pig	Oral	10 g/kg	Timofievskaya *et al.*, 1980

Dibutyl phthalate was evaluated as a peroxisome-proliferating agent in a comparison with six other phthalate esters in groups of five male and five female Fischer 344 rats fed nominal concentrations of 0, 0.6, 1.2, and 2.5% in the diet for 21 days (BIBRA, 1986; Barber *et al.*, 1987). Toxic signs included lower mean body weights (males, 1.2% and 2.5% groups; females, 2.5% group), reduced feed consumption (males and females, 2.5% groups), increased absolute and relative liver weights (all exposed rats), increased relative kidney weights (males, 1.2% and 2.5% groups; females, 2.5% group) and decreased absolute and relative testis weights (2.5% group). Decreases in serum cholesterol (all exposed rats) and serum triglycerides (all exposed males) were considered treatment related but not dose related. Liver biochemistry analyses revealed increases in cyanide-insensitive palmitoyl-CoA oxidation activity (males, 1.2% and 2.5% groups; females, 2.5% group), lauric acid 11- and 12-hydroxylase activity (all exposed males; females, 2.5% group) and total hepatic protein levels (males, 0.6% and 1.2% groups; females, 1.2% and 2.5% groups). There was no consistent dose-response relationship among exposure groups for lipid content in the liver. Severe testicular atrophy was observed in males receiving 2.5% dibutyl phthalate. Additional unpublished data suggests that dibutyl phthalate may weakly induce hepatocellular replication (DeAngelo, personal communication, 1994).

Rats exposed to a nominal air concentration of 0.5, 2.5, or 7.0 ppm of dibutyl phthalate for 5 days exhibited dose-dependent reductions in lung microsomal cytochrome P_{450} values, with maximum reductions of 63% (Walseth and Nilsen, 1984). Liver alterations were also observed in these animals.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

Synthetic organic chemicals, particularly phthalate esters, have been implicated in the apparent decline in human sperm counts in the last 40 years (Murature *et al.*, 1987). The recognized testicular toxicity of the phthalate esters in rodents at high doses and the abundance of these chemicals as low-level environmental contaminants have been cited in support of this implication.

Testicular atrophy and loss of testicular zinc were seen in Sprague-Dawley rats administered 2,000 mg dibutyl phthalate per kilogram body weight per day by gavage for as few as 4 days (Cater *et al.*, 1977; Gosselin *et al.*, 1984) and in JCL:Wistar rats that received 2% dibutyl phthalate in feed for 1 week (Oishi and Hiraga, 1980). Young male Wistar rats receiving 500 or 1,000 mg/kg dibutyl phthalate per day for 15 days exhibited decreased testis weight and degeneration of testicular germ cells (Srivastava *et al.*, 1990).

In addition to the observation of testicular atrophy and testicular germ cell toxicity in standard evaluations (Cater *et al.*, 1977; Oishi and Hiraga, 1980; Gosselin *et al.*, 1984; BIBRA, 1986; Barber, 1987; Srivastava *et al.*, 1990), dibutyl phthalate has been directly evaluated for reproductive and developmental toxicity. The results of reproductive studies in female rats suggested reduced fertility following exposure to dibutyl phthalate (Cummings and Gray, 1987). Reproductive toxicity studies in male rats have detected testicular atrophy/seminiferous tubule degeneration, with the Sertoli cell suggested as the initial target of dibutyl phthalate toxicity (Gray and Gangolli, 1986). In addition, indirect mechanisms such as disruption of the pituitary-testes hormonal (FSH) axis (Heindel and Powell, 1992) or urinary excretion and resultant depletion of testicular zinc (Gangolli, 1982) have been suggested.

Overall, developmental toxicity studies have shown increased incidences of fetal resorption, exencephaly, spina bifida, skeletal abnormalities, and anophthalmia in rodents exposed to dibutyl phthalate at doses that also reduced maternal weight gain (Schardein, 1985; Cummings and Gray, 1987). Intraperitoneal injection of female Sprague-Dawley rats with 1.017 mL/kg dibutyl phthalate (a dose equivalent to one-third of the LD_{50}) on Days 5, 10, and 15 of gestation caused a 23% resorption rate and a 33% incidence of skeletal abnormalities (Singh *et al.*, 1972, 1975). In mice fed 1.0% dibutyl phthalate during gestation, the number of resorptions, external malformations (Shiota

and Nishimura, 1982), and neural tube defects including exencephaly and spina bifida (Shiota *et al.*, 1980) were of borderline significance.

CARCINOGENICITY

No information on the carcinogenicity of dibutyl phthalate in humans was found in a search of the available literature.

No information was found in the literature on long-term studies which were of sufficient duration to evaluate the specific carcinogenic potential of dibutyl phthalate in animals. Long-term studies (approximately 1 year in duration) of dibutyl phthalate at concentrations up to 500 mg/kg in feed did not alter body weight or cause other signs of toxicity or carcinogenicity in male or female Wistar rats (Lefaux, 1968). Dibutyl phthalate is a known peroxisome proliferator, a class of chemicals that are often rodent carcinogens. Peroxisome-proliferating chemicals are thought to exert their carcinogenic activity through a nongenotoxic, promotional, or oxidative stress mechanism or through a combination of these mechanisms.

A number of chronic toxicity and carcinogenicity studies with phthalate esters and phthalate-related chemicals have been conducted. This series of studies has had both positive results (diethylhexyl adipate, butyl benzyl phthalate, and di(2-ethylhexyl) phthalate; NTP, 1982a,b,c) and negative results (dimethyl terephthalate, diallylphthalate, and diethylphthalate; NCI, 1979; NTP, 1985, 1994) for carcinogenic activity in rodents.

In initiation/promotion studies in which dibutyl phthalate was used as the carrier for evaluating methyl ethyl ketone peroxide, there was no evidence of tumor-promoting activity by dibutyl phthalate (as the vehicle control) on the skin of male or female hairless albino mutant mice irradiated with UV-B compared with untreated controls irradiated with UV-B (Logani *et al.*, 1984).

GENETIC TOXICITY

In general, results from bacterial mutagenicity tests with dibutyl phthalate, conducted with and without S9 metabolic activation enzymes, have been negative. No inhibition of growth due to DNA damage was noted in *Bacillus subtilis* (Omori, 1976; Kawachi *et al.*, 1980) or *Escherichia coli* (Omori, 1976), and no mutagenic activity was noted in any of several strains of *Salmonella typhimurium* (Rubin *et al.*, 1979; Florin *et al.*, 1980; Kozumbo *et al.*, 1982; Nohmi *et al.*, 1985; Zeiger *et al.*, 1985). There was a single report of a weakly mutagenic response obtained with *S. typhimurium* strains TA100 and

TA1535 treated with dibutyl phthalate in the absence of S9 (Agarwal *et al.*, 1985). However, in this case the activity noted with strain TA1535 was extremely weak and the data were not clearly presented in the publication; the response noted with strain TA100 was not dose related but was consistent across all administered doses.

Negative results were also reported for dibutyl phthalate in most mutagenicity tests performed with eukaryotic cells. No induction of gene mutations was observed in the yeast *Saccharomyces cerevisiae* with or without S9 activation (Shahin and von Borstel, 1977). No consistent increase in sister chromatid exchanges was noted in Chinese hamster cells treated with graduated doses of dibutyl phthalate (Abe and Sasaki, 1977). Results of tests for induction of chromosomal aberrations were negative in Chinese hamster cell lines (Abe and Sasaki, 1977; Ishidate and Odashima, 1977) and human leukocyte cultures (Tsuchiya and Hattori, 1976). No sex-linked recessive lethal mutations were induced in germ cells of female nematodes (*Panagrellus redivivus*) exposed to various concentrations of dibutyl phthalate in water (Samoiloff *et al.*, 1980). Finally, no mutations were observed in silkworms fed dibutyl phthalate (Kawachi *et al.*, 1980). The only positive response noted in a mammalian cell mutagenicity assay occurred in a mouse lymphoma cell assay for induction of trifluorothymidine resistance (Hazleton Biotechnologies Company, 1986); in this assay, dibutyl phthalate was mutagenic only in the presence of S9 and at concentrations that were highly toxic.

Study Rationale and Design

Dibutyl phthalate, which has been ranked Number 28 on the ATSDR's Priority List of 275 Hazardous Substances, was nominated for study by the NTP, along with several other phthalates, under a Superfund agreement. Some phthalates, including diethylhexyl adipate, butyl benzyl phthalate, and di(2-ethylhexyl) phthalate, have been implicated as animal carcinogens (NTP, 1982a,b,c). Additional studies with dibutyl phthalate have been recommended due to its known potential for human exposure, the continuing uncertainty over the mechanism of carcinogenicity of the phthalates in animals, and the relevance of this response to humans.

Recently, the potential for long-term, low-level human exposure to the phthalates, and particularly the potential effects on subpopulations at higher risk, has been recognized as an area of concern (NRC, 1993). Little is known about the impact of environmental agents such as the phthalates on the very young, before or after birth. Mobilization of chemicals from body fat stores during pregnancy and lactation may lead to abnormally high exposure concentrations of toxicants during a

potentially vulnerable period of histogenesis. Baby bottles and nipples, plastic liners, and toys are potential sources of additional chemical exposure to the very young.

Due to the aforementioned concerns, standard 13-week toxicity studies were conducted in F344/N rats and $B6C3F_1$ mice (see Figure 1). Thirteen-week studies in F344/N rats incorporating prior perinatal exposure were also included to examine the effects of preexposure of animals to dibutyl phthalate *in utero* and during lactation, in addition to exposure as adults. The maximum perinatal exposure (MPE) concentration for rats and mice was determined; in these studies, pregnant dams and nursing pups were exposed to dietary concentrations of dibutyl phthalate ranging from 1,250 to 20,000 ppm. A perinatal exposure concentration of 10,000 ppm was determined to be the MPE concentration in rats, with minimal evidence of maternal or fetal toxicity. Supplemental studies specifically targeting the *in utero* or lactational period (first 4 weeks immediately following birth) were designed to evaluate hepatic peroxisome proliferation activity and testicular toxicity of dibutyl phthalate and the related phthalate, di(2-ethylhexyl) phthalate.

In the 13-week toxicity studies, the endpoints evaluated included histopathology and clinical pathology. The effects of dibutyl phthalate on reproduction were assessed by the evaluation of testicular and spermatozoal parameters and by determination of the length of the estrous cycle. In separate studies, a reproductive assessment with a continuous breeding protocol was conducted in Sprague-Dawley rats and Swiss (CD-1®) mice (Figure 2), the species and strains routinely used by the NTP in reproductive evaluations. In addition, the genetic toxicity of dibutyl phthalate was assessed in studies of *S. typhimurium*, by the determination of TFT resistance in L5178Y mouse lymphoma cells, and by the determination of micronucleated erythrocytes in peripheral blood erythrocytes of mice.

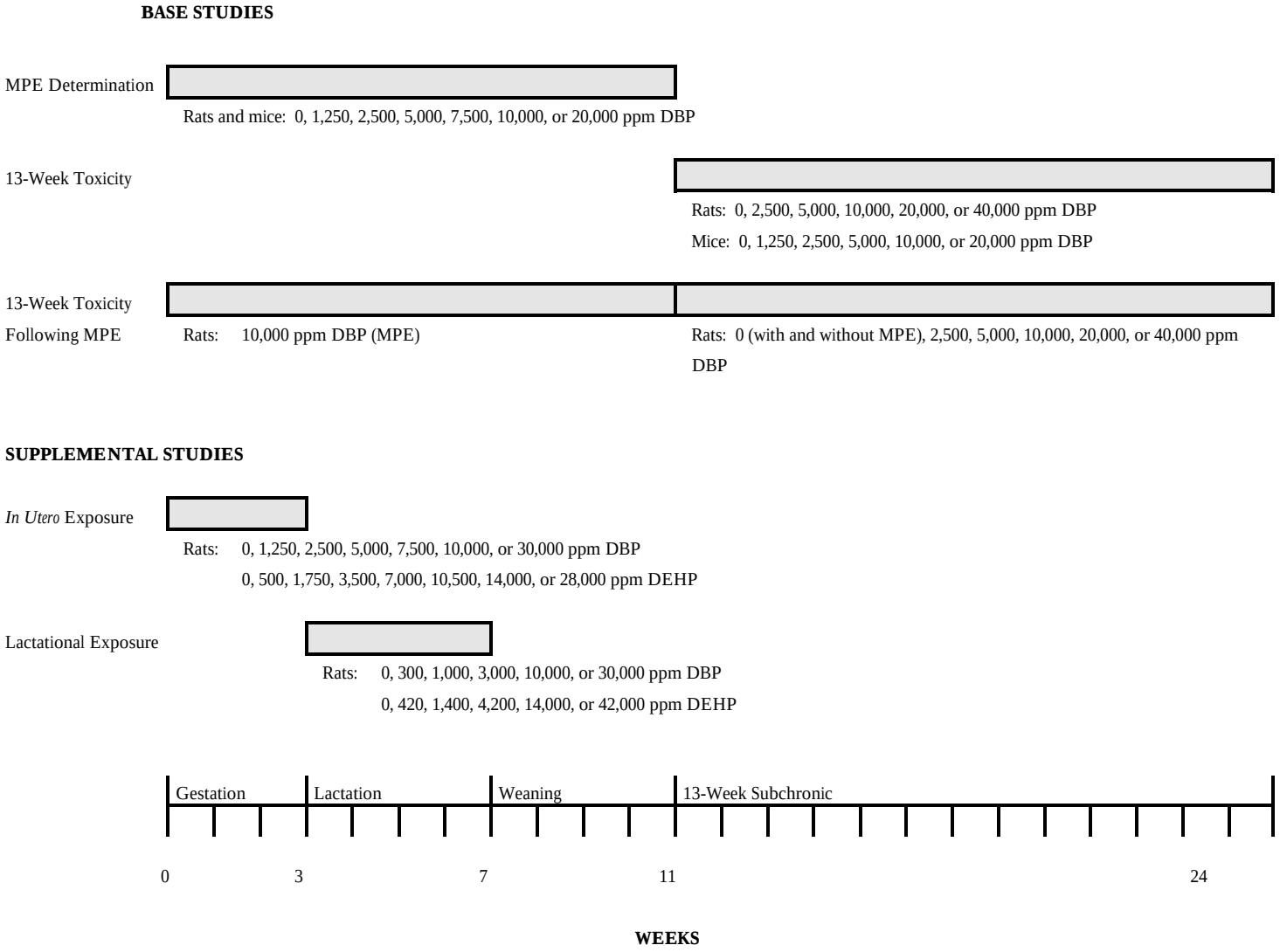


FIGURE 1 Design of Studies Examining the Perinatal and Subchronic Toxicity of Dibutyl Phthalate in F344/N Rats and B6C3F$_1$ Mice

[MPE = maximum perinatal exposure; DBP = dibutyl phthalate; DEHP = di(2-ethylhexyl) phthalate]

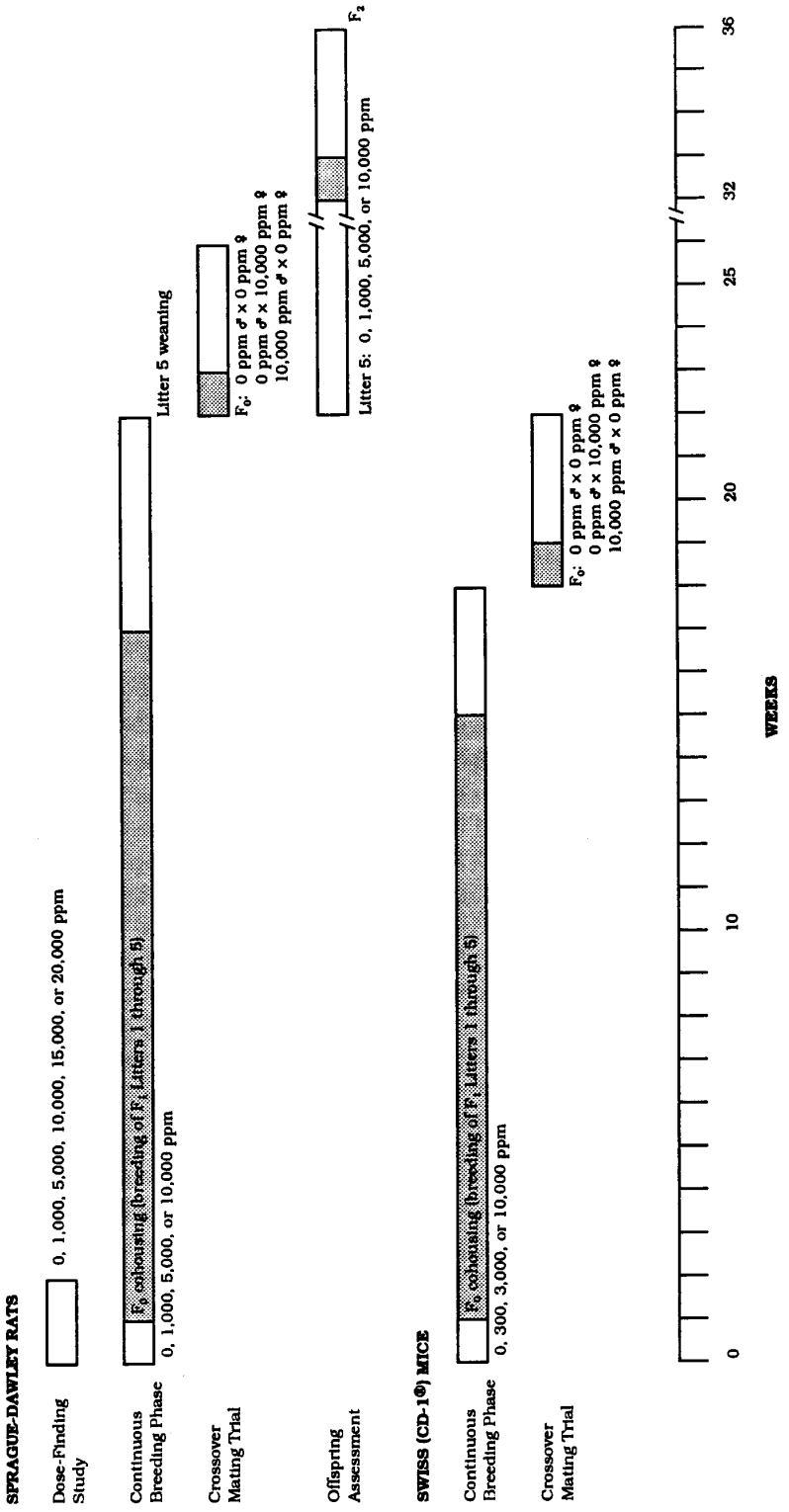


FIGURE 2 Design of Studies Examining Dibutyl Phthalate in Reproductive Assessments by a Continuous Breeding Protocol in Sprague-Dawley Rats and CD-1® Swiss Mice

(Shaded portions represent periods of cohousing; F_0, F_1 and F_2 denote the parental, first, and second generations, respectively)

MATERIALS AND METHODS

Procurement and Characterization of Dibutyl Phthalate

Dibutyl phthalate (Lot L-121 1-83) was obtained from Chem Central (Kansas City, MO). Initial identity and purity analyses were performed by Midwest Research Institute (MRI, Kansas City, MO).

The chemical, a colorless liquid, was identified as dibutyl phthalate by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy; spectra were consistent with those expected for the structure of dibutyl phthalate, with a literature reference (*Sadtler Standard Spectra*), and with a previously analyzed lot of dibutyl phthalate (Lot C100682) that was not used in the current studies. The results of elemental analysis for carbon and hydrogen were in agreement with theoretical values. Karl Fischer analysis indicated 0.106 ± 0.002% water. Free acid titration indicated less than 0.001 mEq acid/g sample; titration of the ester group by hydrolysis followed by back titration with hydrochloric acid indicated a purity of 97.2 ± 0.3%. Thin-layer chromatography (TLC) by two solvent systems indicated a major spot only. Gas chromatographic analysis by two systems with a flame ionization detector (FID) indicated a major peak and three impurities with a combined area of 0.4% relative to the major peak area. Gas chromatographic major peak comparison indicated that samples from two drums of Lot L-121 1-83 were identical within the limits of experimental error; these samples had a purity of 99.8 ± 0.3% relative to a concomitantly analyzed sample of Lot C100682. The cumulative data indicated a purity of 98% or greater for Lot L-121 1-83.

Stability studies performed by MRI on Lot C100682 on a gas chromatographic system with an FID indicated that dibutyl phthalate is stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60° C. Throughout the studies, dibutyl phthalate was stored at room temperature; periodic reanalyses performed by the study laboratory with infrared spectroscopy, gas chromatography, and ester hydrolysis titration indicated no decomposition of the bulk chemical.

Dose Formulations

A premix of dibutyl phthalate and Zeigler NIH-07 Open Formula meal diet (Zeigler Brothers, Inc., Gardners, PA) was prepared for each dosed feed formulation; additional portions of feed were added, and the premix was stirred with a spatula after each addition. For the final preparation, the premix and additional feed were layered in a twin-shell blender with an intensifier bar (Patterson-Kelley, East Stroudsberg, PA) and blended for 15 minutes, with the intensifier bar on for the first 5 minutes.

Homogeneity studies of the dosed feed mixtures were performed at MRI with high-performance liquid chromatography (HPLC); stability studies were performed at MRI with gas chromatography with an FID. Homogeneity was confirmed. The feed mixtures were found to be stable for 3 weeks when stored in the dark at −20° C and for 1 week when stored under animal room conditions.

The feed mixtures were stored in stainless steel buckets in the dark at approximately −20° C throughout the studies. The study laboratory periodically analyzed the dosed feed mixtures by HPLC. All dose formulations administered to rats and mice were within 10% of the theoretical concentrations. Results of referee analyses performed by MRI on the feed mixtures were in agreement with study laboratory results.

Toxicity Study Designs

BASE STUDIES

Male and female F344/N rats, male C3H mice, and female C57BL/6 mice used for breeding in the maximum perinatal exposure (MPE) determination studies and the 13-week study with perinatal exposure were obtained from Charles River Breeding Laboratories (Raleigh, NC for rats and Kingston, NY for mice). Male and female F344/N rats and B6C3F$_1$ mice used in the standard 13-week studies (without perinatal exposure) were obtained from Simonsen Laboratories (Gilroy, CA). The female rats and mice used for breeding were 56 to 70 days old at receipt, and rats and mice in the standard 13-week studies were 29 to 30 days old at receipt. Quarantine periods and ages of rats and mice at the beginning of the studies are given in Table 2. Blood samples were collected from five rats and five mice of each sex at the beginning of each study. Blood was also collected from five male and five female control rats and from 10 female control mice at the end of the 13-week studies; for the MPE determination studies, the final blood collections were taken from five untreated male and five control female parental rats and mice. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). Results for all studies except the MPE determination study in mice were negative; five serum samples collected at the beginning of the mouse study were positive for epizootic diarrhea of infant mice. Additional details concerning the study design are provided in Figure 1 and Tables 2 and 3.

In the MPE determination studies, unexposed rats and mice were housed in breeding groups (one male and two females) for 12 days or until the females were determined to be sperm positive (Gestation Day 0) from examination of vaginal smears or by the presence of a sperm plug. Groups of up to 24 female rats and 20 female mice were then administered 1,250, 2,500, 5,000, 7,500, 10,000,

or 20,000 ppm dibutyl phthalate in feed 7 days a week throughout gestation and lactation; 48 female rats and 20 female mice were maintained on undosed feed as controls. The uteri of female rats and mice exposed to 20,000 ppm that did not litter after 28 to 35 days were stained with ammonium sulfide and examined for implantation sites. After parturition, pups were examined and the number and sex of pups and the litter weight were recorded. On Day 4 postpartum, litters were randomly culled to a maximum of eight (rats) or six (mice) pups per litter; pup body weights were recorded on Days 4, 7, 14, 21, and 28. Pups were weaned on Day 28 postpartum; selected pups (up to 10 per group) received the perinatal exposure concentrations in feed for 4 weeks (no rat pups in the 20,000 ppm groups survived to weaning, and no mouse dams exposed to 20,000 ppm littered).

The perinatal exposure concentration selected for the 13-week study with perinatal exposure was based on the results of the MPE determination studies (Figure 1). In the 13-week study with perinatal exposure, unexposed rats were housed in breeding groups (one male and two females) for 10 days or until the females were observed to be sperm positive. Twelve females were maintained on undosed feed as controls; 72 females were administered 10,000 ppm dibutyl phthalate in feed 7 days a week throughout gestation and lactation. On Lactation Days 0 and 1, the number and sex of pups and the litter weight were recorded. On Day 4 postpartum, litters were culled to a maximum of eight pups; the number and sex of pups and individual body weights were recorded on Days 4, 7, 14, 21, and 28. Pups were weaned on Day 28 postpartum; 15 control pups and 90 exposed pups per sex (no more than three pups per sex from each litter) received the perinatal exposure concentrations in feed for 4 weeks. At approximately 8 weeks of age, groups of 10 male and 10 female pups began receiving the adult dietary concentrations and were continued on these diets 7 days a week for 13 weeks. Control rats continued to receive undosed feed; rats that were exposed to 10,000 ppm perinatally received 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm dibutyl phthalate as adults.

The exposure concentrations selected for the 13-week studies with no perinatal exposure were based on the results for rat dams used in the MPE determination study; in the 13-week studies, groups of 10 rats per sex were administered 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm dibutyl phthalate and groups of 10 mice per sex were administered 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm dibutyl phthalate in feed 7 days a week for 13 weeks.

Female rats and mice were housed individually during gestation and lactation. Rats in the 13-week studies and postweanling rats and mice were housed five per cage by sex; 13-week study mice were housed individually. Water was available *ad libitum*. Animal rooms were maintained at 69° to 75° F

and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and at least 10 room air changes per hour.

In the MPE determination studies, complete necropsies were performed on one rat and one mouse pup of each sex from each litter at weaning and on all animals at the end of the studies. Complete necropsies were performed on all rats in the 13-week exposure phase of the 13-week study with perinatal exposure and on all rats and mice in the 13-week studies without perinatal exposure. The heart, right kidney, liver, lungs, right testis, and thymus were weighed at the end of each study. Organs and tissues were examined for gross lesions and fixed in 10% neutral buffered formalin. Tissues to be examined microscopically were trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on all animals in the control and highest exposure groups in all studies. Because only one mouse pup, a male, survived through weaning in the 10,000 ppm group in the MPE determination study, male mice in the 7,500 ppm group were also examined. Gross lesions and selected organs of rats and mice in lower exposure groups were examined until a no-observed-effect level was determined. Tissues examined microscopically are listed in Table 2. After the completion of the 13-week studies, selected formalin-fixed, praffin-embedded tissues of rats and mice were stained for lipofuscin by Schmorl's method for reducing substances and AFIP's method for lipofuscin (Luna, 1968).

Upon completion of the laboratory pathologist's histologic evaluation, the slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology laboratory where quality assessment was performed. Results were reviewed and evaluated by the NTP Pathology Working Group (PWG); the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985).

SUPPLEMENTAL EVALUATIONS

Summaries of the continuous breeding studies and *in utero* and lactational exposure studies are given in Appendixes E and F, respectively.

Clinical Pathology

Blood for hematology and clinical chemistry evaluations of rats and hematology evaluations of mice was collected from 10 rats and 10 mice per sex and exposure level at the end of the 13-week studies with and without perinatal exposure. Animals were anesthetized with a CO_2:O_2 (70:30) mixture, and blood samples were drawn from the retroorbital sinus. Samples for hematology analysis were placed in microhematocrit capillary tubes containing EDTA; samples for clinical chemistry evaluations were placed in similar tubes devoid of anticoagulant. The latter samples were allowed to clot at room temperature; the samples were then centrifuged and serum was removed.

Hematologic determinations were made on an Ortho ELT-8 Hematology Analyzer (Ortho Instruments, Westwood, MA). The parameters that were evaluated are listed in Table 2. Differential leukocyte counts and morphologic evaluation of blood cells were determined by light microscopy from blood smears stained with Wright-Giemsa. Smears made from blood samples stained with methylene blue were examined microscopically with a Miller disc for quantitative determination of reticulocytes.

Clinical chemistry variables were measured using a Hitachi 704® Analyzer (Boehringer Mannheim, Indianapolis, IN). The parameters that were evaluated are listed in Table 2. Reagents for assays of sorbitol dehydrogenase were obtained from Sigma Chemical Company (St. Louis, MO); other reagents were obtained from the equipment manufacturer.

Sperm Motility and Vaginal Cytology in Rats and Mice

Vaginal cytology and sperm morphology evaluations were performed on rats and mice at the end of the 13-week studies. Ten rats per sex from the control groups (with and without perinatal exposure) and from groups receiving 2,500, 10,000, or 20,000 ppm adult exposure were evaluated in the 13-week study with perinatal exposure. In the 13-week studies without perinatal exposure, 10 male and 10 female rats from the 0, 2,500, 10,000, and 20,000 ppm groups and 10 male and 10 female mice from the 0, 1,250, 5,000, and 20,000 ppm groups were evaluated. The parameters that were evaluated are listed in Table 2. Methods were those outlined in the National Toxicology Program's Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluation in Toxicity Testing for Rats and

Mice (NTP, 1987). Briefly, for the 12 days before sacrifice, the vaginal vaults of 10 females of each species per exposure group were lavaged, and the aspirated vaginal fluid and cells were stained with toluidine blue. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (*i.e.*, diestrus, proestrus, estrus, and metestrus).

Sperm motility was evaluated at necropsy in the following manner. The left testis and epididymis were weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body and weighed. Test yolk (rats) or modified Tyrode's buffer (mice) was applied to slides, and a small incision was made in the cauda. The sperm effluxing from the incision were dispersed in the buffer on the slides and the numbers of motile and nonmotile spermatozoa were counted on two slides for five microscopic fields per slide by two observers.

Following completion of sperm motility estimates, each left cauda was placed in phosphate-buffered saline solution. Caudae were finely minced and the tissue was incubated and then heat fixed. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. The suspension was stained with diluted trypan blue. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Zinc Concentration Analyses

Serum and testis samples from all male rats and mice in the 13-week studies were analyzed for zinc by inductively coupled plasma emission spectroscopy (ICP). Animals were anesthetized with a CO_2:O_2 mixture, and blood samples were drawn from the retroorbital sinus. The blood was centrifuged and serum was collected; serum aliquots were prepared by dry-ashing at 475 ° C. One-fourth of the left testis of each animal was weighed and then digested with a mixture of nitric and perchloric acid. The zinc content of the serum and testis samples was analyzed by ICP at 213.86 nm.

Testosterone Concentration Analyses

Serum and testis samples from all male rats and serum samples from all male mice in the 13-week studies were analyzed for testosterone by a solid-phase ^{125}I radioimmunoassay. Portions of samples collected for the zinc concentration analyses were used for the testosterone analyses. Serum testosterone concentrations were measured with a kit from Diagnostic Products Corporation (Los

Angeles, CA). Serum samples were pipetted into polypropylene tubes coated with antibodies to testosterone. Additional antibody-coated tubes containing testosterone calibrators or zero calibrator and uncoated tubes containing zero calibrator were also prepared. Buffered [^{125}I]-testosterone was added to all prepared tubes and to extra uncoated tubes. The tubes containing calibrator or serum were vortexed, incubated in a water bath, and decanted to separate free testosterone from bound testosterone. All tubes were then counted in a Micromedic ME-Plus 4/440 gamma counter.

A weighed sample of testis tissue from each rat was quick-frozen in an ethanol:dry ice bath. The frozen samples were placed in vials (nitric acid-treated glass or high-density polyethylene) and stored at approximately −70° C. The samples were minced and homogenized in saline. Ether was added to the samples; the samples were shaken and the organic layer was removed and allowed to dry. The samples were extracted with ether twice more; methanol (70%) was then added to the residue from the extractions. The testosterone concentrations in the extracts were then tested by the same procedures used to measure testosterone in serum. For samples that were stored overnight before analysis, aqueous methanol was pipetted from the residue, which was then re-extracted with methanol; and the methanol extracts were combined for analysis.

Liver Peroxisome Analyses

Five male and five female rats from each group in the 13-week studies with and without perinatal exposure and 10 male and 10 female rat weanlings from the 0 and 10,000 ppm groups in the 13-week study with perinatal exposure were analyzed for hepatic peroxisomal proliferation by measuring peroxisomal palmitoyl-CoA oxidase activity. Liver samples were minced and then homogenized in a sucrose/tris-hydrochloride/EDTA buffer. The homogenates were sonicated in an ice bath and centrifuged; the supernatants were removed and stored on ice prior to analysis. The samples were assayed for palmitoyl-CoA oxidase activity with a tris:β-NAD:FAD:DDT:CoA:KCN:Triton X-100:BSA mixture in a reaction initiated with palmitoyl CoA (Lazarow and de Duve, 1976). Supernatants with high enzyme activity were diluted with the homogenization buffer. The reaction was monitored for the formation of β-NADH at 340 nm between 8 and 15 minutes. The data were normalized per gram of tissue. The protein concentration in the supernatant samples was measured by biuret reaction monitored by a Hitachi® 704 microcomputer (Boehringer Mannheim, Indianapolis, IN).

TABLE 2 Experimental Design and Materials and Methods in the Feed Studies of Dibutyl Phthalate

Maximum Perinatal Exposure (MPE) Determination Studies	13-Week Study with Perinatal Exposure	13-Week Studies
EXPERIMENTAL DESIGN		
Study Laboratory Battelle Columbus Laboratories (Columbus, OH)	Same as MPE determination studies	Same as MPE determination studies
Strain and Species Rats: F344/N Parental mice: C3H (males), C57BL/6 (females) Mouse pups: B6C3F_1	F344/N rats	Rats: F344/N Mice: B6C3F_1
Animal Source Parental: Charles River Breeding Laboratories (Raleigh, NC) Pups: Bred at study laboratory from parental animals	Same as MPE determination studies	Simonsen Laboratories (Gilroy, CA)
Size of Study Groups 10 male and 10 female pups	10 males and 10 females	10 males and 10 females
Doses/Duration of Dosing Pups: 0, 1,250, 2,500, 5,000, 7,500, or 10,000 ppm perinatally through weaning, then daily in feed for 4 weeks	Perinatal exposure: 10,000 ppm daily through 8 weeks of age; additional, untreated group maintained for use as untreated controls in adult exposure phase Adult exposure: 0 (with and without 10,000 ppm perinatal exposure), 2,500, 5,000, 10,000, 20,000, or 40,000 ppm daily in feed for 13 weeks	Rats: 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm daily in feed for 13 weeks Mice: 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm daily in feed for 13 weeks
Date of First Dose Rat dams: 17-27 November 1987 Rat weanlings: 5-14 January 1988 Mouse dams: 20-30 October 1987 Mouse weanlings: 4-14 December 1987	Perinatal exposure: Dams: 2-11 May 1989 Weanlings: 26 June 1989 13-Week exposure: 24 July 1989	Rats: 31 July 1989 Mice: 16 August 1989
Date of Last Dose and Necropsy Rat pups: 5-14 January 1988 Postweanling rats: 9-10 February 1988 Mouse pups: 4-14 December 1987 Postweanling mice: 5-6 January 1988	13-Week exposure: 23 October 1989 (males), 24 October 1989 (females)	Rats: 30 October 1989 (males), 31 October 1989 (females) Mice: 16 November 1989 (males), 17 November 1989 (females)

TABLE 2 Experimental Design and Materials and Methods in the Feed Studies of Dibutyl Phthalate (continued)

Maximum Perinatal Exposure (MPE) Determination Studies	13-Week Study with Perinatal Exposure	13-Week Studies
EXPERIMENTAL DESIGN (continued)		
Necropsy Complete necropsies were performed on one rat and one mouse of each sex from each litter at weaning and on all animals at the end of the studies. The following organs were weighed at the end of the studies: heart, right kidney, liver, lungs, right testis, and thymus.	Complete necropsies were performed on all adult rats in the 13-week exposure phase. The following organs were weighed: heart, right kidney, liver, lungs, right testis, and thymus.	Complete necropsies were performed on all animals. The following organs were weighed: heart, right kidney, liver, lungs, right testis, and thymus.
Histologic Examinations Histopathologic evaluations were performed on all animals in the control and highest exposure groups and on male mice in the 7,500 ppm group. The following tissues were examined: adrenal glands, brain (three sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, skin, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurological signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, and uterus. Gross lesions of rats and mice in all lower exposure groups were examined. Tissues examined in the lower exposure groups included: epididymis of rats (2,500, 5,000, and 7,500 ppm groups); and forestomach (all groups), brain, and kidneys (5,000 ppm groups) of mice.	Histopathologic evaluations were performed on all rats in the control and highest exposure groups in the 13-week exposure phase. The following tissues were routinely examined: adrenal glands, brain (three sections), clitoral glands, esophagus, eyes (if grossly abnormal), femur and marrow, gallbladder (mice only), gross lesions and tissue masses, heart, kidneys, large intestine (cecum, colon, rectum), liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial glands, prostate gland, salivary gland, seminal vesicle, small intestine (duodenum, jejunum, ileum), spinal cord/sciatic nerve (if neurological signs were present), spleen, stomach (forestomach and glandular stomach), testes (with epididymis), thigh muscle, thymus, thyroid gland, trachea, urinary bladder, uterus, and vagina (females in vaginal cytology studies only). Gross lesions of rats in all lower exposure groups were examined. Tissues examined in the lower exposure groups included the liver and testes.	Histopathologic evaluations were performed on all animals in the control and highest exposure groups. The tissues routinely examined were the same as in the 13-week study with perinatal exposure. Gross lesions of rats and mice in all lower exposure groups were examined. Tissues examined in the lower exposure groups included the liver and testes of rats and the liver of mice.

TABLE 2 Experimental Design and Materials and Methods in the Feed Studies of Dibutyl Phthalate (continued)

Maximum Perinatal Exposure (MPE) Determination Studies	13-Week Study with Perinatal Exposure	13-Week Studies
EXPERIMENTAL DESIGN (continued)		
Supplemental Evaluations		
Clinical Pathology Studies		
None	Hematology and clinical chemistry evaluations were conducted at the end of the study. Hematology parameters included hematocrit (Hct), hemoglobin (Hgb) concentration, erythrocyte (RBC) count, reticulocyte count, nucleated erythrocyte count, mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelet count, and leukocyte (WBC) count and differential. Clinical chemistry parameters included urea nitrogen, creatinine, glucose, total protein, albumin, cholesterol, triglycerides, alanine aminotransferase (ALT), alkaline phosphatase, creatine kinase (CK), sorbitol dehydrogenase (SDH), and total bile acids.	Hematology evaluations for rats and mice and clinical chemistry evaluations for rats were conducted at the end of the studies. Hematology and clinical chemistry parameters evaluated were the same as in the 13-week study with perinatal exposure.
Serum and Testis Zinc and Testosterone Concentration Analyses		
None	Zinc and testosterone levels were measured in sera and testes of all male rats in the 13-week exposure phase.	Zinc and testosterone levels were measured in sera and testes of all male rats. Serum and testis zinc levels and serum testosterone levels were measured in all male mice.
Sperm Morphology and Vaginal Cytology Evaluations		
None	Sperm motility and vaginal cytology evaluations were performed on rats in the 0 (with and without perinatal exposure), 2,500, 10,000, and 20,000 ppm groups in the 13-week exposure phase. Male rats were evaluated for necropsy body and reproductive tissue weights, spermatozoal data, and spermatogenesis. Females were evaluated for necropsy body weight, estrous cycle length, and the percent of cycle spent in the various stages.	Sperm motility and vaginal cytology evaluations were performed on rats in the 0, 2,500, 10,000, and 20,000 ppm groups and mice in the 0, 1,250, 5,000, and 20,000 ppm groups. Parameters evaluated were the same as in the 13-week study with perinatal exposure.

TABLE 2 Experimental Design and Materials and Methods in the Feed Studies of Dibutyl Phthalate (continued)

Maximum Perinatal Exposure (MPE) Determination Studies	13-Week Study with Perinatal Exposure	13-Week Studies
EXPERIMENTAL DESIGN (continued)		
Supplemental Evaluations (continued)		
Liver Peroxisome and Enlargement Analyses		
None	Ten male and 10 female rat pups per exposure level (no more than two pups per sex per litter) were evaluated for liver peroxisomal proliferation and liver enlargement analyses at weaning. The livers of five male and five female rats per group were also analyzed for hepatic peroxisomal proliferation and liver enlargement by monitoring increases in peroxisomal palmitoyl-CoA oxidase activity and liver weight.	The livers of five male and five female rats per group were analyzed for hepatic peroxisomal proliferation and liver enlargement by monitoring increases in peroxisomal palmitoyl-CoA oxidase activity and liver weight.
ANIMAL MAINTENANCE		
Time Held Before Study		
Parental: 14 days	Parental: 10 days	Rats: 10 days Mice: 12 days
Age When Study Began		
Parental: 11 weeks Postweanling: 4 weeks	Perinatal exposure: Parental: 9-12 weeks Postweanling: 4 weeks 13-Week exposure: 8 weeks	6 weeks
Age When Killed		
Pups: 4 weeks Postweanling: 8 weeks	Perinatal exposure: 4 weeks 13-Week exposure: 21 weeks	19 weeks
Method of Animal Distribution		
Animals were weighed and were randomized using a computer program.	Same as MPE determination studies	Same as MPE determination studies
Diet		
NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in meal form, undosed during breeding and containing the appropriate doses of dibutyl phthalate thereafter, was available *ad libitum*; water (City of Columbus) was available *ad libitum*.	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in meal form, undosed during breeding and containing the appropriate doses of dibutyl phthalate thereafter, was available *ad libitum*; water (City of Columbus) was available *ad libitum*.	NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) in meal form, containing the appropriate doses of dibutyl phthalate, and water (City of Columbus) were available *ad libitum*.
Animal Room Environment		
One male and two females were housed together during breeding; females were housed individually during gestation and lactation. Postweanling rats and mice were housed five animals per cage by sex. The temperature was maintained at 69° to 75° F and relative humidity at 35% to 65%, with at least 10 air changes per hour. Fluorescent light was provided for 12 hours per day.	One male and two females were housed together during breeding; females were housed individually during gestation and lactation. Postweanling rats were housed five animals per cage by sex. Temperature, relative humidity, and fluorescent light were maintained as in the MPE determination studies.	Rats were housed five animals per cage by sex; mice were housed individually. Temperature, relative humidity, and fluorescent light were maintained as in the MPE determination studies.

TABLE 3 Type and Frequency of Observations in the Feed Studies of Dibutyl Phthalate[1]

Parameter	Study		
	MPE Determination	13-Week with Perinatal Exposure	13-Week
Observation			
Breeding	Daily[2]	Daily[2]	NA
Gestation	2x/day	2x/day	NA
Lactation	2x/day	2x/day	NA
Postweaning	2x/day	2x/day	NA
Adult exposure	NA	2x/day	2x/day
Observations Recorded			
Gestation	As necessary	As necessary	NA
Lactation	Weekly or as necessary	Weekly	NA
Postweaning	Weekly	Weekly	NA
Adult exposure	NA	Weekly	Weekly
Litter Weight	LD 0 & 1	LD 0 & 1	NA
Body Weight			
Gestation	GD 0 & 17 (mice) or 18 (rats)	GD 0 & 20	NA
Lactation (dams)	Weekly from LD 0	Weekly from LD 0	NA
Lactation (pups)	LD 4, 7, weekly thereafter	LD 4, 7, weekly thereafter	NA
Postweaning	Weekly	Initially and weekly	NA
Adult exposure	NA	Initially, on Day 1, weekly thereafter	Initially, on Day 1, weekly thereafter
Feed Consumption			
Gestation	GD 7, 14, (rats & mice) & 21 (rats only)	GD 0, 4, 7, 10, 14, 17, 20	NA
Lactation	Weekly	2x/week	NA
Postweaning	Weekly	Weekly	NA
Adult exposure	NA	Measured 2x/week, recorded weekly	Measured 2x/week, recorded weekly

[1] MPE = Maximum Perinatal Exposure; LD = Lactation Day; GD = Gestation Day; NA = not applicable.
[2] Vaginal smear for sperm (rats and mice) or examination for sperm plug (mice).

Genetic Toxicity Studies

SALMONELLA TYPHIMURIUM MUTAGENICITY TEST PROTOCOL

Testing was performed as reported by Zeiger *et al.* (1985). Dibutyl phthalate was sent to the testing laboratory as a coded aliquot and was incubated with the *S. typhimurium* tester strains (TA98, TA100, TA1535, and TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37 ° C. Top agar supplemented with *l*-histidine and *d*-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37 ° C.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of dibutyl phthalate. In the absence of toxicity, a high dose of 10,000 µg/plate was

selected. All assays were repeated. Because the data are published (Zeiger *et al.*, 1985), only one trial is presented for each test in Appendix F, Table F1.

MOUSE LYMPHOMA MUTAGENICITY TEST PROTOCOL

The experimental protocol is presented in detail by Myhr *et al.* (1985). Dibutyl phthalate was supplied as a coded aliquot. The high dose was limited by toxicity. L5178Y mouse lymphoma cells were maintained at 37° C as suspension cultures in supplemented Fischer's medium; normal cell cycling time was approximately 10 hours. To reduce the number of spontaneously occurring trifluorothymidine-resistant cells, subcultures were exposed once to medium containing THMG (thymidine, hypoxanthine, methotrexate, and glycine) for 1 day, to medium containing THG for 1 day, and to normal medium for 3 to 5 days. For cloning, horse serum content was increased and Noble agar was added.

All treatment levels within an experiment, including concurrent positive and solvent controls, were replicated. Treated cultures contained 6×10^6 cells in 10 mL of medium. Incubation with dibutyl phthalate continued for 4 hours, at which time the medium plus chemical was removed and the cells were resuspended in fresh medium and incubated for an additional 2 days to express the mutant phenotype. Cell density was monitored so that log phase growth was maintained. After the 48-hour expression period, cells were plated in medium and soft agar supplemented with trifluorothymidine (TFT) for selection of TFT-resistant cells ($TK^{-/-}$) and plated in nonselective medium and soft agar to determine cloning efficiency. Plates were incubated at 37° C in 5% CO_2 for 10 to 12 days.

PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL

A detailed discussion of this assay is presented in MacGregor *et al.* (1990). At the end of the 13-week mouse study, blood samples were taken from male and female mice and smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Two thousand normochromatic erythrocytes were scanned in each of five mice per exposure group. The criteria of Schmid (1976) were used to define micronuclei.

Statistical Methods

ANALYSIS OF CONTINUOUS VARIABLES

In the MPE determination and the standard 13-week studies, two approaches were employed to assess the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). The following parameters, which typically have skewed distributions, were analyzed with the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964): hematology, clinical chemistry, spermatid and epididymal spermatozoal data, palmitoyl-CoA oxidase activity data, zinc and testosterone concentrations, gestation length, and live pups per litter (number and percentage).

Because two control groups per sex were included in the 13-week exposure phase of the 13-week study with perinatal exposure, the methods of Dunnett and Dunn were considered the most appropriate in assessing the significance of pairwise comparisons between exposed and control groups in the analysis of continuous variables. Organ and body weight data were analyzed using the parametric multiple comparisons procedures of Dunnett; palmitoyl-CoA oxidase activities were analyzed with Dunnett's test after square root transformation. Hematology, clinical chemistry, spermatid and epididymal spermatozoal data, and zinc and testosterone concentration were analyzed with the nonparametric multiple comparisons methods of Dunn.

For all studies, Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at a P-value less than 0.01.

Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the study laboratory personnel indicated as being inadequate due to technical problems were eliminated from the analysis.

ANALYSIS OF VAGINAL CYTOLOGY DATA

Because the data are proportions (the proportion of the observation period that an animal was in a given estrous state), an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

ANALYSIS OF MUTAGENICITY IN *SALMONELLA TYPHIMURIUM*

A positive response in the *Salmonella typhimurium* assay is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that was not dose related, not reproducible, or not of sufficient magnitude to support a determination of mutagenicity. A negative response was obtained when no increase in revertant colonies was observed following chemical treatment. There was no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

ANALYSIS OF MOUSE LYMPHOMA MUTAGENICITY DATA

Minimum criteria for accepting an experiment as valid and a detailed description of the statistical analysis and data evaluation are presented in Caspary *et al.* (1988). All data were evaluated statistically for both trend and peak responses. Both responses had to be significant ($P \leq 0.05$) for a chemical to be considered capable of inducing TFT resistance; a single significant response led to a "questionable" conclusion, and the absence of both a trend and a peak response resulted in a "negative" call.

ANALYSIS OF PERIPHERAL BLOOD MICRONUCLEUS DATA

The frequency of micronucleated cells among normochromatic erythrocytes was analyzed by a statistical software package (ILS, 1990) that employed a one-tailed trend test across exposure groups and a *t*-test for pairwise comparisons of each exposure group to the concurrent control.

Quality Assurance

The animal studies of dibutyl phthalate were performed in compliance with USFDA Good Laboratory Practices regulations (21 CFR, Part 58). The Quality Assurance Unit of Battelle Columbus Laboratories performed audits and inspections of protocols, procedures, data, and reports throughout the course of the studies.

RESULTS

Maximum Perinatal Exposure Determination Feed Study in F344/N Rats

The number of females in the maximum perinatal exposure (MPE) determination study delivering at least one live pup (gestation index) was significantly lower in the 5,000 and 20,000 ppm groups than in the control group (Table 4). The length of gestation was also shorter for dams in the 5,000 ppm group than for the controls. The Gestation Day 18 mean body weight and the mean body weight gain of females in the 20,000 ppm group were significantly lower than those of the controls. Females in the 10,000 ppm group gained slightly more weight during gestation and had a significantly greater mean body weight on the day of delivery than control females, but gained less weight during lactation than the controls. Only 4 of 19 sperm-positive females in the 20,000 ppm group delivered at least one live pup. All females survived until the pups were weaned.

On the day of delivery, the number of pups per litter and number of live pups per litter were significantly lower for females in the 20,000 ppm group than for control females; furthermore, no pups in the 20,000 ppm group survived beyond Day 1 postpartum (Table 4). The percentage of live pups per litter in the 10,000 ppm group was lower than that in the control group on Day 1 and on Day 4 before culling. The mean body weights of pups exposed to 7,500 or 10,000 ppm were significantly lower than the body weight of the controls throughout lactation; the mean body weight of pups exposed to 5,000 ppm was also lower than that of the controls from Lactation Day 1 through weaning on Day 28. The mean body weight of pups in the 2,500 ppm group was lower than that of the controls on Lactation Day 21 and at weaning.

No clinical signs in the dams or pups were considered related to dibutyl phthalate administration; necropsy of one male and one female pup per litter on Day 28 of lactation revealed no lesions attributable to dibutyl phthalate administration.

TABLE 4 Reproductive Performance and Body Weights of F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)						
	0	1,250	2,500	5,000	7,500	10,000	20,000
Number of breeding groups[2]	19	10	10	9	10	11	3
DAM DATA							
Gestation index[3]	28/30 (93%)	15/19 (79%)	15/18 (83%)	13/19 (68%)*	14/18 (78%)	16/18 (89%)	4/19 (21%)**
Gestation length (days)	21.42 ± 0.08	21.38 ± 0.11	21.13 ± 0.08	21.08 ± 0.06*	21.20 ± 0.09	21.14 ± 0.07	22.04 ± 0.23[4]
Dam weight during gestation (g)							
Day 0	156 ± 2	157 ± 2	155 ± 2	156 ± 2	158 ± 3	159 ± 2	157 ± 2[5]
Day 18	223 ± 3	218 ± 6	224 ± 2	227 ± 3	226 ± 2[6]	234 ± 3*	200 ± 3**[5]
Weight gain (Days 0-18)	67 ± 3	61 ± 6	69 ± 2	71 ± 2	71 ± 3[6]	75 ± 3	43 ± 2**[5]
Dam weight during lactation (g)							
Day 0	193 ± 2[7]	193 ± 5	196 ± 2	200 ± 2	197 ± 2	203 ± 2*	193 ± 6[8]
Day 7	207 ± 2	209 ± 3[6]	203 ± 2	207 ± 2	208 ± 3	208 ± 2	)[9]
Day 14	216 ± 3	227 ± 3*[6]	225 ± 2	228 ± 3*	224 ± 3	224 ± 2	)
Day 21	219 ± 2	221 ± 3[6]	221 ± 2	224 ± 2	223 ± 3	222 ± 2	)
Day 28	203 ± 2	203 ± 2[6]	201 ± 2	206 ± 2	208 ± 2	206 ± 2	)
Weight gain (Days 0-28)	10 ± 1[7]	5 ± 2[6]	6 ± 2	6 ± 2	11 ± 1	4 ± 2*	)
LITTER DATA							
Number of pups per litter	9.24 ± 0.53[10]	8.40 ± 1.02	9.80 ± 0.48	10.39 ± 0.46	10.55 ± 0.48	10.18 ± 0.65	2.57 ± 0.69**[4]
Number of live pups per litter							
Day 0	9.18 ± 0.53[10]	8.30 ± 1.01	9.80 ± 0.48	10.39 ± 0.46	10.55 ± 0.48	10.09 ± 0.65	0.64 ± 0.36**[4]
Day 1	8.87 ± 0.54	7.50 ± 1.32	9.75 ± 0.48	10.17 ± 0.45	10.45 ± 0.51	9.32 ± 0.67	0.00 ± 0.00
Day 4 (precull)	8.87 ± 0.54	7.50 ± 1.32	9.75 ± 0.48	9.72 ± 0.64	10.20 ± 0.48	9.27 ± 0.68	0.00 ± 0.00
Day 4 (postcull)	7.24 ± 0.30	5.95 ± 0.87	7.80 ± 0.20	7.72 ± 0.22	7.80 ± 0.15	7.18 ± 0.42	0.00 ± 0.00*
Day 7	7.08 ± 0.30	5.95 ± 0.87	7.60 ± 0.22	7.50 ± 0.33	7.70 ± 0.17	7.18 ± 0.42	0.00 ± 0.00*
Day 14	7.08 ± 0.30	5.95 ± 0.87	7.60 ± 0.22	7.39 ± 0.44	7.70 ± 0.17	7.18 ± 0.42	0.00 ± 0.00*
Day 21	7.08 ± 0.30	5.95 ± 0.87	7.60 ± 0.22	7.39 ± 0.44	7.70 ± 0.17	7.14 ± 0.42	0.00 ± 0.00*
Day 28	7.08 ± 0.30	5.90 ± 0.87	7.60 ± 0.22	7.39 ± 0.44	7.70 ± 0.17	7.09 ± 0.41	0.00 ± 0.00*
Percentage of live pups per litter[11]							
Day 0	99 ± 1[10]	99 ± 1	100 ± 0	100 ± 0	100 ± 0	99 ± 1	29 ± 15**[4]
Day 1	96 ± 3[10]	90 ± 10	100 ± 1	98 ± 2	99 ± 1	90 ± 4**	0 ± 0**
Day 4	96 ± 3[10]	90 ± 10	100 ± 1	94 ± 3	97 ± 2	89 ± 4**	0 ± 0**
Day 7	95 ± 3	90 ± 10	98 ± 2	97 ± 2	99 ± 1	95 ± 5	0 ± 0**
Day 14	95 ± 3	90 ± 10	98 ± 2	95 ± 4	99 ± 1	95 ± 5	0 ± 0**
Day 21	95 ± 3	90 ± 10	98 ± 2	95 ± 4	99 ± 1	94 ± 5	0 ± 0**
Day 28	95 ± 3	89 ± 10	98 ± 2	95 ± 4	99 ± 1	94 ± 5	0 ± 0**

TABLE 4 Reproductive Performance and Body Weights of F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate (continued)

	Concentration (ppm)						
	0	1,250	2,500	5,000	7,500	10,000	20,000
LITTER DATA (continued)							
Pup weight (g)							
Day 0	4.92 ± 0.12[10]	4.75 ± 0.21	4.67 ± 0.07	4.59 ± 0.03	4.47 ± 0.05**	4.47 ± 0.07**	4.02 ± 0.25**
Day 1	5.34 ± 0.07	5.37 ± 0.14	5.14 ± 0.11	4.98 ± 0.05**	4.86 ± 0.09**	4.69 ± 0.08**	)
Day 4	8.16 ± 0.15	8.15 ± 0.35	7.83 ± 0.19	7.48 ± 0.09*	7.12 ± 0.19**	6.92 ± 0.19**	)
Day 7	11.82 ± 0.22	12.10 ± 0.33	11.49 ± 0.21	10.99 ± 0.12*	10.60 ± 0.25**	10.59 ± 0.22**	)
Day 14	21.66 ± 0.28	21.87 ± 0.40	21.21 ± 0.25	20.73 ± 0.16*	20.39 ± 0.35**	20.24 ± 0.24**	)
Day 21	31.56 ± 0.43	31.03 ± 0.45	30.39 ± 0.41*	30.22 ± 0.25*	28.99 ± 0.43**	29.20 ± 0.25**	)
Day 28	55.96 ± 0.51	55.02 ± 0.89	53.46 ± 0.95**	51.54 ± 0.29**	50.37 ± 0.71**	50.32 ± 0.58**	)

[1] All data except gestation indices are given as mean ± standard error for averages of two dams per breeding group; for breeding groups in which only one dam delivered pups, data for that dam only are included.
[2] One male and two females per breeding group; number of groups in which at least one female was impregnated.
[3] Females that delivered at least one live pup/sperm-positive females.
[4] n=7 dams and litters.
[5] n=11 dams.
[6] n=9 dams.
[7] n=18 dams.
[8] n=2 dams.
[9] n=0 dams and litters.
[10] n=17 litters.
[11] Number of live pups/total number of pups.
* Significantly different ($P \leq 0.05$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.

Selected pups from each exposure group were weaned onto feed containing dibutyl phthalate on Day 28. All weanlings survived until the end of the study (Table 5). Male rats receiving 5,000 ppm or greater had notably lower mean body weights at weaning, at Week 4 (Table 5), and at necropsy (Table 6) than the controls; the mean body weight change of males receiving 7,500 or 10,000 ppm was also less than that of the controls (Table 5). The initial mean body weights of female rats in the 7,500 and 10,000 ppm groups were less than the mean body weight of the controls; however, mean body weights at Week 4 and at necropsy were similar to those of the controls. Mean body weight gains of exposed females were generally similar to or greater than the mean body weight gain of the controls. No treatment-related clinical signs were observed. Feed and compound consumption are shown in Table 5; feed consumption by exposed rats was similar to that by the controls.

TABLE 5 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for Postweanling F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate

Dose (ppm)	Survival[1]	Mean Body Weight[2] (grams) Week 1[3]	Week 4	Change	Final Weight Relative to Controls[4] (%)	Average Feed Consumption[5] (g/day)	Average Dose[6] (mg/kg/day)
MALE							
0	10/10	79 ± 1	195 ± 4	116 ± 3		15.1	
1,250	10/10	77 ± 3	194 ± 3	117 ± 1	100	15.5	143
2,500	10/10	78 ± 1	189 ± 2	111 ± 2	97	15.2	284
5,000	10/10	72 ± 2*	182 ± 3**	110 ± 2	94	14.6	579
7,500	10/10	68 ± 3*	169 ± 3**	101 ± 3**	87	13.7	879
10,000	10/10	76 ± 1*	179 ± 3**	103 ± 2**	92	14.8	1,165
FEMALE							
0	10/10	73 ± 2	130 ± 2	58 ± 1		11.0	
1,250	10/10	67 ± 1	130 ± 2	63 ± 1*	100	10.9	133
2,500	10/10	67 ± 3	128 ± 3	61 ± 1	98	11.0	275
5,000	10/10	68 ± 2	131 ± 2	63 ± 1**	101	10.2	500
7,500	10/10	63 ± 3*	125 ± 2	61 ± 2	96	10.7	836
10,000	10/10[7]	64 ± 3*	123 ± 3	60 ± 1	95	10.6	1,104

[1] Number surviving at Week 4 postweaning/number of animals per group.
[2] Weights and weight changes are given as mean ± standard error.
[3] Mean body weight at the end of the first week of exposure postweaning.
[4] (Dose group mean/control group mean) x 100.
[5] Average of individual consumption values for Weeks 1 through 4 postweaning; not corrected for feed spillage.
[6] Average dose based on average mean body weight for Weeks 1 through 4 postweaning.
[7] The initial body weight of one rat was not recorded.
* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

The absolute liver weights of male rats receiving 5,000 ppm or greater and relative liver weights of all groups of exposed males were significantly greater than those of the controls (Table 6 and Appendix B, Table B1); female rats administered 2,500 ppm or greater also had significantly greater absolute and relative liver weights than the controls. Relative right kidney weights were greater in male rats in all exposed groups and female rats receiving 5,000 ppm or greater than in the controls. The relative right testis weight of males in the 10,000 ppm group was lower than that of the controls. Other differences in organ weights between exposed and control groups were considered secondary to body weight changes.

TABLE 6 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	1,250	2,500	5,000	7,500	10,000
MALE						
n	10	10	10	10	10	10
Necropsy body wt	237 ± 4	237 ± 2	234 ± 3	226 ± 3*	215 ± 3**	225 ± 4**
Right kidney						
Absolute	1.056 ± 0.029	1.116 ± 0.015	1.091 ± 0.014	1.111 ± 0.027	1.051 ± 0.029	1.110 ± 0.023
Relative	4.45 ± 0.08	4.71 ± 0.05*	4.67 ± 0.05*	4.91 ± 0.10**	4.88 ± 0.09**	4.94 ± 0.07**
Liver						
Absolute	12.128 ± 0.387	13.044 ± 0.272	13.145 ± 0.370	14.935 ± 0.351**	15.803 ± 0.377**	17.106 ± 0.485**
Relative	51.01 ± 1.07	55.15 ± 1.26*	56.17 ± 1.29**	65.90 ± 0.99**	73.43 ± 0.92**	76.04 ± 1.24**
Right testis						
Absolute	1.330 ± 0.014	1.339 ± 0.012	1.367 ± 0.033	1.295 ± 0.014	1.162 ± 0.033**	1.116 ± 0.029**
Relative	5.61 ± 0.06	5.66 ± 0.04	5.86 ± 0.18	5.72 ± 0.06	5.40 ± 0.11	4.97 ± 0.11**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	154 ± 2	152 ± 2	153 ± 3	156 ± 2	148 ± 2	146 ± 3
Right kidney						
Absolute	0.688 ± 0.011	0.704 ± 0.009	0.706 ± 0.016	0.756 ± 0.013**	0.692 ± 0.016	0.692 ± 0.012
Relative	4.49 ± 0.07	4.63 ± 0.07	4.61 ± 0.07	4.84 ± 0.07*	4.68 ± 0.07*	4.74 ± 0.09*
Liver						
Absolute	6.441 ± 0.138	6.618 ± 0.074	6.819 ± 0.120*	7.434 ± 0.129**	7.205 ± 0.174**	7.803 ± 0.107**
Relative	41.96 ± 0.64	43.46 ± 0.47	44.49 ± 0.62*	47.63 ± 0.76**	48.72 ± 0.81**	53.39 ± 0.72**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

No treatment-related gross lesions were identified at necropsy. The only lesion identified microscopically as being treatment related was hypospermia in the epididymal tubules of males. All males in the 7,500 and 10,000 ppm groups had a mild to marked hypospermia. Four of ten males exposed to 5,000 ppm had minimal hypospermia. Mild hypospermia (severity = 2) was characterized by relatively few degenerating cells and a minor apparent decrease in the number of intact sperm. Marked hypospermia (severity = 4) was characterized by degenerative cells with relatively no intact sperm. A review of the testes from all groups revealed no appreciable difference in germinal cell production or maturation in rats receiving perinatal concentrations of up to 10,000 ppm dibutyl phthalate.

Based on the results of the MPE determination study, an MPE concentration of 10,000 ppm dibutyl phthalate was selected for use in the 13-week study with perinatal exposure. Minor decreases in pup weights, persistent in males and transient in females, were possibly attributable in part to lower maternal body weight gain. No other signs of maternal toxicity were noted at the 10,000 ppm concentration. Differences in organ weights at this concentration were considered to result from the pharmacologic/toxicologic effects of dibutyl phthalate but were not considered to be life threatening or exposure concentration limiting. Hypospermia of the epididymis, observed in male postweanling rats in the 10,000 ppm group at the end of the study, was considered a significant toxicologic finding. However, without concomitant evidence of a testicular lesion, the epididymal lesion was also not considered to be exposure concentration limiting.

13-Week Feed Study with Perinatal Exposure in F344/N Rats

MPE Phase: All dams survived until the scheduled termination. The length of gestation for dams receiving 10,000 ppm was slightly shorter than that of the controls (Table 7). The gestational mean body weights and mean body weight gains of control and exposed dams were similar. During lactation, the mean body weight of exposed dams was similar to that of the controls at all time points except for Day 14, when the mean body weight of exposed dams was slightly lower than that of the controls. No treatment-related clinical signs were observed.

The numbers of pups born to control and exposed dams were similar (Table 7). However, the percentage of live pups per litter for exposed dams was significantly lower than that for control dams from the day of parturition through culling on Day 4. The number of live pups per litter was significantly lower for exposed females than for the controls on Day 1 postpartum and remained lower throughout lactation. The mean body weight of pups in the 10,000 ppm group was significantly less than that of control pups from the day of parturition through weaning on Day 28 (Table 7). No clinical signs were noted during gestation that were considered related to dibutyl phthalate administration. In 10 pups per sex evaluated at weaning, the liver weights of male and female pups from dams receiving 10,000 ppm were significantly greater than those of control pups (Table 8). Further, the palmitoyl-CoA oxidase activities in the liver of male and female weanling pups exposed to 10,000 ppm were markedly greater than in the controls.

Selected pups were weaned onto feed containing 10,000 ppm dibutyl phthalate on Day 28. All weanlings survived through the 4-week postweaning period. The mean body weight of male weanlings administered 10,000 ppm dibutyl phthalate was significantly less than that of the controls from Day 8 through Day 22 of the postweaning period (Table 9); the mean body weight of exposed female weanlings was less than that of the controls throughout the postweaning period. No clinical signs observed during the postweaning period were considered treatment related.

TABLE 7 Fertility, Reproductive Performance, and Body Weights of F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)	
	0	10,000
Number of breeding groups[2]	6	35
DAM DATA		
Mating index[3]	12/12 (100%)	71/72 (98.6%)
Fertility index[4]	12/12 (100%)	63/71 (88.7%)
Gestation index[5]	12/12 (100%)	63/71 (88.7%)
Gestation length (days)	22.00 ± 0.00	21.85 ± 0.04*
Dam weight during gestation (g)		
Day 0	154 ± 2	154 ± 1
Day 20	241 ± 2	236 ± 2
Weight gain (Days 0-20)	87 ± 3	81 ± 1
Dam weight during lactation (g)		
Day 0	186 ± 2	185 ± 1
Day 7	205 ± 1	201 ± 1
Day 14	220 ± 3	213 ± 1*
Day 21	216 ± 3	213 ± 1
Day 28	197 ± 2	201 ± 1
Weight gain (Days 0-28)	12 ± 1	16 ± 1
LITTER DATA		
Number of pups per litter	9.67 ± 0.48	9.60 ± 0.32
Number of live pups per litter		
Day 0	9.67 ± 0.48	8.90 ± 0.31
Day 1	9.67 ± 0.48	7.71 ± 0.29**
Day 4 (precull)	9.67 ± 0.48	7.53 ± 0.30**
Day 4 (postcull)	7.83 ± 0.17	6.93 ± 0.22*
Day 7	7.83 ± 0.17	6.91 ± 0.22*
Day 14	7.83 ± 0.17	6.91 ± 0.22*
Day 21	7.83 ± 0.17	6.90 ± 0.22*
Day 28	7.83 ± 0.17	6.90 ± 0.22*
Percentage of live pups per litter[6]		
Day 0	100 ± 0	93 ± 1*
Day 1	100 ± 0	88 ± 2*
Day 4	100 ± 0	86 ± 2**
Day 7	100 ± 0	100 ± 0
Day 14	100 ± 0	100 ± 0
Day 21	100 ± 0	100 ± 0
Day 28	100 ± 0	100 ± 0

TABLE 7 Fertility, Reproductive Performance, and Body Weights of F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

	Concentration (ppm)	
	0	10,000
LITTER DATA (continued)		
Pup weight (g)		
Day 0	4.74 ± 0.05	4.37 ± 0.05**
Day 1	5.16 ± 0.06	4.70 ± 0.05**
Day 4	8.08 ± 0.08	7.25 ± 0.08**
Day 7	11.48 ± 0.12	10.21 ± 0.13**
Day 14	21.22 ± 0.09	19.53 ± 0.24**
Day 21	31.00 ± 0.32	28.33 ± 0.32**
Day 28	56.37 ± 0.62	50.01 ± 0.49**

[1] All data except mating, fertility, and gestation indices are given as mean ± standard error for averages of two dams per breeding group; for breeding groups in which only one dam delivered pups, data for that dam only are included.
[2] One male and two females per breeding group; number of groups in which at least one female was impregnated.
[3] Sperm-positive or pregnant females/total females.
[4] Females delivering at least one pup/sperm-positive or pregnant females.
[5] Females delivering at least one live pup/sperm-positive or pregnant females.
[6] Number of live pups/total number of pups.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's (body weight only) or Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' (body weight only) or Shirley's test.

TABLE 8 Liver Weights and Palmitoyl-CoA Oxidase Activity Data for F344/N Rats at Weaning in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)	
	0	10,000
n	10	10
Liver weight (g)		
Male	2.79 ± 0.07	3.19 ± 0.12**
Female	2.66 ± 0.10	3.32 ± 0.10**
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)		
Male	3.2 ± 0.2	61.8 ± 1.2**
Female	3.4 ± 0.2	63.9 ± 1.8**

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test (liver weight only) or Shirley's test.

TABLE 9 Body Weights of Postweanling F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)	
	0	10,000
MALE		
n	15	90
Day 1	69 ± 1	66 ± 1
Day 8	105 ± 1	98 ± 1**
Day 15	143 ± 2	132 ± 1**
Day 22	183 ± 2	166 ± 1**
FEMALE		
n	15	90
Day 1	66 ± 1	60 ± 1**
Day 8	93 ± 1	86 ± 1**
Day 15	115 ± 1	106 ± 1**
Day 22	132 ± 1	123 ± 1**

[1] Data are given as mean ± standard error. Time points are given as number of days postweaning.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

13-Week Feed Study Phase: Selected rats from the postweanling groups were continued on dosed feed for 13 weeks. All rats survived to the end of the study (Table 10). Mean body weights of male and female control rats that were not exposed perinatally were greater at the beginning of the adult exposure than those of all groups of males and females that received 10,000 ppm perinatally (MPE:0 ppm groups). Female rats receiving dibutyl phthalate only during the perinatal period recovered from their initially lower body weight, with a greater mean body weight gain than control rats, and at the end of the study had a final mean body weight similar to that of the controls. The mean body weights of all groups of males exposed perinatally and of females that received 20,000 or 40,000 ppm as adults remained less than those of unexposed controls at the end of the study (Table 10 and Figure 3). The final mean body weights and mean body weight gains of males and females receiving 10,000 ppm or greater as adults were significantly less than those of the MPE:0 ppm group. Males in the 40,000 ppm group lost weight during the study.

All male and female rats that received 40,000 ppm as adults were emaciated. Males in the 40,000 ppm group also had abnormal posture and ruffled fur and appeared hypoactive during Week 2 through Week 4, and males in this group had a higher incidence of nasal discharge (8/10) than the controls (2/10) or the MPE:0 ppm group (3/10). No other clinical signs were considered related to exposure. Feed consumption by males and females receiving 40,000 ppm was lower than that by the controls (Table 10).

TABLE 10 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for F_1 F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure

Dose[1] (ppm)	Survival[2]	Mean Body Weight[3] (grams) Week 1[4]	Final	Change	Final Weight Relative to Controls[5] (%)	Average Feed Consumption[6] (g/day)	Average Dose[6] (mg/kg/day)
MALE							
0:0[7]	10/10	219 ± 2	370 ± 3	151 ± 3		16.0	
MPE:0	10/10	203 ± 2**	352 ± 3**	149 ± 3	95	15.4	
MPE:2,500	10/10	201 ± 2**	353 ± 5**	152 ± 3	95	15.9	138
MPE:5,000	10/10	202 ± 2**	350 ± 4**	149 ± 3	95	15.9	279
MPE:10,000	10/10	202 ± 2**	341 ± 4**▲	139 ± 2*▲	92	16.1	571
MPE:20,000	10/10	202 ± 3**	300 ± 3**▲▲	98 ± 3**▲▲	81	16.1	1,262
MPE:40,000	10/10	201 ± 2**	187 ± 3**▲▲	−14 ± 2**▲▲	51	11.1	2,495
FEMALE							
0:0[7]	10/10	146 ± 1	204 ± 2	58 ± 2		10.2	
MPE:0	10/10	139 ± 1**	205 ± 3	67 ± 2*	101	10.2	
MPE:2,500	10/10	139 ± 1**	202 ± 2	63 ± 2	99	10.4	147
MPE:5,000	10/10	138 ± 1**	201 ± 2	63 ± 2	98	10.3	294
MPE:10,000	10/10	140 ± 1**	197 ± 2▲▲	57 ± 2▲▲	96	10.3	593
MPE:20,000	10/10	137 ± 2**	185 ± 2**▲▲	48 ± 2**▲▲	91	9.8	1,182
MPE:40,000	10/10	137 ± 1**	151 ± 2**▲▲	14 ± 2**▲▲	74	8.6	2,445

[1] Maximum perinatal exposure (MPE) = 10,000 ppm; administered to dams through gestation and lactation and to pups for 4 weeks (until the beginning of the 13-week adult exposure phase).
[2] Number surviving at 13 weeks/number of animals per group.
[3] Weights and weight changes are given as mean ± standard error.
[4] Mean body weight during first week of adult exposure.
[5] (Dose group mean/0:0 ppm control group mean) x 100.
[6] Average of individual consumption values for Weeks 1-13.
[7] Perinatal and adult exposure = 0 ppm.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunnett's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunnett's test.
▲ Significantly different ($P \leq 0.05$) from the MPE:0 ppm group by Williams' test.
▲▲ Significantly different ($P \leq 0.01$) from the MPE:0 ppm group by Williams' test.

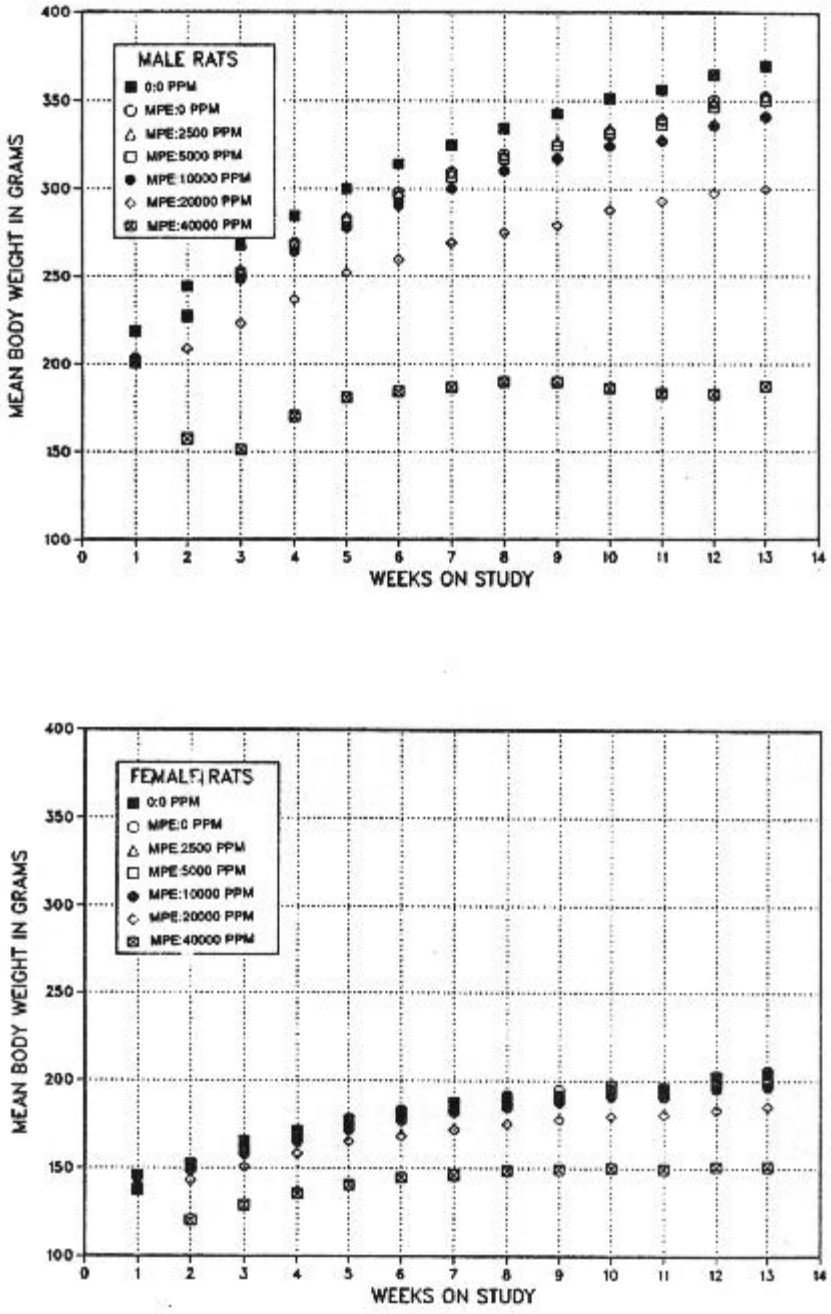


FIGURE 3 Body Weights of F344/N Rats Administered Dibutyl Phthalate Perinatally and in Feed for 13 Weeks

The relative testis weight of male rats that were exposed only perinatally was significantly greater than that of control males (Tables 11 and B2). This was considered secondary to the lower mean body weight of this group. No other significant differences in organ weights were noted between males in the MPE:0 ppm group and the controls. The organ weights of females that were exposed only perinatally and control females were similar.

Among groups exposed to dibutyl phthalate as adults, absolute and relative liver weights were generally significantly greater in males receiving 5,000 ppm or greater than those of the perinatally exposed and unexposed controls. Absolute and relative liver weights were greater in females that received 10,000 ppm or greater than in the perinatally exposed controls. Absolute liver weights of females receiving 5,000 ppm or greater and relative liver weights of females receiving 2,500 ppm or greater as adults were greater than those of the unexposed controls. In general, differences in kidney weights reflected body weight changes, with greater relative kidney weights and lower absolute kidney weights occurring at the higher exposure concentrations. Relative kidney weights of males administered 5,000 ppm or greater were significantly greater than the relative kidney weight of the unexposed controls. Relative kidney weights were greater in all groups of females receiving adult exposure than in the perinatally exposed controls. In males in the 20,000 and 40,000 ppm groups, absolute and relative testis weights were lower than those of males in the MPE:0 ppm group and reflected the markedly decreased mean body weights in these groups. At lower exposure concentrations, relative testis weights were greater in all groups of males exposed perinatally than in the unexposed controls. Other statistically significant differences in organ weights were considered secondary to body weight changes (Table B2).

TABLE 11 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
MALE							
n	10	10	10	10	10	10	10
Necropsy body wt	378 ± 4	358 ± 5**	359 ± 6*	358 ± 4*	348 ± 4**	306 ± 3**▲▲	187 ± 3**▲▲
Right kidney							
Absolute	1.291 ± 0.014	1.241 ± 0.019	1.268 ± 0.029	1.310 ± 0.037	1.355 ± 0.017▲▲	1.264 ± 0.019	0.890 ± 0.015**▲▲
Relative	3.42 ± 0.03	3.47 ± 0.04	3.53 ± 0.05	3.65 ± 0.07*	3.90 ± 0.06**▲▲	4.13 ± 0.05**▲▲	4.75 ± 0.05**▲▲
Liver							
Absolute	14.136 ± 0.283	13.589 ± 0.357	14.318 ± 0.390	15.428 ± 0.435*▲▲	16.850 ± 0.193**▲▲	18.157 ± 0.286**▲▲	12.375 ± 0.250**▲
Relative	37.41 ± 0.64	37.94 ± 0.77	39.82 ± 0.69	43.04 ± 0.92**▲▲	48.42 ± 0.62**▲▲	59.33 ± 0.86**▲▲	66.04 ± 0.83**▲▲
Right testis							
Absolute	1.568 ± 0.014	1.610 ± 0.019	1.613 ± 0.022	1.684 ± 0.025**	1.638 ± 0.021	1.114 ± 0.039**▲▲[3]	0.498 ± 0.022**▲▲
Relative	4.15 ± 0.05	4.50 ± 0.07*	4.50 ± 0.07*	4.70 ± 0.06**	4.71 ± 0.09**	3.62 ± 0.13**▲▲[3]	2.66 ± 0.12**▲▲
FEMALE							
n	10	10	10	10	10	10	10
Necropsy body wt	204 ± 2	206 ± 2	201 ± 3	202 ± 3	199 ± 3	187 ± 2**▲▲	149 ± 2**▲▲
Right kidney							
Absolute	0.703 ± 0.011	0.688 ± 0.010	0.721 ± 0.014	0.708 ± 0.010	0.706 ± 0.011	0.682 ± 0.011	0.610 ± 0.009**▲▲
Relative	3.45 ± 0.06	3.33 ± 0.04	3.58 ± 0.04▲▲	3.51 ± 0.03▲	3.55 ± 0.05▲▲	3.65 ± 0.04*▲▲	4.08 ± 0.04**▲▲
Liver							
Absolute	6.532 ± 0.066	6.860 ± 0.163	7.022 ± 0.227	7.142 ± 0.180*	7.543 ± 0.092**▲	7.945 ± 0.106**▲▲	8.373 ± 0.226**▲▲
Relative	32.03 ± 0.23	33.22 ± 0.53	34.91 ± 1.18*	35.33 ± 0.59**	37.90 ± 0.37**▲▲	42.53 ± 0.60**▲▲	55.99 ± 0.91**▲▲

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.

[3] n=9.

* Significantly different (P≤0.05) from the 0:0 ppm group by Dunnett's test.

** Significantly different (P≤0.01) from the 0:0 ppm group by Dunnett's test.

▲ Significantly different (P≤0.05) from the MPE:0 ppm group by Dunnett's test.

▲▲ Significantly different (P≤0.01) from the MPE:0 ppm group by Dunnett's test.

Hematology results for the 13-week feed study of dibutyl phthalate with perinatal exposure are presented in Table 12 and Appendix C, Table C1. In comparing the hematology results of the two control groups, values for perinatally exposed and unexposed rats were similar. In male rats also receiving 10,000 or 40,000 ppm dibutyl phthalate as adults, changes indicating a normocytic, normochromic anemia occurred; this was evidenced by lower hematocrit (Hct) values, hemoglobin (Hgb) concentrations, and erythrocyte (RBC) counts in exposed groups than in the controls. However, mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) indexes of exposed and control groups were similar. The anemia was moderate and responsive in males in the MPE:40,000 ppm group, as evidenced by greater numbers of reticulocytes and nucleated erythrocytes (NRBCs), and was mild and nonresponsive (with no increase in the number of circulating reticulocytes) in males in the MPE:10,000 and MPE:20,000 ppm groups. In males receiving 10,000 or 40,000 ppm as adults, platelet counts were slightly greater than in the controls; this change is consistent with a reactive thrombocytosis. A mild, nonresponsive anemia was evident in females that received 40,000 ppm as adults. Differences between exposed and control group values for other hematology parameters were inconsistent and minor and were not considered to be treatment related.

Results of the clinical chemistry analyses are provided in Tables 13 and C1. In comparing clinical chemistry results between the two control groups, values for perinatally exposed and unexposed rats were similar. Several changes in clinical chemistry parameters occurred in groups administered dibutyl phthalate as adults; most of these changes involved male and female rats in the MPE:20,000 and MPE:40,000 ppm groups, although in some instances lower exposure groups were affected. Hypotriglyceridemia and hypocholesterolemia were consistently observed effects in male and female rats in the MPE:20,000 and MPE:40,000 ppm groups; hypotriglyceridemia also occurred in males receiving 10,000 ppm as adults. Triglyceride and cholesterol concentrations in exposed groups of rats were approximately 20% to 80% lower than those in the control groups that did not receive perinatal exposure. Triglyceride concentrations appeared to be slightly higher in perinatally exposed controls than in unexposed controls.

TABLE 12 Selected Hematology Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
MALE							
n	9	9	10	8	7	6	9
Hematocrit (%)	50.3 ± 0.5	50.7 ± 0.5	49.4 ± 0.4	50.1 ± 0.5	48.1 ± 0.4▲	48.4 ± 0.6	44.7 ± 0.3**▲▲
Hemoglobin (g/dL)	15.27 ± 0.15	15.47 ± 0.13	14.91 ± 0.18	15.01 ± 0.15	14.59 ± 0.11▲	14.90 ± 0.21	13.60 ± 0.06**▲▲
Erythrocytes (10^6/µL)	9.53 ± 0.09	9.60 ± 0.10	9.41 ± 0.09	9.44 ± 0.09	9.08 ± 0.08▲	9.14 ± 0.11	8.33 ± 0.08**▲▲
Reticulocytes (10^6/µL)	0.17 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	0.17 ± 0.01	0.20 ± 0.02	0.25 ± 0.02▲▲
Nucleated erythrocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.04	0.26 ± 0.04**▲▲
Mean cell volume (fL)	52.6 ± 0.3	52.9 ± 0.3	52.7 ± 0.4	53.0 ± 0.2	52.9 ± 0.3	53.0 ± 0.3	53.6 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	16.1 ± 0.1	16.3 ± 0.1	16.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.4 ± 0.2	30.5 ± 0.2	30.2 ± 0.2	30.0 ± 0.2	30.3 ± 0.2	30.8 ± 0.1	30.4 ± 0.1
Platelets (10^3/µL)	637.1 ± 7.7	620.3 ± 16.9	642.8 ± 36.3	633.6 ± 9.3	706.9 ± 14.3▲	688.2 ± 9.6	749.2 ± 11.2**▲▲
FEMALE							
n	8	10	8	9	10	7	8
Hematocrit (%)	49.6 ± 0.8	50.0 ± 0.3	51.0 ± 0.4	51.1 ± 0.5	50.4 ± 0.3	50.1 ± 0.5	46.2 ± 0.3▲
Hemoglobin (g/dL)	15.0 ± 0.2	15.1 ± 0.1	15.2 ± 0.2	15.2 ± 0.1	15.2 ± 0.1	15.1 ± 0.2	14.1 ± 0.1*▲▲
Erythrocytes (10^6/µL)	8.68 ± 0.12	8.66 ± 0.06	8.83 ± 0.10	8.92 ± 0.09	8.78 ± 0.05	8.75 ± 0.09	8.27 ± 0.07
Reticulocytes (10^6/µL)	0.13 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.16 ± 0.02	0.17 ± 0.01	0.18 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01*	0.06 ± 0.03	0.08 ± 0.01**▲▲
Mean cell volume (fL)	57.3 ± 0.5	57.8 ± 0.2	57.8 ± 0.3	57.4 ± 0.3	57.3 ± 0.3	57.1 ± 0.3	56.0 ± 0.2▲▲
Mean cell hemoglobin (pg)	17.3 ± 0.1	17.4 ± 0.1	17.2 ± 0.1	17.1 ± 0.1▲	17.3 ± 0.1	17.3 ± 0.1	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.3 ± 0.3	30.2 ± 0.1	29.8 ± 0.1	29.8 ± 0.2	30.1 ± 0.2	30.2 ± 0.3	30.6 ± 0.2
Platelets (10^3/µL)	623.8 ± 33.1	637.2 ± 11.4	659.3 ± 44.1	628.9 ± 35.0	636.5 ± 10.3	631.6 ± 6.6	634.5 ± 8.2

[1] Data are given as mean ± standard error.
[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunn's test.
▲ Significantly different ($P \leq 0.05$) from the MPE:0 ppm group by Dunn's test.
▲▲ Significantly different ($P \leq 0.01$) from the MPE:0 ppm group by Dunn's test.

TABLE 13 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
MALE							
n	10	9	10	10	10	10	10
Total protein (g/dL)	7.0 ± 0.1	7.1 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	6.9 ± 0.1	6.3 ± 0.1**▲▲
Albumin (g/dL)	4.7 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	5.0 ± 0.1	5.2 ± 0.1**▲	5.6 ± 0.1**▲▲	5.2 ± 0.1**▲
Cholesterol (mg/dL)	85 ± 2	80 ± 3	80 ± 3	89 ± 2	79 ± 3	51 ± 1**▲	43 ± 2**▲▲
Triglycerides (mg/dL)	161 ± 7	194 ± 13	128 ± 7	129 ± 7	92 ± 5*▲▲	35 ± 1**▲▲	35 ± 3**▲▲
Alkaline phosphatase (IU/L)	411 ± 6	438 ± 13	419 ± 12	423 ± 14	447 ± 13	696 ± 17**▲	920 ± 29**▲▲
Bile acids (µmol/L)	11.1 ± 1.8	16.1 ± 1.2	13.9 ± 1.2	15.5 ± 1.3	15.7 ± 1.5	21.7 ± 2.3*	35.2 ± 2.9**▲▲
FEMALE							
n	10	10	9	10	10	10	10
Total protein (g/dL)	7.0 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	6.8 ± 0.1	6.3 ± 0.0**▲▲
Albumin (g/dL)	4.9 ± 0.1	4.9 ± 0.1	5.1 ± 0.1	5.1 ± 0.1*	5.3 ± 0.1**▲▲	5.2 ± 0.0**▲	5.1 ± 0.0
Cholesterol (mg/dL)	112 ± 3	114 ± 3	111 ± 4	111 ± 2	101 ± 3	87 ± 2**▲▲	58 ± 2**▲▲
Triglycerides (mg/dL)	79 ± 5	104 ± 10	79 ± 8	73 ± 5	73 ± 6	49 ± 2*▲▲	35 ± 1**▲▲
Alkaline phosphatase (IU/L)	316 ± 13	313 ± 11	347 ± 14	362 ± 13	353 ± 21	457 ± 10**▲▲	670 ± 22**▲▲
Bile acids (µmol/L)	33.3 ± 2.8	30.9 ± 2.5	29.8 ± 2.9	34.7 ± 2.5	36.0 ± 2.2	40.5 ± 3.4	50.3 ± 7.5▲

[1] Data are given as mean ± standard error.
[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunn's test.
▲ Significantly different ($P \leq 0.05$) from the MPE:0 ppm group by Dunn's test.
▲▲ Significantly different ($P \leq 0.01$) from the MPE:0 ppm group by Dunn's test.

Serum total protein concentrations in male and female rats in the MPE:40,000 ppm groups were lower than in the controls. In males, this difference was counterbalanced by higher serum albumin concentrations, which also occurred in exposed groups that did not have the lower protein concentrations. Lower total protein concentrations in conjunction with normal or higher albumin concentrations would suggest that the globulin protein fractions were reduced. It is of note that higher albumin concentrations are limited to conditions causing dehydration, and a rise in albumin concentration would indicate hemoconcentration. This suggests that the total protein values and globulin fractions in exposed rats may be tempered by the hemoconcentration and could be lower, and affect more exposure groups, than the results indicate. Hemoconcentration may also impact the hemogram values, indicating that the anemia may be more severe than is suggested by the data.

Alkaline phosphatase activities and bile salt concentrations were higher in male and female rats in the MPE:20,000 and MPE:40,000 ppm groups than in the controls; these changes are consistent with cholestasis. Differences in other clinical chemistry parameters were inconsistent and minor and were not considered to be treatment related.

There were no significant differences in liver palmitoyl-CoA activities between controls that were exposed perinatally (MPE:0 ppm group) and those that were not (0:0 ppm group) (Table 14). Palmitoyl-CoA oxidase activities were significantly higher in male rats exposed as adults to 5,000 ppm or greater than in the controls; females exposed to 10,000 ppm or greater as adults also had a higher activity than the controls. In males and females in the highest exposure groups (MPE:40,000 ppm), palmitoyl-CoA oxidase activities were over 20-fold higher than those of the controls.

There were no significant differences in either serum or testis zinc concentrations between the two control groups. Serum zinc concentrations in male rats receiving 20,000 or 40,000 ppm were higher than in the perinatally exposed controls (Table 15); the testis zinc concentration was lower in the 40,000 ppm group than in either control group. The serum testosterone concentration was significantly lower in control males that received 10,000 ppm perinatally than in control males that were not exposed perinatally. The serum testosterone concentration was also lower in males that received 40,000 ppm as adults than in the controls receiving no perinatal exposure. Serum testosterone concentrations in exposed males were low and variable and appeared unrelated to dibutyl phthalate treatment.

TABLE 14 Liver Peroxisome Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
MALE							
n	5	5	5	5	5	5	5
Palmitoyl-CoA oxidase activity	3.2 ± 0.3	2.8 ± 0.2	3.1 ± 0.2	5.4 ± 0.3**▲▲	13.1 ± 1.0**▲▲	36.3 ± 0.9**▲▲	65.5 ± 2.9**▲▲
FEMALE							
n	5	5	5	5	5	5	5
Palmitoyl-CoA oxidase activity	3.3 ± 0.1	2.7 ± 0.2	3.7 ± 0.2	4.0 ± 0.2	5.8 ± 0.3**▲▲	16.5 ± 1.4**▲▲	64.2 ± 1.7**▲▲

[1] Data are given as mean ± standard error (nmol/minute per mg protein). Statistical tests were performed on unrounded data.
[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups for 4 weeks (until the beginning of the 13-week adult exposure phase).
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunnett's test with a square root transformation.
▲▲ Significantly different ($P \leq 0.01$) from the MPE:0 ppm group by Dunnett's test with a square root transformation.

TABLE 15 Serum and Testis Zinc and Testosterone Concentrations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
n	10	9	10	10	10	10	10
Zinc (µg/g)							
Serum	1.20 ± 0.14	1.16 ± 0.05	1.29 ± 0.05	1.39 ± 0.09	1.36 ± 0.06	1.60 ± 0.04*▲▲	1.64 ± 0.14▲▲
Testis	31.90 ± 0.70	32.12 ± 0.98	31.30 ± 0.33	32.02 ± 0.64	32.07 ± 0.85	30.24 ± 1.42[3]	16.37 ±0.84**▲▲
Testosterone							
Serum (ng/mL)	4.09 ± 0.32	1.81 ± 0.34**	2.94 ± 0.49	3.25 ± 0.47	4.04 ± 0.56▲	2.40 ± 0.48	1.27 ± 0.28**
Testis (ng/g)	164.0 ± 26.0	195.1 ± 18.3	232.6 ± 43.8	184.6 ± 27.5	177.0 ± 25.0	228.0 ± 49.4	253.3 ± 32.7

[1] Data are given as mean ± standard error.
[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.
[3] n=9.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunn's test.
▲ Significantly different ($P \leq 0.05$) from the MPE:0 ppm group by Dunn's test.
▲▲ Significantly different ($P \leq 0.01$) from the MPE:0 ppm group by Dunn's test.

The liver was identified as a site of dibutyl phthalate toxicity in rats exposed perinatally and as adults. Hepatomegaly was evident, with livers of males and females noted at necropsy to be generally enlarged with increasing exposure concentration. Microscopic examination revealed a lesion which was characterized as cytoplasmic alteration and which occurred with minimal to moderate severity in the livers of all male and female rats receiving 10,000 ppm or greater as adults (Table 16 and Appendix A, Tables A1 and A2). The lesion consisted of hepatocytes with a more intensely staining eosinophilic cytoplasm and fewer small, clear vacuoles than in the controls. Subsequent staining of adjacent sections with PAS, with and without diastase, confirmed the presence of glycogen within these vacuoles and thus the apparent lowering of glycogen content in the liver of rats receiving 10,000 ppm or greater as adults. The no-effect level for the cytoplasmic alterations in the liver was determined to be MPE:5,000 ppm for both sexes. Small, fine, eosinophilic granules were more commonly observed in the cytoplasm of hepatocytes from the livers of rats in the MPE:40,000 ppm groups than in the controls. In a subset of animals that were examined for ultrastructural changes, peroxisome proliferation was clearly evident in males and females in the highest exposure groups (MPE:40,000 ppm); electron-dense, membrane-bound structures, consistent with tertiary phagolysomal bodies or lipofuscin, were also observed. Subsequent staining for lipofuscin was conducted on formalin-fixed, paraffin-embedded tissue. The presence of dark green, granular staining of the cytoplasm of hepatocytes, consistent with lipofuscin, was observed in Schmorl's-stained sections. The staining intensity increased with increasing exposure concentration, becoming more diffusely distributed, and the staining was generally more severe in males than in females. Rats that received 10,000 ppm or greater as adults had greater staining than the controls; in the MPE/10,000 ppm groups, the lesion was restricted to focal accumulations of increased Schmorl's-positive staining (4/5 males and 1/5 females). In all males receiving 20,000 or 40,000 ppm as adults and in all females receiving 40,000 ppm as adults, the lipofuscin accumulation was diffuse and more severe, with fine to coarse Schmorl's-positive granules outlining bile canaliculi. Although the positive control tissue (cardiac muscle) stained intensely, no positive staining was detected with the AFIP method.

TABLE 16 Incidence and Severity of Selected Lesions in F_1 F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

	Concentration (ppm)						
Perinatal	**0**	**10,000[2]**	**10,000**	**10,000**	**10,000**	**10,000**	**10,000**
Adult	**0**	**0**	**2,500**	**5,000**	**10,000**	**20,000**	**40,000**
MALE							
n	10	10	10	10	10	10	10
Liver							
Cytoplasmic alteration	0	0	0	0	10** (1.0)	10** (3.0)	10** (3.0)
Testes							
Germinal epithelium, atrophy	0	0	0	0	4* (1.0)	10** (2.3)	10** (4.0)
FEMALE							
n	10	10	10	10	10	10	10
Liver							
Cytoplasmic alteration	0	0	0	0	10** (1.0)	10** (3.0)	10** (2.7)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
[2] 10,000 ppm = maximum perinatal exposure (MPE); administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.
* Significantly different ($P \leq 0.05$) from the control groups by the Fisher exact test.
** Significantly different ($P \leq 0.01$) from the control groups by the Fisher exact test.

The testis was also identified as a site of dibutyl phthalate toxicity, based on degeneration of the germinal epithelium (Table 16). In males in the MPE:40,000 ppm group, the lesion was diffuse and consisted of almost complete loss of germinal epithelial cells in all seminiferous tubules, with no spermatogenesis occurring. The tubules were lined only with Sertoli cells, many of which contained vacuolated cytoplasm. This lesion also occurred in all rats in the MPE:20,000 ppm group; however, the atrophy was focal in distribution and less severe. Four of ten rats in the MPE:10,000 ppm group were minimally affected with focal atrophy of seminiferous tubules; the PWG determined the no-effect level to be 5,000 ppm. Hypospermia of the epididymis was present in males receiving 20,000 or 40,000 ppm as adults; 10,000 ppm was considered the no-effect level for this lesion.

Additional male and female reproductive system parameters were assessed in rats in the control groups and the 2,500, 10,000, and 20,000 ppm adult-exposure groups (Appendix D, Tables D1 and D2). The left cauda epididymal weight of control males that were exposed perinatally was significantly greater than that of unexposed control males. In general, the results for males exposed as adults are consistent with the loss of germinal epithelium observed histologically (Table 16). Left epididymal and cauda epididymal weights of males receiving 2,500 ppm or greater as adults were generally lower than those of the perinatally exposed controls. Despite the similar body weights of males receiving 2,500 or 10,000 ppm as adults, left testis weights in these groups were greater than the testis weight of the perinatally exposed controls (Table D1), a finding that was also observed at the 5,000 ppm exposure level for the right testis weight (Table 11). Left epididymal, cauda epididymal, and testis weights, the number of spermatid heads per testis, and the spermatid count of males administered 20,000 ppm as adults were lower than those of unexposed control males, consistent with hypospermia of the epididymis observed histologically at this exposure concentration. Among the females, there were no significant differences in estrous cycle length or in the percentage of time spent in the various estrous stages between the two control groups or between the control groups and females exposed as adults (Table D2).

13-Week Feed Study in F344/N Rats

All rats survived to the end of the study (Table 17). Males receiving 10,000 ppm or greater and females receiving 20,000 or 40,000 ppm had lower final mean body weights and mean body weight gains than the respective controls (Table 17 and Figure 4). All male and female rats that received 40,000 ppm were emaciated. No other clinical signs were considered related to exposure. Feed consumption by males and females receiving 40,000 ppm was lower than that by the controls (Table 17).

TABLE 17 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate

Dose (ppm)	Survival[1]	Mean Body Weight[2] (grams)			Final Weight Relative to Controls[3] (%)	Average Feed Consumption[4] (g/day)	Average Dose[4] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	118 ± 3	360 ± 6	242 ± 4		17.2	
2,500	10/10	115 ± 3	349 ± 4	235 ± 4	97	16.8	176
5,000	10/10	116 ± 3	354 ± 3	238 ± 4	98	17.5	359
10,000	10/10	117 ± 3	332 ± 6**	215 ± 5**	92	16.9	720
20,000	10/10	116 ± 4	300 ± 7**	184 ± 6**	83	17.0	1,540
40,000	10/10	118 ± 4	161 ± 4**	43 ± 1**	45	10.5	2,964
FEMALE							
0	10/10	103 ± 2	197 ± 3	93 ± 2		11.2	
2,500	10/10	101 ± 2	191 ± 3	90 ± 2	97	10.8	177
5,000	10/10	102 ± 2	200 ± 3	98 ± 2	102	11.4	356
10,000	10/10	102 ± 2	191 ± 3	89 ± 2	97	11.0	712
20,000	10/10	103 ± 2	182 ± 2**	78 ± 2**	92	10.6	1,413
40,000	10/10	103 ± 2	144 ± 3**	42 ± 2**	73	9.3	2,943

[1] Number surviving at 13 weeks/number of animals per group.
[2] Weights and weight changes are given as mean ± standard error.
[3] (Dose group mean/control group mean) x 100.
[4] Average of individual consumption values for Weeks 1-13.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

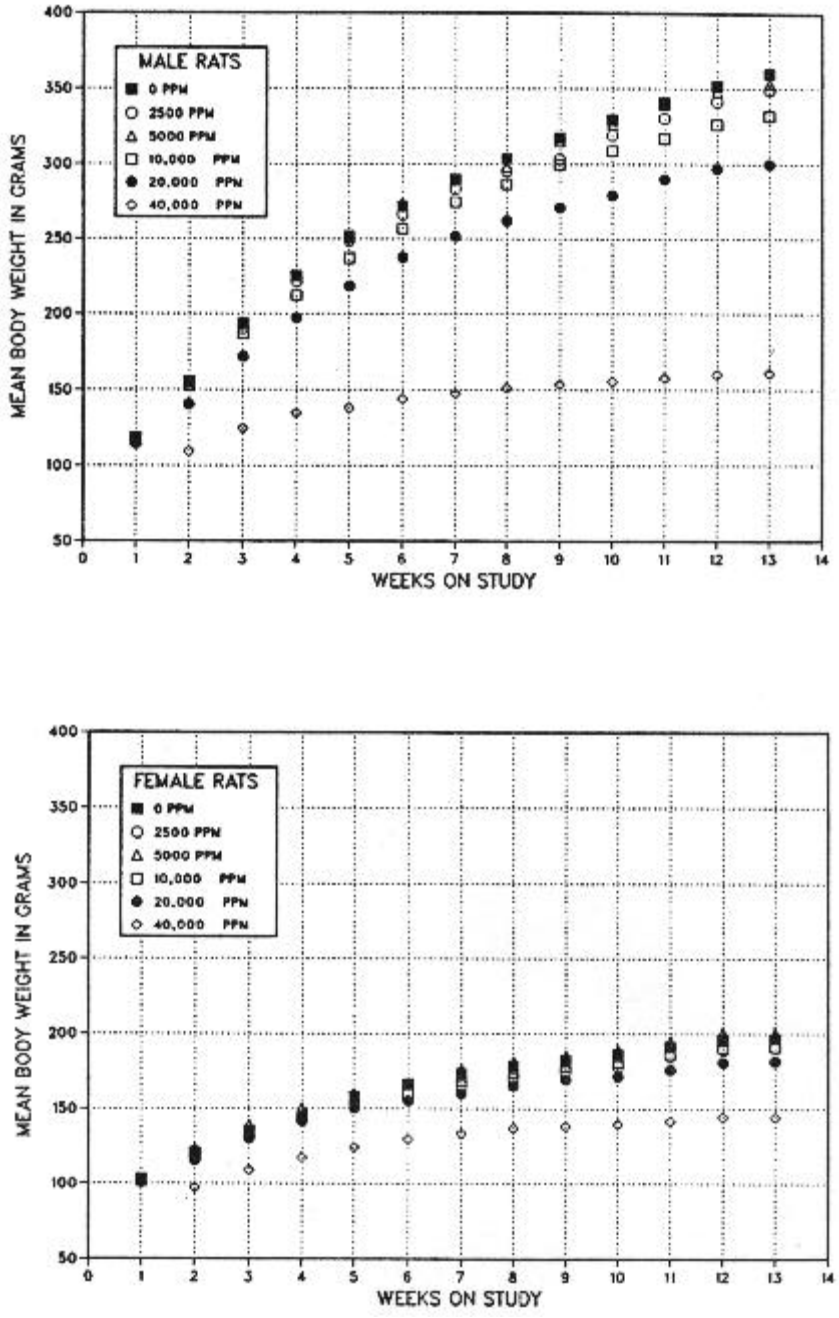


FIGURE 4 Body Weights of F344/N Rats Administered Dibutyl Phthalate in Feed for 13 Weeks

Relative liver and kidney weights were significantly greater in male rats administered 5,000 ppm dibutyl phthalate or greater than in the controls (Tables 18 and B3). Absolute liver weights were greater in male rats receiving 5,000, 10,000, or 20,000 ppm than in the controls. Absolute and relative liver weights and relative kidney weights were greater in females receiving 10,000 ppm or greater than in the controls. In male rats that received 20,000 or 40,000 ppm, absolute and relative testis weights were significantly less than those of the controls. Other statistically significant differences in organ weights were considered secondary to body weight changes.

TABLE 18 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
MALE						
n	10	10	10	10	10	10
Necropsy body wt	374 ± 6	363 ± 5	369 ± 3	345 ± 6**	310 ± 8**	163 ± 4**
Right kidney						
Absolute	1.353 ± 0.028	1.362 ± 0.028	1.442 ± 0.016	1.404 ± 0.030	1.325 ± 0.042	0.804 ± 0.026**
Relative	3.62 ± 0.06	3.75 ± 0.06	3.91 ± 0.03**	4.07 ± 0.04**	4.27 ± 0.05**	4.92 ± 0.09**
Liver						
Absolute	15.660 ± 0.356	16.150 ± 0.446	18.263 ± 0.284**	19.069 ± 0.498**	19.984 ± 0.748**	11.633 ± 0.478**
Relative	41.87 ± 0.72	44.45 ± 0.80	49.57 ± 0.72**	55.29 ± 1.09**	64.33 ± 1.08**	71.07 ± 1.66**
Right testis						
Absolute	1.495 ± 0.029	1.494 ± 0.023	1.490 ± 0.010	1.455 ± 0.017	0.521 ± 0.054**	0.321 ± 0.012**
Relative	4.00 ± 0.04	4.12 ± 0.03	4.05 ± 0.04	4.22 ± 0.04	1.68 ± 0.17**	1.96 ± 0.04**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	202 ± 3	197 ± 3	207 ± 3	197 ± 3	185 ± 2**	148 ± 3**
Right kidney						
Absolute	0.744 ± 0.020	0.732 ± 0.014	0.789 ± 0.018	0.788 ± 0.015	0.746 ± 0.015	0.674 ± 0.017*
Relative	3.68 ± 0.06	3.71 ± 0.05	3.80 ± 0.05	4.00 ± 0.08**	4.03 ± 0.06**	4.55 ± 0.06**
Liver						
Absolute	7.238 ± 0.175	7.089 ± 0.203	7.708 ± 0.182	7.872 ± 0.134*	8.293 ± 0.189**	9.431 ± 0.237**
Relative	35.84 ± 0.77	35.89 ± 0.61	37.17 ± 0.79	39.91 ± 0.58**	44.74 ± 0.91**	63.78 ± 1.35**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

Hematology and clinical chemistry results are provided in Tables 19, 20, and C2. Treatment-related hematology changes occurred only in male rats and were characterized primarily by a minimal anemia in males receiving 5,000 ppm or greater (Table 19). This anemia was evidenced by lower Hct values, Hgb concentrations, and RBC counts in exposed males than in the controls. Hemoconcentration by dehydration was evidenced by higher albumin concentrations in exposed groups than in the controls (Table 20), and the anemia may have been more severe than the data indicate. Reticulocyte counts of exposed and control males were similar, suggesting no bone marrow response to the minimal anemia. However, MCV values were slightly greater in exposed groups than in the controls, suggesting that young cells were being released from the bone marrow maturation pool. NRBC counts in males and females in the 40,000 ppm groups were four to five times higher than control values, possibly indicating an inappropriate release of erythroid precursors from the bone marrow. The number of platelets in males receiving 5,000 ppm or greater were higher than in the controls; this would be compatible with a reactive thrombocytosis. Other differences in hematology parameters were not consistent and did not indicate a treatment-related response.

As was mentioned previously, minimally higher concentrations of albumin occurred in all treated groups of male rats (Table 20); this would be compatible with hemoconcentration by dehydration. In male rats in the 40,000 ppm group, the change was counterbalanced by a lower total protein concentration than in the controls, suggesting that the globulin protein fraction in this group was lowered. The globulin fraction may have been reduced in female rats as well; total protein concentrations were lower in female rats in the 20,000 and 40,000 ppm groups than in the controls, with no changes in albumin concentration evident. Triglyceride and cholesterol concentrations were lower in exposed male and female rats than in the controls; these differences were most pronounced in the 20,000 and 40,000 ppm groups and were more pronounced in males than females. Triglyceride concentrations were more affected than cholesterol concentrations, as evidenced by the greater percentage differences and the higher number of exposure groups affected. Cholestasis was evidenced by higher alkaline phosphatase activities and bile salt concentrations in exposed groups than in the controls; this change primarily involved males and females in the 20,000 and 40,000 ppm groups, though females in the 5,000 and 10,000 ppm groups also had similar but milder changes.

TABLE 19 Selected Hematology Data for Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
n	10	9	10	10	9	9
Hematocrit (%)	49.3 ± 0.4	48.8 ± 0.6	48.0 ± 0.4	48.0 ± 0.4	46.0 ± 1.0**	46.2 ± 0.5**
Hemoglobin (g/dL)	14.7 ± 0.1	14.5 ± 0.1	14.2 ± 0.1**	14.3 ± 0.1*	14.0 ± 0.3**	14.0 ± 0.1**
Erythrocytes (10^6/µL)	9.15 ± 0.08	9.08 ± 0.12	8.89 ± 0.07*	8.80 ± 0.08*	8.24 ± 0.16**	8.32 ± 0.07**
Reticulocytes (10^6/µL)	0.19 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.24 ± 0.03
Nucleated erythrocytes (10^3/µL)	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.13 ± 0.04*
Mean cell volume (fL)	54.0 ± 0.2	53.9 ± 0.3	53.9 ± 0.1	54.6 ± 0.2*	55.8 ± 0.3**	55.3 ± 0.3**
Platelets (10^3/µL)	568.6 ± 11.9[2]	570.0 ± 16.4	628.6 ± 11.5**	648.8 ± 14.5**	650.8 ± 11.6**	638.2 ± 19.3**

[1] Data are given as mean ± standard error.
[2] n=9.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

TABLE 20 Selected Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
MALE						
n	10	10	10	10	10	10
Total protein (g/dL)	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	5.9 ± 0.1**
Albumin (g/dL)	4.59 ± 0.05	4.80 ± 0.05*	5.00 ± 0.03**	5.25 ± 0.05**	5.45 ± 0.08**	4.82 ± 0.05**
Cholesterol (mg/dL)	79 ± 2	82 ± 2	83 ± 2	75 ± 1	52 ± 1**	37 ± 2**
Triglycerides (mg/dL)	233 ± 19	171 ± 12*	168 ± 14*	119 ± 5**	49 ± 2**	32 ± 1**
Alkaline phosphatase (IU/L)	537 ± 24	528 ± 12	509 ± 8	552 ± 15	826 ± 32**	939 ± 17**
Bile acids (µmol/L)	11.9 ± 3.3	10.0 ± 1.4	13.4 ± 2.3	15.8 ± 3.2	28.7 ± 5.6**	46.5 ± 2.6**
FEMALE						
n	10	10	10	10	10	10
Total protein (g/dL)	7.3 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.1**	6.2 ± 0.1**
Albumin (g/dL)	5.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.1
Cholesterol (mg/dL)	102 ± 3	101 ± 2	100 ± 2	94 ± 3	77 ± 2**	52 ± 1**
Triglycerides (mg/dL)	109 ± 10	115 ± 13	108 ± 11	71 ± 9*	57 ± 2**	38 ± 1**
Alkaline phosphatase (IU/L)	409 ± 15	405 ± 16	439 ± 14	522 ± 39**	536 ± 23**	787 ± 21**
Bile acids (µmol/L)	22.2 ± 2.1	30.8 ± 3.3	35.9 ± 4.4*	35.2 ± 4.0*	40.0 ± 4.4**	67.6 ± 5.2**

[1] Data are given as mean ± standard error.
* Significantly different (P≤0.05) from the control group by Shirley's test.
** Significantly different (P≤0.01) from the control group by Shirley's test.

Liver palmitoyl-CoA oxidase activities were significantly higher in male and female rats receiving 5,000 ppm or greater than in the controls (Table 21). Liver palmitoyl-CoA oxidase activities were 13-fold higher in males and 32-fold higher in females administered 40,000 ppm than in the controls. The serum concentration of zinc in male rats in the 40,000 ppm group was slightly lower than that in the controls (Table 22); testis zinc was lower in males in the 20,000 and 40,000 ppm groups than in the controls. Serum testosterone was also lower in males in the 20,000 and 40,000 ppm groups than in the controls; however, testis testosterone concentrations in exposed and control rats were similar.

TABLE 21 Liver Palmitoyl-CoA Oxidase Activity Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
MALE						
n	5	5	5	5	5	5
Palmitoyl-CoA oxidase activity	3.1 ± 0.2	3.3 ± 0.2	6.0 ± 0.2**	17.7 ± 0.6**	30.0 ± 2.7**	40.6 ± 7.0**
FEMALE						
n	5	5	5	5	5	5
Palmitoyl-CoA oxidase activity	1.6 ± 0.1	2.1 ± 0.2	2.7 ± 0.2**	4.1 ± 0.3**	17.6 ± 4.1**	51.9 ± 5.1**

[1] Data are given as mean ± standard error (nmol/minute per mg protein). Statistical tests were performed on unrounded data.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

TABLE 22 Serum and Testis Zinc and Testosterone Concentrations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
n	10	10	10	10	10	10
Zinc (µg/g)						
Serum	1.52 ± 0.07	1.43 ± 0.04	1.38 ± 0.06	1.47 ± 0.03	1.79 ± 0.04	1.25 ± 0.06*
Testis	30.77 ± 1.13	29.26 ± 0.46	30.95 ± 1.06	28.43 ± 0.49	17.05 ± 0.77**	14.13 ± 0.83**
Testosterone						
Serum (ng/mL)	6.49 ± 0.74	5.30 ± 0.50	5.39 ± 0.51	4.73 ± 0.48	2.50 ± 0.33**	1.00 ± 0.22**
Testis (ng/g)	211.5 ± 26.4	135.6 ± 22.6	150.9 ± 26.9	171.7 ± 21.3	273.4 ± 34.1	288.5 ± 66.2

[1] Data are given as mean ± standard error.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

As in the 13-week study with perinatal exposure (Table 18), the liver and testes in rats in the standard 13-week study were identified as sites of dibutyl phthalate toxicity (Table 23). Microscopic examination revealed a liver lesion that was characterized as cytoplasmic alteration (Table 23 and Plates 1 and 2) and that consisted of hepatocytes with a more intensely staining eosinophilic cytoplasm and fewer small, clear vacuoles than in the controls. Subsequent staining of adjacent sections with PAS, with and without diastase, confirmed this to be a decrease in the number of glycogen-containing vacuoles. Small, fine, eosinophilic granules were more commonly observed in the cytoplasm of hepatocytes from livers of rats in the 40,000 ppm groups than in the controls. The no-effect level for the cytoplasmic alterations in the liver was considered to be 5,000 ppm for males and females. In a subset of animals examined for ultrastructural changes, peroxisome proliferation was clearly evident in males and females in the 40,000 ppm groups. Electron-dense, membrane-bound structures, consistent with tertiary phagolysomal bodies or lipofuscin, were also observed. Subsequent staining for lipofuscin was conducted on formalin-fixed, paraffin-embedded tissue. The presence of dark green, granular staining of the cytoplasm of hepatocytes, consistent with lipofuscin, was observed in Shmorl's-stained sections. The staining intensity increased with increasing exposure concentration, becoming more diffusely distributed, and the staining was generally more severe in males than in females. Rats that received 20,000 ppm or greater had greater staining than the controls; in the 20,000 ppm groups, the lesion was mild and diffusely distributed in all males and was restricted to focal accumulations of increased Schmorl's-positive staining in four of five females. In all males and females receiving 40,000 ppm, the lipofuscin accumulation was diffuse and more severe,

with fine to coarse Schmorl's-positive granules outlining bile canaliculi. Although the positive control tissue (cardiac muscle) stained intensely, no positive staining was detected with the AFIP method.

TABLE 23 Incidence and Severity of Selected Lesions in F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	2,500	5,000	10,000	20,000	40,000
MALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	0	0	10** (2.8)	10** (3.0)	10** (3.0)
Testes						
Germinal epithelium, atrophy	0	0	0	4* (1.8)	10** (3.8)	10** (4.0)
FEMALE						
n	10	10	10	10	10	10
Liver						
Cytoplasmic alteration	0	0	0	10** (1.4)	10** (3.1)	10** (3.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=marked.
* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test.
** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

The testicular lesion was characterized by degeneration of the germinal epithelium (Table 23 and Plates 3 and 4). In males exposed to 40,000 ppm, the lesion was diffuse and consisted of an almost complete loss of germinal epithelial cells in all seminiferous tubules, with no spermatogenesis occurring. The tubules were lined only with Sertoli cells, many of which contained vacuolated cytoplasm. This lesion also occurred in the 20,000 ppm group; however, in this group the atrophy was focal in distribution. Four of 10 rats in the 10,000 ppm group were also considered to have focal atrophy of seminiferous tubules. The no-effect level was considered to be 5,000 ppm. Marked hypospermia of the epididymis was present in the 20,000 and 40,000 ppm groups; the no-effect level for this lesion was determined to be 10,000 ppm.

Additional reproductive parameters were evaluated in males and females in the 0, 2,500, 10,000, and 20,000 ppm groups (Appendix D, Tables D3 and D4). Left epididymal, cauda epididymal, and testis weights, the number of spermatid heads per testis and per gram testis, spermatid count, and epididymal spermatozoal motility and concentration were significantly lower in males in the

20,000 ppm group than in the controls (Table D3). There were no significant differences in estrous cycle length or in the percentage of time spent in the various estrous stages between exposed and control female rats (Table D4).

Comparison of untreated controls in the 13-week rat study to untreated controls in the 13-week rat study with perinatal exposure: The most noteworthy difference between the two studies was the initial body weights, which differed due to a difference in the age of the rats at the beginning of the 13-week treatment phase (Tables 10 and 17). The rats of the standard 13-week study were approximately 2 to 3 weeks younger than the rats in the study with perinatal exposure and were near the end of the rapid growth phase of young rats. Therefore, in this standard 13-week study, the males weighed 45% less and females 30% less than their respective counterparts in the study with perinatal exposure. Due to the leveling off of adult weights, this differential was not observed between the untreated controls of the two studies by the end of the 13-week exposures.

Triglyceride concentrations differed considerably between the controls of the two studies, with males and females from the standard 13-week study having values roughly 50% higher than their counterparts in the 13-week study with perinatal exposure. This additional elevation in the younger rats may have artificially impacted the apparent reduction of triglyceride concentrations in the standard 13-week study. Significant hypotriglyceridemia was observed in males receiving 2,500 ppm in the standard study; this finding was only observed at 10,000 ppm and higher concentrations in the females in the same study and in male and female rats in the 13-week study with perinatal exposure. Other minor differences were noted between the two sets of controls; however, these were not as consistent and were considered to be more likely the result of normal variation between animals.

Continuous Breeding Study in Sprague-Dawley Rats

Toxic and reproductive effects of dibutyl phthalate on Sprague-Dawley rats and effects on survival and body weights of pups were evaluated in a continuous breeding study which included crossover mating and offspring assessment phases. This study is summarized in Figure 2 and Appendix E, and only clearly treatment-related effects are noted here.

Although there were no effects on the fertility (ability to deliver any young) of F_0 rats exposed to dibutyl phthalate during the continuous breeding phase (Table E1), the average number of live pups per litter was significantly lower in all exposed groups than in the controls (Table E2). The mean body weight of dams in the 10,000 ppm group was 6% to 13% lower than that of the controls at delivery and during lactation (Table E1), and pup weights in the 10,000 ppm group were less than the control value at birth and during lactation (Tables E2 and E3).

In the crossover mating trial, there were no differences in fertility between control rats and males or females receiving 10,000 ppm (Table E4). The live pup weight, when adjusted for litter size, was significantly less for litters from exposed dams. The absolute liver weight of exposed male F_0 rats and relative liver and kidney weights of exposed male and female F_0 rats were significantly greater than those of the controls (Table E5). There were no significant differences in sperm parameters or in estrous cycle lengths between control and exposed F_0 rats (Table E6).

In the offspring assessment phase, the fifth litter of F_1 rats were treated *in utero*, during lactation and weaning, and through delivery of the F_2 generation. In contrast to the F_0 rats, mating, pregnancy, and fertility indices were significantly lower in F_1 rats in the 10,000 ppm group than in the controls; only one F_2 litter was produced in this group of 20 mating pairs (Table E7). The mating, pregnancy, and fertility alterations appeared to involve males and females exposed to 10,000 ppm. In F_1 males, absolute and relative right epididymal, right cauda epididymal, right testis, seminal vesicle, and prostate gland weights in the 10,000 ppm group were significantly lower than those of the controls (Table E8). In females, the absolute right ovary weight in the 10,000 ppm group was lower than in the controls (Table E8). Spermatid measurements and epididymal sperm concentration were lower in males exposed to 10,000 ppm than in the controls (Table E9). Germinal epithelial degeneration of the testis and underdevelopment and/or hypospermia of the epididymis were noted histopathologically in exposed males in the 10,000 ppm group (Table E10). Mild interstitial cell hyperplasia was noted in 7 of 10 males in the 10,000 ppm group. There were no significant differences in estrous cycle length between control and exposed F_1 females; however, diestrus

appeared shorter and estrus appeared to be extended in the 10,000 ppm group (Table E9). Female F_2 pup weights and total and adjusted live pup weights were lower in all exposed groups, including the single litter produced to F_1 rats in the 10,000 ppm group, than in the controls (Table E7).

Perinatal Peroxisome Determination Studies in F344/N Rats

The effects of dibutyl phthalate and di(2-ethylhexyl) phthalate on hepatic peroxisome proliferation *in utero* or during lactation were assessed in breeding studies in which F344/N rats were administered dibutyl phthalate or di(2-ethylhexyl) phthalate in feed for up to 20 days during gestation or for 22 days during lactation. These studies are presented in Appendix F.

In the *in utero* exposure study of dibutyl phthalate, the terminal body weight of dams receiving 20,000 ppm was less than that of the controls (Table F1). The liver weight of dams receiving 10,000 ppm was greater than that of the control dams. The hepatic palmitoyl-CoA oxidase (peroxisomal enzyme) activity was higher in dams receiving 1,250, 2,500, or 5,000 ppm dibutyl phthalate than in the controls; however, the activity was low and highly variable. In the 20,000 ppm group, the average litter size was smaller than in the controls (Table F1). The pooled absolute liver weight of fetuses from female rats administered 20,000 ppm dibutyl phthalate was significantly lower than that of fetuses from the controls. The peroxisomal enzyme activity of each group of exposed pups was similar to the control value.

Among dams exposed to di(2-ethylhexyl) phthalate in the *in utero* exposure study, the terminal body weight in the 28,000 ppm group was less than that of the controls (Table F2). Liver weights of all groups of exposed dams except the 28,000 ppm group were greater than the liver weight of the controls. The peroxisomal enzyme activity of each group of exposed dams was similar to that of the control dams. The liver weight of pups from dams receiving 7,000 ppm di(2-ethylhexyl) phthalate was greater than that of the control pups (Table F2). No fetuses were recovered in the 28,000 ppm group. In the highest exposure group in which fetuses developed (14,000 ppm), the peroxisomal enzyme activity of the fetuses was twice that of the controls.

In the lactational exposure study, the terminal body weight of dams that received 30,000 ppm dibutyl phthalate was less than that of the controls (Table F3). The liver weight of dams receiving 10,000 or 30,000 ppm was greater than that of the controls. The peroxisomal enzyme activity of each treated group of dams was low; values for exposed groups varied and were not highly significant. The body weights of male and female pups from dams receiving 30,000 ppm dibutyl phthalate was lower than those of the control pups on Days 7, 14, and 21 (Table F3). The liver weights of male and female pups in this group were significantly lower than those of the controls at all time points. The peroxisomal enzyme activity in male pups in the 30,000 ppm group was significantly higher than in the controls on Days 7 and 21; lower exposure groups also had slightly higher or lower peroxisomal

enzyme activities than the controls on Days 14 and 21. The peroxisomal enzyme activity in female pups exposed to 3,000 or 30,000 ppm was higher than in the controls on Day 7; however, the peroxisomal enzyme activity in each group of female pups throughout treatment was low, and these values varied and were not considered highly significant.

In dams exposed to di(2-ethylhexyl) phthalate during the lactational exposure study, lower body weights were noted in the 4,200, 14,000, and 42,000 ppm groups (Table F4). The liver weights of dams in the 1,400, 4,200, and 14,000 ppm groups were greater than in the controls. Peroxisomal enzyme activity generally increased with increasing exposure concentration, and the increase was significant in the 14,000 ppm group. The survival of pups in the highest exposure group of di(2-ethylhexyl) phthalate, 42,000 ppm, was decreased; in this group, no pups survived to Day 14. Body weight differences were noted in all exposed groups (Table F4). Male and female pups from dams receiving 14,000 ppm had lower body weights than the controls on Days 7, 14, and 21. On Day 14, female pups in the 420, 1,400, and 4,200 ppm groups also had significantly lower body weights than the controls; on Day 21, male pups exposed to 4,200 ppm and female pups exposed to 1,400 or 4,200 ppm had lower body weights than the controls. The liver weight of male and female pups in the 42,000 ppm group was significantly lower than in the controls on Day 7. At all time points, the liver weights of male and female pups exposed to 14,000 ppm were lower than in the controls. The peroxisomal enzyme activity was higher in male and female pups in the 42,000 ppm group than in the controls on Day 7. In general, the peroxisomal enzyme activity in exposed male and female pups was low and variable and was not considered elevated in response to treatment.

Maximum Perinatal Exposure Determination Feed Study in B6C3F$_1$ Mice

One female in each of the 0, 5,000, and 7,500 ppm groups died during the gestation period. Females receiving 2,500 ppm dibutyl phthalate or greater had a longer gestation than control females, and gestation length generally increased with increasing exposure concentration (Table 24). Gestation Day 17 mean body weights and mean body weight gains of dams were generally decreased with increasing exposure concentration, and the decreases were significant for females receiving 7,500 ppm or greater. No live pups were delivered in the 20,000 ppm group, and only 5 of 20 females in the 10,000 ppm group delivered a live pup. All females in the 20,000 ppm group that did not deliver were killed and examined for implantations by uterine staining with ammonium sulfide during the fifth and sixth weeks after breeding.

On the day of delivery, the number of pups per litter and the number of live pups per litter in the 7,500 and 10,000 ppm groups were significantly lower than in the controls (Table 24); only a single pup in the 10,000 ppm group survived beyond Day 1 postpartum. The number of live pups per litter in the 7,500 ppm group remained lower than that in the controls throughout the study. The mean body weight of pups in the 10,000 ppm group was significantly lower than that of the controls at delivery.

The incidence of cannibalization of pups was greater in the 7,500 and 10,000 ppm groups than in the controls. No other clinical signs in pups or dams were considered related to dibutyl phthalate administration.

TABLE 24 Reproductive Performance and Body Weights of B6C3F$_1$ Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)						
	0	1,250	2,500	5,000	7,500	10,000	20,000
Number of breeding groups[2]	8	9	8	8	8	4	5
DAM DATA							
Gestation index[3]	11/20 (55%)	10/19 (53%)	12/19 (63%)	9/19 (47%)	11/18 (61%)	5/20 (25%)	0/20 (0%)**
Gestation length (days)	18.19 ± 0.13	18.31 ± 0.08	18.53 ± 0.14*	18.72 ± 0.13**	19.13 ± 0.23**[4]	19.36 ± 0.16**[5]	19.00[6]
Dam weight during gestation (g)							
Day 0	20.2 ± 0.3	20.7 ± 0.2	20.3 ± 0.3	21.1 ± 0.3	20.4 ± 0.4[4]	20.3 ± 0.3[5]	20.6 ± 0.2
Day 17	39.7 ± 1.2	39.6 ± 0.9	39.1 ± 0.9	39.7 ± 0.8	36.3 ± 1.2*[4]	33.1 ± 0.8**[5]	26.3 ± 1.0**
Weight gain (Days 0-17)	19.5 ± 1.0	19.0 ± 0.9	18.8 ± 0.7	18.6 ± 0.7	15.9 ± 1.4*[4]	12.8 ± 0.7**[5]	5.7 ± 1.2**
Dam weight during lactation (g)							
Day 0	26.4 ± 0.5	28.1 ± 0.5	26.4 ± 0.6	27.5 ± 0.5	26.4 ± 0.4	26.7 ± 0.7	)[7]
Day 7	28.5 ± 0.9	30.3 ± 0.2	28.5 ± 0.6[8]	30.3 ± 0.6	28.0 ± 0.7[9]	26.6[6]	)
Day 14	29.6 ± 0.8	31.3 ± 0.6	29.4 ± 0.3[8]	28.0 ± 1.3	28.0 ± 1.3[9]	25.8[6]	)
Day 21	26.6 ± 0.4	26.6 ± 0.3	25.7 ± 0.5[8]	27.2 ± 0.5	26.8 ± 0.6[9]	24.8[6]	)
Day 28	24.8 ± 0.4	26.0 ± 0.3	24.7 ± 0.4[8]	24.8 ± 0.5	24.6 ± 0.5[9]	25.4[6]	)
Weight gain (Days 0-28)	−1.6 ± 0.2	−2.1 ± 0.3	−1.6 ± 0.5[8]	−2.6 ± 0.5	−2.2 ± 0.2[9]	0.2[6]	)
LITTER DATA							
Number of pups per litter	8.50 ± 0.70	8.94 ± 0.29	8.56 ± 0.47	9.19 ± 0.40	6.10 ± 0.76*[4]	4.44 ± 0.67**[5]	1.00[6]
Number of live pups per litter							
Day 0	8.50 ± 0.70	8.89 ± 0.31	8.44 ± 0.50	9.13 ± 0.40	3.60 ± 0.95**[4]	0.50 ± 0.24**[5]	0.00[6]
Day 1	8.50 ± 0.70	8.78 ± 0.32	7.56 ± 0.82	8.50 ± 0.63	4.81 ± 1.07*	0.38 ± 0.24**	)
Day 4 (precull)	8.19 ± 0.76	8.78 ± 0.32	6.19 ± 1.16	8.38 ± 0.60	4.00 ± 1.17**	0.13 ± 0.13**	)
Day 4 (postcull)	5.75 ± 0.25	6.00 ± 0.00	4.81 ± 0.73	5.88 ± 0.13	3.25 ± 0.84**	0.13 ± 0.13**	)
Day 7	5.50 ± 0.33	6.00 ± 0.00	4.81 ± 0.73	5.88 ± 0.13	3.25 ± 0.84*	0.13 ± 0.13**	)
Day 14	5.50 ± 0.33	6.00 ± 0.00	4.81 ± 0.73	5.88 ± 0.13	3.13 ± 0.85*	0.13 ± 0.13**	)
Day 21	5.31 ± 0.34	6.00 ± 0.00	4.81 ± 0.73	5.88 ± 0.13	3.13 ± 0.85	0.13 ± 0.13**	)
Day 28	5.31 ± 0.34	6.00 ± 0.00	4.81 ± 0.73	5.88 ± 0.13	3.13 ± 0.85	0.13 ± 0.13**	)
Percentage of live pups per litter[10]							
Day 0	100 ± 0	99 ± 1	98 ± 2	99 ± 1	47 ± 11**[4]	11 ± 7**[5]	0[6]
Day 1	100 ± 0	99 ± 1	88 ± 6*	93 ± 5	90 ± 6*	31 ± 24**	)
Day 4	96 ± 3	99 ± 1	74 ± 12	91 ± 5	59 ± 15*	6 ± 6**	)
Day 7	96 ± 4	100 ± 0	88 ± 13	100 ± 0	69 ± 16	13 ± 13**	)
Day 14	96 ± 4	100 ± 0	88 ± 13	100 ± 0	65 ± 16*	13 ± 13**	)
Day 21	93 ± 5	100 ± 0	88 ± 13	100 ± 0	65 ± 16	13 ± 13**	)
Day 28	93 ± 5	100 ± 0	88 ± 13	100 ± 0	65 ± 16	13 ± 13**	)

TABLE 24 Reproductive Performance and Body Weights of B6C3F$_1$ Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate (continued)

	Concentration (ppm)						
	0	1,250	2,500	5,000	7,500	10,000	20,000
LITTER DATA (continued)							
Pup weight (g)							
Day 0	1.43 ± 0.05	1.44 ± 0.02	1.37 ± 0.04	1.33 ± 0.02	1.37 ± 0.03	1.23 ± 0.05**	)
Day 1	1.52 ± 0.06	1.56 ± 0.04	1.39 ± 0.04	1.48 ± 0.03	1.51 ± 0.06	1.40 ± 0.20[11]	)
Day 4	2.58 ± 0.13	2.86 ± 0.11	2.62 ± 0.09[8]	2.71 ± 0.06	2.89 ± 0.24[9]	2.20[6]	)
Day 7	4.33 ± 0.21	4.91 ± 0.13	4.48 ± 0.15[8]	4.68 ± 0.08	4.63 ± 0.31[9]	3.10[6]	)
Day 14	7.69 ± 0.25	7.96 ± 0.10	7.55 ± 0.16[8]	7.45 ± 0.21	7.78 ± 0.46[9]	6.10[6]	)
Day 21	10.11 ± 0.42	10.83 ± 0.20	10.11 ± 0.23[8]	9.83 ± 0.32	10.01 ± 0.60[9]	8.80[6]	)
Day 28	13.61 ± 0.39	14.55 ± 0.34	13.80 ± 0.20[8]	12.84 ± 0.24	13.23 ± 0.74[9]	13.60[6]	)

[1] All data except gestation indices are given as mean ± standard error for averages of two dams per breeding group; for breeding groups in which only one dam delivered pups, data for that dam only are included. Dam weights and weight gains during lactation are not significant by Dunn's test.
[2] One male and two females per breeding group; number of groups in which at least one female was impregnated.
[3] Females that delivered at least one live pup/sperm-positive females.
[4] n=10 dams and litters.
[5] n=9 dams and litters.
[6] n=1 dam and litter.
[7] n=0 dams and litters.
[8] n=7 dams and litters.
[9] n=6 dams and litters.
[10] Number of live pups/total number of pups.
[11] n=2 litters.
* Significantly different ($P \leq 0.05$) from the control group by Williams' (body weight only) or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' (body weight and gestation index) or Shirley's test.

Selected pups from each exposure group were weaned onto feed containing dibutyl phthalate on Day 28. All weanlings survived to the end of the study. The initial (weaning) and final mean body weights of males receiving 2,500 ppm or greater were notably lower than those of the controls (Table 25). The mean body weight gains of control and exposed male mice were generally similar; the weight gain of the single male receiving 10,000 ppm was notably lower than the mean body weight gain of the controls. The initial and final mean body weights and mean body weight gains of exposed females were similar to those of the controls. Feed consumption by exposed mice was generally similar to that by the controls. No clinical signs related to dibutyl phthalate administration were observed.

TABLE 25 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for Postweanling B6C3F$_1$ Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate

Dose (ppm)	Survival[1]	Mean Body Weight[2] (grams) Week 1[3]	Week 4	Change	Final Weight Relative to Controls[4] (%)	Average Feed Consumption[5] (g/day)	Average Dose[6] (mg/kg/day)
MALE							
0	10/10	16.9 ± 0.5	24.1 ± 0.4	7.2 ± 0.4		3.3	
1,250	10/10	16.2 ± 0.6	23.5 ± 0.4	7.4 ± 0.4	97	3.2	199
2,500	10/10	15.0 ± 0.5*	22.7 ± 0.5*	7.7 ± 0.3	94	3.3	437
5,000	10/10	14.7 ± 0.5**	21.6 ± 0.5**	6.9 ± 0.3	90	2.7	750
7,500	10/10[7]	13.3 ± 0.6**	21.1 ± 0.6**	7.9 ± 0.6	87	3.0	1,286
10,000	1/1	13.6	17.8	4.2	74	6.2	3,804
FEMALE							
0	10/10	13.0 ± 0.3	17.6 ± 0.5	4.7 ± 0.3		2.1	
1,250	10/10	13.9 ± 0.3	18.5 ± 0.2	4.6 ± 0.3	105	2.2	170
2,500	10/10	12.8 ± 0.3	18.0 ± 0.3	5.2 ± 0.2	102	2.5	399
5,000	10/10	13.1 ± 0.4	17.3 ± 0.4	4.2 ± 0.2	98	2.2	714
7,500	10/10	12.0 ± 0.5	17.6 ± 0.2	5.6 ± 0.3	100	2.1	1,060

[1] Number surviving at Week 4 postweaning/number of animals per group.
[2] Weights and weight changes are given as mean ± standard error.
[3] Mean body weight taken during the first week of exposure postweaning.
[4] (Exposure group mean/control group mean) x 100.
[5] Average of individual consumption values for Weeks 1 through 4 postweaning; not corrected for feed spillage.
[6] Average dose based on average mean body weight for Week 1 through 4 postweaning.
[7] The initial weight of one mouse was not recorded.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

The absolute liver weight of males in the 7,500 ppm group was significantly greater than that of the controls (Tables 26 and B4); relative liver weights were greater in all groups of exposed male mice than in the controls. The absolute kidney weights of males administered 5,000 or 7,500 ppm were significantly less than the control value. In contrast, absolute kidney weights of female mice in all but the 7,500 ppm group were greater than in the controls, and relative kidney weights were significantly greater in all exposure groups than in the controls. Other statistically significant differences in organ weights were considered secondary to body weight changes.

No treatment-related gross lesions were identified at necropsy. No histopathologic lesions were observed in either male or female mice in the highest surviving exposure group (7,500 ppm) that could be definitively associated with treatment with dibutyl phthalate. In the one surviving male pup from the 10,000 ppm group, cytoplasmic alteration in the liver was the only histopathologic abnormality noted. This liver lesion consisted of hepatocytes with a more intensely staining eosinophilic cytoplasm and fewer small, clear vacuoles than in the liver of control mice. Small, fine eosinophilic granules were more commonly observed in the cytoplasm of hepatocytes from the liver of this pup than in the controls. This hepatocellular cytoplasmic alteration is consistent with peroxisome proliferation, a known effect of this chemical in rodents. This lesion was not definitively identified in mice in lower exposure groups.

Based on the results of the MPE determination study, 5,000 ppm was considered a reasonable MPE concentration. At this concentration, maternal effects were minor and were not considered life threatening. Effects on pups at this concentration were restricted to body and organ weight effects, responses considered to be part of the pharmacologic action of dibutyl phthalate and not life threatening. Higher exposure concentrations resulted in marked fetal and neonatal toxicity and lethality. Positive titers for epizootic diarrhea of infant mice led to the decision to discontinue plans for conducting a 13-week study with perinatal exposure in mice.

TABLE 26 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling B6C3F$_1$ Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm[2]
MALE						
n[3]	10	10	10	10	10	1
Necropsy body wt	26.3 ± 0.3	25.7 ± 0.4	24.4 ± 0.5**	23.5 ± 0.5**	23.1 ± 0.5**	18.8
Right kidney						
Absolute	0.261 ± 0.006	0.260 ± 0.006	0.249 ± 0.009	0.229 ± 0.005**	0.230 ± 0.006**	0.193
Relative	9.91 ± 0.19	10.13 ± 0.18	10.20 ± 0.21	9.76 ± 0.22	9.92 ± 0.12	10.26
Liver						
Absolute	1.380 ± 0.022	1.427 ± 0.028	1.379 ± 0.033	1.443 ± 0.025	1.494 ± 0.038*	1.293
Relative	52.49 ± 1.03	55.53 ± 0.57*	56.69 ± 1.25**	61.48 ± 0.95**	64.59 ± 1.11**	68.76
FEMALE						
n	10	10	10	10	10	
Necropsy body wt	18.8 ± 0.4	19.8 ± 0.3	18.6 ± 0.2	18.4 ± 0.3	16.7 ± 0.5**	
Right kidney						
Absolute	0.156 ± 0.005	0.183 ± 0.003**	0.179 ± 0.003**	0.177 ± 0.005**	0.167 ± 0.005	
Relative	8.28 ± 0.15	9.25 ± 0.14**	9.61 ± 0.18**	9.65 ± 0.22**	10.00 ± 0.11**	
Liver						
Absolute	0.958 ± 0.028	1.099 ± 0.019	1.064 ± 0.017	1.101 ± 0.026	0.907 ± 0.110	
Relative	51.06 ± 0.97	55.45 ± 0.82	57.13 ± 0.72	59.93 ± 1.19	53.38 ± 5.78	

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] A single male mouse and no female mice exposed to 10,000 ppm survived through weaning; no statistical comparisons between the organ weights of the male mouse and those of the control males were made.
[3] Number surviving at 4 weeks postweaning.
* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

13-Week Feed Study in B6C3F$_1$ Mice

All male and female mice survived until the end of the study (Table 27). Final mean body weights and mean body weight gains were lower in male and female mice that received 5,000 ppm or greater than in the controls (Table 27 and Figure 5). No clinical signs were considered related to exposure. Feed consumption by mice in exposed groups was higher than that by the controls (Table 27); this was attributed to unusually high feed consumption by a few animals in the higher exposure groups and was associated with feed spillage.

TABLE 27 Survival, Body Weight, Feed Consumption, and Compound Consumption Data for B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate

Dose (ppm)	Survival[1]	Mean Body Weight[2] (grams)			Final Weight Relative to Controls[3] (%)	Average Feed Consumption[4] (g/day)	Average Dose[4] (mg/kg/day)
		Initial	Final	Change			
MALE							
0	10/10	22.1 ± 0.4	32.9 ± 1.0	10.8 ± 0.6		3.4	
1,250	10/10	21.4 ± 0.4	32.9 ± 0.9	11.5 ± 0.7	100	3.5	163
2,500	10/10	21.9 ± 0.4	31.3 ± 0.7	9.4 ± 0.6	95	3.8	353
5,000	10/10	21.8 ± 0.4	29.7 ± 0.8**	7.9 ± 0.6**	90	4.2	812
10,000	10/10	21.7 ± 0.3	30.2 ± 0.7**	8.5 ± 0.5**	92	4.2	1,601
20,000	10/10	21.3 ± 0.5	27.9 ± 0.4**	6.6 ± 0.4**	85	4.7	3,689
FEMALE							
0	10/10	17.7 ± 0.1	29.0 ± 0.8	11.3 ± 0.8		4.3	
1,250	10/10	17.7 ± 0.3	29.2 ± 0.7	11.5 ± 0.7	101	4.5	238
2,500	10/10	18.0 ± 0.2	30.0 ± 0.6	11.9 ± 0.7	103	4.7	486
5,000	10/10	17.8 ± 0.2	27.2 ± 0.4*	9.4 ± 0.5*	94	4.5	971
10,000	10/10	17.7 ± 0.4	27.2 ± 0.6*	9.5 ± 0.4*	94	4.9	2,137
20,000	10/10	17.7 ± 0.2	24.6 ± 0.4**	6.9 ± 0.4**	85	4.7	4,278

[1] Number surviving at 13 weeks/number of animals per group.
[2] Weights and weight changes are given as mean ± standard error.
[3] (Dose group mean/control group mean) x 100.
[4] Average of individual consumption values for Weeks 1-13.
* Significantly different ($P \leq 0.05$) from the control group by Williams' test.
** Significantly different ($P \leq 0.01$) from the control group by Williams' test.

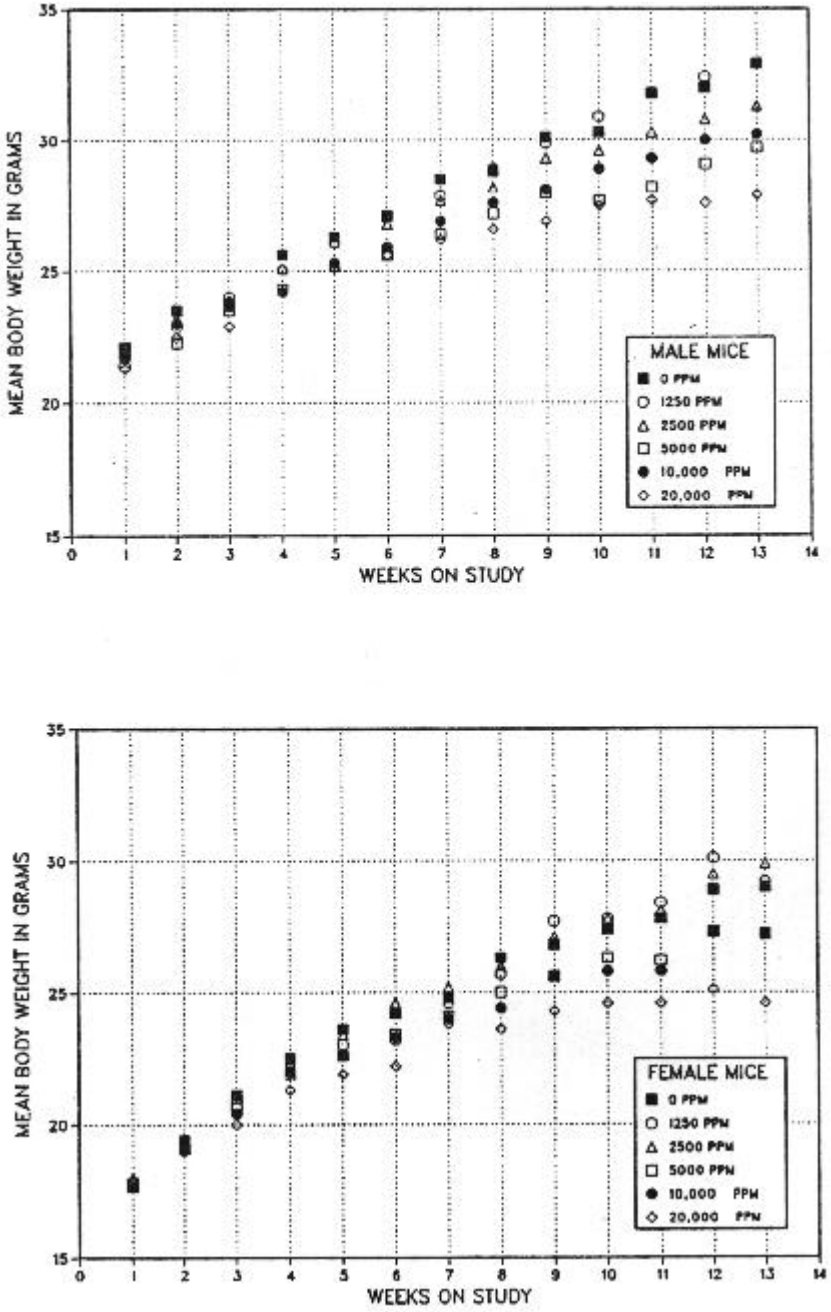


FIGURE 5 Body Weights of B6C3F$_1$ Mice Administered Dibutyl Phthalate in Feed for 13 Weeks

Absolute liver weights were significantly greater in male mice receiving 20,000 ppm and in female mice receiving 10,000 or 20,000 ppm than in the controls (Tables 28 and B5); relative liver weights were also greater in males and females administered 5,000 ppm or greater than in the controls. Absolute and relative kidney weights in all groups of exposed females were greater than those in the controls, and all of these differences except the absolute kidney weight in the 20,000 ppm group were significant. Other statistically significant differences in organ weights were considered secondary to body weight changes.

TABLE 28 Selected Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	32.9 ± 1.0	33.1 ± 0.8	32.2 ± 0.7	30.0 ± 0.8*	30.3 ± 0.6*	28.5 ± 0.4**
Right kidney						
Absolute	0.297 ± 0.007	0.303 ± 0.008	0.295 ± 0.006	0.288 ± 0.010	0.282 ± 0.010	0.252 ± 0.004**
Relative	9.06 ± 0.23	9.17 ± 0.20	9.18 ± 0.21	9.61 ± 0.26	9.27 ± 0.18	8.88 ± 0.17
Liver						
Absolute	1.470 ± 0.041	1.433 ± 0.039	1.525 ± 0.037	1.438 ± 0.042	1.574 ± 0.027	1.756 ± 0.025**
Relative	44.81 ± 1.13	43.40 ± 0.83	47.37 ± 0.85	48.02 ± 1.13*	52.02 ± 1.01**	61.85 ± 1.27**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	30.0 ± 0.8	31.2 ± 0.9	31.3 ± 0.6	27.6 ± 0.5	28.3 ± 0.8	26.2 ± 0.4**
Right kidney						
Absolute	0.203 ± 0.006	0.235 ± 0.004**	0.230 ± 0.005**	0.236 ± 0.006**	0.233 ± 0.004**	0.221 ± 0.005
Relative	6.81 ± 0.24	7.57 ± 0.27*	7.38 ± 0.13*	8.55 ± 0.15**	8.29 ± 0.25**	8.42 ± 0.14**
Liver						
Absolute	1.369 ± 0.025	1.472 ± 0.049	1.464 ± 0.024	1.372 ± 0.049	1.545 ± 0.047**	1.836 ± 0.045**
Relative	45.93 ± 1.18	47.27 ± 1.38	46.86 ± 0.34	49.63 ± 1.32*	54.67 ± 1.15**	70.01 ± 1.48**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

There were few changes in hematologic parameters in mice (Appendix C, Table C3). The Hct value in female mice in the 20,000 ppm group was slightly lower than in the controls; this would be compatible with the development of a minimal anemia, similar to that occurring in the rat studies.

Testis zinc concentrations in males receiving 5,000 ppm dibutyl phthalate or greater were higher than in the controls (Table 29). Serum testosterone was generally greater in all exposed groups than in the controls; however, testosterone values were highly variable, and the only statistically significant difference was in the 1,250 ppm group.

TABLE 29 Serum and Testis Zinc and Serum Testosterone Concentrations in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	1,250	2,500	5,000	10,000	20,000
n	10	10	10	10	10	10
Zinc (µg/g)						
Serum	1.42 ± 0.15[2]	1.70 ± 0.25	2.01 ± 0.31	1.63 ± 0.21	1.30 ± 0.04	1.67 ± 0.20[2]
Testis	27.75 ± 0.52	30.89 ± 2.16	27.27 ± 0.49	31.91 ± 1.47*	30.95 ± 1.36*	33.20 ± 2.20**
n	9	7	9	9	10	9
Serum testosterone (ng/mL)	1.11 ± 0.42	16.22 ± 3.42**	6.22 ± 2.46	2.98 ± 0.92	5.31 ± 1.92	6.44 ± 2.02

[1] Data are given as mean ± standard error.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

The liver was the only organ in mice that was identified as a site of dibutyl phthalate toxicity. No gross lesions were noted at necropsy in male or female mice; however, microscopic examination of the liver of exposed mice revealed a lesion that was characterized as cytoplasmic alteration (Table 30 and Plates 5 and 6) and which consisted of hepatocytes with a more intensely staining eosinophilic cytoplasm and fewer small, clear vacuoles (presumably glycogen-containing) than in the controls. Small, fine eosinophilic granules were more commonly observed in the cytoplasm of hepatocytes from the livers of mice in the 20,000 ppm groups than in the controls. The no-effect level for the cytoplasmic alterations in the liver was considered to be 5,000 ppm in male mice and 10,000 ppm in female mice. Subsequent staining for lipofuscin was conducted on formalin-fixed, paraffin-embedded tissue. The presence of dark green, granular staining of the cytoplasm of hepatocytes, consistent with lipofuscin, was observed in Shmorl's-stained sections. The incidence and staining intensity increased

with increasing exposure concentration and were more diffusely distributed and severe in males than in females. Mice that received 10,000 ppm or greater had greater staining than the controls; in the 10,000 ppm groups, the lesion was minimal and restricted to focal accumulations of increased Schmorl's-positive staining (3/5 males, 3/5 females). In all males receiving 20,000 ppm, the lipofuscin accumulation was diffuse and more severe, with fine Schmorl's-positive granules detected. Although the positive control tissue (cardiac muscle) stained intensely, no positive staining was detected with the AFIP method.

TABLE 30 Incidence and Severity of Selected Liver Lesions in B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	Concentration (ppm)					
	0	1,250	2,500	5,000	10,000	20,000
MALE						
n	10	10	10	10	10	10
Hepatocyte						
Cytoplasmic alteration	0	0	0	0	6** (1.0)	10** (2.8)
FEMALE						
n	10	10	10	10	10	10
Hepatocyte						
Cytoplasmic alteration	0	0	0	0	0	10** (2.7)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, and 3=moderate.
** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test.

An extended evaluation of the reproductive tissues was conducted in selected groups of mice (0, 1,250, 5,000, and 20,000 ppm). In males in the 20,000 ppm group, the body weight and left epididymal weight were significantly lower than in the controls (Table D5). In contrast, the number of spermatid heads per gram testis in males in this group was significantly higher than that of the controls. No testicular alterations were noted at necropsy or microscopically. No significant differences in estrous cycle length or the percentage of time spent in the various estrous stages occurred between exposed and control female mice (Table D6).

Continuous Breeding Study in Swiss (CD-1®) Mice

Toxic and reproductive effects of dibutyl phthalate on Swiss (CD-1®) mice and effects on survival and body weights of pups were evaluated in a continuous breeding study (at dietary concentrations of 0, 300, 3,000, and 10,000 ppm) with a crossover mating trial (at a dietary concentration of 10,000 ppm); no offspring assessment phase was conducted. These studies are summarized in Figure 2 and Appendix E. In F_0 mice that received 10,000 ppm dibutyl phthalate during the continuous breeding phase, the fertility index, average number of litters per breeding pair, live male pups, live female pups, and live pups per litter were significantly lower than in the controls. The ratio of live male pups to total live pups in the 10,000 ppm group was greater than in the controls.

The results of the subsequent crossover mating trial at 10,000 ppm indicate that females were the affected sex in the F_0 mice. The fertility index, numbers of live male, live female, and total live pups per litter, and total and adjusted live pup weights were significantly lower for F_0 females exposed to 10,000 ppm (bred with control males) than for the control females bred with control males; the female pup weight in litters from control females bred with exposed males was also lower than that of control females bred with unexposed males. In the 10,000 ppm group, three dams produced one live pup each; one of the three also delivered one dead pup, and a fourth dam delivered a single dead pup. In exposed females, the liver weight was greater and the uterus weight was less than in control females. No differences in sperm parameters or estrous cycle length were noted. The incidences of lesions in the reproductive organs in exposed males and females were similar to the control incidences.

Genetic Toxicity

Dibutyl phthalate (100 to 10,000 µg/plate) was not mutagenic in any of four strains of *Salmonella typhimurium* tested with a preincubation protocol in the presence and in the absence of Aroclor-induced rat or hamster liver S9 (Table G1; Zeiger *et al.*, 1985). Treatment of L5178Y mouse lymphoma cells in the absence of S9 activation enzymes led to a significant increase in mutant colonies and a marked decrease in cell survival at doses of 46 µg dibutyl phthalate/mL and greater (Table G2). Analyses of peripheral blood samples taken from male and female mice at the end of the 13-week study revealed no increased incidences of micronucleated normochromatic erythrocytes in males or females (Table G3).

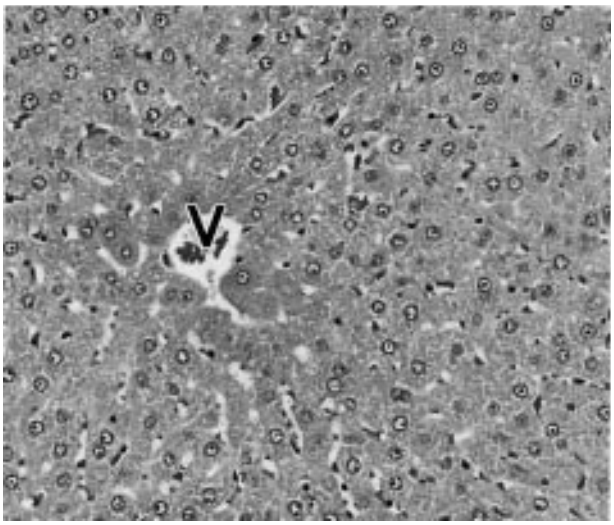


PLATE 1

Liver of a male rat administered 20,000 ppm dibutyl phthalate in feed for 13 weeks. Note the increased staining intensity of the cytoplasm, particularly around the central vein (V) and the absence of the typical cytoplasmic vacuolization of hepatocytes compared to the control male shown in Plate 2. H&E 155×.

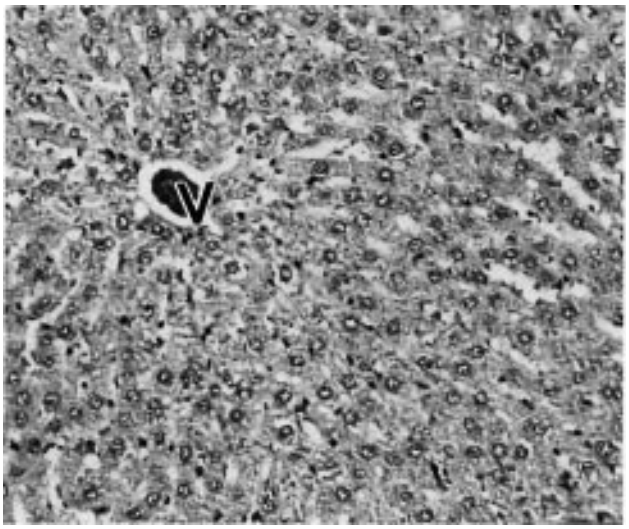


PLATE 2

Liver from a control male rat for comparison with the liver of a rat treated with dibutyl phthalate, shown in Plate 1. H&E 155×.

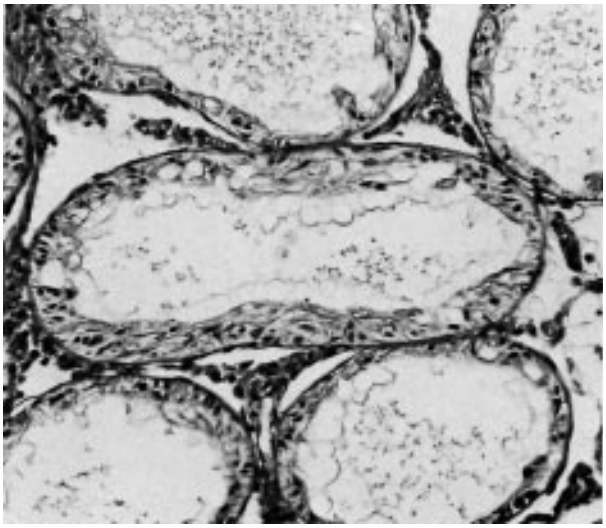


PLATE 3

Testis of a rat administered 40,000 ppm dibutyl phthalate in feed for 13 weeks. Marked degeneration of the germinal epithelium has resulted in the absence of spermatogenesis within seminiferous tubules. Compare these tubules, lined by only Sertoli cells, to those of the control rat in Plate 4. H&E 155×.

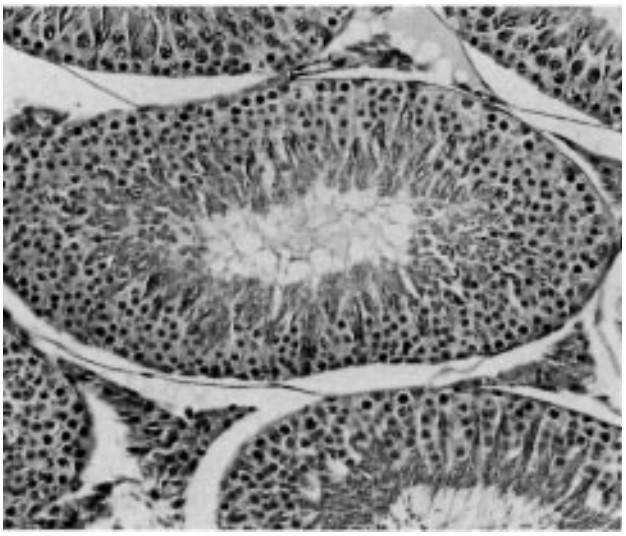


PLATE 4

Testis of a control rat for comparison with the testis of a rat treated with dibutyl phthalate, shown in Plate 3. H&E 155×.

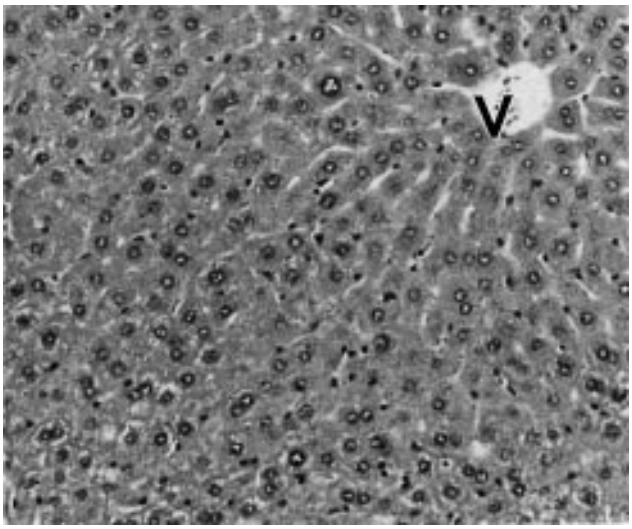


PLATE 5

Liver of a female mouse administered 20,000 ppm dibutyl phthalate in feed for 13 weeks. Note the increased staining intensity of the hepatocyte cytoplasm around the central vein (V) compared to the more vacuolated cytoplasm of the hepatocytes of the control female shown in Plate 6. H&E 155×.

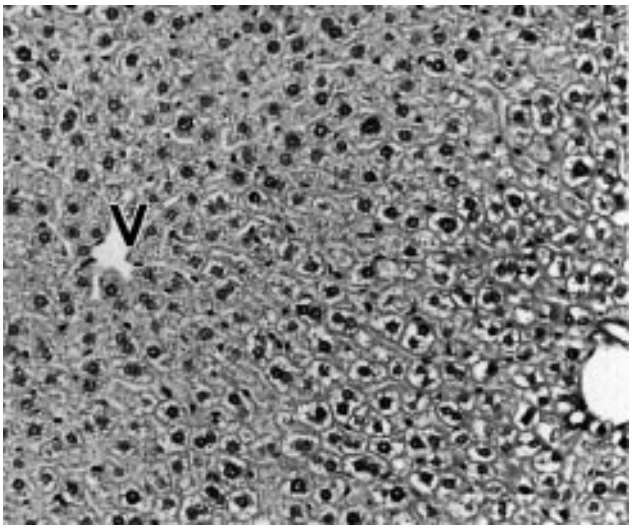


PLATE 6

Liver of a control female mouse for comparison with the liver of a mouse treated with dibutyl phthalate, shown in Plate 5. H&E 155×.

DISCUSSION

As the complexity and coordination required to create the adult organism is recognized, concern has increased over the potential effects of chemical exposure to the very young (NRC, 1993). Past experience with human tragedies such as *in utero* exposure to alcohol, thalidomide, and diethylstilbestrol have led to a greater appreciation of the sensitivity of the developing organism to both teratogenic and carcinogenic effects of chemical exposure. In an attempt to prevent such tragedies in the future, considerable effort is now expended in evaluating chemicals for their potential to induce reproductive and teratogenic effects in animals. Refined protocols such as the continuous breeding studies have allowed investigators to not only identify potential hazards but more accurately identify the causative effects and hopefully circumvent these effects in future products. In contrast to the advances in teratology, few studies have examined the long-term effects of chemicals following exposure during the perinatal period. Of the transplacental (multigenerational) carcinogenesis studies that have been conducted, DNA-reactive or genotoxic carcinogens have been emphasized (Ivankovic, 1984). Despite the clear evidence of transplacental human carcinogenicity of diethylstilbestrol (Metzler, 1984; Vessey, 1989), other chemicals without significant DNA reactivity have not been as well characterized. Chemicals exerting a toxic response during the perinatal period may be recognized early (teratogenicity) or may not be recognized until a significant amount of time has passed (impaired physical or mental performance, decreased immunocompetence, or carcinogenicity). Most insidious are these latter possibilities, such as is the case with diethylstilbestrol, where the detrimental effects of the chemical are often not present in the acute phase of exposure in the parent or offspring and go unrecognized until later in the life of the second generation (Preston-Martin, 1989).

The reproductive toxicity and hepatocarcinogenicity of phthalate ester plasticizers in rodents, and their prevalence in the environment, are of particular concern (NTP, 1982c; Srivastava *et al.*, 1990). Little is known about the effects of phthalates on the developing organism. The plasticizer di(2-ethylhexyl) phthalate has been detected in normal human placenta from full-term births (Poole and Wibberley, 1977). Di(2-ethylhexyl) phthalate has been shown to cross the rodent placenta and accumulate in the fetus (Singh, 1975). Peroxisome proliferation has been demonstrated in the liver of suckling pups of dams exposed to the related chemicals nafenopin and Wy-14,643 (Fahl, 1983). Dibutyl phthalate, a reproductive toxicant found in significant quantities in foodstuffs and as an environmental contaminant, was considered an ideal candidate for examining perinatal effects of a non-DNA reactive chemical.

SUBCHRONIC TOXICITY OF DIBUTYL PHTHALATE

Dibutyl phthalate, a plasticizer with considerable potential for human exposure, was confirmed in these studies to be a hepatic and testicular toxicant in F344/N rats and B6C3F$_1$ mice. At dietary concentrations as low as 1,250 ppm, dibutyl phthalate induced hepatomegaly in male rats and male mice in the MPE determination studies; in the 13-week study with perinatal exposure, increased liver weights and peroxisome proliferation were clearly evident at weaning in male and female pups of dams that received 10,000 ppm. Consistent with the hepatomegaly, peroxisomal enzyme activity was increased in adult rats treated with 5,000 ppm or greater (males) or 10,000 ppm or greater (females).

In the standard 13-week study, dibutyl phthalate clearly reduced lipids (triglycerides and cholesterol) in rats, and the effects on male rats (specifically triglyceride concentrations) were greater than those on female rats. The effect on triglyceride concentrations was noted in male rats at concentrations as low as 2,500 ppm and was most significant in the three highest exposure groups. The mechanism for the hypotriglyceridemia and hypocholesterolemia was not evident. However, the liver is the major site of cholesterol biosynthesis in the rat and is a target for dibutyl phthalate toxicity. Factors affecting the activity of liver HMG-CoA reductase (the rate-limiting enzyme of cholesterol synthesis), such as decreased HMG-CoA reductase production, production of a biologically inactive enzyme, and increased degradation or inhibition of the enzyme would affect circulating levels of cholesterol. The uncoupling of oxidative phosphorylation and lowering of available ATP stores by chemicals such as dibutyl phthalate (Gosselin *et al.*, 1984) have also been implicated in decreased enzymatic activity (Keller *et al.*, 1993). Considering that high-density lipoprotein (HDL) carries about 60% of the circulating cholesterol in the rat, a decrease in HDL levels may play a part in the total cholesterol decrease. Very-low-density lipoprotein and low-density lipoprotein fractions of rodents are known to be higher in triglycerides and lower in cholesterol than their human counterparts (Carroll and Feldman, 1989). Mechanisms involving alterations in production or metabolism of the circulating lipoprotein fractions could impact triglyceride and cholesterol concentrations. Androgens have also been reported to decrease HDL in humans.

Decreased serum protein values can be caused by several factors, including overhydration, albumin and protein loss in renal or intestinal disease, impaired protein synthesis (liver or lymphocyte dysfunction), increased catabolism, and poor nutritional status. Often, a decrease in serum total protein is related to a decrease in serum albumin. In the current studies, this was not the case. These data suggest that other protein fractions, *i.e.*, liver-produced globulins or lymphocyte-produced immunoglobulins, were affected.

The testicular toxicity of dibutyl phthalate, confirmed in these studies, was a phenomenon primarily detected at the higher exposure concentrations. Morphologic indicators of toxicity, primarily germinal epithelial degeneration, were observed at similar concentrations as alterations in sperm counts and infertility. One noteworthy exception may be the epididymal hypospermia noted in weanling rats receiving concentrations as low as 5,000 ppm in the MPE determination study. This may represent a subtle delay in the maturation of the germinal epithelium during early development. However, if this represents a true enhanced testicular toxicity following perinatal exposure, it raises concerns regarding the potential for long-term exposure of males to dibutyl phthalate. Some, but not all, studies have suggested a lowering of sperm counts in the human population since the mid 1950s (Carlsen *et al.*, 1992; Stone, 1994), a period of time in which the use of many chemicals, including the phthalates, has significantly risen.

The effects of dibutyl phthalate in the continuous breeding studies conducted with rats and mice suggest that females also are sensitive to dibutyl phthalate toxicity, with reproductive failure and decreases in the numbers of live pups linked to dibutyl phthalate exposure. Failure to ovulate has been implicated in the ovarian toxicity in rats following exposure to di(2-ethylhexyl) phthalate (Davis, 1994). Some epidemiological studies in women have implicated plasticizers in maladies ranging from anovulatory anestrus to miscarriage (Klinefelter and Gray, 1993).

PERINATAL TOXICITY OF DIBUTYL PHTHALATE TO RATS AND MICE

In the maximum perinatal exposure (MPE) determination studies, fewer numbers of pups born and early deaths of pups were evident at exposure concentrations that also compromised maternal body weight gains in rats (20,000 ppm) and mice (7,500 ppm and greater). Further, in the 13-week and continuous breeding studies in rats, fewer live pups were born to dams exhibiting no maternal toxicity; this difference was observed in F344/N rats receiving 10,000 ppm and in Sprague-Dawley rats receiving 1,000 ppm or greater. Body weights of exposed rat and mouse pups were less at birth (7,500 ppm and greater for rats, 10,000 ppm for mice) than those of the controls, and body weights of male rats and male mice remained less than the control values through the postweaning phase. In contrast, female rats and mice were less sensitive to body weight effects, and in the MPE determination studies, only the highest exposure groups with survivors (20,000 ppm) were affected. Liver weights of pups were increased at exposure concentrations as low as 1,250 ppm in male rats and male mice and at 2,500 ppm in female rats. Decreased testis weights were noted in rat pups at exposure concentrations as low as 7,500 ppm. These studies suggest that dibutyl phthalate is lethal to rat fetuses and rat and mouse neonates and in older pups causes effects similar to those occurring in the adult rodent.

EFFECT OF PERINATAL EXPOSURE ON THE SUBCHRONIC TOXICITY OF DIBUTYL PHTHALATE

Although some minor differences between the standard 13-week study and the 13-week study with perinatal exposure were observed, the results of these studies suggest that the perinatal rat is neither resistant nor hypersensitive to the short-term toxic effects of dibutyl phthalate. Rats additively exposed to 10,000 ppm dibutyl phthalate during the perinatal period exhibited body weight gain depressions, hepatomegaly, peroxisome proliferation, and testicular toxicity, with a response pattern very similar to the subchronic toxicity of young adults. In comparing the effects observed in the standard 13-week toxicity study with the 13-week exposure immediately following a maximum perinatal exposure of dibutyl phthalate, few differences were noteworthy and most of these suggested simply the additive effect of animals receiving an extra 11 weeks of treatment. This suggests that the short-term toxicity and teratogenic effects of dibutyl phthalate are not synergistic, and short-term consequences of exposures *in utero* or during lactation may be minimal.

No significant reproductive effects were observed at 10,000 ppm in male or female F344/N rats during the MPE determination study, nor was the reproductive performance of dams in the 13-week study with perinatal exposure adversely affected before the beginning of the subchronic phase. These findings are consistent with the results in male and female Sprague-Dawley rats receiving 10,000 ppm during the continuous cohabitation phase and crossover mating trials. In contrast, however, the offspring assessment phase of the continuous breeding protocol detected considerable reproductive toxicity at 10,000 ppm, with decreased mating, fertility, and pregnancy indices in male and female F_1 rats that were exposed *in utero* and during lactation. However, the differences between these studies examining perinatal effects in rats are considerable. One of the differences that may be important is the rat strain; the F344/N rat was used in the MPE determination studies, and the Sprague-Dawley rat was used in the reproductive assessments. A particularly noteworthy difference between the two perinatal studies was the length of time for which the dams were exposed. In the MPE determination study, treatment began as the dams became pregnant (sperm positive). In the continuous breeding study, dams were treated during the cohabitation phase and during their pregnancies with four litters. The fifth litters of dams that had thus received long-term exposure were used for offspring assessment. These male and female pups, reared separately to presumed sexual maturity, were then cohoused. Hormonal disruption has been suggested to play a role in the testicular carcinogenicity of related chemicals (Biegel *et al.*, 1992); similar hormonal disruption in this study could have altered the growth and sexual development of the offspring. Of note, increased, rather than decreased, relative testicular weights were a consistent finding in rats receiving the lower exposure concentrations, as well as in the perinatally exposed (MPE:0 ppm) control group. The spectrum of

effects observed in the F_1 rats, and particularly in the testes and accessory sex glands of F_1 males, are not unlike similar effects observed in estrogen-exposed human infants (Sharpe and Skakkebaek, 1993).

Despite the suggestion that perinatal exposure does not markedly alter the toxicity of dibutyl phthalate, the long-term effects of treatment with dibutyl phthalate remain unknown. Dibutyl phthalate is a confirmed peroxisome proliferator and a male and female reproductive toxicant. The peroxisome proliferators as a chemical class are often hepatic carcinogens and, less frequently, testicular and/or pancreatic carcinogens. These chemicals, routinely negative for DNA reactivity, are thought to induce tumors through one of several potential mechanisms. Indirect genotoxicity has been implicated in the induction of liver cancer, through an oxidative stress mechanism linked to the observed proliferation of peroxisomes (Reddy *et al.*, 1980; Cattley and Glover, 1993). Induction of cell replication may also play a central role in the development of neoplasia following extended peroxisome proliferator treatment (Ames and Swirsky Gold, 1991). Several investigators have attributed the majority of effects of cell replication to a specific subpopulation of cells uniquely sensitive to the effects of chemical treatment (Marsman and Popp, 1994). Of note, studies with peroxisome proliferators have shown that susceptibility to the carcinogenic effects of these chemicals increases with age (Cattley *et al.*, 1991; Kraupp-Grasl *et al.*, 1991). These observations may have relevance for susceptibility of rats and mice to the carcinogenic effects of peroxisome proliferators following transplacental or perinatal exposure.

Conclusions

Together, these studies in rodents suggest that the very young respond in a manner qualitatively similar to the response of adult rats and mice. There was some evidence to that rodent fetuses and neonates are more sensitive to the short-term effects of dibutyl phthalate treatment than adults. Dibutyl phthalate induced toxic effects in rodent pups *in utero* and/or during the lactational phases of development, as evidenced by fetal and neonatal lethality, body weight gain reductions, increased liver weights, female reproductive toxicity, testicular toxicity, and hepatic peroxisome proliferation.

REFERENCES

ABE, S., AND SASAKI, M. (1977). Chromosome aberrations and sister chromatid exchanges in Chinese hamster cells exposed to various chemicals. *J. Natl. Cancer Inst.* **58**, 1635-1641.

AGARWAL, D. K., LAWRENCE, W. H., NUNEZ, L. J., AND AUTIAN, J. (1985). Mutagenicity evaluation of phthalic acid esters and metabolites in *Salmonella typhimurium* cultures. *J. Toxicol. Environ. Health* **16**, 61-69.

ALBRO, P. W., AND MOORE, B. (1974). Identification of the metabolites of simple phthalate diesters in rat urine. *J. Chromatogr.* **94**, 209-218.

AMERICAN CONFERENCE OF GOVERNMENTAL INDUSTRIAL HYGIENISTS (ACGIH) (1986). *Documentation of the Threshold Limit Values and Biological Exposure Indices*, 5th ed, pp. 176.3-177. Cincinnati, OH.

AMES, B. N., AND SWIRSKY GOLD, L. (1991). Mitogenesis, mutagenesis, and rodent cancer tests. In *Origins of Human Cancer: A Comprehensive Review* (J. Brugge, T. Curran, E. Harlow, and F. McCormick, Eds.), pp. 125-135. Cold Spring Harbor Laboratory Press, Plainview, NY.

ANTONYUK, O. K., AND ALDYREVA, M. V. (1973). Substantion of maximum permissible concentration of dibutylphthalate in the air of industrial premises (in Russian, English abstract). *Gig. Tr. Prof. Zabol.* **17**, 26-30.

ARMITAGE, P. (1971). *Statistical Methods in Medical Research.* John Wiley and Sons, New York.

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

BARBER, E. D., ASTILL, B. D., MORAN, E. J., SCHNEIDER, B. F., GRAY, T. J. B., LAKE, B. G., AND EVANS, J. G. (1987). Peroxisome induction studies on seven phthalate esters. *Toxicol. Ind. Health* **3**, 7-24.

BEDFORD, C. T. (1977). Biotransformations. Part II: Agricultural and industrial chemicals; miscellaneous organics. In *Foreign Compound Metabolism in Mammals. Volume 4. A Review of the Literature Published during 1974 and 1975*, p. 239. The Chemical Society, London.

BIEGEL, L. B., HURTT, M. E., FRAME, S. R., APPLEGATE, M., O'CONNOR, J. C., AND COOK, J. C. (1992). Comparison of the effects of Wyeth-14,643 in Crl:CD BR and Fischer-344 rats. *Fundam. Appl. Toxicol.* **19**, 590-597.

BOORMAN, G. A., MONTGOMERY, C. A., JR., EUSTIS, S. L., WOLFE, M. J., MCCONNELL, E. E., AND HARDISTY, J. F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H. A. Milman and E. K. Weisberger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

BOORMAN, G. A., HICKMAN, R. L., DAVIS, G. W., RHODES, L. S., WHITE, N. W., GRIFFIN, T. A., MAYO, J., AND HAMM, T. E., JR. (1986). Serological titers to murine viruses in 90-day and 2-year studies. In *Complications of Viral and Mycoplasmal Infections in Rodents to Toxicology Research and Testing* (T. E. Hamm, Jr., Ed.), pp. 11-23. Hemisphere, New York.

BRITISH INDUSTRIAL BIOLOGICAL RESEARCH ASSOCIATION (BIBRA) (1986). A 21-Day Feeding Study of Di-n-butyl Phthalate to Rats: Effects on the Liver and Liver Lipids. USEPA Document No. 40+8626209 **(?)**, Fiche No. OTS0509545. United States Environmental Protection Agency, **Washington, DC?**

CALLEY, D., AUTIAN, J., AND GUESS, W. L. (1966). Toxicology of a series of phthalate esters. *J. Pharm. Sci.* **55**, 158-162.

CARLSEN, E., GIWERCMAN, A., KEIDING, N., AND SKAKKEBÆK, N. E. (1992). Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.* **305**, 609-613.

CARROLL, R. M., AND FELDMAN, E. B. (1989). Lipids and lipoproteins. In *The Clinical Chemistry of Laboratory Animals* (W. F. Loeb and F. W. Quimby, Eds.), pp. 95-116. Pergamon Press, New York.

CASPARY, W. J., LEE, Y. J., POULTON, S., MYHR, B. C., MITCHELL, A. D., AND RUDD, C. J. (1988). Evaluation of the L5178Y mouse lymphoma cell mutagenesis assay: Quality-control guidelines and response categories. *Environ. Mol. Mutagen.* **12** (Suppl. 13), 19-36.

CATER, B. R., COOK, M. W., GANGOLLI, S. D., AND GRASSO, P. (1977). Studies on dibutyl phthalate-induced testicular atrophy in the rat: Effect on zinc metabolism. *Toxicol. Appl. Pharmacol.* **41**, 609-618.

CATTLEY, R. C., AND GLOVER, S. E. (1993). Elevated 8-hydroxydeoxyguanosine in hepatic DNA of rats following exposure to peroxisome proliferators: Relationship to carcinogenesis and nuclear localization. *Carcinogenesis* **14**, 2495-2499.

CATTLEY, R. C., MARSMAN, D. S., AND POPP, J. A. (1991). Age-related susceptibility to the carcinogenic effect of the peroxisome proliferator Wy-14,643 in rat liver. *Carcinogenesis* **12**, 469-473.

CHING, N. P. H., JHAM, G. N., SUBBARAYAN, C., BOWEN, D. V., SMIT, A. L. C., JR., GROSSI, C. E., HICKS, R. G., FIELD, F. H., AND NEALON, T. F., JR. (1981). Gas chromatographic-mass spectrometric detection of circulating plasticizers in surgical patients. *J. Chromatogr.* **222**, 171-177.

CODE OF FEDERAL REGULATIONS (CFR) **21**, Part 58. Good Laboratory Practice for Nonclinical Laboratory Studies.

CONOVER, W. J. (1971). *Practical Nonparametric Statistics.* John Wiley and Sons, New York.

CUMMINGS, A. M., AND GRAY, L. E., JR. (1987). Dibutyl phthalate: Maternal effects versus fetotoxicity. *Toxicol. Lett.* **39**, 43-50.

DAVIS, B. J., MARONPOT, R. R., AND HEINDEL, J. J. (1994). Di-(2-ethylhexyl) Phthalate suppresses estradiol and ovulation in cycling rats. *Toxicol. Appl. Pharmacol.* **128**, 216-223.

DIXON, W. J., AND MASSEY, F. J., JR. (1951). *Introduction to Statistical Analysis*, 1st ed., pp. 145-147. McGraw-Hill Book Company, New York.

DOSTAL, L. A., WEAVER, R. P., AND SCHWETZ, B. A. (1987). Transfer of di(2-ethylhexyl) phthalate through rat milk and effects on milk composition and the mammary gland. *Toxicol. Appl. Pharmacol.* **91**, 315-325.

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

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

ELSISI, A. E., CARTER, D. E., AND SIPES, I. G. (1989). Dermal absorption of phthalate diesters in rats. *Fundam. Appl. Toxicol.* **12**, 70-77.

FAHL, W. E., LALWANI, N. D., REDDY, M. K., AND REDDY, J. K. (1983). Induction of peroxisomal enzymes in livers of neonatal rats exposed to lactating mothers treated with hypolipidaemic drugs. *Biochem. J.* **210**, 875-883.

FLORIN, I., RUTBERG, L., CURVALL, M., AND ENZELL, C. R. (1980). Screening of tobacco smoke constituents for mutagenicity using the Ames' test. *Toxicology* **15**, 219-232.

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

GOLDEMBERG, R. L., AND SAFRIN, L. (1977). Reduction of topical irritation. *J. Soc. Cosmet. Chem.* **28**, 667-679.

GOSSELIN, R. E., SMITH, R. P., HODGE, H. C., AND BRADDOCK, J. E. (1984). *Clinical Toxicology of Commercial Products*, 5th ed., p. II-204. Williams and Wilkins, Baltimore, MD.

GRANT, W. M. (1986). *Toxicology of the Eye*, 3rd ed., pp. 317-318. Charles C. Thomas Publisher, Springfield, IL.

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

GUESS, W. L., JACOB, J., AND AUTIAN, J. (1967). A study of polyvinyl chloride blood bag assemblies. I. Alteration or contamination of ACD solutions. *Drug Intell.* **1**, 120-127.

HAZLETON BIOTECHNOLOGIES COMPANY (1986). Mutagenicity of 1C in a mouse lymphoma mutation assay. Final report. HB Project No. 20989. Submitted to Chemical Manufacturers Association.

HEINDEL, J. J., AND POWELL, C. J. (1992). Phthalate ester effects on rat Sertoli cell function *in vitro*: Effects of phthalate side chain and age of animal. *Toxicol. Appl. Pharmacol.* **115**, 116-123.

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

ISHIDATE, M., JR., AND ODASHIMA, S. (1977). Chromosome tests with 134 compounds on Chinese hamster cells in vitro - a screening for chemical carcinogens. *Mutat. Res.* **48**, 337-354.

ISSEMANN, I., AND GREEN, S. (1990). Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. *Nature* **347**, 645-649.

IVANKOVIC, S. (1984). Perinatal carcinogenesis in rats. In *Comparative Perinatal Carcinogenesis* (H. M. Schuller, Ed.), pp. 5-11. CRC Press, Boca Raton, FL.

IZMEROV, N. F., ***et al.*** (1982). *Toxicometric Parameters of Industrial Toxic Chemicals Under Single Exposure*, **GKNT 44?**. Centre of International Projects, Moscow.

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

KAWACHI, T., KOMATSU, T., KADA, T., ISHIDATE, M., SASAKI, M., SUGIYAMA, T., AND TAZIMA, Y. (1980). Results of recent studies on the relevance of various short-term screening tests in Japan. *Appl. Methods Oncol.* **3**, 253-267.

KELLER, B. J., BRADFORD, B. U., MARSMAN, D. S., CATTLEY, R. C., POPP, J. A., BOJES, H. K., AND THURMAN, R. G. (1993). The nongenotoxic hepatocarcinogen Wy-14,643 is an uncoupler of oxidative phosphorylation *in vivo*. *Toxicol. Appl. Pharmacol.* **119**, 52-58.

KLINEFELTER, G., AND GRAY, L. E., JR. (1993). The clinical relevance of animal models. Animal studies that assess the potential for drugs and environmental agents to cause reproductive disorders in humans. In *Reproductive Toxicology and Infertility* (A. R. Scialli and M. J. Zinaman, Eds.), pp. 219-282. McGraw-Hill, New York.

KONAROVA, E. N. (1979). **Article title?** *Toksikol. Sanit. Khim. Plastmass.* **3**, 12-15.

KOZUMBO, W. J., KROLL, R., AND RUBIN, R. J. (1982). Assessment of the mutagenicity of phthalate esters. *Environ. Health Perspect.* **45**, 103-109.

KRAUPP-GRASL, B., HUBER, W., TAPER, H., AND SCHULTE-HERMANN, R. (1991). Increased susceptibility of aged rats to hepatocarcinogenesis by the peroxisome proliferator nafenopin and the possible involvement of altered liver foci occurring spontaneously. *Cancer Res.* **51**, 666-671.

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

KRUSKAL, W. H., AND WALLIS, W. A. (1952). Use of ranks in one-criterion variance analysis. *J. Am. Stat. Assoc.* **47**, 583-621.

LAMB, J. C., IV, CHAPIN, R. E., TEAGUE, J., LAWTON, A. D., AND REEL, J. R. (1987). Reproductive effects of four phthalic acid esters in the mouse. *Toxicol. Appl. Pharmacol.* **88**, 255-269.

LAZAROW, P. B., AND DE DUVE, C. (1976). A fatty acyl-CoA oxidizing system in rat liver peroxisomes; enhancement by clofibrate, a hypolipidemic drug. *Proc. Natl. Acad. Sci. U. S. A.* **73**, 2043-2046.

LEFAUX, R. (1968). *Practical Toxicology of Plastics*, p. 348. CRC Press, Cleveland, OH.

LEHMAN, A. J. (1955). Insect repellents. *Quart. Bull. Assoc. Food Drug Officials U.S.* **19**, 87-99.

LOGANI, M. K., SAMBUCO, C. P., FORBES, P. D., AND DAVIES, R. E. (1984). Skin-tumour promoting activity of methyl ethyl ketone peroxide - a potent lipid-peroxidizing agent. *Food Chem. Toxicol.* **22**, 879-882.

LUNA, L. G., ED. (1968). *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*, 3rd ed., pp. 185-186. McGraw-Hill Book Company, New York.

MACGREGOR, J. T., WEHR, C. M., HENIKA, P. R., AND SHELBY, M. D. (1990). The *in vivo* erythrocyte micronucleus test: Measurement at steady state increases assay efficiency and permits integration with toxicity studies. *Fundam. Appl. Toxicol.* **14**, 513-522.

MANN, H. B., AND WHITNEY, D. R. (1947). On a test of whether one of two random variables is stochastically larger than the other. *Ann. Math. Stat.* **18**, 50-60.

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

MARSMAN, D. S., AND POPP, J. A. (1994). Biological potential of basophilic hepatocellular foci and hepatic adenoma induced by the peroxisome proliferator, Wy-14,643. *Carcinogenesis* **15**, 111-117.

MARTIN, H., AND WORTHING, C. R. (EDS.) (1974). *Pesticide Manual*, 4th ed., p. 167. Publication of the British Crop Protection Council, England. Boots Company Ltd., Nottingham, England.

MELNICK, R. L., AND SCHILLER, C. M. (1985). Effect of phthalate esters on energy coupling and succinate oxidation in rat liver mitochondria. *Toxicology* **34**, 13-27.

THE MERCK INDEX (1983). 10th ed. (M. Windholz, Ed.), p. 1561. Merck and Company, Rahway, NJ.

MES, J., AND CAMPBELL, D. S. (1976). Extraction efficiency of polychlorinated biphenyl, organochlorine pesticides and phthalate esters from human adipose tissue. *Bull. Environ. Contam. Toxicol.* **16**, 53-60.

MES, J., COFFIN, D. E., AND CAMPBELL, D. S. (1974). Di-n-butyl- and di-2-ethylhexyl phthalate in human adipose tissue. *Bull. Environ. Contam. Toxicol.* **12**, 721-725.

METZLER, M. (1984). Mechanisms of carcinogenesis induced by diethylstilbestrol. In *Comparative Perinatal Carcinogenesis* (H. M. Schuller, Ed.), pp. 137-150. CRC Press, Boca Raton, FL.

MIYAHARA, C., KOGI, K., SATOH, S., SHIMIZU, H., KISHI, M., TANI, T., ARAI, K., HORIGUCHI, Y., AND WADA, Y. (1973). Studies on the toxicity of phthalate plasticizers [in Japanese, English synopsis]. *Bull. Kanagawa P. H. Lab.* **3**, 19-34.

MORRISON, D. F. (1976). *Multivariate Statistical Methods*, 2nd ed., pp. 170-179. McGraw-Hill Book Company, New York.

MURATURE, D. A., TANG, S. Y., STEINHARDT, G., AND DOUGHERTY, R. C. (1987). Phthalate esters and semen quality parameters. *Biomed. Environ. Mass Spectrom.* **14**, 473-477.

MYHR, B., BOWERS, L., AND CASPARY, W. J. (1985). Assays for the induction of gene mutations at the thymidine kinase locus in L5178Y mouse lymphoma cells in culture. *Prog. Mutat. Res.* **5**, 555-568.

NATIONAL CANCER INSTITUTE (NCI) (1979). Bioassay of Dimethyl Terephthalate for possible Carcinogenicity (CAS No. 120-61-6). Technical Report Series No. 121. NIH Publication No. 79-1376. U.S. Department of Health, Education, and Welfare, Public Health Service, National Institutes of Health, Bethesda, MD.

NATIONAL INSTITUTE FOR OCCUPATIONAL SAFETY AND HEALTH (NIOSH) (1994). National Occupational Hazard Survey (1981 to 1983), unpublished provisional data as of January 31, 1994. Cincinnati, OH.

NATIONAL RESEARCH COUNCIL (NRC) (1993). *Pesticides in the Diets of Infants and Children*. Committee on Pesticides in the Diets of Infants and Children, Board on Agriculture and Board on Environmental Studies and Toxicology, Commission on Life Sciences. National Academy Press, Washington, DC.

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

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

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

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

NATIONAL TOXICOLOGY PROGRAM (NTP) (1987). Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version (updated December 1987). U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NATIONAL TOXICOLOGY PROGRAM (NTP) (1991). Final Report on the Reproductive Toxicity of Di-n-butyl Phthalate in Sprague-Dawley Rats. **NTIS No. ?** U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC.

NATIONAL TOXICOLOGY PROGRAM (NTP) (1994). Toxicology and Carcinogenesis Studies of Diethylphthalate (CAS No. 84-66-2) in F344/N Rats and B6C3F$_1$ Mice (Dermal Studies) with Dermal Initiation/Promotion Study of Diethylphthalate and Dimethylphthalate (CAS No. 131-11-3) in Male Swiss (CD-1®) Mice. Technical Report Series No. 429. NIH Publication No. 94-3356. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Research Triangle Park, NC. (in press)

NETER, J., AND WASSERMAN, W. (1974). *Applied Linear Statistical Models. Regression, Analysis of Variance, and Experimental Designs,* pp. 685-721. Richard D. Irwin, Homewood, IL.

NIKONOROW, M., MAZUR, H., AND PIEKACZ, H. (1973). Effect of orally administered plasticizers and polyvinyl chloride stabilizers in the rat. *Toxicol. Appl. Pharmacol*. **26**, 253-259.

NOHMI, T., MIYATA, R., YOSHIKAWA, K., AND ISHIDATE, M., JR. (1985). Mutagenicity tests on organic chemical contaminants in city water and related compounds. I. Bacterial mutagenicity tests [in Japanese, English summary]. *Eisei Shikenjo Hokoku* **103**, 60-64.

OISHI, S., AND HIRAGA, K. (1980). Testicular atrophy induced by phthalic acid esters: Effect on testosterone and zinc concentrations. *Toxicol. Appl. Pharmacol.* **53**, 35-41.

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

OVERCASH, M. R., ***et al.*** (1982). Behavior of organic priority pollutants in the terrestrial system: Di-n-butyl phthalate ester, toluene and 2,4-dinitrophenol, p. 104. NTIS PB82-224-544.

PLUNKETT, E. R. (1976). *Handbook of Industrial Toxicology*. Chemical Publishing Co., New York.

POOLE, C. F., AND WIBBERLEY, D. G. (1977). Determination of di(2-ethylhexyl)phthalate in human placenta. *J. Chromatogr.* **132**, 511-518.

PRESTON-MARTIN, S. (1989). Epidemiological studies of perinatal carcinogenesis. In *Perinatal and Multigeneration Carcinogenesis* (N. P. Napalkov, J. M. Rice, L. Tomatis, and H. Yamasaki, Eds.), pp. 289-314. International Agency for Research on Cancer, Lyon.

RAO, G. N., HASEMAN, J. K., AND EDMONDSON, J. (1989a). Influence of viral infections on body weight, survival, and tumor prevalence in Fischer 344/NCr rats on two-year studies. *Lab. Anim. Sci.* **39**, 389-393.

RAO, G. N., PIEGORSCH, W. W., CRAWFORD, D. D., EDMONDSON, J., AND HASEMAN, J. K. (1989b). Influence of viral infections on body weight, survival, and tumor prevalence of B6C3F 1 (C57BL/6N × C3H/HeN) mice in carcinogenicity studies. *Fundam. Appl. Toxicol.* **13**, 156-164.

REDDY, J. K., AZARNOFF, D. L., AND HIGNITE, C. E. (1980). Hypolipidaemic hepatic peroxisome proliferators form a novel class of chemical carcinogens. *Nature* **283**, 397-398.

REGISTRY OF TOXIC EFFECTS OF CHEMICAL SUBSTANCES (RTECS) (1994). National Institute for Occupational Safety and Health, RTECS database available through the National Library of Medicine MEDLARS System.

RESEARCH TRIANGLE INSTITUTE (RTI) (1984). Di(n-butyl) Phthalate: Reproduction and Fertility Assessment in CD-1 Mice when Administered in the Feed. Research Triangle Park, NC.

ROWLAND, I. R., COTTRELL, R. C., AND PHILLIPS, J. C. (1977). Hydrolysis of phthalate esters by the gastro-intestinal contents of the rat. *Food Cosmet. Toxicol.* **15**, 17-21.

RUBIN, R. J., KOZUMBO, W., AND KROLL, R. (1979). Ames mutagenic assay of a series of phthalic acid esters: Positive response of the dimethyl and diethyl esters in TA 100. *Toxicol. Appl. Pharmacol.* **48**, A133. (Abstr.)

SADTLER STANDARD SPECTRA. IR No. 1902, UV No. 529UV, NMR No. 721M (dibutyl phthalate); IR No. 28, UV No. 22080, NMR No. 9392 (diethylhexyl phthalate). Sadtler Research Laboratories, Philadelphia, PA.

SAMOILOFF, M. R., SCHULZ, S., JORDAN, Y., DENICH, K., AND ARNOTT, E. (1980). A rapid simple long-term toxicity assay for aquatic contaminants using the nematode *Panagrellus redivivus*. *Can. J. Fish. Aquat. Sci.* **37**, 1167-1174.

SANDMEYER, E. E., AND KIRWIN, C. J., JR. (1981). Esters. In *Patty's Industrial Hygiene and Toxicology* (G. D. Clayton and F. E. Clayton, Eds.), 3rd ed., Vol. 2A: Toxicology, pp. 2259-2412. John Wiley and Sons, New York.

SAX, N. I. (1984). *Dangerous Properties of Industrial Materials*, 6th ed., p. 926. Van Nostrand Reinhold Company, New York.

SCHARDEIN, J. L. (1985). *Chemically Induced Birth Defects* (F. J. DiCarlo and F. W. Oehme, Eds.), pp. 669-678. Marcel Dekker, New York.

SCHMID, W. (1976). The micronucleus test for cytogenetic analysis. In *Chemical Mutagens. Principles and Methods for Their Detection*, Vol. 4 (A. Hollaender, Ed.), pp. 31-53. Plenum Press, New York.

SCOTT, R. C., DUGARD, P. H., RAMSEY, J. D., AND RHODES, C. (1987). *In vitro* absorption of some *o*-phthalate diesters through human and rat skin. *Environ. Health Perspect.* **74**, 223-227.

SHAHIN, M. M., AND VON BORSTEL, R. C. (1977). Mutagenic and lethal effects of α-benzene hexachloride, dibutyl phthalate and trichloroethylene in *Saccharomyces cerevisiae*. *Mutat. Res.* **48**, 173-180.

SHARPE, R. M., AND SKAKKEBAEK, N. E. (1993). Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* **341**, 1392-1395.

SHIBATA, K., TANAKA, K., AND MURATA K. (1984). Effect of dibutylphthalate on tryptophan-NAD pathway in rat [in Japanese, English abstract]. *Nippon Eiyō Shokuryō Gakkaishi (J. Jpn. Soc. Nutr. Food Sci.* **37**, 513-518.

SHIOTA, K., AND NISHIMURA, H. (1982). Teratogenicity of di(2-ethylhexyl) phthalate (DEHP) and di(*n*-butyl) phthalate (DBP) in mice. *Environ. Health Perspect.* **45**, 65-70.

SHIOTA, K., CHOU, M. J., AND NISHIMURA, H. (1980). Embryotoxic effects of di-2-ethylhexyl phthalate (DEHP) and di-*n*-butyl phthalate (DBP) in mice. *Environ. Res.* **22**, 245-253.

SHIRLEY, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* **33**, 386-389.

SINGH, A. R., LAWRENCE, W. H., AND AUTIAN, J. (1972). Teratogenicity of phthalate esters in rats. *J. Pharm. Sci.* **61**, 51-55.

SINGH, A. R., LAWRENCE, W. H., AND AUTIAN, J. (1975). Maternal-fetal transfer of ^{14}C-di-2-ethylhexyl phthalate and ^{14}C-diethyl phthalate in rats. *J. Pharm. Sci.* **64**, 1347-1350.

SMITH, C. C. (1953). Toxicity of butyl stearate, dibutyl sebacate, dibutyl phthalate, and methoxyethyl oleate. *Arch. Ind. Hyg. Occup. Med.* **7**, 310-318.

SRIVASTAVA, S. P., SRIVASTAVA, S., SAXENA, D. K., CHANDRA, S. V., AND SETH, P. K. (1990). Testicular effects of di-*n*-butyl phthalate (DBP): Biochemical and histopathological alterations. *Arch. Toxicol.* **64**, 148-152.

STONE, R. (1994). Causes sought for sperm-count drop. *Science* **265**, 309.

TANAKA, A., MATSUMOTO, A., AND YAMAHA, T. (1978). Biochemical studies on phthalic esters. III. Metabolism of dibutyl phthalate (DBP) in animals. *Toxicology* **9**, 109-123.

TIMOFIEVSKAYA, L. A., IVANOVA, N. I., AND BALYNINA, E. S. (1980). Toxicology of esters of o-phthalic acid and hygienic standards for them (in Russian, English abstract). *Gig. Tr. Prof. Zabol.* **24**, 25-28.

TOMITA, I., NAKAMURA, Y., AND YAGI, Y. (1977). Phthalic acid esters in various foodstuffs and biological materials. *Ecotoxicol. Environ. Safety* **1**, 275-287.

TSUCHIYA, K., AND HATTORI, K. (1976). Chromosomal study on human leucocytes cultures treated with phthalate acid ester [in Japanese, English summary table]. *Hokkaid oritsu Eisei Kenky ujo h o (Rep. Hokkaido Inst. Publ. Health)* **26**, 114.

UNION CARBIDE (1971). **Title?** Data Sheet **12**, 29.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (1954). Preliminary Acute Toxicity Tests and Short Term Feeding Tests of Rats and Dogs Given Di-isobutyl Phthalate and Di-butyl Phthalate. USEPA Document No. 878210833, Fiche No. OTS0205995. University of Rochester School of Medicine and Dentistry, Division of Pharmacology and Toxicology **(wrong year, perhaps?)**.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY (USEPA) (1985). Monograph on dibutyl phthalate. Washington, DC.

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY/ENVIRONMENTAL CRITERIA AND ASSESSMENT OFFICE (USEPA/ECAO) (1980). Phthalate Atlas Report. **Washington, DC?**

VESSEY, M. P. (1989). Epidemiological studies of the effects of diethylstilboestrol. In *Perinatal and Multigeneration Carcinogenesis* (N. P. Napalkov, J. M. Rice, L. Tomatis, and H. Yamasaki), pp. 335-348. International Agency for Research on Cancer, Lyon.

WALSETH, F., AND NILSEN, O. G. (1984). Phthalate esters. II. Effects of inhaled dibutylphthalate on cytochrome P-450 mediated metabolism in rat liver and lung. *Arch. Toxicol.* **55**, 132-136.

WALSETH, F., AND NILSEN, O. G. (1986). Phthalate esters: Effects of orally administered dibutylphthalate on cytochrome P-450 mediated metabolism in rat liver and lung. *Acta Pharmacol. Toxicol.* **59**, 263-269.

WALSETH, F., TOFTGÅRD, R., AND NILSEN, O. G. (1982). Phthalate esters I: Effects on cytochrome P-450 mediated metabolism in rat liver and lung, serum enzymatic activities and serum protein levels. *Arch. Toxicol.* **50**, 1-10.

WHITE, R. D., CARTER, D. E., EARNEST, D., AND MUELLER, J. (1980). Absorption and metabolism of three phthalate diesters by the rat small intestine. *Food Cosmet. Toxicol.* **18**, 383-386.

WILLIAMS, D. A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* **27**, 103-117.

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

WILLIAMS, D. T., AND BLANCHFIELD, B. J. (1975). The retention, distribution, excretion, and metabolism of dibutyl phthalate-*7*-^{14}C in the rat. *J. Agric. Food Chem.* **23**, 854-858.

ZEIGER, E., HAWORTH, S., MORTELMANS, K., AND SPECK, W. (1985). Mutagenicity testing of di(2-ethylhexyl)phthalate and related chemicals in *Salmonella*. *Environ. Mutagen.* **7**, 213-232.

APPENDIX A

Summary of Nonneoplastic Lesions in Rats and Mice

Table A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure A-2

Table A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure A-6

Table A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate A-10

Table A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate A-12

Table A5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F_1 Mice in the 13-Week Feed Study of Dibutyl Phthalate A-14

Table A6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F_1 Mice in the 13-Week Feed Study of Dibutyl Phthalate A-16

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm[2] 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration					10 (100%)	10 (100%)
Hepatodiaphragmatic nodule			1 (10%)			1 (10%)
Bile duct, hyperplasia			1 (10%)			
Pancreas	(10)	(10)				
Acinus, atrophy						
Cardiovascular System						
Heart	(10)	(10)	(9)	(10)	(9)	(7)
Degeneration, chronic	10 (100%)	10 (100%)	9 (100%)	10 (100%)	9 (100%)	7 (100%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)	(10)				
Inflammation, chronic active	5 (50%)	6 (60%)				
Prostate	(10)	(10)				
Inflammation, chronic active						
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Granuloma sperm		1 (10%)				
Germinal epithelium, atrophy					4 (40%)	10 (100%)
Hematopoietic System						
Spleen	(10)	(10)			(1)	
Fibrosis					1 (100%)	
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration	**10,000 ppm**
Adult Concentration	**40,000 ppm**
DISPOSITION SUMMARY	
Animals initially in study	10
Survivors	
Terminal sacrifice	10
Animals examined microscopically	10
Alimentary System	
Liver	(10)
Cytoplasmic alteration	10 (100%)
Hepatodiaphragmatic nodule	
Bile duct, hyperplasia	
Pancreas	(10)
Acinus, atrophy	1 (10%)
Cardiovascular System	
Heart	(10)
Degeneration, chronic	5 (50%)
Endocrine System	
None	
General Body System	
None	
Genital System	
Preputial gland	(10)
Inflammation, chronic active	2 (20%)
Prostate	(10)
Inflammation, chronic active	1 (10%)
Testes	(10)
Granuloma sperm	
Germinal epithelium, atrophy	10 (100%)
Hematopoietic System	
Spleen	(10)
Fibrosis	
Integumentary System	
None	
Musculoskeletal System	
None	

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(9)	(8)	(8)	(7)
Nephropathy, chronic	9 (90%)	10 (100%)	9 (100%)	8 (100%)	8 (100%)	7 (100%)
Renal tubule, dilatation						
Urinary bladder	(10)	(10)				
Calculus microscopic observation only						
Transitional epithelium, hyperplasia						

TABLE A1 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration	**10,000 ppm**
Adult Concentration	**40,000 ppm**
Nervous System	
None	
Respiratory System	
None	
Special Senses System	
None	
Urinary System	
Kidney	(10)
Nephropathy, chronic	2 (20%)
Renal tubule, dilatation	1 (10%)
Urinary bladder	(10)
Calculus microscopic observation only	1 (10%)
Transitional epithelium, hyperplasia	1 (10%)

[1] Number of animals examined microscopically at site and number of animals with lesion.
[2] 10,000 ppm = maximum perinatal exposure; administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm[2] 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Angiectasis	1 (10%)					
Cytoplasmic alteration					10 (100%)	10 (100%)
Mesentery				(1)		
Fat, inflammation, chronic active				1 (100%)		
Cardiovascular System						
Heart	(10)	(10)				
Degeneration, chronic	1 (10%)	1 (10%)				
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)	(10)				
Inflammation, chronic active	3 (30%)					
Ovary	(10)	(10)	(1)	(2)		
Periovarian tissue, cyst		2 (20%)	1 (100%)	2 (100%)		
Uterus	(10)	(10)				
Dilatation, bilateral						
Dilatation		2 (20%)				
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration	**10,000 ppm**
Adult Concentration	**40,000 ppm**
DISPOSITION SUMMARY	
Animals initially in study	10
Survivors	
Terminal sacrifice	10
Animals examined microscopically	10
Alimentary System	
Liver	(10)
Angiectasis	
Cytoplasmic alteration	10 (100%)
Mesentery	
Fat, inflammation, chronic active	
Cardiovascular System	
Heart	(10)
Degeneration, chronic	
Endocrine System	
None	
General Body System	
None	
Genital System	
Clitoral gland	(10)
Inflammation, chronic active	
Ovary	(10)
Periovarian tissue, cyst	1 (10%)
Uterus	(10)
Dilatation, bilateral	3 (30%)
Dilatation	
Hematopoietic System	
None	
Integumentary System	
None	
Musculoskeletal System	
None	

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)				
Nephropathy, chronic	1 (10%)	1 (10%)				

TABLE A2 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration	**10,000 ppm**
Adult Concentration	**40,000 ppm**
Nervous System	
None	
Respiratory System	
None	
Special Senses System	
None	
Urinary System	
Kidney	(10)
Nephropathy, chronic	

[1] Number of animals examined microscopically at site and number of animals with lesion.
[2] 10,000 ppm = maximum perinatal exposure; administered to dams through gestation and lactation and to pups until the beginning of the 13-week adult exposure phase.

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration				10 (100%)	10 (100%)	10 (100%)
Fatty change			1 (10%)			
Hepatodiaphragmatic nodule		1 (10%)				
Cardiovascular System						
Heart	(10)	(6)	(8)	(10)	(9)	(10)
Degeneration, chronic	10 (100%)	6 (100%)	8 (100%)	10 (100%)	9 (100%)	5 (50%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Preputial gland	(10)					(10)
Inflammation, chronic active	5 (50%)					3 (30%)
Prostate	(10)					(10)
Inflammation, chronic active						3 (30%)
Testes	(10)	(10)	(10)	(10)	(10)	(10)
Germinal epithelium, atrophy				4 (40%)	10 (100%)	10 (100%)
Hematopoietic System						
Thymus	(10)			(1)		(10)
Congestion				1 (100%)		
Integumentary System						
None						
Musculoskeletal System						
None						

TABLE A3 Summary of the Incidence of Nonneoplastic Lesions in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(8)	(5)	(10)
Nephropathy, chronic	10 (100%)	10 (100%)	10 (100%)	8 (100%)	5 (100%)	5 (50%)
Renal tubule, dilatation						2 (20%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Cytoplasmic alteration				10 (100%)	10 (100%)	10 (100%)
Hepatodiaphragmatic nodule	1 (10%)			2 (20%)		1 (10%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)					(10)
Inflammation, chronic active	2 (20%)					2 (20%)
Ovary	(10)				(1)	(10)
Periovarian tissue, cyst	1 (10%)				1 (100%)	
Uterus	(10)					(10)
Bilateral, dilatation	1 (10%)					2 (20%)
Hematopoietic System						
None						
Integumentary System						
Skin	(10)				(1)	(10)
Cyst epithelial inclusion					1 (100%)	
Musculoskeletal System						
None						

TABLE A4 Summary of the Incidence of Nonneoplastic Lesions in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Nervous System						
None						
Respiratory System						
Nose	(10)					(10)
Respiratory epithelium, inflammation, chronic active	1 (10%)					
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Hepatocyte, cytoplasmic alteration					6 (60%)	10 (100%)
Pancreas	(10)					(10)
Necrosis, coagulative						1 (10%)
Stomach, glandular	(10)					(10)
Cyst	1 (10%)					
Cardiovascular System						
None						
Endocrine System						
Pituitary gland	(10)					(10)
Pars distalis, cyst	1 (10%)					
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						

TABLE A5 Summary of the Incidence of Nonneoplastic Lesions in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate (continued)

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Respiratory System						
None						
Special Senses System						
Eye	(1)		(2)		(2)	
Lens, cataract					1 (50%)	
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
DISPOSITION SUMMARY						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)	(10)	(10)	(10)	(10)	(10)
Necrosis, coagulative	1 (10%)			1 (10%)		
Hepatocyte, cytoplasmic alteration						10 (100%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

TABLE A6 Summary of the Incidence of Nonneoplastic Lesions in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate (continued)

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Special Senses System						
None						
Urinary System						
None						

[1] Number of animals examined microscopically at site and number of animals with lesion.

APPENDIX B

Organ Weights and Organ-Weight-to-Body-Weight Ratios

Table B1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate B-2

Table B2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure B-3

Table B3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate B-5

Table B4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling B6C3F_1 Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate B-6

Table B5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F_1 Mice in the 13-Week Feed Study of Dibutyl Phthalate B-7

TABLE B1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling F344/N Rats in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	237 ± 4	237 ± 2	234 ± 3	226 ± 3*	215 ± 3**	225 ± 4**
Heart						
Absolute	0.856 ± 0.023	0.846 ± 0.013	0.849 ± 0.016	0.813 ± 0.011	0.826 ± 0.023	0.810 ± 0.018
Relative	3.61 ± 0.08	3.57 ± 0.05	3.63 ± 0.07	3.59 ± 0.03	3.84 ± 0.06*	3.61 ± 0.05
Right kidney						
Absolute	1.056 ± 0.029	1.116 ± 0.015	1.091 ± 0.014	1.111 ± 0.027	1.051 ± 0.029	1.110 ± 0.023
Relative	4.45 ± 0.08	4.71 ± 0.05*	4.67 ± 0.05*	4.91 ± 0.10**	4.88 ± 0.09**	4.94 ± 0.07**
Liver						
Absolute	12.128 ± 0.387	13.044 ± 0.272	13.145 ± 0.370	14.935 ± 0.351**	15.803 ± 0.377**	17.106 ± 0.485**
Relative	51.01 ± 1.07	55.15 ± 1.26*	56.17 ± 1.29**	65.90 ± 0.99**	73.43 ± 0.92**	76.04 ± 1.24**
Lungs						
Absolute	1.525 ± 0.059	1.596 ± 0.046	1.527 ± 0.053	1.482 ± 0.047	1.547 ± 0.100	1.413 ± 0.053
Relative	6.42 ± 0.21	6.75 ± 0.22	6.54 ± 0.24	6.55 ± 0.21	7.20 ± 0.47	6.29 ± 0.21
Right testis						
Absolute	1.330 ± 0.014	1.339 ± 0.012	1.367 ± 0.033	1.295 ± 0.014	1.162 ± 0.033**	1.116 ± 0.029**
Relative	5.61 ± 0.06	5.66 ± 0.04	5.86 ± 0.18	5.72 ± 0.06	5.40 ± 0.11	4.97 ± 0.11**
Thymus						
Absolute	0.427 ± 0.014	0.450 ± 0.011	0.457 ± 0.010	0.436 ± 0.014	0.453 ± 0.017	0.444 ± 0.012
Relative	1.80 ± 0.06	1.90 ± 0.06	1.95 ± 0.03	1.92 ± 0.06	2.11 ± 0.07**	1.97 ± 0.04**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	154 ± 2	152 ± 2	153 ± 3	156 ± 2	148 ± 2	146 ± 3
Heart						
Absolute	0.553 ± 0.012	0.552 ± 0.013	0.564 ± 0.013	0.582 ± 0.009	0.538 ± 0.011	0.554 ± 0.015
Relative	3.60 ± 0.05	3.62 ± 0.06	3.68 ± 0.05	3.73 ± 0.07	3.64 ± 0.06	3.79 ± 0.08
Right kidney						
Absolute	0.688 ± 0.011	0.704 ± 0.009	0.706 ± 0.016	0.756 ± 0.013**	0.692 ± 0.016	0.692 ± 0.012
Relative	4.49 ± 0.07	4.63 ± 0.07	4.61 ± 0.07	4.84 ± 0.07*	4.68 ± 0.07*	4.74 ± 0.09*
Liver						
Absolute	6.441 ± 0.138	6.618 ± 0.074	6.819 ± 0.120*	7.434 ± 0.129**	7.205 ± 0.174**	7.803 ± 0.107**
Relative	41.96 ± 0.64	43.46 ± 0.47	44.49 ± 0.62*	47.63 ± 0.76**	48.72 ± 0.81**	53.39 ± 0.72**
Lungs						
Absolute	1.031 ± 0.027	1.071 ± 0.038	1.119 ± 0.025	1.074 ± 0.034	1.141 ± 0.053	1.071 ± 0.032
Relative	6.72 ± 0.15	7.02 ± 0.20	7.30 ± 0.15	6.87 ± 0.16	7.71 ± 0.32**	7.32 ± 0.18
Thymus						
Absolute	0.367 ± 0.011	0.370 ± 0.011	0.361 ± 0.013	0.374 ± 0.008	0.356 ± 0.008	0.362 ± 0.012
Relative	2.39 ± 0.07	2.43 ± 0.06	2.36 ± 0.08	2.40 ± 0.07	2.41 ± 0.06	2.48 ± 0.09

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE B2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm
MALE					
n	10	10	10	10	10
Necropsy body wt	378 ± 4	358 ± 5**	359 ± 6*	358 ± 4*	348 ± 4**
Heart					
Absolute	1.120 ± 0.022	1.031 ± 0.024	1.034 ± 0.025	1.065 ± 0.019	1.149 ± 0.060▲
Relative	2.96 ± 0.05	2.88 ± 0.04	2.88 ± 0.05	2.97 ± 0.05	3.31 ± 0.19*▲▲
Right kidney					
Absolute	1.291 ± 0.014	1.241 ± 0.019	1.268 ± 0.029	1.310 ± 0.037	1.355 ± 0.017▲▲
Relative	3.42 ± 0.03	3.47 ± 0.04	3.53 ± 0.05	3.65 ± 0.07*	3.90 ± 0.06**▲▲
Liver					
Absolute	14.136 ± 0.283	13.589 ± 0.357	14.318 ± 0.390	15.428 ± 0.435*▲▲	16.850 ± 0.193**▲▲
Relative	37.41 ± 0.64	37.94 ± 0.77	39.82 ± 0.69	43.04 ± 0.92**▲▲	48.42 ± 0.62**▲▲
Lungs					
Absolute	1.700 ± 0.063	1.786 ± 0.061	1.770 ± 0.053	1.781 ± 0.054	1.692 ± 0.048
Relative	4.50 ± 0.16	4.99 ± 0.15	4.93 ± 0.15	4.97 ± 0.15	4.86 ± 0.15
Right testis					
Absolute	1.568 ± 0.014	1.610 ± 0.019	1.613 ± 0.022	1.684 ± 0.025**	1.638 ± 0.021
Relative	4.15 ± 0.05	4.50 ± 0.07*	4.50 ± 0.07*	4.70 ± 0.06**	4.71 ± 0.09**
Thymus					
Absolute	0.342 ± 0.025	0.327 ± 0.011	0.302 ± 0.021	0.332 ± 0.017	0.341 ± 0.017
Relative	0.90 ± 0.06	0.91 ± 0.02	0.84 ± 0.05	0.93 ± 0.04	0.98 ± 0.05
FEMALE					
n	10	10	10	10	10
Necropsy body wt	204 ± 2	206 ± 2	201 ± 3	202 ± 3	199 ± 3
Heart					
Absolute	0.697 ± 0.015	0.702 ± 0.013	0.707 ± 0.007	0.681 ± 0.019	0.676 ± 0.017
Relative	3.42 ± 0.09	3.41 ± 0.08	3.52 ± 0.04	3.37 ± 0.08	3.40 ± 0.07
Right kidney					
Absolute	0.703 ± 0.011	0.688 ± 0.010	0.721 ± 0.014	0.708 ± 0.010	0.706 ± 0.011
Relative	3.45 ± 0.06	3.33 ± 0.04	3.58 ± 0.04▲▲	3.51 ± 0.03▲	3.55 ± 0.05▲▲
Liver					
Absolute	6.532 ± 0.066	6.860 ± 0.163	7.022 ± 0.227	7.142 ± 0.180*	7.543 ± 0.092**▲
Relative	32.03 ± 0.23	33.22 ± 0.53	34.91 ± 1.18*	35.33 ± 0.59**	37.90 ± 0.37**▲▲
Lungs					
Absolute	1.272 ± 0.032	1.252 ± 0.042	1.198 ± 0.025	1.183 ± 0.029	1.133 ± 0.043*
Relative	6.24 ± 0.17	6.07 ± 0.19	5.96 ± 0.16	5.87 ± 0.17	5.68 ± 0.19
Thymus					
Absolute	0.253 ± 0.007	0.276 ± 0.016	0.276 ± 0.009	0.270 ± 0.011	0.278 ± 0.010
Relative	1.24 ± 0.03	1.33 ± 0.07	1.37 ± 0.04	1.33 ± 0.04	1.40 ± 0.04

TABLE B2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration Adult Concentration	10,000 ppm 20,000 ppm	10,000 ppm 40,000 ppm
MALE		
n	10	10
Necropsy body wt	306 ± 3**▲▲	187 ± 3**▲▲
Heart		
Absolute	0.950 ± 0.022**	0.766 ± 0.015**▲▲
Relative	3.10 ± 0.07	4.09 ± 0.07**▲▲
Right kidney		
Absolute	1.264 ± 0.019	0.890 ± 0.015**▲▲
Relative	4.13 ± 0.05**▲▲	4.75 ± 0.05**▲▲
Liver		
Absolute	18.157 ± 0.286**▲▲	12.375 ± 0.250**▲
Relative	59.33 ± 0.86**▲▲	66.04 ± 0.83**▲▲
Lungs		
Absolute	1.528 ± 0.031▲▲	1.140 ± 0.025**▲▲
Relative	4.99 ± 0.09	6.10 ± 0.16**▲▲
Right testis		
Absolute	1.114 ± 0.039**▲▲[2]	0.498 ± 0.022**▲▲
Relative	3.62 ± 0.13**▲▲[2]	2.66 ± 0.12**▲▲
Thymus		
Absolute	0.279 ± 0.014	0.192 ± 0.012**▲▲
Relative	0.91 ± 0.05	1.03 ± 0.06
FEMALE		
n	10	10
Necropsy body wt	187 ± 2**▲▲	149 ± 2**▲▲
Heart		
Absolute	0.639 ± 0.010*▲	0.593 ± 0.014**▲▲
Relative	3.42 ± 0.05	3.97 ± 0.08**▲▲
Right kidney		
Absolute	0.682 ± 0.011	0.610 ± 0.009**▲▲
Relative	3.65 ± 0.04*▲▲	4.08 ± 0.04**▲▲
Liver		
Absolute	7.945 ± 0.106**▲▲	8.373 ± 0.226**▲▲
Relative	42.53 ± 0.60**▲▲	55.99 ± 0.91**▲▲
Lungs		
Absolute	1.171 ± 0.033	1.009 ± 0.024**▲▲
Relative	6.27 ± 0.17	6.75 ± 0.10▲
Thymus		
Absolute	0.267 ± 0.010	0.213 ± 0.005*▲▲
Relative	1.43 ± 0.05*	1.42 ± 0.03*

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).
[2] n=9.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunnett's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunnett's test.
▲ Significantly different ($P \leq 0.05$) from the 10,000:0 ppm group by Dunnett's test.
▲▲ Significantly different ($P \leq 0.01$) from the 10,000:0 ppm group by Dunnett's test.

TABLE B3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	374 ± 6	363 ± 5	369 ± 3	345 ± 6**	310 ± 8**	163 ± 4**
Heart						
Absolute	1.144 ± 0.024	1.096 ± 0.029	1.103 ± 0.022	1.083 ± 0.022	0.916 ± 0.057**	0.679 ± 0.020**
Relative	3.06 ± 0.07	3.02 ± 0.06	3.00 ± 0.07	3.14 ± 0.05	2.96 ± 0.17	4.16 ± 0.06**
Right kidney						
Absolute	1.353 ± 0.028	1.362 ± 0.028	1.442 ± 0.016	1.404 ± 0.030	1.325 ± 0.042	0.804 ± 0.026**
Relative	3.62 ± 0.06	3.75 ± 0.06	3.91 ± 0.03**	4.07 ± 0.04**	4.27 ± 0.05**	4.92 ± 0.09**
Liver						
Absolute	15.660 ± 0.356	16.150 ± 0.446	18.263 ± 0.284**	19.069 ± 0.498**	19.984 ± 0.748**	11.633 ± 0.478**
Relative	41.87 ± 0.72	44.45 ± 0.80	49.57 ± 0.72**	55.29 ± 1.09**	64.33 ± 1.08**	71.07 ± 1.66**
Lungs						
Absolute	1.889 ± 0.061	1.898 ± 0.100	1.941 ± 0.053	1.837 ± 0.085	1.637 ± 0.074*	1.043 ± 0.032**
Relative	5.04 ± 0.10	5.23 ± 0.25	5.26 ± 0.11	5.33 ± 0.26	5.28 ± 0.20	6.39 ± 0.13**
Right testis						
Absolute	1.495 ± 0.029	1.494 ± 0.023	1.490 ± 0.010	1.455 ± 0.017	0.521 ± 0.054**	0.321 ± 0.012**
Relative	4.00 ± 0.04	4.12 ± 0.03	4.05 ± 0.04	4.22 ± 0.04	1.68 ± 0.17**	1.96 ± 0.04**
Thymus						
Absolute	0.403 ± 0.011	0.390 ± 0.012	0.407 ± 0.011	0.400 ± 0.019	0.351 ± 0.012**	0.209 ± 0.008**
Relative	1.08 ± 0.03	1.07 ± 0.03	1.10 ± 0.03	1.15 ± 0.04	1.13 ± 0.03	1.28 ± 0.05**
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	202 ± 3	197 ± 3	207 ± 3	197 ± 3	185 ± 2**	148 ± 3**
Heart						
Absolute	0.700 ± 0.015	0.701 ± 0.018	0.703 ± 0.015	0.682 ± 0.016	0.666 ± 0.022	0.623 ± 0.019**
Relative	3.47 ± 0.06	3.55 ± 0.07	3.39 ± 0.06	3.46 ± 0.08	3.59 ± 0.12	4.22 ± 0.12**
Right kidney						
Absolute	0.744 ± 0.020	0.732 ± 0.014	0.789 ± 0.018	0.788 ± 0.015	0.746 ± 0.015	0.674 ± 0.017*
Relative	3.68 ± 0.06	3.71 ± 0.05	3.80 ± 0.05	4.00 ± 0.08**	4.03 ± 0.06**	4.55 ± 0.06**
Liver						
Absolute	7.238 ± 0.175	7.089 ± 0.203	7.708 ± 0.182	7.872 ± 0.134*	8.293 ± 0.189**	9.431 ± 0.237**
Relative	35.84 ± 0.77	35.89 ± 0.61	37.17 ± 0.79	39.91 ± 0.58**	44.74 ± 0.91**	63.78 ± 1.35**
Lungs						
Absolute	1.239 ± 0.059	1.218 ± 0.031	1.279 ± 0.036	1.246 ± 0.042	1.209 ± 0.026	1.011 ± 0.051**
Relative	6.12 ± 0.23	6.17 ± 0.11	6.17 ± 0.17	6.31 ± 0.19	6.52 ± 0.11	6.81 ± 0.27*
Thymus						
Absolute	0.278 ± 0.010	0.303 ± 0.010	0.327 ± 0.015*	0.315 ± 0.018	0.301 ± 0.011	0.259 ± 0.005
Relative	1.38 ± 0.04	1.54 ± 0.05*	1.57 ± 0.06*	1.59 ± 0.08*	1.62 ± 0.05**	1.75 ± 0.05**

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE B4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for Postweanling B6C3F$_1$ Mice in the Maximum Perinatal Exposure Determination Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm[2]
MALE						
n	10	10	10	10	10	1
Necropsy body wt	26.3 ± 0.3	25.7 ± 0.4	24.4 ± 0.5**	23.5 ± 0.5**	23.1 ± 0.5**	18.8
Heart						
Absolute	0.145 ± 0.003	0.144 ± 0.005	0.137 ± 0.007	0.132 ± 0.004	0.129 ± 0.003*	0.099
Relative	5.49 ± 0.10	5.60 ± 0.18	5.61 ± 0.27	5.60 ± 0.14	5.56 ± 0.07	5.24
Right kidney						
Absolute	0.261 ± 0.006	0.260 ± 0.006	0.249 ± 0.009	0.229 ± 0.005**	0.230 ± 0.006**	0.193
Relative	9.91 ± 0.19	10.13 ± 0.18	10.20 ± 0.21	9.76 ± 0.22	9.92 ± 0.12	10.26
Liver						
Absolute	1.380 ± 0.022	1.427 ± 0.028	1.379 ± 0.033	1.443 ± 0.025	1.494 ± 0.038*	1.293
Relative	52.49 ± 1.03	55.53 ± 0.57*	56.69 ± 1.25**	61.48 ± 0.95**	64.59 ± 1.11**	68.76
Lungs						
Absolute	0.213 ± 0.006	0.236 ± 0.010	0.228 ± 0.009	0.224 ± 0.004	0.228 ± 0.007	0.157
Relative	8.10 ± 0.24	9.17 ± 0.31**	9.35 ± 0.32**	9.55 ± 0.20**	9.86 ± 0.27**	8.37
Right testis						
Absolute	0.107 ± 0.002	0.109 ± 0.001	0.110 ± 0.003	0.106 ± 0.002	0.107 ± 0.002	0.094
Relative	4.08 ± 0.09	4.26 ± 0.04	4.54 ± 0.16**	4.53 ± 0.13**	4.64 ± 0.12**	4.98
Thymus						
Absolute	0.047 ± 0.003	0.050 ± 0.003	0.051 ± 0.002	0.065 ± 0.004**	0.061 ± 0.002**	0.036
Relative	1.80 ± 0.12	1.94 ± 0.14	2.10 ± 0.07	2.79 ± 0.22**	2.66 ± 0.14**	1.91
FEMALE						
n	10	10	10	10	10	
Necropsy body wt	18.8 ± 0.4	19.8 ± 0.3	18.6 ± 0.2	18.4 ± 0.3	16.7 ± 0.5**	
Heart						
Absolute	0.111 ± 0.006	0.125 ± 0.005	0.118 ± 0.005	0.110 ± 0.004	0.106 ± 0.004	
Relative	5.91 ± 0.24	6.35 ± 0.27	6.32 ± 0.26	5.95 ± 0.15	6.36 ± 0.23	
Right kidney						
Absolute	0.156 ± 0.005	0.183 ± 0.003**	0.179 ± 0.003**	0.177 ± 0.005**	0.167 ± 0.005	
Relative	8.28 ± 0.15	9.25 ± 0.14**	9.61 ± 0.18**	9.65 ± 0.22**	10.00 ± 0.11**	
Liver						
Absolute	0.958 ± 0.028	1.099 ± 0.019	1.064 ± 0.017	1.101 ± 0.026	0.907 ± 0.110	
Relative	51.06 ± 0.97	55.45 ± 0.82	57.13 ± 0.72	59.93 ± 1.19	53.38 ± 5.78	
Lungs						
Absolute	0.202 ± 0.012	0.243 ± 0.016	0.224 ± 0.015	0.199 ± 0.010	0.173 ± 0.005	
Relative	10.75 ± 0.50	12.28 ± 0.86	12.03 ± 0.75	10.83 ± 0.44	10.40 ± 0.30	
Thymus						
Absolute	0.065 ± 0.002	0.065 ± 0.003	0.065 ± 0.002	0.065 ± 0.004	0.059 ± 0.002	
Relative	3.49 ± 0.08	3.29 ± 0.15	3.51 ± 0.08	3.52 ± 0.18	3.54 ± 0.11	

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] A single male mouse and no female mice exposed to 10,000 ppm survived through weaning; no statistical comparisons between the organ weights of the male mouse and those of the control males were made.

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

TABLE B5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
MALE						
n	10	10	10	10	10	10
Necropsy body wt	32.9 ± 1.0	33.1 ± 0.8	32.2 ± 0.7	30.0 ± 0.8*	30.3 ± 0.6*	28.5 ± 0.4**
Heart						
Absolute	0.173 ± 0.006	0.172 ± 0.006	0.183 ± 0.009	0.159 ± 0.005	0.175 ± 0.006	0.170 ± 0.007
Relative	5.29 ± 0.20	5.24 ± 0.23	5.70 ± 0.27	5.32 ± 0.17	5.81 ± 0.25	5.99 ± 0.25
Right kidney						
Absolute	0.297 ± 0.007	0.303 ± 0.008	0.295 ± 0.006	0.288 ± 0.010	0.282 ± 0.010	0.252 ± 0.004**
Relative	9.06 ± 0.23	9.17 ± 0.20	9.18 ± 0.21	9.61 ± 0.26	9.27 ± 0.18	8.88 ± 0.17
Liver						
Absolute	1.470 ± 0.041	1.433 ± 0.039	1.525 ± 0.037	1.438 ± 0.042	1.574 ± 0.027	1.756 ± 0.025**
Relative	44.81 ± 1.13	43.40 ± 0.83	47.37 ± 0.85	48.02 ± 1.13*	52.02 ± 1.01**	61.85 ± 1.27**
Lungs						
Absolute	0.265 ± 0.014	0.249 ± 0.009	0.282 ± 0.013	0.299 ± 0.014	0.257 ± 0.012	0.281 ± 0.015
Relative	8.08 ± 0.37	7.54 ± 0.23	8.79 ± 0.47	10.03 ± 0.58	8.47 ± 0.37	9.88 ± 0.50**
Right testis						
Absolute	0.117 ± 0.002	0.121 ± 0.003	0.120 ± 0.003	0.118 ± 0.002	0.118 ± 0.004	0.112 ± 0.002
Relative	3.59 ± 0.12	3.65 ± 0.06	3.72 ± 0.08	3.94 ± 0.11*	3.89 ± 0.11*	3.94 ± 0.05*
Thymus						
Absolute	0.046 ± 0.002	0.049 ± 0.003	0.044 ± 0.002	0.041 ± 0.002	0.044 ± 0.002	0.044 ± 0.002
Relative	1.41 ± 0.07	1.47 ± 0.06	1.38 ± 0.06	1.38 ± 0.06	1.45 ± 0.08	1.55 ± 0.06
FEMALE						
n	10	10	10	10	10	10
Necropsy body wt	30.0 ± 0.8	31.2 ± 0.9	31.3 ± 0.6	27.6 ± 0.5	28.3 ± 0.8	26.2 ± 0.4**
Heart						
Absolute	0.140 ± 0.004	0.154 ± 0.008	0.148 ± 0.005	0.143 ± 0.003	0.149 ± 0.003	0.155 ± 0.007
Relative	4.68 ± 0.12	4.97 ± 0.30	4.75 ± 0.20	5.20 ± 0.12	5.30 ± 0.15*	5.91 ± 0.25**
Right kidney						
Absolute	0.203 ± 0.006	0.235 ± 0.004**	0.230 ± 0.005**	0.236 ± 0.006**	0.233 ± 0.004**	0.221 ± 0.005
Relative	6.81 ± 0.24	7.57 ± 0.27*	7.38 ± 0.13*	8.55 ± 0.15**	8.29 ± 0.25**	8.42 ± 0.14**
Liver						
Absolute	1.369 ± 0.025	1.472 ± 0.049	1.464 ± 0.024	1.372 ± 0.049	1.545 ± 0.047**	1.836 ± 0.045**
Relative	45.93 ± 1.18	47.27 ± 1.38	46.86 ± 0.34	49.63 ± 1.32*	54.67 ± 1.15**	70.01 ± 1.48**
Lungs						
Absolute	0.257 ± 0.013	0.288 ± 0.017	0.307 ± 0.014	0.254 ± 0.011	0.267 ± 0.015	0.302 ± 0.015
Relative	8.64 ± 0.52	9.26 ± 0.55	9.82 ± 0.39	9.22 ± 0.43	9.46 ± 0.53	11.52 ± 0.55**
Thymus						
Absolute	0.062 ± 0.003	0.065 ± 0.003	0.068 ± 0.003	0.055 ± 0.002	0.055 ± 0.002	0.056 ± 0.002
Relative	2.06 ± 0.08	2.10 ± 0.09	2.17 ± 0.10	2.00 ± 0.07	1.96 ± 0.08	2.13 ± 0.07

[1] Organ weights and body weights are given in grams; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P \leq 0.05$) from the control group by Williams' test.

** Significantly different ($P \leq 0.01$) from the control group by Williams' or Dunnett's test.

APPENDIX C

Hematology and Clinical Chemistry Results

Table C1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure C-2

Table C2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate C-6

Table C3 Hematology Data for B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate C-8

TABLE C1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm
MALE					
Hematology					
n	9	9	10	8	7
Hematocrit (%)	50.3 ± 0.5	50.7 ± 0.5	49.4 ± 0.4	50.1 ± 0.5	48.1 ± 0.4▲
Hemoglobin (g/dL)	15.27 ± 0.15	15.47 ± 0.13	14.91 ± 0.18	15.01 ± 0.15	14.59 ± 0.11▲
Erythrocytes (10^6/µL)	9.53 ± 0.09	9.60 ± 0.10	9.41 ± 0.09	9.44 ± 0.09	9.08 ± 0.08▲
Reticulocytes (10^6/µL)	0.17 ± 0.02	0.16 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	0.17 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.01
Mean cell volume (fL)	52.6 ± 0.3	52.9 ± 0.3	52.7 ± 0.4	53.0 ± 0.2	52.9 ± 0.3
Mean cell hemoglobin (pg)	16.0 ± 0.1	16.1 ± 0.1	15.8 ± 0.1	15.9 ± 0.1	16.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.4 ± 0.2	30.5 ± 0.2	30.2 ± 0.2	30.0 ± 0.2	30.3 ± 0.2
Platelets (10^3/µL)	637.1 ± 7.7	620.3 ± 16.9	642.8 ± 36.3	633.6 ± 9.3	706.9 ± 14.3▲
Leukocytes (10^3/µL)	4.51 ± 0.22	4.51 ± 0.32	4.48 ± 0.26	5.06 ± 0.29	5.71 ± 0.38
Segmented neutrophils (10^3/µL)	1.30 ± 0.11	0.94 ± 0.10	1.06 ± 0.13	1.25 ± 0.12	1.72 ± 0.32
Lymphocytes (10^3/µL)	3.14 ± 0.23	3.53 ± 0.24	3.33 ± 0.20	3.77 ± 0.18	3.90 ± 0.21
Monocytes (10^3/µL)	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.03 ± 0.01[2]
Eosinophils (10^3/µL)	0.04 ± 0.02	0.02 ± 0.01	0.08 ± 0.02	0.04 ± 0.01	0.06 ± 0.03
Clinical Chemistry					
n	10	9	10	10	10
Urea nitrogen (mg/dL)	21.1 ± 0.4	21.4 ± 0.4	21.4 ± 0.6	21.2 ± 0.4	20.9 ± 0.5
Creatinine (mg/dL)	0.68 ± 0.02	0.72 ± 0.02	0.68 ± 0.03	0.69 ± 0.02	0.70 ± 0.02
Glucose (mg/dL)	120 ± 2	122 ± 2	120 ± 2	123 ± 2	125 ± 2
Total protein (g/dL)	7.0 ± 0.1	7.1 ± 0.1	6.9 ± 0.1	7.1 ± 0.1	7.1 ± 0.1
Albumin (g/dL)	4.7 ± 0.0	4.7 ± 0.1	4.7 ± 0.1	5.0 ± 0.1	5.2 ± 0.1**▲
Cholesterol (mg/dL)	85 ± 2	80 ± 3	80 ± 3	89 ± 2	79 ± 3
Triglycerides (mg/dL)	161 ± 7	194 ± 13	128 ± 7	129 ± 7	92 ± 5*▲▲
Alanine aminotransferase (IU/L)	68 ± 6	57 ± 3	58 ± 3	55 ± 2	53 ± 4
Alkaline phosphatase (IU/L)	411 ± 6	438 ± 13	419 ± 12	423 ± 14	447 ± 13
Creatine kinase (IU/L)	429 ± 39	456 ± 63	412 ± 24	439 ± 50	470 ± 67
Sorbitol dehydrogenase (IU/L)	26 ± 4	23 ± 2	22 ± 3	19 ± 2	20 ± 3
Bile acids (µmol/L)	11.1 ± 1.8	16.1 ± 1.2	13.9 ± 1.2	15.5 ± 1.3	15.7 ± 1.5

TABLE C1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration Adult Concentration	10,000 ppm 20,000 ppm	10,000 ppm 40,000 ppm
MALE (continued)		
Hematology		
n	6	9
Hematocrit (%)	48.4 ± 0.6	44.7 ± 0.3**▲▲
Hemoglobin (g/dL)	14.90 ± 0.21	13.60 ± 0.06**▲▲
Erythrocytes (10^6/µL)	9.14 ± 0.11	8.33 ± 0.08**▲▲
Reticulocytes (10^6/µL)	0.20 ± 0.02	0.25 ± 0.02▲▲
Nucleated erythrocytes (10^3/µL)	0.05 ± 0.04	0.26 ± 0.04**▲▲
Mean cell volume (fL)	53.0 ± 0.3	53.6 ± 0.3
Mean cell hemoglobin (pg)	16.3 ± 0.1	16.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.8 ± 0.1	30.4 ± 0.1
Platelets (10^3/µL)	688.2 ± 9.6	749.2 ± 11.2**▲▲
Leukocytes (10^3/µL)	5.10 ± 0.25	5.38 ± 0.49
Segmented neutrophils (10^3/µL)	1.09 ± 0.12	0.77 ± 0.09*
Lymphocytes (10^3/µL)	3.93 ± 0.22	4.54 ± 0.44*
Monocytes (10^3/µL)	0.02 ± 0.01	0.04 ± 0.02
Eosinophils (10^3/µL)	0.07 ± 0.02	0.03 ± 0.02
Clinical Chemistry		
n	10	10
Urea nitrogen (mg/dL)	21.6 ± 0.4	21.9 ± 1.1
Creatinine (mg/dL)	0.76 ± 0.02	0.63 ± 0.02▲
Glucose (mg/dL)	127 ± 3	124 ± 3
Total protein (g/dL)	6.9 ± 0.1	6.3 ± 0.1**▲▲
Albumin (g/dL)	5.6 ± 0.1**▲▲	5.2 ± 0.1**▲
Cholesterol (mg/dL)	51 ± 1**▲	43 ± 2**▲▲
Triglycerides (mg/dL)	35 ± 1**▲▲	35 ± 3**▲▲
Alanine aminotransferase (IU/L)	43 ± 1**▲▲	56 ± 2
Alkaline phosphatase (IU/L)	696 ± 17**▲	920 ± 29**▲▲
Creatine kinase (IU/L)	559 ± 54	185 ± 14**▲▲[3]
Sorbitol dehydrogenase (IU/L)	13 ± 1**▲▲	16 ± 1*
Bile acids (µmol/L)	21.7 ± 2.3*	35.2 ± 2.9**▲▲

TABLE C1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 5,000 ppm	10,000 ppm 10,000 ppm
FEMALE					
Hematology					
n	8	10	8	9	10
Hematocrit (%)	49.6 ± 0.8	50.0 ± 0.3	51.0 ± 0.4	51.1 ± 0.5	50.4 ± 0.3
Hemoglobin (g/dL)	15.0 ± 0.2	15.1 ± 0.1	15.2 ± 0.2	15.2 ± 0.1	15.2 ± 0.1
Erythrocytes (10^6/µL)	8.68 ± 0.12	8.66 ± 0.06	8.83 ± 0.10	8.92 ± 0.09	8.78 ± 0.05
Reticulocytes (10^6/µL)	0.13 ± 0.02	0.14 ± 0.01	0.15 ± 0.01	0.13 ± 0.01	0.16 ± 0.02
Nucleated erythrocytes (10^3/µL)	0.01 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.02 ± 0.01	0.05 ± 0.01*
Mean cell volume (fL)	57.3 ± 0.5	57.8 ± 0.2	57.8 ± 0.3	57.4 ± 0.3	57.3 ± 0.3
Mean cell hemoglobin (pg)	17.3 ± 0.1	17.4 ± 0.1	17.2 ± 0.1	17.1 ± 0.1▲	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.3 ± 0.3	30.2 ± 0.1	29.8 ± 0.1	29.8 ± 0.2	30.1 ± 0.2
Platelets (10^3/µL)	623.8 ± 33.1	637.2 ± 11.4	659.3 ± 44.1	628.9 ± 35.0	636.5 ± 10.3
Leukocytes (10^3/µL)	3.23 ± 0.37	3.63 ± 0.19	3.39 ± 0.41	4.28 ± 0.40	4.10 ± 0.30
Segmented neutrophils (10^3/µL)	0.79 ± 0.06	1.14 ± 0.12	0.81 ± 0.07	1.17 ± 0.11	1.08 ± 0.10
Lymphocytes (10^3/µL)	2.38 ± 0.33	2.40 ± 0.12	2.53 ± 0.42	3.04 ± 0.33	2.94 ± 0.26
Monocytes (10^3/µL)	0.03 ± 0.01	0.05 ± 0.01	0.01 ± 0.00▲▲	0.03 ± 0.01	0.04 ± 0.01
Eosinophils (10^3/µL)	0.02 ± 0.01	0.04 ± 0.01	0.05 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
Clinical Chemistry					
n	10	10	9	10	10
Urea nitrogen (mg/dL)	18.9 ± 0.9	18.9 ± 0.9	18.4 ± 0.4	19.3 ± 0.7	19.3 ± 0.7
Creatinine (mg/dL)	0.60 ± 0.02	0.63 ± 0.02	0.59 ± 0.01[2]	0.61 ± 0.02	0.66 ± 0.02
Glucose (mg/dL)	116 ± 3	113 ± 2	115 ± 3	118 ± 4	125 ± 6
Total protein (g/dL)	7.0 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1
Albumin (g/dL)	4.9 ± 0.1	4.9 ± 0.1	5.1 ± 0.1	5.1 ± 0.1*	5.3 ± 0.1**▲▲
Cholesterol (mg/dL)	112 ± 3	114 ± 3	111 ± 4	111 ± 2	101 ± 3
Triglycerides (mg/dL)	79 ± 5	104 ± 10	79 ± 8	73 ± 5	73 ± 6
Alanine aminotransferase (IU/L)	59 ± 5	62 ± 6	55 ± 5	54 ± 4	54 ± 2
Alkaline phosphatase (IU/L)	316 ± 13	313 ± 11	347 ± 14	362 ± 13	353 ± 21
Creatine kinase (IU/L)	256 ± 34	301 ± 35	307 ± 36	267 ± 23	278 ± 48
Sorbitol dehydrogenase (IU/L)	23 ± 4	22 ± 3	16 ± 2	16 ± 2	16 ± 1
Bile acids (µmol/L)	33.3 ± 2.8	30.9 ± 2.5	29.8 ± 2.9	34.7 ± 2.5	36.0 ± 2.2

TABLE C1 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure (continued)

Perinatal Concentration **Adult Concentration**	**10,000 ppm** **20,000 ppm**	**10,000 ppm** **40,000 ppm**
FEMALE (continued)		
Hematology		
n	7	8
Hematocrit (%)	50.1 ± 0.5	46.2 ± 0.3▲
Hemoglobin (g/dL)	15.1 ± 0.2	14.1 ± 0.1*▲▲
Erythrocytes (10^6/µL)	8.75 ± 0.09	8.27 ± 0.07
Reticulocytes (10^6/µL)	0.17 ± 0.01	0.18 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.06 ± 0.03	0.08 ± 0.01**▲▲
Mean cell volume (fL)	57.1 ± 0.3	56.0 ± 0.2▲▲
Mean cell hemoglobin (pg)	17.3 ± 0.1	17.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	30.2 ± 0.3	30.6 ± 0.2
Platelets (10^3/µL)	631.6 ± 6.6	634.5 ± 8.2
Leukocytes (10^3/µL)	4.70 ± 0.35	4.90 ± 0.32*
Segmented neutrophils (10^3/µL)	1.29 ± 0.13*	0.82 ± 0.14
Lymphocytes (10^3/µL)	3.31 ± 0.25	4.04 ± 0.31*▲▲
Monocytes (10^3/µL)	0.06 ± 0.02	0.03 ± 0.01
Eosinophils (10^3/µL)	0.04 ± 0.01	0.01 ± 0.01
Clinical Chemistry		
n	10	10
Urea nitrogen (mg/dL)	18.6 ± 0.5	20.3 ± 0.8
Creatinine (mg/dL)	0.67 ± 0.02*	0.63 ± 0.02
Glucose (mg/dL)	115 ± 2	116 ± 3
Total protein (g/dL)	6.8 ± 0.1	6.3 ± 0.0**▲▲
Albumin (g/dL)	5.2 ± 0.0**▲	5.1 ± 0.0
Cholesterol (mg/dL)	87 ± 2**▲▲	58 ± 2**▲▲
Triglycerides (mg/dL)	49 ± 2*▲▲	35 ± 1**▲▲
Alanine aminotransferase (IU/L)	51 ± 1	51 ± 1
Alkaline phosphatase (IU/L)	457 ± 10**▲▲	670 ± 22**▲▲
Creatine kinase (IU/L)	270 ± 29	216 ± 28
Sorbitol dehydrogenase (IU/L)	19 ± 1	16 ± 1
Bile acids (µmol/L)	40.5 ± 3.4	50.3 ± 7.5▲

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=8.
[3] n=9.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunn's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunn's test.
▲ Significantly different ($P \leq 0.05$) from the 10,000:0 ppm group by Dunn's test.
▲▲ Significantly different ($P \leq 0.01$) from the 10,000:0 ppm group by Dunn's test.

TABLE C2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
MALE						
Hematology						
n	10	9	10	10	9	9
Hematocrit (%)	49.3 ± 0.4	48.8 ± 0.6	48.0 ± 0.4	48.0 ± 0.4	46.0 ± 1.0**	46.2 ± 0.5**
Hemoglobin (g/dL)	14.7 ± 0.1	14.5 ± 0.1	14.2 ± 0.1**	14.3 ± 0.1*	14.0 ± 0.3**	14.0 ± 0.1**
Erythrocytes (10^6/µL)	9.15 ± 0.08	9.08 ± 0.12	8.89 ± 0.07*	8.80 ± 0.08*	8.24 ± 0.16**	8.32 ± 0.07**
Reticulocytes (10^6/µL)	0.19 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.20 ± 0.02	0.24 ± 0.03
Nucleated erythrocytes (10^3/µL)	0.03 ± 0.01	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.04 ± 0.01	0.13 ± 0.04*
Mean cell volume (fL)	54.0 ± 0.2	53.9 ± 0.3	53.9 ± 0.1	54.6 ± 0.2*	55.8 ± 0.3**	55.3 ± 0.3**
Mean cell hemoglobin (pg)	16.1 ± 0.1	16.0 ± 0.1	16.0 ± 0.1	16.2 ± 0.1	17.0 ± 0.1**	16.9 ± 0.1**
Mean cell hemoglobin concentration (g/dL)	29.9 ± 0.2	29.7 ± 0.2	29.6 ± 0.1	29.7 ± 0.1	30.4 ± 0.2	30.4 ± 0.1*
Platelets (10^3/µL)	568.6 ± 11.9[2]	570.0 ± 16.4	628.6 ± 11.5**	648.8 ± 14.5**	650.8 ± 11.6**	638.2 ± 19.3**
Leukocytes (10^3/µL)	4.64 ± 0.31	5.60 ± 0.68	6.49 ± 0.62	6.32 ± 0.42	4.50 ± 0.43	4.22 ± 0.28
Segmented neutrophils (10^3/µL)	0.84 ± 0.07	0.94 ± 0.12	0.98 ± 0.18	1.06 ± 0.10	0.72 ± 0.07	0.59 ± 0.09
Lymphocytes (10^3/µL)	3.67 ± 0.25	4.49 ± 0.61	5.32 ± 0.46*	5.04 ± 0.35	3.65 ± 0.38	3.55 ± 0.28
Monocytes (10^3/µL)	0.07 ± 0.01	0.14 ± 0.03	0.10 ± 0.03	0.12 ± 0.02	0.06 ± 0.01	0.05 ± 0.02
Eosinophils (10^3/µL)	0.05 ± 0.02	0.03 ± 0.01	0.09 ± 0.02	0.09 ± 0.03	0.05 ± 0.02	0.03 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	20.9 ± 0.4	21.1 ± 0.5	20.6 ± 0.7	20.5 ± 0.7	21.8 ± 0.9	22.7 ± 0.9
Creatinine (mg/dL)	0.70 ± 0.02	0.73 ± 0.02	0.72 ± 0.02	0.75 ± 0.02	0.73 ± 0.02	0.66 ± 0.03
Glucose (mg/dL)	131 ± 2	136 ± 2	140 ± 3	132 ± 2	154 ± 8**	139 ± 5
Total protein (g/dL)	6.8 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	5.9 ± 0.1**
Albumin (g/dL)	4.6 ± 0.1	4.8 ± 0.1*	5.0 ± 0.0**	5.3 ± 0.1**	5.5 ± 0.1**	4.8 ± 0.1**
Cholesterol (mg/dL)	79 ± 2	82 ± 2	83 ± 2	75 ± 1	52 ± 1**	37 ± 2**
Triglycerides (mg/dL)	233 ± 19	171 ± 12*	168 ± 14*	119 ± 5**	49 ± 2**	32 ± 1**
Alanine aminotransferase (IU/L)	51 ± 2	51 ± 4	45 ± 2	48 ± 3	41 ± 3	61 ± 3
Alkaline phosphatase (IU/L)	537 ± 24	528 ± 12	509 ± 8	552 ± 15	826 ± 32**	939 ± 17**
Creatine kinase (IU/L)	312 ± 30	296 ± 43	275 ± 44	383 ± 64	477 ± 61	301 ± 26
Sorbitol dehydrogenase (IU/L)	25 ± 2	25 ± 2	23 ± 2	21 ± 2	17 ± 2**	19 ± 1*
Bile acids (µmol/L)	11.9 ± 3.3	10.0 ± 1.4	13.4 ± 2.3	15.8 ± 3.2	28.7 ± 5.6**	46.5 ± 2.6**

TABLE C2 Hematology and Clinical Chemistry Data for F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate (continued)

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
FEMALE						
Hematology						
n	10	10	10	9	10	10
Hematocrit (%)	45.8 ± 0.4	46.1 ± 0.4	45.4 ± 0.4	46.9 ± 0.4	46.3 ± 0.4	43.9 ± 0.5
Hemoglobin (g/dL)	14.4 ± 0.1	14.4 ± 0.1	14.2 ± 0.1	14.5 ± 0.1	14.4 ± 0.1	13.9 ± 0.1
Erythrocytes (10^6/µL)	8.31 ± 0.07	8.31 ± 0.07	8.23 ± 0.07	8.49 ± 0.09	8.38 ± 0.08	8.07 ± 0.09
Reticulocytes (10^6/µL)	0.14 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.17 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.02 ± 0.02	0.06 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.02	0.11 ± 0.03*
Mean cell volume (fL)	55.0 ± 0.2	55.5 ± 0.2	55.3 ± 0.2	55.6 ± 0.2	55.2 ± 0.1	54.5 ± 0.2
Mean cell hemoglobin (pg)	17.3 ± 0.1	17.3 ± 0.1	17.2 ± 0.1	17.1 ± 0.1	17.2 ± 0.1	17.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.4 ± 0.2	31.2 ± 0.2	31.2 ± 0.2	30.9 ± 0.2	31.2 ± 0.2	31.7 ± 0.1
Platelets (10^3/µL)	591.2 ± 12.8	654.3 ± 34.9	593.7 ± 17.7	603.3 ± 13.1	593.8 ± 11.9	588.1 ± 5.9
Leukocytes (10^3/µL)	5.84 ± 0.27	6.12 ± 0.43	5.61 ± 0.52	6.79 ± 0.51	6.79 ± 0.62	8.28 ± 0.39**
Segmented neutrophils (10^3/µL)	1.46 ± 0.20	1.19 ± 0.20	1.20 ± 0.23	1.37 ± 0.13	1.31 ± 0.16	1.03 ± 0.10
Lymphocytes (10^3/µL)	4.28 ± 0.26	4.83 ± 0.27	4.32 ± 0.33	5.29 ± 0.38*	5.35 ± 0.53	7.17 ± 0.38**
Monocytes (10^3/µL)	0.05 ± 0.02	0.06 ± 0.01	0.03 ± 0.01	0.04 ± 0.02	0.06 ± 0.02	0.06 ± 0.01
Eosinophils (10^3/µL)	0.05 ± 0.01	0.05 ± 0.02	0.06 ± 0.02	0.09 ± 0.03	0.08 ± 0.03	0.02 ± 0.01
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)	18.2 ± 0.7	20.0 ± 0.8	20.8 ± 0.8	20.0 ± 0.9	20.0 ± 0.6	21.0 ± 0.9
Creatinine (mg/dL)	0.72 ± 0.01	0.72 ± 0.03	0.76 ± 0.03	0.77 ± 0.03	0.77 ± 0.02	0.74 ± 0.02
Glucose (mg/dL)	119 ± 4	126 ± 4	128 ± 3	124 ± 4	126 ± 2	123 ± 2
Total protein (g/dL)	7.3 ± 0.1	7.1 ± 0.1	7.1 ± 0.1	7.2 ± 0.1	6.8 ± 0.1**	6.2 ± 0.1**
Albumin (g/dL)	5.3 ± 0.1	5.2 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.3 ± 0.1	5.1 ± 0.1
Cholesterol (mg/dL)	102 ± 3	101 ± 2	100 ± 2	94 ± 3	77 ± 2**	52 ± 1**
Triglycerides (mg/dL)	109 ± 10	115 ± 13	108 ± 11	71 ± 9*	57 ± 2**	38 ± 1**
Alanine aminotransferase (IU/L)	45 ± 4	40 ± 2	43 ± 3	44 ± 2[2]	46 ± 2	51 ± 2*
Alkaline phosphatase (IU/L)	409 ± 15	405 ± 16	439 ± 14	522 ± 39**	536 ± 23**	787 ± 21**
Creatine kinase (IU/L)	211 ± 24	196 ± 27	260 ± 54	253 ± 37	249 ± 31	142 ± 20
Sorbitol dehydrogenase (IU/L)	27 ± 1	25 ± 2	26 ± 2	28 ± 2	28 ± 1	27 ± 1
Bile acids (µmol/L)	22.2 ± 2.1	30.8 ± 3.3	35.9 ± 4.4*	35.2 ± 4.0*	40.0 ± 4.4**	67.6 ± 5.2**

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.
[2] n=9.
* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test.
** Significantly different ($P \leq 0.01$) from the control group by Dunn's or Shirley's test.

TABLE C3 Hematology Data for B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
MALE						
n	10	10	10	10	10	10
Hematocrit (%)	50.5 ± 0.5	49.3 ± 0.5	50.1 ± 0.5	50.7 ± 0.8	49.7 ± 0.8	48.7 ± 0.5
Hemoglobin (g/dL)	16.2 ± 0.1	15.9 ± 0.1	16.1 ± 0.1	16.3 ± 0.2	16.0 ± 0.2	15.8 ± 0.1
Erythrocytes (10^6/µL)	10.76 ± 0.11	10.49 ± 0.12	10.71 ± 0.09	10.87 ± 0.16	10.61 ± 0.15	10.50 ± 0.12
Reticulocytes (10^6/µL)	0.17 ± 0.02	0.18 ± 0.02	0.17 ± 0.01	0.17 ± 0.01	0.21 ± 0.02	0.16 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	47.0 ± 0.2	47.1 ± 0.2	46.7 ± 0.2	46.6 ± 0.2	46.8 ± 0.3	46.4 ± 0.2*
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.2 ± 0.1	15.1 ± 0.1	15.0 ± 0.1	15.1 ± 0.1	15.1 ± 0.1
Mean cell hemoglobin concentration (g/dL)	32.1 ± 0.2	32.2 ± 0.2	32.2 ± 0.2	32.1 ± 0.2	32.2 ± 0.1	32.5 ± 0.1
Platelets (10^3/µL)	920.5 ± 40.1	940.5 ± 44.8	906.9 ± 22.0	849.1 ± 63.3	882.2 ± 52.2	887.8 ± 62.1
Leukocytes (10^3/µL)	5.90 ± 0.49	4.57 ± 0.71	6.50 ± 0.63	7.51 ± 0.59	5.24 ± 0.85	3.77 ± 0.67
Segmented neutrophils (10^3/µL)	1.17 ± 0.22	0.78 ± 0.24	1.27 ± 0.16	1.88 ± 0.28	1.40 ± 0.33	0.84 ± 0.27
Lymphocytes (10^3/µL)	4.59 ± 0.38	3.76 ± 0.61	5.16 ± 0.58	5.50 ± 0.48	3.76 ± 0.64	2.91 ± 0.44
Monocytes (10^3/µL)	0.07 ± 0.02	0.01 ± 0.01	0.03 ± 0.01	0.06 ± 0.03	0.04 ± 0.02	0.01 ± 0.01
Eosinophils (10^3/µL)	0.07 ± 0.02	0.03 ± 0.01	0.03 ± 0.02	0.08 ± 0.05	0.05 ± 0.03	0.01 ± 0.01
FEMALE						
n	10	10	10	10	10	10
Hematocrit (%)	51.8 ± 0.6	51.5 ± 0.6	51.4 ± 0.4	50.4 ± 1.3	50.6 ± 0.7	48.9 ± 0.8**
Hemoglobin (g/dL)	16.2 ± 0.2	16.2 ± 0.2	16.1 ± 0.1	16.0 ± 0.4	16.0 ± 0.2	15.5 ± 0.2
Erythrocytes (10^6/µL)	10.72 ± 0.15	10.58 ± 0.15	10.59 ± 0.08	10.50 ± 0.27	10.50 ± 0.13	10.14 ± 0.17
Reticulocytes (10^6/µL)	0.11 ± 0.01	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.11 ± 0.01
Nucleated erythrocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Mean cell volume (fL)	48.3 ± 0.3	48.6 ± 0.4	48.7 ± 0.3	47.9 ± 0.3	48.2 ± 0.4	48.3 ± 0.3
Mean cell hemoglobin (pg)	15.1 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.3 ± 0.1	15.3 ± 0.1
Mean cell hemoglobin concentration (g/dL)	31.3 ± 0.2	31.4 ± 0.2	31.3 ± 0.2	31.7 ± 0.2	31.7 ± 0.2	31.7 ± 0.2
Platelets (10^3/µL)	884.5 ± 61.3	809.9 ± 53.8	817.1 ± 18.4	793.8 ± 45.1	863.3 ± 41.6	803.9 ± 43.8
Leukocytes (10^3/µL)	4.48 ± 0.29	4.12 ± 0.15	3.65 ± 0.27	4.81 ± 0.44	4.43 ± 0.34	4.21 ± 0.44
Segmented neutrophils (10^3/µL)	0.71 ± 0.09	0.61 ± 0.11	0.41 ± 0.03	0.94 ± 0.29	0.72 ± 0.08	0.72 ± 0.13
Lymphocytes (10^3/µL)	3.70 ± 0.27	3.43 ± 0.14	3.17 ± 0.25	3.80 ± 0.26	3.61 ± 0.29	3.41 ± 0.33
Monocytes (10^3/µL)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Eosinophils (10^3/µL)	0.07 ± 0.02	0.09 ± 0.03	0.08 ± 0.02	0.07 ± 0.02	0.11 ± 0.04	0.09 ± 0.02

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Shirley's test.

APPENDIX D

Reproductive Tissue Evaluations and Estrous Cycle Characterization

Table D1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure D-2

Table D2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure D-2

Table D3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate D-3

Table D4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate D-3

Table D5 Summary of Reproductive Tissue Evaluations in Male B6C3F_1 Mice in the 13-Week Feed Study of Dibutyl Phthalate D-4

Table D6 Summary of Estrous Cycle Characterization in Female B6C3F_1 Mice in the 13-Week Feed Study of Dibutyl Phthalate D-4

TABLE D1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
n	10	9	10	10	9
Weights (g)					
Necropsy body weight	378 ± 4	358 ± 5**[2]	359 ± 6*	348 ± 4**	306 ± 3**▲▲[2]
Left epididymis	0.429 ± 0.004	0.442 ± 0.003	0.422 ± 0.004▲	0.433 ± 0.005	0.341 ± 0.008**▲▲
Left cauda epididymis	0.136 ± 0.004	0.152 ± 0.005*	0.127 ± 0.003▲▲	0.130 ± 0.004▲▲	0.110 ± 0.003**▲▲
Left testis	1.69 ± 0.03	1.61 ± 0.01	1.70 ± 0.02▲	1.72 ± 0.02▲▲	1.19 ± 0.03**▲▲
Spermatid measurements					
Spermatid heads (10^7/g testis)	17.20 ± 0.61	16.16 ± 1.14	16.34 ± 0.64	17.56 ± 0.76	17.63 ± 1.31
Spermatid heads (10^7/testis)	13.59 ± 0.57	11.83 ± 0.72	11.77 ± 0.54	13.93 ± 0.83	9.28 ± 0.85**
Spermatid count (mean/10^{-4} mL suspension)	67.95 ± 2.85	59.14 ± 3.58	58.83 ± 2.69	69.63 ± 4.14	46.42 ± 4.26**
Epididymal spermatozoal measurements					
Motility (%)	70.02 ± 1.51	71.33 ± 1.24	70.27 ± 1.38	73.46 ± 1.90	70.46 ± 1.26
Concentration (10^6/g cauda epididymal tissue)	751 ± 45	804 ± 30	818 ± 48	900 ± 46	581 ± 44▲

[1] Data are presented as mean ± standard error. Differences from the control groups for spermatid heads per gram testis and epididymal spermatozoal motility are not significant by Dunn's test.
[2] n=10.
* Significantly different ($P \leq 0.05$) from the 0:0 ppm group by Dunnett's test.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunnett's or Dunn's test.
▲ Significantly different ($P \leq 0.05$) from the 10,000:0 ppm group by Dunnett's or Dunn's test.
▲▲ Significantly different ($P \leq 0.01$) from the 10,000:0 ppm group by Dunnett's test.

TABLE D2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate with Perinatal Exposure[1]

Perinatal Concentration Adult Concentration	0 ppm 0 ppm	10,000 ppm 0 ppm	10,000 ppm 2,500 ppm	10,000 ppm 10,000 ppm	10,000 ppm 20,000 ppm
n	10	10	10	10	10
Necropsy body weight (g)	204 ± 2	206 ± 2	201 ± 3	199 ± 3	187 ± 2**▲▲
Estrous cycle length (days)	5.00 ± 0.00	5.00 ± 0.00	4.95 ± 0.05	4.95 ± 0.05	5.35 ± 0.30
Estrous stages (% of cycle)					
Diestrus	35.0	41.7	41.7	40.0	43.3
Proestrus	17.5	16.7	11.7	14.2	19.2
Estrus	26.7	24.2	28.3	23.3	19.2
Metestrus	20.8	17.5	18.3	22.5	18.3

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences between the two control groups (with and without perinatal exposure) and between the exposed groups and either control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, there are no significant differences between the control groups or between the exposed groups and either control group in the relative length of time spent in the estrous stages.
** Significantly different ($P \leq 0.01$) from the 0:0 ppm group by Dunnett's test.
▲▲ Significantly different ($P \leq 0.01$) from the 10,000:0 ppm group by Dunnett's test.

TABLE D3 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	10,000 ppm	20,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	374 ± 6	363 ± 5	345 ± 6**	310 ± 8**
Left epididymis	0.434 ± 0.008	0.427 ± 0.008	0.429 ± 0.003	0.217 ± 0.005**
Left cauda epididymis	0.156 ± 0.005	0.152 ± 0.006	0.154 ± 0.003	0.072 ± 0.002**
Left testis	1.523 ± 0.028	1.529 ± 0.022	1.515 ± 0.018	0.523 ± 0.021**
Spermatid measurements				
Spermatid heads (10^7/g testis)	9.262 ± 0.759	9.377 ± 0.536	9.111 ± 0.479	0.878 ± 0.216**
Spermatid heads (10^7/testis)	6.515 ± 0.392	6.725 ± 0.303	6.790 ± 0.426	0.183 ± 0.042**
Spermatid count (mean/10^{-4} mL suspension)	65.13 ± 3.93	67.23 ± 3.03	67.88 ± 4.27	1.83 ± 0.42**
Epididymal spermatozoal measurements				
Motility (%)	75.68 ± 1.32	73.90 ± 1.52	73.67 ± 1.91	0.00 ± 0.00**
Concentration (10^6/g cauda epididymal tissue)	690 ± 41	803 ± 60	687 ± 61	1 ± 1**

[1] Data are presented as mean ± standard error.
** Significantly different ($P \leq 0.01$) from the control group by Williams' (necropsy body weight only) or Shirley's test.

TABLE D4 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	2,500 ppm	10,000 ppm	20,000 ppm
n	10	10	10	10
Necropsy body weight (g)	202 ± 3	197 ± 3	197 ± 3	185 ± 2**
Estrous cycle length (days)	4.90 ± 0.19	4.85 ± 0.11	5.00 ± 0.00	5.15 ± 0.21
Estrous stages (% of cycle)				
Diestrus	36.7	36.7	40.0	38.3
Proestrus	16.7	15.8	19.2	18.3
Estrus	25.8	26.7	23.3	21.7
Metestrus	20.8	20.8	17.5	21.7

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
** Significantly different ($P \leq 0.05$) from the control group by Williams' test.

TABLE D5 Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	5,000 ppm	20,000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	32.9 ± 1.0	33.1 ± 0.8	30.0 ± 0.8*	28.5 ± 0.4**
Left epididymis	0.041 ± 0.001	0.041 ± 0.001	0.040 ± 0.001	0.037 ± 0.000**
Left cauda epididymis	0.014 ± 0.001	0.015 ± 0.001	0.013 ± 0.001	0.013 ± 0.000
Left testis	0.113 ± 0.002	0.114 ± 0.003	0.112 ± 0.003	0.105 ± 0.003
Spermatid measurements				
Spermatid heads (10^7/g testis)	13.63 ± 0.89	16.21 ± 1.47	15.95 ± 1.11	18.68 ± 0.70**
Spermatid heads (10^7/testis)	0.767 ± 0.050	0.881 ± 0.062	0.861 ± 0.049	0.941 ± 0.043
Spermatid count (mean/10^{-4} mL suspension)	48.03 ± 3.12	55.00 ± 3.91	53.80 ± 3.05	58.83 ± 2.69
Epididymal spermatozoal measurements				
Motility (%)	68.17 ± 1.99	72.75 ± 1.22	69.41 ± 2.87	72.07 ± 2.17
Concentration (10^6/g cauda epididymal tissue)	1042 ± 100	1133 ± 164	946 ± 135	1181 ± 134

[1] Data are presented as mean ± standard error. Differences from the control group for cauda epididymal and testis weights, spermatid heads per testis, spermatid count, and spermatozoal measurements are not significant by Dunn's test.
* Significantly different ($P \le 0.05$) from the control group by Williams' test.
** Significantly different ($P \le 0.01$) from the control group by Williams' (necropsy body weight only) or Shirley's test.

TABLE D6 Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Dibutyl Phthalate[1]

	0 ppm	1,250 ppm	5,000 ppm	20,000 ppm
n	10	10	10	10
Necropsy body weight (g)	30.0 ± 0.8	31.2 ± 0.9	27.6 ± 0.5	26.2 ± 0.4**
Estrous cycle length (days)	4.15 ± 0.11	4.20 ± 0.13	4.15 ± 0.11	4.90 ± 0.61
Estrous stages (% of cycle)				
Diestrus	32.5	29.2	30.0	37.5
Proestrus	15.8	14.2	12.5	15.0
Estrus	33.3	36.7	35.0	28.3
Metestrus	17.5	20.0	22.5	19.2
Uncertain diagnoses	0.8	0.0	0.0	0.0

[1] Necropsy body weight and estrous cycle length data are presented as mean ± standard error. Differences from the control group for estrous cycle length are not significant by Dunn's test. By multivariate analysis of variance, exposed groups do not differ significantly from the control group in the relative length of time spent in the estrous stages.
** Significantly different ($P \le 0.05$) from the control group by Williams' test.

APPENDIX E

Continuous Breeding Studies

Introduction E-2
Materials and Methods E-2
Results E-5

Table E1 Fertility Data, Length of Gestation, and Body Weights for F_0 Sprague-Dawley Rats in the Continuous Breeding Study of Dibutyl Phthalate E-9

Table E2 Litter Data and Body Weights of F_1 Sprague-Dawley Rat Pups in the Continuous Breeding Study of Dibutyl Phthalate E-10

Table E3 Survival and Body Weights of F_1 Sprague-Dawley Rat Pups (Final Litter) in the Continuous Breeding Study of Dibutyl Phthalate E-12

Table E4 Fertility, Reproductive Performance, and Body Weight Data for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate E-13

Table E5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate E-14

Table E6 Sperm Parameters and Estrous Cycle Characterization for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate E-15

Table E7 Fertility, Reproductive Performance, and Body Weight Data for F_1 and F_2 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate E-16

Table E8 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate E-17

Table E9 Sperm Parameters and Estrous Cycle Characterization for F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate E-18

Table E10 Incidence and Severity of Selected Lesions in Male F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate E-19

Table E11 Fertility, Reproductive Performance, and Body Weight Data for F_0 and F_1 Swiss (CD-1®) Mice in the Continuous Breeding Study of Dibutyl Phthalate E-20

Table E12 Fertility, Reproductive Performance, and Body Weight Data for F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate E-21

Table E13 Organ Weights and Sperm Parameters for F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate E-22

Table E14 Incidence and Severity of Selected Lesions in F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate E-23

CONTINUOUS BREEDING STUDIES

Introduction

The effects of exposure to dibutyl phthalate on reproduction were assessed by the performance of continuous breeding studies in Sprague-Dawley rats and Swiss (CD-1®) mice administered dibutyl phthalate in feed. Reproductive assessment consists of four phases: dose finding, continuous breeding, identification of the affected sex, and offspring assessment.

A 2-week dose-finding phase is conducted to determine doses for the continuous breeding phase. During the continuous breeding phase, the effects of the maximum tolerated exposure level estimated in the dose-finding phase and two lower exposure levels on fertility and reproduction are determined. If fertility is significantly affected during the continuous breeding phase, crossover mating trials are performed to determine if males, females, or both sexes are affected. Offspring assessment includes evaluation of reproductive performance of second-generation (F_1) animals from the final litters of the continuous breeding phase. The F_1 animals are raised to sexual maturity while receiving the same exposure concentrations as their parents, are mated, and are allowed to deliver the third-generation (F_2) offspring.

In the dibutyl phthalate studies, crossover mating trials were performed in rats and mice, and rat pups from all breeding pairs were maintained for offspring assessment. Complete results of these studies are available (RTI, 1984; NTP, 1991), and preliminary results of the studies in mice have been reported elsewhere (Lamb *et al.*, 1987). The design of these studies is summarized in Figure 2 of this Toxicity Study Report.

Materials and Methods

CONTINUOUS BREEDING STUDY DESIGNS

Dibutyl phthalate was obtained from Chem Central (Kansas City, MO). The cumulative results of elemental analyses, Karl Fischer water analysis, free acid and ester hydrolysis titration, thin-layer chromatography, and gas chromatography indicated a purity of 98% or greater. Stability studies indicated that dibutyl phthalate is stable as a bulk chemical for 2 weeks when stored protected from light at temperatures up to 60 ° C.

Male and female VAF Crl:CD BR outbred Sprague-Dawley albino rats and COBS® CD-1® (ICR)BR outbred Swiss albino mice were used in the 2-week dose-setting and continuous breeding studies; rats were obtained from Charles River Breeding Laboratories (Portage, MI) and mice were obtained from Charles River Breeding Laboratories (Kingston, NY). Rats in the continuous breeding study were approximately 8 weeks old at receipt; mice were

approximately 6 weeks old at receipt. Rats were quarantined for 2 to 3 weeks and mice were quarantined for 5 weeks before the start of the studies. Blood samples were periodically collected from sentinel rats and mice and were also collected from study rats; these samples were analyzed for antibody titers to rodent viruses. A single serum sample from a sentinel rat showed a positive antibody response to mouse encephalomyelitis virus; however, no clinical signs of disease were detected in the study rats.

For the 2-week dose-setting phase, groups of 8 rats per sex received 0, 1,000, 5,000, 10,000, 15,000, or 20,000 ppm dibutyl phthalate in feed. The exposure levels for the continuous breeding phase in mice were based on results of studies reported in the literature; groups of up to 20 breeding pairs of mice received 300, 3,000, or 10,000 ppm dibutyl phthalate in feed and 40 breeding pairs were maintained as controls. The exposure levels for the continuous breeding phase in rats were based on the results of the 2-week phase and were also chosen to facilitate comparison of the results of the rat study with those of the mouse study. For the continuous breeding phase in rats, groups of 20 breeding pairs received 1,000, 5,000, or 10,000 ppm dibutyl phthalate in feed, and 40 breeding pairs were maintained as controls.

During the continuous breeding phase, rats were housed separately for 7 days and in breeding pairs for 112 days while being exposed to dibutyl phthalate; mice were housed separately for 7 days and in breeding pairs for 98 days while being exposed. After the continuous breeding period, animals were housed separately while exposure was continued to allow delivery of the final litter of pups. Deionized water and NIH-07 Open Formula meal diet (Zeigler Brothers, Inc., Gardners, PA) containing the appropriate concentrations of dibutyl phthalate were available *ad libitum*. Clinical signs of toxicity, body weights, feed consumption, fertility, number of litters per pair, number of live pups per litter, proportion of pups born alive, sex ratio of live pups, and pup body weights were recorded at birth.

After the last litter of pups was weaned, crossover mating trials with the F_0 adult animals were performed. The following groups were mated: control males × control females (20 rat pairs, 19 mouse pairs); control males × females exposed to 10,000 ppm (20 pairs per species); and males exposed to 10,000 ppm × control females (20 rat pairs, 19 mouse pairs). Animal pairs were housed together for 7 days or until a vaginal plug or sperm in lavage fluid was noted. Clinical signs of toxicity, body weights, pregnancy and fertility data, feed consumption (rats), and litter data were recorded. Before necropsy, estrous cycle data were collected. At necropsy, sperm data were collected and the following organs were weighed: liver, kidneys, right cauda epididymis, right epididymis, right testis, right ovary, prostate gland, and seminal vesicles (rats); brain, liver, pituitary gland, testes and epididymides, prostate gland, seminal vesicles, ovaries with oviducts, and uterus (mice). Selected organs were fixed in 10% neutral buffered formalin or Bouin's fixative and embedded in glycol methacrylate or paraffin. Sections were stained with PAS and hematoxylin or hematoxylin and eosin.

To assess the offspring of treated animals, the final litter of rat pups born to each breeding pair in the holding period following the continuous breeding phase was reared. Pup number and body weight data were collected during lactation. After weaning, siblings were housed two per cage by sex and were exposed to the same concentrations of dibutyl phthalate as their parents. At sexual maturity (at approximately 77 days of age), 20 nonsibling F_1 rats of each sex in the same exposure group were cohoused for 7 days. Rats were examined for the presence of a copulatory plug or vaginal sperm and then housed singly through the delivery of pups. Clinical signs of toxicity, body weights, feed consumption, fertility, number of pairs delivering a litter, number of live (F_2) pups per litter, proportion of pups born alive, sex ratio of live pups, and pup body weights were recorded. At the end of the study, F_1 rats were necropsied; organ weights (kidney, liver, right cauda epididymis, right epididymis, right testis, prostate gland, seminal vesicles, and right ovary) and body weights were determined, and sperm morphology and vaginal cytology evaluations were made for 12 days prior to necropsy. Selected organs were fixed in 10% neutral buffered formalin or Bouin's fixative and embedded in glycol methacrylate or paraffin. Sections were stained with PAS and hematoxylin or hematoxylin and eosin.

STATISTICAL METHODS

For data expressed as proportions (fertility, mating, and pregnancy indexes), the Cochran-Armitage test (Armitage, 1971) was used to test for dose-related trends. Each dose group was compared to the control group with a chi-square test (Conover, 1971) or Fisher's exact test. A chi-square test for homogeneity was used to identify overall differences in fertility and for pairwise comparisons in the crossover mating trials. The number of litters and the number of live pups per litter were determined per fertile pair and then treatment group means were determined. The proportion of live pups was defined as the number of pups born alive divided by the total number of pups produced by each pair. The sex ratio was expressed as the number of male pups born alive divided by the total number of live pups born to each fertile pair.

Dose group means for data with skewed distributions were analyzed by the nonparametric multiple comparisons methods of Shirley (1977), Dunn (1964), or Mann and Whitney (1947). Jonckheere's test (Jonckheere, 1954) or Wilcoxon's test (Conover, 1971) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunn). In the crossover mating trials, parameters were tested for overall differences by the Kruskal-Wallis test (Kruskal and Wallis, 1952), and multiple comparisons were made with Dunn's test or the Mann-Whitney U test.

Analyses of covariance (Neter and Wasserman, 1974), with average litter size as the covariate, were performed to remove the potential effect of number of pups per litter on average pup weight. Least-square estimates of dose group means adjusted for litter size were tested for overall equality by an F-test and for pairwise equality by Dunnett's test

(Dunnett, 1955) or a *t*-test; these tests were performed on males, females, and males and females combined to analyze potential sex differences.

For vaginal cytology data, an arcsine transformation was used to bring the data into closer conformance with normality assumptions. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across dose levels.

Results

RATS

2-Week Dose-Finding Phase

All rats survived to the end of the 2-week phase. The mean body weight gain of males in the 20,000 ppm group was 10% less than that of control males, and females exposed to 20,000 ppm lost weight. Feed consumption by males exposed to 10,000 ppm or greater was decreased during Week 1. Feed consumption by females decreased with increasing exposure concentration during Week 1, and feed consumption by females exposed to 20,000 ppm was also decreased during Week 2. No clinical signs related to dibutyl phthalate exposure were noted.

Continuous Breeding Phase

One male and one female exposed to 5,000 ppm died during the continuous breeding period; the male was killed due to paralysis of undetermined etiology, and the female due to renal failure. The mean body weight of females in the 10,000 ppm group was 11% lower than that of the controls at 17 weeks (the end of the continuous breeding period). Feed consumption by exposed animals was generally similar to that by the controls.

All control and exposed pairs were fertile (Table E1). The average number of litters per pair and cumulative days to litter for exposed pairs were similar to those of the controls. The mean body weight of dams in the 10,000 ppm group was significantly lower than that of the controls at the delivery of each litter and throughout lactation of the final litter; the mean body weights of dams in the 1,000 and 5,000 ppm groups were also slightly lower than the mean body weight of control dams during lactation, and the decreases were significant on Days 14 and 21 for dams in the 5,000 ppm group and on Day 21 for dams in the 1,000 ppm group (Table E1). The number of live pups per litter generally decreased with increasing exposure concentration (Table E2). Male pup weights in three of five litters in the 5,000 and 10,000 ppm groups and female pup weights in all litters in the 10,000 ppm group were significantly lower than those of control pups. Total and adjusted live pup weights of all litters in the 10,000 ppm group and two litters in the 5,000 ppm group were significantly lower than in the controls; two other litters in the 5,000 ppm group also had lower adjusted live pup weights than the controls. The average ratio of live male pups to live pups was decreased in the first and third litters in the 10,000 ppm group. No biologically significant clinical signs of toxicity were noted.

For the final litter of pups reared to weaning, the weights of male and female pups from breeding pairs in the 10,000 ppm group were less than those of control pups through postnatal Day 21 (Table E3). The number of live pups per breeding pair and pup survival in exposed groups were similar to those of the controls.

Crossover Mating Trial

During the crossover mating trial, one control female died due to lymphoma and one female from the 10,000 ppm group died due to cardiac failure. These deaths were not attributed to exposure to dibutyl phthalate. There were no significant differences in mating, pregnancy, or fertility indexes or number of days to litter between the groups (Table E4). During the week of breeding, the mean body weight of females exposed to 10,000 ppm was significantly less than that of control dams; at delivery, the mean body weight of exposed dams remained slightly less than that of the controls. No significant clinical signs of toxicity were noted.

Relative kidney and liver weights of exposed males and females and the absolute liver weight of exposed males were significantly greater than those of the controls (Table E5). The relative right cauda epididymal weight of exposed males was also significantly increased. There were no significant differences in spermatid or epididymal spermatozoal measurements in exposed males or in estrous cycle lengths in exposed females (Table E6). These trials suggest that fertility and gametogenesis were unaffected in the F_0 rats that received 10,000 ppm.

Offspring Assessment Phase

All F_1 rats survived to the end of the offspring assessment phase. The mean body weights of male and female F_1 rats exposed to 10,000 ppm were less than those of the controls at weaning (males, 15%; females, 7%) and remained less throughout breeding; however, mean body weights of control and exposed dams were similar at delivery (Table E7). During the week of breeding, feed consumption by rats in the 10,000 ppm group was less than that by the control group. Mating, pregnancy, and fertility indexes of rats in the 10,000 ppm group were significantly decreased, and only a single litter was produced by dams in this group. These data suggest that adverse reproductive effects were present in both male and female F_1 rats receiving 10,000 ppm. Female (F_2) pup weights, total live pup weights, and adjusted live pup weights were decreased in all exposed groups (Table E7), and the adjusted live male pup weight for litters in the 5,000 ppm group was also decreased. There were no clinical signs of toxicity in F_1 rats or F_2 rat pups.

At necropsy, the relative kidney weights of F_1 males in the 5,000 and 10,000 ppm groups and the relative liver weight of males in the 10,000 ppm group were significantly increased (Table E8); absolute and relative right epididymal, cauda epididymal, and testis weights, prostate gland weights, and seminal vesicle weights were decreased in males exposed to 10,000 ppm. Absolute kidney, liver, and right ovary weights of females in the 10,000 ppm group were decreased. Spermatid heads per testis and per gram testis, spermatid count, and sperm concentration were significantly decreased

in F_1 males (Table E9). Epididymides were absent or poorly developed in 12 of 20 males in the 10,000 ppm group and in 1 of 20 males in each of the two lower exposure groups. The testes of four males exposed to 10,000 ppm and one male exposed to 5,000 ppm were atrophied. The testes of three males in the 10,000 ppm group were not descended into the scrotal sacs; four males in this group had poorly developed seminal vesicles and four had an underdeveloped prepuce or penis. No significant differences in estrous cycle length or in the percent of time spent in the various estrous stages were noted in females, although an extended estrous cycle (primarily due to a longer estrus) was suggested by the data (Table E9). These necropsy data also suggest that the reproductive system was a target of dibutyl phthalate toxicity in male and female F_1 rats receiving 10,000 ppm. Delayed maturation is suggested as well in male F_1 rats receiving 5,000 ppm or greater, based upon the testicular and accessory gland findings.

MICE

Continuous Breeding Phase

One male died during the 1-week exposure period prior to cohousing. Therefore, 39 mouse pairs were available for breeding. Additionally, two control males and one male and one female from the 3,000 ppm groups died during the continuous breeding phase. Mean body weights of control and exposed mice were similar throughout this period. Feed consumption by control and exposed mice was also similar.

The fertility index and the average litters per pair were significantly decreased in mice exposed to 10,000 ppm (Table E11). The numbers of live male pups, live female pups, and total live pups per litter and the percent live pups per litter were also decreased in this exposure group. The ratio of live male pups to live pups was significantly increased in the 10,000 ppm group. The male and female pup weights and total and adjusted live pup weight from exposed and control breeding pairs were similar (Table E11); however, the adjusted weight of male pups in the 10,000 ppm group was slightly but significantly lower than that of control male pups.

Crossover Mating Trial

All male and female mice survived to the end of the crossover mating trial. Mean body weights of control and exposed males and females were similar throughout breeding and during the 3-week segregation period. Females exposed to 10,000 ppm had a slightly lower mating index and a significantly lower fertility index than the control females (Table E12). Three dams in the 10,000 ppm group had one live pup each, and one of these three also delivered one dead pup. A fourth dam in this group delivered a single dead pup. The numbers of male, female, and total live pups per litter, the percent live pups per litter, and total and adjusted live pup weights were significantly decreased in the 10,000 ppm group (Table E12); female pup weight was also decreased in litters from control females bred with males exposed to 10,000 ppm.

Organ weights of exposed and control male mice were similar (Table E13). When adjusted for body weight, however, the liver weight of exposed males was significantly increased. The liver weight was increased and the uterus weight was decreased in females exposed to 10,000 ppm. There were no significant differences in sperm parameters in exposed males (Table E13) or in estrous cycle length in exposed females (data not shown). Incidences and severity of selected histopathologic lesions are shown in Table E14; no lesions were considered related to dibutyl phthalate exposure.

TABLE E1 Fertility Data, Length of Gestation, and Body Weights for F_0 Sprague-Dawley Rats in the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
Pregnancy Index (pregnant females/cohabiting pairs)				
Litter 1	40/40 (100%)	20/20 (100%)	19/19 (100%)	20/20 (100%)
Litter 2	40/40 (100%)	20/20 (100%)	19/19 (100%)	20/20 (100%)
Litter 3	40/40 (100%)	20/20 (100%)	19/19 (100%)	20/20 (100%)
Litter 4	38/40 (95%)	20/20 (100%)	19/19 (100%)	20/20 (100%)
Litter 5	34/40 (85%)	19/20 (95%)	18/19 (95%)	18/20 (90%)
Average Litters per Pair	4.8 ± 0.1	5.0 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
Cumulative Days to Litter				
Litter 1	25.2 ± 0.8	23.9 ± 0.3	24.9 ± 1.1	26.8 ± 1.6
Litter 2	47.8 ± 0.8	46.8 ± 0.6	47.1 ± 1.1	49.2 ± 1.7
Litter 3	71.4 ± 1.0	70.1 ± 0.9	71.2 ± 1.5	72.0 ± 1.8
Litter 4	93.7 ± 0.8	94.4 ± 1.6	93.7 ± 1.5	95.0 ± 1.8
Litter 5	116.0 ± 0.7	116.5 ± 0.9	115.4 ± 1.3	115.6 ± 0.9
Dam Weight at Delivery (g)				
Litter 1	285 ± 4	276 ± 3	279 ± 5	267 ± 4*
Litter 2	311 ± 5	299 ± 4	301 ± 5	287 ± 5*
Litter 3	335 ± 6	321 ± 4	320 ± 6	303 ± 5*
Litter 4	355 ± 7	340 ± 6	336 ± 6	314 ± 5*
Litter 5	369 ± 9	352 ± 5	351 ± 7	323 ± 5*
Dam Weight During Lactation of Litter 5 (g)				
n	35	20	19	20
Lactation Day 0	371 ± 9	355 ± 6	352 ± 6	324 ± 5*
Lactation Day 4	365 ± 8	348 ± 5	353 ± 8	321 ± 5*
Lactation Day 7	371 ± 8	353 ± 5	350 ± 6[2]	320 ± 5*[2]
Lactation Day 14	374 ± 6	357 ± 4	354 ± 6*	326 ± 5*
Lactation Day 21	355 ± 5	333 ± 4*[3]	335 ± 5*	321 ± 4*

[1] Data for litters per pair, cumulative days to litter, and dam weights are given as mean ± standard error. Differences from the control group for pregnancy indexes are not significant by a chi-square test; differences from the control group for litters per pair are not significant by Dunn's test.

[2] n=18.

[3] n=19.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE E2 Litter Data and Body Weights of F_1 Sprague-Dawley Rat Pups in the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
Litter 1				
Number of pairs delivering	40	20	19	20
Live pups/litter[2]	14.9 ± 0.3	13.2 ± 0.8	12.8 ± 0.9*	13.1 ± 0.6*
Live pups/litter[2] (%)	99 ± 0	96 ± 3	98 ± 1	93 ± 3
Sex ratio[3] (%)	51 ± 2	47 ± 5	50 ± 4	41 ± 4*
Male pup weight (g)	5.71 ± 0.06	5.83 ± 0.10[4]	5.69 ± 0.08[5]	5.29 ± 0.11*[4]
Female pup weight (g)	5.36 ± 0.06	5.51 ± 0.09	5.45 ± 0.10	5.01 ± 0.10*
Total live pup weight (g)	5.54 ± 0.06	5.66 ± 0.09	5.59 ± 0.08	5.13 ± 0.09*
Adjusted live pup weight[6] (g)	5.59 ± 0.06	5.63 ± 0.08	5.52 ± 0.08	5.11 ± 0.08*
Litter 2				
Number of pairs delivering	40	20	19	20
Live pups/litter	13.5 ± 0.5	13.9 ± 0.4	12.6 ± 0.9	12.0 ± 0.7
Live pups/litter (%)	98 ± 1	99 ± 1	98 ± 1	92 ± 4
Sex ratio (%)	48 ± 2	54 ± 3	54 ± 4	43 ± 4
Male pup weight (g)	6.22 ± 11[7]	6.09 ± 0.11	5.74 ± 0.13*	5.48 ± 0.13[4]
Female pup weight (g)	5.87 ± 0.10	5.66 ± 0.10	5.53 ± 0.15*[5]	5.24 ± 0.11*
Total live pup weight (g)	6.04 ± 0.10	5.90 ± 0.10	5.66 ± 0.14*	5.37 ± 0.12*
Adjusted live pup weight (g)	6.06 ± 0.09	5.94 ± 0.12	5.62 ± 0.12*	5.33 ± 0.12*
Litter 3				
Number of pairs delivering	40	20	19	20
Live pups/litter	13.6 ± 0.4	11.8 ± 0.6*	10.4 ± 0.8*	10.5 ± 0.7*
Live pups/litter (%)	97 ± 1	94 ± 2	97 ± 2	98 ± 1
Sex ratio (%)	51 ± 2	51 ± 2	51 ± 3	42 ± 4*
Male pup weight (g)	6.22 ± 0.09	6.22 ± 0.09	6.12 ± 0.13	5.47 ± 0.14[4]
Female pup weight (g)	5.88 ± 0.09	5.80 ± 0.11	5.82 ± 0.13	5.29 ± 0.14*
Total live pup weight (g)	6.04 ± 0.09	6.01 ± 0.09	5.95 ± 0.12	5.40 ± 0.13*
Adjusted live pup weight (g)	6.19 ± 0.08	6.02 ± 0.10	5.79 ± 0.11*	5.24 ± 0.11*
Litter 4				
Number of pairs delivering	38	20	19	20
Live pups/litter	11.1 ± 0.5	10.1 ± 0.5	10.1 ± 0.8	9.3 ± 0.6*
Live pups/litter (%)	94 ± 1	96 ± 2	96 ± 2	98 ± 1
Sex ratio (%)	48 ± 2	45 ± 3	48 ± 4	45 ± 4
Male pup weight (g)	6.51 ± 0.11	6.45 ± 0.13	6.01 ± 0.13*	5.71 ± 0.18*[4]
Female pup weight (g)	6.14 ± 0.09	6.25 ± 0.13	5.72 ± 0.12*	5.53 ± 0.12*
Total live pup weight (g)	6.31 ± 0.09	6.34 ± 0.12	5.86 ± 0.11*	5.61 ± 0.14*
Adjusted live pup weight (g)	6.43 ± 0.08	6.30 ± 0.10	5.82 ± 0.10*	5.46 ± 0.10*
Litter 5				
Number of pairs delivering	34	19	18	18[8]
Live pups/litter	10.8 ± 0.5	10.4 ± 0.5	9.2 ± 0.6	8.6 ± 0.9*
Live pups/litter (%)	93 ± 2	95 ± 2	95 ± 3	93 ± 6
Sex ratio (%)	51 ± 2	52 ± 3	51 ± 4	50 ± 3
Male pup weight (g)	6.46 ± 0.12	6.52 ± 0.16	6.09 ± 0.15*	5.85 ± 0.18*
Female pup weight (g)	6.15 ± 0.11	6.17 ± 0.13	5.96 ± 0.15	5.67 ± 0.22*
Total live pup weight (g)	6.31 ± 0.11	6.34 ± 0.13	6.02 ± 0.13	5.76 ± 0.19*
Adjusted live pup weight (g)	6.41 ± 0.10	6.38 ± 0.13	5.92 ± 0.13*	5.61 ± 0.14*

TABLE E2 Litter Data and Body Weights of F_1 Sprague-Dawley Rat Pups in the Continuous Breeding Study of Dibutyl Phthalate (continued)

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
Litters 1 through 5				
Average live pups/litter	12.9 ± 0.2	11.9 ± 0.3*	11.0 ± 0.5*	10.7 ± 0.4*
Average live pups/litter (%)	97 ± 1	96 ± 1	97 ± 1	95 ± 1
Average sex ratio (%)	50 ± 1	50 ± 1	51 ± 2	45 ± 2
Average male pup weight (g)	6.12 ± 0.07	6.15 ± 0.07	5.84 ± 0.07*	5.53 ± 0.11*
Average female pup weight (g)	5.81 ± 0.06	5.82 ± 0.06	5.63 ± 0.07	5.26 ± 0.10*
Total live pup weight (g)	5.96 ± 0.06	5.99 ± 0.06	5.74 ± 0.07*	5.38 ± 0.10*
Adjusted live pup weight (g)	6.04 ± 0.06	5.99 ± 0.08	5.66 ± 0.08*	5.30 ± 0.08*

[1] Data for live pups/litter, pups/breeding pair, sex ratios, and pup weights are given as mean ± standard error. Differences from the control group for percent live pups/litter are not significant by Dunn's or Shirley's test.
[2] Mean of average number of live pups per litter for each fertile pair.
[3] Live male pups/live pups.
[4] n=19.
[5] n=18.
[6] Least-squares estimate of mean pup weight adjusted for average litter size.
[7] n=39.
[8] Because no live pups were born in one litter, n=17 for pup weights and sex ratio.
* Significantly different ($P \leq 0.05$) from the control group by Dunnett's (adjusted live pup weights only) or Shirley's test.

TABLE E3 Survival and Body Weights of F_1 Sprague-Dawley Rat Pups (Final Litter) in the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
Day 0				
Number of litters	35	20	19	19
Live pups/breeding pair (%)	93 ± 2	95 ± 2	95 ± 3	93 ± 5[2]
Male pup weight (g)	6.49 ± 0.12	6.53 ± 0.15	6.15 ± 0.15*	5.68 ± 0.20*
Female pup weight (g)	6.16 ± 0.10	6.18 ± 0.12	6.01 ± 0.15	5.61 ± 0.20*
Day 4				
Male survival (%)	96 ± 2	97 ± 2	100 ± 0	93 ± 3
Female survival (%)	96 ± 2	98 ± 1	97 ± 2	98 ± 1
Total survival (%)	97 ± 2	97 ± 1	98 ± 1	94 ± 3
Male pup weight (g)	10.91 ± 0.31	11.36 ± 0.38	10.82 ± 0.35	10.00 ± 0.49
Female pup weight (g)	10.44 ± 0.31	10.74 ± 0.30	10.55 ± 0.34	9.76 ± 0.55
Day 7				
Male survival (%)	96 ± 2	97 ± 2	98 ± 2	92 ± 4
Female survival (%)	96 ± 2	98 ± 1	97 ± 2	98 ± 1
Total survival (%)	97 ± 2	97 ± 1	98 ± 1	94 ± 3
Male pup weight (g)	16.08 ± 0.48	16.69 ± 0.53	16.85 ± 0.48[3]	15.06 ± 0.73[4]
Female pup weight (g)	15.58 ± 0.44	15.91 ± 0.42	16.47 ± 0.55[3]	14.55 ± 0.87[4]
Day 14				
Male survival (%)	96 ± 2	97 ± 2	98 ± 2	92 ± 4
Female survival (%)	95 ± 2	98 ± 1	97 ± 2	97 ± 1
Total survival (%)	96 ± 2	97 ± 1	98 ± 1	94 ± 3
Male pup weight (g)	30.57 ± 0.72	31.20 ± 0.74	30.18 ± 0.75	26.10 ± 1.17*
Female pup weight (g)	29.62 ± 0.72	30.24 ± 0.64	29.95 ± 0.82	26.54 ± 1.48*
Day 21				
Male survival (%)	96 ± 2	93 ± 4	98 ± 2	87 ± 6
Female survival (%)	95 ± 2	96 ± 2	97 ± 2	97 ± 1
Total survival (%)	96 ± 2	95 ± 3	98 ± 1	92 ± 3
Male pup weight (g)	49.10 ± 1.28	50.33 ± 1.19	47.74 ± 1.27	41.45 ± 1.75*[3]
Female pup weight (g)	46.86 ± 1.19	47.76 ± 1.14	46.45 ± 1.26	41.29 ± 2.12*

[1] Survival and pup weight data are given as mean ± standard error. Differences from the control group for number of live pups/breeding pair and pup survival are not significant by Dunn's test.

[2] n=20; no live pups were born in one litter.

[3] n=18.

[4] n=17.

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.

TABLE E4 Fertility, Reproductive Performance, and Body Weight Data for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate[1]

Male Exposure Group Female Exposure Group	0 ppm 0 ppm	10,000 ppm 0 ppm	0 ppm 10,000 ppm
F_0 Adult Data			
Mating index[2]	16/19 (84%)	19/20 (95%)	16/19 (84%)
Pregnancy index[3]	12/18 (67%)	18/20 (90%)	15/19 (79%)
Fertility index[4]	12/15 (80%)	18/19 (95%)	15/16 (94%)
Dam weight at delivery (g)	364 ± 13	377 ± 13	343 ± 6
Days to litter	22.1 ± 0.1	22.3 ± 0.1	21.9 ± 0.2
F_1 Pup Data			
Live male pups/litter	6.1 ± 0.8	7.1 ± 0.5	5.7 ± 0.6
Live female pups/litter	6.6 ± 1.0	6.3 ± 0.6	7.3 ± 0.6
Total live pups/litter	12.7 ± 1.3	13.4 ± 0.6	12.9 ± 0.7
Total live pups/litter (%)	85 ± 8	99 ± 1	98 ± 1
Sex ratio[5] (%)	53 ± 6	54 ± 3	44 ± 4
Male pup weight (g)	6.01 ± 0.17	6.34 ± 0.14	5.38 ± 0.16
Female pup weight (g)	5.55 ± 0.15[6]	5.97 ± 0.15	5.30 ± 0.15
Total live pup weight (g)	5.84 ± 0.18	6.18 ± 0.14	5.36 ± 0.14
Adjusted live pup weight[7] (g)	5.96 ± 0.16	6.16 ± 0.12	5.28 ± 0.14*

[1] Days to litter, live pups/litter, sex ratio, and body weight data are given as mean ± standard error. Differences from the 0:0 ppm group for mating, pregnancy, and fertility indexes are not significant by a chi-square test. Differences from the 0:0 ppm group for live pups per litter, body weights, and days to litter are not significant by Dunn's test.
[2] Females with sperm plug/cohabiting pairs.
[3] Pregnant females/cohabiting pairs.
[4] Pregnant females/females with sperm plug.
[5] Live male pups/live pups.
[6] n=11.
[7] Least-squares estimate of mean pup weight adjusted for average litter size.
* Significantly different ($P<0.05$) from the 0:0 ppm group by a *t*-test.

TABLE E5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate[1]

	0 ppm	10,000 ppm
MALE		
n	40	20
Necropsy body wt	684 ± 8	657 ± 15
Kidneys		
Absolute	4.5 ± 0.1	4.8 ± 0.1
Relative	6.6 ± 0.1	7.3 ± 0.2*
Liver		
Absolute	25.7 ± 0.5	28.5 ± 1.1*
Relative	37.6 ± 0.6	43.4 ± 1.1*
Prostate gland		
Absolute	1.6 ± 0.1	1.4 ± 0.1
Relative	2.3 ± 0.1	2.2 ± 0.1
Seminal vesicles		
Absolute	3.1 ± 0.1	3.2 ± 0.1
Relative	4.6 ± 0.1	5.0 ± 0.2
Right testis		
Absolute	1.8 ± 0.0	1.7 ± 0.1
Relative	2.7 ± 0.0	2.7 ± 0.1
Right epididymis		
Absolute	0.7 ± 0.0	0.7 ± 0.0
Relative	1.0 ± 0.0	1.0 ± 0.0
Right cauda epididymis		
Absolute	0.3 ± 0.0	0.3 ± 0.0
Relative	0.4 ± 0.0	0.5 ± 0.0*
FEMALE		
n	38	19
Necropsy body wt	379 ± 10	326 ± 7*
Kidneys		
Absolute	2.6 ± 0.1	2.5 ± 0.1
Relative	7.0 ± 0.1	7.6 ± 0.1*
Liver		
Absolute	12.8 ± 0.3	12.7 ± 0.3
Relative	34.0 ± 0.6	38.9 ± 0.7*
Right ovary		
Absolute	53.3 ± 2.3	49.5 ± 2.3
Relative	0.1 ± 0.0	0.2 ± 0.0

[1] Organ weights and body weights are given in grams unless otherwise specified; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

* Significantly different ($P<0.05$) from the control group by Wilcoxon's test.

TABLE E6 Sperm Parameters and Estrous Cycle Characterization for F_0 Sprague-Dawley Rats in the Crossover Mating Trial of Dibutyl Phthalate[1]

	0 ppm	10,000 ppm
MALE		
n	40	20
Spermatid measurements		
Spermatid heads (10^7/g testis)	7.23 ± 0.20	6.93 ± 0.47
Spermatid heads (10^7/testis)	13.28 ± 0.39	12.42 ± 0.89
Epididymal spermatozoal measurements		
Motility (%)	76.8 ± 1.0	75.1 ± 1.4[2]
Concentration (10^6/g cauda epididymal tissue)	617 ± 15	649 ± 32
Abnormal sperm (%)	1.0 ± 0.1	1.5 ± 0.3[2]
FEMALE		
n	33	17
Estrous cycle length (days)	4.20 ± 0.07[3]	4.35 ± 0.20[4]
Estrous stages (% of cycle)		
Diestrus	37.9	30.3
Proestrus	18.6	20.6
Estrus	26.3	28.1
Metestrus	17.1	21.1

[1] Data are presented as mean ± standard error. Differences from the control group for spermatid and epididymal spermatozoal measurements are not significant by Wilcoxon's test. By multivariate analysis of variance, exposed groups of females do not differ significantly from control females in cycle length or in the relative length of time spent in the estrous stages.

[2] n=19.

[3] Estrous cycle longer than 12 days or unclear in 5 of 38 animals.

[4] Estrous cycle longer than 12 days or unclear in 2 of 19 animals.

TABLE E7 Fertility, Reproductive Performance, and Body Weight Data for F_1 and F_2 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
F_1 Adult Data				
Mating index[2]	20/20 (100%)	19/20 (95%)	18/20 (90%)	6/20 (30%)*
Pregnancy index[3]	19/20 (95%)	17/20 (85%)	17/20 (85%)	1/20 (5%)*
Fertility index[4]	19/20 (95%)	17/19 (89%)	17/18 (94%)	1/6 (17%)*
Dam weight at delivery (g)	323 ± 6	307 ± 8	317 ± 9	279
Days to litter	22.3 ± 0.2	21.6 ± 0.1	21.9 ± 0.1	21.0
F_2 Pup Data				
Live male pups/litter	7.1 ± 0.5	7.5 ± 0.4	5.9 ± 0.7	6.0
Live female pups/litter	6.9 ± 0.6	8.1 ± 0.5	6.8 ± 0.5	7.0
Total live pups/litter	14.0 ± 0.8	15.5 ± 0.4	12.8 ± 0.8	13.0
Live pups/litter (%)	98 ± 1	100 ± 0	99 ± 1	100
Sex ratio[5] (%)	52 ± 3	49 ± 3	45 ± 4	46
Male pup weight (g)	6.13 ± 0.13	5.79 ± 0.11	5.83 ± 0.11	5.10
Female pup weight (g)	5.81 ± 0.11	5.43 ± 0.08*	5.42 ± 0.08*	4.91
Total live pup weight (g)	5.97 ± 0.11	5.60 ± 0.09*	5.60 ± 0.09*	5.00
Adjusted live pup weight[6] (g)	5.98 ± 0.08	5.69 ± 0.09*	5.50 ± 0.09*	)[7]

[1] Days to litter, live pups/litter, sex ratio, and body weight data are given as mean ± standard error. Differences from the control group for dam and male pup body weights, days to litter, live pups/litter, and sex ratio are not significant by Dunn's test.
[2] Females with sperm plug/cohabiting pairs.
[3] Pregnant females/cohabiting pairs.
[4] Pregnant females/females with sperm plug.
[5] Live male pups/live pups.
[6] Least-squares estimate of mean pup weight adjusted for average litter size.
[7] Because only one litter was produced in this exposure group, no adjusted live pup weight was calculated.
* Significantly different ($P<0.05$) from the control group by the chi-square test (reproductive indexes) or Shirley's test.

TABLE E8 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
MALE				
n	20	20	20	20
Necropsy body wt	506 ± 9	509 ± 14	497 ± 12	467 ± 10*
Kidneys				
Absolute	3.9 ± 0.1	4.0 ± 0.1	4.0 ± 0.1	3.8 ± 0.1
Relative	7.7 ± 0.1	7.9 ± 0.1	8.2 ± 0.1*	8.2 ± 0.1*
Liver				
Absolute	20.6 ± 0.6	19.8 ± 0.7	20.1 ± 0.8	22.1 ± 0.6
Relative	40.7 ± 0.9	38.9 ± 0.7	40.2 ± 1.0	47.4 ± 0.7*
Prostate gland				
Absolute	0.8 ± 0.0	0.8 ± 0.1	0.7 ± 0.0	0.6 ± 0.1*[2]
Relative	1.7 ± 0.1	1.6 ± 0.1	1.5 ± 0.1	1.3 ± 0.1*[2]
Seminal vesicles				
Absolute	2.5 ± 0.1	2.6 ± 0.1	2.5 ± 0.1	1.8 ± 0.1*
Relative	5.0 ± 0.2	5.1 ± 0.2	5.0 ± 0.2	3.9 ± 0.3*
Right testis				
Absolute	1.8 ± 0.1	1.8 ± 0.0	1.8 ± 0.1	1.1 ± 0.1*
Relative	3.5 ± 0.1	3.5 ± 0.1	3.6 ± 0.2	2.4 ± 0.3*
Right epididymis				
Absolute	0.6 ± 0.0	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.0*
Relative	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	0.9 ± 0.1*
Right cauda epididymis				
Absolute	0.3 ± 0.0	0.3 ± 0.0	0.3 ± 0.0	0.1 ± 0.0*[3]
Relative	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.3 ± 0.0*[3]
FEMALE				
n				
Necropsy body wt	323 ± 5	312 ± 9	319 ± 9	281 ± 9*
Kidneys				
Absolute	2.4 ± 0.0	2.4 ± 0.0	2.5 ± 0.1	2.2 ± 0.1*
Relative	7.4 ± 0.1	7.7 ± 0.1	7.8 ± 0.3	7.8 ± 0.1
Liver				
Absolute	11.9 ± 0.3	11.3 ± 0.3*	12.0 ± 0.5	10.6 ± 0.4*
Relative	37.0 ± 0.8	36.3 ± 0.8	37.4 ± 1.0	37.9 ± 0.6
Right ovary				
Absolute (mg)	52.8 ± 2.1	58.3 ± 1.9	56.5 ± 2.1	41.1 ± 2.4*[3]
Relative	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0[3]

[1] Organ weights and body weights are given in grams unless otherwise specified; relative organ weights (organ-weight-to-body-weight ratios) are given as mg organ weight/g body weight (mean ± standard error).

[2] n=17.

[3] n=19.

* Significantly different (P<0.05) from the control group by Shirley's test.

TABLE E9 Sperm Parameters and Estrous Cycle Characterization for F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
MALE				
n	20	20	20	20
Spermatid measurements				
Spermatid heads (10^7/g testis)	8.29 ± 0.58	9.64 ± 0.41	8.29 ± 0.54	4.37 ± 0.98*
Spermatid heads (10^7/testis)	14.52 ± 0.95	16.84 ± 0.61	15.26 ± 0.99	6.69 ± 1.73*
Epididymal spermatozoal measurements				
Motility (%)	71.0 ± 1.6[2]	72.0 ± 2.0	69.8 ± 1.5	72.4 ± 3.2[3]
Concentration (10^6/g cauda epididymal tissue)	575 ± 38[2]	576 ± 22	548 ± 37[2]	295 ± 85*[2]
Abnormal sperm (%)	1.0 ± 0.2[4]	1.0 ± 0.1	1.0 ± 0.1[2]	0.9 ± 0.1[5]
FEMALE				
n	20	20	19	19
Estrous cycle length (days)	4.11 ± 0.06	4.17 ± 0.14	4.08 ± 0.10[6]	4.42 ± 0.13
Estrous stages (% of cycle)				
Diestrus	30.8	27.9	34.2	27.2
Proestrus	22.5	22.5	21.2	18.9
Estrus	24.2	25.8	24.6	32.0
Metestrus	22.5	23.7	20.0	21.9

[1] Data are presented as mean ± standard error. Differences from the control group for spermatozoal motility and abnormal sperm are not significant by Dunn's test. By multivariate analysis of variance, exposed groups of females do not differ significantly from control females in cycle length or in the relative length of time spent in the estrous stages.
[2] n=19.
[3] n=9.
[4] n=18.
[5] n=7.
[6] Estrous cycle longer than 12 days or unclear in 1 of 20 animals.
* Significantly different (P<0.05) from the control group.

TABLE E10 Incidence and Severity of Selected Lesions in Male F_1 Sprague-Dawley Rats in the Offspring Assessment Phase of the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	1,000 ppm	5,000 ppm	10,000 ppm
MALE				
n	10	0	10	10
Liver				
Degeneration, hepatocellular	3 (1.7)		1 (2.0)	
Hepatitis, focal	6 (1.0)		1 (1.0)	4 (1.0)
Testis				
Degeneration, seminiferous tubules	1 (4.0)		3 (1.7)	8 (2.9)
Interstitial cell hyperplasia	1 (2.0)		1 (1.0)	7 (2.1)
Granuloma	1 (1.0)			
Epididymis				
Degeneration, epithelial cell	1 (2.0)		2 (1.0)	1 (2.0)
Epididymitis, interstitial	1 (1.0)		1 (1.0)	1 (1.0)
Granuloma, focal				1 (4.0)
Underdeveloped epididymis				5 (3.4)
Defective epididymis				5 (3.4)
Presence of fluid/degenerated cells	1 (4.0)		1 (1.0)	2 (2.5)
Sperm content reduction	1 (4.0)		1 (3.0)	3 (3.3)
Seminal vesicles				
Vesiculitis				1 (4.0)
Inspissated secretion				1 (2.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=mild, 3=moderate, and 4=severe.

TABLE E11 Fertility, Reproductive Performance, and Body Weight Data for F_0 and F_1 Swiss (CD-1®) Mice in the Continuous Breeding Study of Dibutyl Phthalate[1]

	0 ppm	300 ppm	3,000 ppm	10,000 ppm
F_0 Adult Data				
Fertility index[2]	39/39 (100%)	20/20 (100%)	18/18 (100%)	15/20 (75%)**
Average litters per pair	4.9 ± 0.1	5.0 ± 0.1	4.7 ± 0.2	1.8 ± 0.3**
F_1 Pup Data				
Live male pups/litter	6.3 ± 0.2	6.5 ± 0.3	6.3 ± 0.3	1.1 ± 0.3**
Live female pups/litter	5.8 ± 0.2	5.8 ± 0.2	5.4 ± 0.3	0.6 ± 0.2**
Total live pups/litter	12.1 ± 0.4	12.3 ± 0.4	11.7 ± 0.4	1.7 ± 0.5**
Live pups/litter (%)	100 ± 0	100 ± 0	99 ± 1	50 ± 11**
Sex ratio[3] (%)	52 ± 1	53 ± 1	54 ± 2	68 ± 5**[4]
Male pup weight (g)	1.61 ± 0.02	1.59 ± 0.02	1.60 ± 0.02	1.65 ± 0.07[4]
Female pup weight (g)	1.56 ± 0.02	1.53 ± 0.02	1.54 ± 0.02	1.59 ± 0.09[5]
Total live pup weight (g)	1.58 ± 0.02	1.56 ± 0.02	1.57 ± 0.02	1.64 ± 0.07[4]
Adjusted live pup weight[6] (g)	1.60 ± 0.02	1.58 ± 0.03	1.59 ± 0.03	1.50 ± 0.06[4]

[1] Litters per pair, live pups/litter, sex ratio, and body weight data are given as mean ± standard error. Differences from the control group for pup body weights are not significant by the Mann-Whitney U test.
[2] Pairs producing one or more live or dead pups/cohabiting pairs.
[3] Live male pups/live pups.
[4] n=10; five breeding pairs produced no live pups.
[5] n=9.
[6] Adjusted for total number of live and dead pups per litter by analysis of covariance.
** Significantly different ($P \leq 0.01$) from the control group by Fisher's exact test or the Mann-Whitney U test.

TABLE E12 Fertility, Reproductive Performance, and Body Weight Data for F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate[1]

Male Exposure Group Female Exposure Group	0 ppm 0 ppm	10,000 ppm 0 ppm	0 ppm 10,000 ppm
F_0 Adult Data			
Mating index[2]	16/19 (84%)	18/20 (90%)	12/19 (63%)
Fertility index[3]	14/19 (74%)	17/20 (85%)	4/19 (21%)**
F_1 Pup Data			
Live male pups/litter	4.1 ± 0.7	4.8 ± 0.6	0.5 ± 0.3*
Live female pups/litter	3.6 ± 0.6	4.6 ± 0.5	0.3 ± 0.3**
Total live pups/litter	7.7 ± 0.9	9.4 ± 0.9	0.8 ± 0.3**
Total live pups/litter (%)	95 ± 5	99 ± 1	63 ± 24*
Sex ratio[4] (%)	50 ± 7	51 ± 5	67 ± 33[5]
Male pup weight (g)	1.86 ± 0.12[6]	1.74 ± 0.04	1.40 ± 0.19[7]
Female pup weight (g)	1.80 ± 0.07	1.63 ± 0.04*[8]	1.44[9]
Total live pup weight (g)	1.83 ± 0.10	1.69 ± 0.04	1.41 ± 0.11*[5]
Adjusted live pup weight[10] (g)	1.82 ± 0.06	1.76 ± 0.05	1.07 ± 0.15**[5]

[1] Days to litter, live pups/litter, sex ratio, and body weight data are given as mean ± standard error. Differences from the 0:0 ppm group for mating indexes are not significant by Fisher's exact test. Differences from the 0:0 ppm group for sex ratios and male and female pup body weights and days to litter are not significant by the Mann-Whitney U test.
[2] Females with sperm plug/cohabiting pairs.
[3] Pairs producing one or more live or dead pups/cohabiting pairs.
[4] Live male pups/live pups.
[5] n=3; no live pups were born in one litter.
[6] n=13.
[7] n=2.
[8] n=16.
[9] n=1.
[10] Adjusted for total number of live and dead pups per litter by analysis of covariance.
* Significantly different (P<0.05) from the 0:0 ppm group by the Mann-Whitney U test.
** Significantly different (P<0.01) from the 0:0 ppm group by Fisher's exact test or the Mann-Whitney U test.

TABLE E13 Organ Weights and Sperm Parameters for F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate[1]

	0 ppm	10,000 ppm
MALE		
n	38	20
Necropsy body wt	42.5 ± 0.7[2]	39.0 ± 0.7**
Organ Weights		
Brain	0.48 ± 0.01	0.47 ± 0.01
Liver	2.23 ± 0.05	2.27 ± 0.06
Pituitary gland (mg)	2.3 ± 0.1[3]	2.2 ± 0.1[4]
Prostate gland	0.05 ± 0.00	0.05 ± 0.00
Seminal vesicles	0.44 ± 0.02	0.40 ± 0.01
Left testis with epididymis	0.20 ± 0.01	0.19 ± 0.01
Right testis	0.15 ± 0.00	0.14 ± 0.01
Right epididymis	0.06 ± 0.00	0.06 ± 0.00
Sperm Parameters		
Motility (%)	55.6 ± 3.4	57.2 ± 5.7
Concentration (10^6/g cauda epididymal tissue)	744 ± 26	713 ± 46
Abnormal sperm[5] (%)	6 ± 1	5 ± 1
Tailless sperm[5] (%)	20 ± 1	20 ± 2
FEMALE		
n	39	19
Necropsy body wt	39.2 ± 0.8	37.9 ± 0.6
Brain	0.50 ± 0.01	0.49 ± 0.00
Liver	2.08 ± 0.04	2.43 ± 0.05**
Ovaries with oviducts	0.04 ± 0.00	0.04 ± 0.00
Pituitary gland (mg)	3.8 ± 1.1	3.9 ± 0.2
Uterus	0.34 ± 0.02	0.25 ± 0.02**

[1] Organ weights and body weights are given in grams unless otherwise specified (mean ± standard error). Differences from the control group for sperm parameters are not significant by the Mann-Whitney U test.
[2] n=37.
[3] n=36.
[4] n=19.
[5] Percent abnormal sperm does not include tailless sperm.
** Significantly different (P<0.01) from the control group by a *t*-test.

TABLE E14 Incidence and Severity of Selected Lesions in F_0 Swiss (CD-1®) Mice in the Crossover Mating Trial of Dibutyl Phthalate[1]

	0 ppm	10,000 ppm
MALE		
n	38	20
Testis		
Seminiferous tubule, atrophy	8 (1.6)	6 (1.8)
Epididymis		
Aspermia	1 (5.0)	1 (5.0)
Hypospermia		1 (3.0)
Prostate gland		
Chronic inflammation	13 (1.2)	2 (1.0)
FEMALE		
n	39	19
Ovary with oviduct		
Cyst[2]	2	1
Uterus		
Stromal hemosiderosis	38 (2.1)	19 (1.9)
Cystic hyperplasia	1 (2.0)	1 (1.0)
Multifocal thrombosis	4 (1.0)	2 (2.0)
Mineralized thrombus	1 (1.0)	5 (1.0)

[1] Average severity (in parentheses) is based on the number of animals with lesions: 1=minimal, 2=slight, 3=moderate, 4=moderately severe/high, and 5=severe/high.
[2] No severity grades available.

APPENDIX F

Perinatal Peroxisome Determination Studies

Introduction F-2
Materials and Methods F-2
Results F-4

Table F1 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Dibutyl Phthalate in the *In Utero* Peroxisome Study F-7

Table F2 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Di(2-ethylhexyl) Phthalate in the *In Utero* Peroxisome Study F-8

Table F3 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Dibutyl Phthalate in the Lactational Peroxisome Study F-9

Table F4 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Di(2-ethylhexyl) Phthalate in the Lactational Peroxisome Study F-11

PERINATAL PEROXISOME DETERMINATION STUDIES

Introduction

Dibutyl phthalate is structurally related to di(2-ethylhexyl) phthalate, a peroxisome proliferator which induces liver tumors in rats and mice. Further, following metabolism to its monoester, di(2-ethylhexyl) phthalate has been shown to be transferable to pups during lactation (Dostal *et al.*, 1987). The effects of dibutyl phthalate and di(2-ethylhexyl) phthalate on hepatic peroxisome proliferation *in utero* and during lactation were assessed by breeding studies of F344/N rats administered dibutyl phthalate or di(2-ethylhexyl) phthalate in feed.

Materials and Methods

PROCUREMENT AND CHARACTERIZATION OF DIBUTYL PHTHALATE AND DI(2-ETHYLHEXYL) PHTHALATE

Dibutyl phthalate was obtained from Chem Central (Kansas City, MO). Di(2-ethylhexyl) phthalate was obtained from Hatco Chemical Corporation (Fords, NJ). The cumulative results of elemental analyses for carbon and hydrogen, Karl Fischer water analysis, free acid and ester hydrolysis titrations, thin-layer chromatography, and gas chromatography indicated a purity of 98% or greater for dibutyl phthalate and a purity greater than 99% for di(2-ethylhexyl) phthalate. Stability studies indicated that dibutyl phthalate and di(2-ethylhexyl) phthalate are both stable as bulk chemicals for 2 weeks when stored protected from light at temperatures up to 60° C. Throughout the studies, bulk dibutyl phthalate and di(2-ethylhexyl) phthalate were stored at room temperature.

Stability studies performed with gas chromatography on the dosed feed mixtures indicated that dibutyl phthalate mixtures were stable for 3 weeks when stored in the dark at −20° C and for 1 week when stored under animal room conditions; di(2-ethylhexyl) phthalate mixtures were found to be stable for 3 weeks when stored in the dark at room temperature and for 1 week when stored under animal room conditions. During the studies, dibutyl phthalate feed mixtures were stored in stainless steel buckets in the dark at approximately −20° C and di(2-ethylhexyl) phthalate feed mixtures were stored at room temperature.

STUDY DESIGNS

Male and female F344/N rats used for breeding in the *in utero* and lactational exposure studies were obtained from Charles River Breeding Laboratories (Raleigh, NC). The female rats were 56 to 70 days old at receipt. Rats in the *in utero* exposure studies were quarantined 14 days and were 11 to 12 weeks old when the studies began; rats in the lactational exposure studies were quarantined 42 days and were 12 weeks old when the studies began. Blood samples

were collected from five rats of each sex at the beginning of the studies. The sera were analyzed for antibody titers to rodent viruses (Boorman *et al.*, 1986; Rao *et al.*, 1989a,b). All results were negative.

Groups of female rats bred at the beginning of the maximum perinatal exposure determination study were used for fetal hepatic peroxisome determinations. Groups of five pregnant females received 1,250, 2,500, 5,000, 7,500, 10,000, or 20,000 ppm dibutyl phthalate in feed 7 days a week for up to 20 days. Groups of 10 pregnant females received 500, 1,750, 3,500, 7,000, 10,500, 14,000, or 28,000 ppm di(2-ethylhexyl) phthalate in feed 7 days a week and 40 pregnant females were maintained on undosed feed as controls for up to 20 days. During the interval between Days 17 and 20 of gestation, maternal livers and pooled fetal livers were weighed and peroxisomal palmitoyl-CoA oxidase activities were measured for all rats exposed to dibutyl phthalate, five rats per group exposed to di(2-ethylhexyl) phthalate, and five control rats per evaluation day (a total of 15 control rats per chemical).

In the lactational exposure studies, unexposed rats were housed in breeding groups (one male and two females) for 12 days or until the females were observed to be sperm positive. Females were assigned to groups and were maintained on undosed feed throughout gestation. Groups of 12 female rats were then administered 300, 1,000, 3,000, 10,000, or 30,000 ppm dibutyl phthalate or 420, 1,400, 4,200, 14,000, or 42,000 ppm di(2-ethylhexyl) phthalate in feed 7 days a week on Days 1 through 22 of lactation; 120 female rats were maintained on undosed feed as controls. The number and sex of pups and the litter weights were recorded on Lactation Days 0 and 1; the number, sex, and individual body weights of pups were recorded on Days 4, 7, 14, and 21. On Day 4 postpartum, litters were culled to a maximum of eight pups. On Days 7, 14, and 21 of lactation, one male and one female pup per litter from six dams per exposure group per chemical were evaluated for liver weights and peroxisomal enzyme activities. Because the day of parturition varied between the exposure groups, the day of lactation also varied; therefore, two (di(2-ethylhexyl) phthalate) or three (dibutyl phthalate) sets of analyses were performed for each of these days of evaluation. On Day 22 of lactation, the dams were evaluated for liver weights and peroxisomal enzyme activities. Six dams and their litters served as controls for both the dibutyl phthalate and the di(2-ethylhexyl) phthalate studies; a total of 24 control rat dams and litters were evaluated.

Female rats were housed individually during gestation and lactation. Water (City of Columbus) was available *ad libitum*. Undosed NIH-07 Open Formula Diet (Zeigler Brothers, Inc., Gardners, PA) was available *ad libitum* during breeding and during the gestation portion of the lactational exposure studies; feed containing the appropriate doses of dibutyl phthalate or di(2-ethylhexyl) phthalate were available during the gestation period of the *in utero* studies and the lactation period of the lactational exposure studies. Animal rooms were maintained at 69° to 75° F and 35% to 65% relative humidity, with 12 hours of fluorescent light per day and at least 10 room air changes per hour. Animals were observed daily during breeding, twice daily during gestation, and weekly thereafter (lactational exposure study). Observations were recorded as necessary.

LIVER PEROXISOME ANALYSES

Liver samples were minced and then homogenized in a sucrose/tris-hydrochloride/EDTA buffer. The homogenates were sonicated in an ice bath and centrifuged; the supernatants were removed and stored on ice prior to analysis. The samples were assayed for palmitoyl-CoA oxidase activity with a tris:β-NAD:FAD:DDT:CoA:KCN:Triton X-100:BSA mixture (Lazarow and de Duve, 1976) in a reaction initiated with palmitoyl CoA. Supernatants with high enzyme activity were diluted with the homogenization buffer. The reaction was monitored with ultraviolet spectroscopy for the formation of β-NADH at 340 nm between 8 and 15 minutes. The data were normalized per gram of protein. The protein concentration in the supernatant samples was measured by biuret reaction monitored by a Hitachi® 704 microcomputer (Boehringer Mannheim, Indianapolis, IN).

STATISTICAL METHODS

Number of fetuses per breeding group and liver and body weight data, which are approximately normally distributed, were analyzed using the parametric multiple comparisons procedures of Williams (1971, 1972) or Dunnett (1955). Palmitoyl-CoA oxidase activity data, which typically have a skewed distribution, were analyzed by the nonparametric multiple comparisons methods of Shirley (1977) or Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams, Shirley) was more appropriate for pairwise comparisons than a test capable of detecting departures from monotonic dose response (Dunnett, Dunn).

Results

IN UTERO EXPOSURE STUDY

The terminal body weight of dams exposed to 20,000 ppm dibutyl phthalate was less than that of the controls (Table F1). The liver weight of dams exposed to 10,000 ppm was greater than that of the control dams. The hepatic palmitoyl-CoA oxidase (peroxisomal enzyme) activity was higher in dams exposed to 1,250, 2,500, or 5,000 ppm dibutyl phthalate than in the controls.

Fewer fetuses were recovered from the 20,000 ppm group than from the controls (Table F1). The pooled absolute liver weight of fetuses from female rats exposed to 20,000 ppm dibutyl phthalate was significantly less than that of fetuses from the controls. Peroxisomal enzyme activities of exposed pups were similar to the control value.

Among dams exposed to di(2-ethylhexyl) phthalate, the terminal body weight in the 28,000 ppm group was less than that of the controls (Table F2). Liver weights of all groups of exposed dams except the 28,000 ppm group were greater than in the controls. Peroxisomal enzyme activities of exposed and control dams were similar.

The liver weight of pups from dams exposed to 7,000 ppm di(2-ethylhexyl) phthalate was greater than that of the control pups (Table F2). No fetuses were recovered from the 28,000 ppm group. In the highest exposure group in which fetuses developed (14,000 ppm), the peroxisomal enzyme activity of the fetuses was significantly higher than in the controls.

LACTATIONAL EXPOSURE STUDY

The terminal body weight of dams exposed to 30,000 ppm dibutyl phthalate was less than that of the controls (Table F3). The liver weight of dams exposed to 10,000 or 30,000 ppm was greater than that of the controls. On Day 22, the peroxisomal enzyme activity in dams in the 3,000 ppm group was higher than in the controls.

The body weights of male and female pups from dams exposed to 30,000 ppm dibutyl phthalate was lower than those of the control pups on Days 7, 14, and 21 (Table F3). The liver weights of male and female pups in this group were significantly lower than those of the controls at all time points. The peroxisomal enzyme activity in male pups in the 30,000 ppm group was significantly higher than in the controls on Days 7 and 21; other differences also occurred in lower exposure groups on Days 14 and 21. The peroxisomal enzyme activity in female pups exposed to 3,000 or 30,000 ppm was significantly higher than in the controls on Day 7; however, the peroxisomal enzyme activity in female pups in the 10,000 ppm group was lower than that in the controls at this time point.

In dams exposed to di(2-ethylhexyl) phthalate, lower body weights were noted in the 4,200, 14,000, and 42,000 ppm groups (Table F4). The liver weights of dams in the 1,400, 4,200, and 14,000 ppm groups were greater than in the controls. Peroxisomal enzyme activities generally increased with increasing exposure concentration, and the increase was significant in the 14,000 ppm group.

The survival of pups in the highest exposure group of di(2-ethylhexyl) phthalate, 42,000 ppm, was decreased; in this group, no pups survived to Day 14. Body weight differences were noted in all exposed groups (Table F4). Male and female pups from dams exposed to 14,000 ppm had lower body weights than the controls on Days 7, 14, and 21. On Day 14, female pups in the 420, 1,400, and 4,200 ppm groups also had significantly lower body weights than the controls; on Day 21, male pups exposed to 4,200 ppm and female pups exposed to 1,400 or 4,200 ppm had lower body weights than the controls. The liver weight of pups in the 42,000 ppm group was significantly less and the peroxisomal enzyme activity was higher than in the controls on Day 7. At all time points, the liver weights of male and female pups

exposed to 14,000 ppm were less than in the controls. The peroxisomal enzyme activity was significantly higher in female pups in the 14,000 ppm group than in the controls on Day 14.

TABLE F1 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Dibutyl Phthalate in the *In Utero* Exposure Study[1]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	7,500 ppm	10,000 ppm	20,000 ppm
Number of breeding groups[2]	15	5	5	5	5	5	5
Number of fetuses/ breeding group	9.9 ± 0.5	10.4 ± 0.4	8.8 ± 1.2	10.2 ± 0.2	10.0 ± 0.7	11.2 ± 0.7	4.2 ± 0.6**
Terminal body weight (g)							
Dams	242 ± 2	243 ± 2	235 ± 4	241 ± 4	243 ± 7	241 ± 4	214 ± 4**
Liver weight (g)							
Dams	11.34 ± 0.21	11.70 ± 0.19	10.93 ± 0.31	11.47 ± 0.25	12.17 ± 0.37	12.53 ± 0.36*	11.53 ± 0.34
Fetuses	1.433 ± 0.089	1.637 ± 0.064	1.329 ± 0.178	1.475 ± 0.088	1.550 ± 0.252	1.494 ± 0.116	0.366 ± 0.063**
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)							
Dams	0.5 ± 0.1	1.6 ± 0.2**	1.7 ± 0.3**	1.3 ± 0.0*	0.7 ± 0.1	0.8 ± 0.1	1.1 ± 0.3
Fetuses	0.3 ± 0.1	0.4 ± 0.1	0.5 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2

[1] Data for dams and fetuses are given as mean ± standard error for averages of two dams per breeding group; data for fetuses are from pooled fetal livers. For breeding groups in which one female was used for the maximum perinatal exposure determination study, only data for the dam in the *in utero* exposure study are included. Statistical tests were performed on unrounded data.

[2] One male and two females per breeding group.

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's or Dunn's test.

** Significantly different ($P \leq 0.01$) from the control group by Dunnett's or Dunn's test.

TABLE F2 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Di(2-ethylhexyl) Phthalate in the *In Utero* Exposure Study[1]

	0 ppm	500 ppm	1,750 ppm	3,500 ppm	7,000 ppm	10,500 ppm	14,000 ppm	28,000 ppm
Number of breeding groups[2]	15	5	5	5	5	5	5	5
Number of fetuses/breeding group	9.9 ± 0.6	11.0 ± 1.2	9.8 ± 1.2	12.0 ± 1.3	11.4 ± 0.2	11.8 ± 0.4	10.2 ± 0.9	0.0 ± 0.0**
Terminal body weight (g)								
Dams	226 ± 2	233 ± 8	233 ± 2	242 ± 6*	236 ± 3	224 ± 4	215 ± 5	154 ± 3**
Liver weight (g)								
Dams	10.88 ± 0.21	11.72 ± 0.44*	12.04 ± 0.30**	13.24 ± 0.37**	13.67 ± 0.22**	13.70 ± 0.30**	13.47 ± 0.25**	10.07 ± 0.49**
Fetuses	0.880 ± 0.050	1.034 ± 0.298	0.939 ± 0.124	1.131 ± 0.215	1.704 ± 0.437**	1.067 ± 0.136	0.824 ± 0.164	)[3]
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)								
Dams	1.2 ± 0.1	1.3 ± 0.1	0.5 ± 0.1*	0.9 ± 0.2	1.2 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.5 ± 0.3
Fetuses	0.4 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.7 ± 0.1	0.5 ± 0.1	1.0 ± 0.1**	)

[1] Data for dams and fetuses are given as mean ± standard error for averages of two dams per breeding group; data for fetuses are from pooled fetal livers. For breeding groups in which one female was used for the maximum perinatal exposure determination study, only data for the dam in the *in utero* exposure study are included. Differences from the control group for number of fetuses per breeding group are not significant by Dunn's test. Statistical tests were performed on unrounded data.

[2] One male and two females per breeding group.

[3] No fetuses were present in females exposed to 28,000 ppm.

* Significantly different ($P \leq 0.05$) from the control group by Williams', Dunnett's, or Dunn's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams', Dunnett's, or Dunn's test.

TABLE F3 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Dibutyl Phthalate in the Lactational Exposure Study[1]

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm	30,000 ppm
DAM DATA						
n	18	6	6	6	6	6
Terminal body weight (g)	225 ± 2	213 ± 15	228 ± 4	218 ± 4	221 ± 3	187 ± 1**
Liver weight (g)	11.82 ± 0.21	11.16 ± 1.23	12.37 ± 0.37	12.15 ± 0.32	13.24 ± 0.55*	13.29 ± 0.15*
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
n	6	6	6	6	5	6
Group 1	1.5 ± 0.2			2.5 ± 0.2**		1.8 ± 0.2
Group 2	1.9 ± 0.2	1.7 ± 0.1				
Group 3	1.8 ± 0.1		1.3 ± 0.2		1.9 ± 0.1	
PUP DATA						
n	18	6	6	6	6	6
Day 0 pup weight (g)	4.89 ± 0.08	4.84 ± 0.06	4.99 ± 0.05	4.95 ± 0.05	4.96 ± 0.09	4.88 ± 0.05
Male						
Body weight (g)						
n	18	6	6	6	6	6
Day 7	12.12 ± 0.16	12.02 ± 0.33	11.96 ± 0.24	11.78 ± 0.19	11.58 ± 0.28	7.28 ± 0.21**
Day 14	23.18 ± 0.30	24.77 ± 0.87	23.17 ± 0.35	22.50 ± 0.42	22.41 ± 0.47	12.87 ± 0.52**
Day 21	35.77 ± 0.62	34.01 ± 2.95	35.88 ± 0.28	34.17 ± 0.69	35.57 ± 0.90	20.64 ± 0.59**
Liver weight (g)						
n	18	6	6	6	6	6
Day 7	0.244 ± 0.009	0.224 ± 0.014	0.247 ± 0.011	0.239 ± 0.014	0.221 ± 0.008	0.169 ± 0.004**
Day 14	0.687 ± 0.028	0.694 ± 0.052	0.655 ± 0.031	0.640 ± 0.026	0.693 ± 0.054	0.282 ± 0.025**
Day 21	1.343 ± 0.042	1.325 ± 0.111	1.283 ± 0.025	1.254 ± 0.070	1.269 ± 0.071	0.730 ± 0.026**
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
n	6	6	6	6	6	6
Day 7						
Group 1	1.0 ± 0.1			1.0 ± 0.1		2.6 ± 0.3**
Group 2	0.9 ± 0.1	0.9 ± 0.1				
Group 3	1.2 ± 0.2		1.7 ± 0.4		0.8 ± 0.2	
Day 14						
Group 1	2.3 ± 0.2			1.0 ± 0.4*		2.0 ± 0.2
Group 2	1.0 ± 0.1	1.3 ± 0.1				
Group 3	1.3 ± 0.1		0.6 ± 0.0*[2]		1.2 ± 0.2	
Day 21						
Group 1	1.8 ± 0.1			2.2 ± 0.1*		2.6 ± 0.1**
Group 2	1.5 ± 0.1	2.0 ± 0.2				
Group 3	2.5 ± 0.2		1.9 ± 0.1		1.7 ± 0.3	

TABLE F3 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Dibutyl Phthalate in the Lactational Exposure Study (continued)

	0 ppm	300 ppm	1,000 ppm	3,000 ppm	10,000 ppm	30,000 ppm
PUP DATA (continued)						
Female						
Body weight (g)						
n	18	6	6	6	6	6
Day 7	11.47 ± 0.15	11.61 ± 0.34	11.54 ± 0.20	11.45 ± 0.17	11.30 ± 0.19	7.06 ± 0.21**
Day 14	22.51 ± 0.23	23.97 ± 0.55*	22.92 ± 0.27	22.26 ± 0.43	22.07 ± 0.46	12.55 ± 0.48**
Day 21	34.39 ± 0.54	33.72 ± 3.14	34.66 ± 0.68	33.49 ± 0.68	33.77 ± 0.72	19.98 ± 0.64**
Liver weight (g)						
n	18	6	6	6	6	6
Day 7	0.285 ± 0.008	0.275 ± 0.013	0.281 ± 0.006	0.286 ± 0.013	0.262 ± 0.010	0.161 ± 0.016**
Day 14	0.653 ± 0.026	0.612 ± 0.020	0.662 ± 0.022	0.637 ± 0.018	0.702 ± 0.042	0.318 ± 0.013**
Day 21	1.296 ± 0.041	1.359 ± 0.115	1.213 ± 0.045	1.216 ± 0.053	1.260 ± 0.038	0.726 ± 0.034**
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
n	6	6	6	6	6	6
Day 7						
Group 1	0.7 ± 0.1			1.5 ± 0.0**		1.3 ± 0.1*
Group 2	0.9 ± 0.1	1.1 ± 0.1				
Group 3	1.5 ± 0.2		1.1 ± 0.0		0.9 ± 0.2*	
Day 14						
Group 1	1.1 ± 0.2			0.5 ± 0.1		1.9 ± 0.1
Group 2	1.0 ± 0.1	1.2 ± 0.2				
Group 3	1.0 ± 0.1		0.8 ± 0.0[2]		1.2 ± 0.2	
Day 21						
Group 1	2.4 ± 0.1			2.2 ± 0.1		2.5 ± 0.2
Group 2	1.8 ± 0.3	1.5 ± 0.2				
Group 3	2.3 ± 0.2		1.2 ± 0.3*		1.5 ± 0.2[2]	

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data. Dams were evaluated on Lactation Day 22. Because the day of parturition varied between the exposure groups, the day of lactation also varied; therefore, three sets of analyses were performed for each of these days of evaluation.

[2] n=5.

* Significantly different ($P \leq 0.05$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.

TABLE F4 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Di(2-ethylhexyl) Phthalate in the Lactational Exposure Study[1]

	0 ppm	420 ppm	1,400 ppm	4,200 ppm	14,000 ppm	42,000 ppm
DAM DATA						
n	6	6	6	6	6	6
Terminal body weight (g)	229 ± 3	221 ± 5	228 ± 3	215 ± 4**	182 ± 2**	141 ± 4**
Liver weight (g)	11.83 ± 0.45	11.66 ± 0.29	13.56 ± 0.40*	14.51 ± 0.59**	14.32 ± 0.27**	10.63 ± 0.51
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
Group 1	1.9 ± 0.2	1.2 ± 0.1				3.3 ± 0.2
Group 2	2.9 ± 0.1		2.1 ± 0.1	4.5 ± 0.7	5.5 ± 0.4*	
PUP DATA						
n	6	6	6	6	6	6
Day 0 pup weight (g)	4.64 ± 0.09	4.93 ± 0.07	4.63 ± 0.08	4.76 ± 0.17	4.82 ± 0.11	4.62 ± 0.12
Male						
n	6	6	6	6	6	5
Body weight (g)						
Day 7	11.33 ± 0.30	12.05 ± 0.29	11.89 ± 0.24	11.65 ± 0.34	9.75 ± 0.30**	5.63 ± 0.18**
Day 14	23.51 ± 0.36	22.73 ± 0.67	23.18 ± 0.81	21.93 ± 0.67	16.97 ± 0.52**	)[2]
Day 21	36.23 ± 0.68	34.43 ± 1.24	33.96 ± 1.26	33.21 ± 0.95*	23.51 ± 0.56**	)
Liver weight (g)						
Day 7	0.276 ± 0.016	0.215 ± 0.013**	0.234 ± 0.013	0.304 ± 0.012	0.222 ± 0.007*	0.122 ± 0.008**
Day 14	0.663 ± 0.024	0.662 ± 0.022	0.626 ± 0.036	0.738 ± 0.020	0.453 ± 0.017**	)
Day 21	1.441 ± 0.025	1.328 ± 0.052	1.333 ± 0.078	1.387 ± 0.081	0.916 ± 0.050**	)
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
Day 7						
Group 1	0.9 ± 0.1	1.8 ± 0.2**				1.7 ± 0.1*
Group 2	1.4 ± 0.3		0.8 ± 0.1	0.8 ± 0.1	1.9 ± 0.3	
Day 14						
Group 1	1.0 ± 0.1	1.3 ± 0.1				)
Group 2	1.0 ± 0.1		0.7 ± 0.1[3]	0.8 ± 0.2[3]	2.0 ± 0.2	
Day 21						
Group 1	1.5 ± 0.1	1.5 ± 0.2				)
Group 2	2.3 ± 0.2		1.7 ± 0.3	2.5 ± 0.4	2.3 ± 0.1	

TABLE F4 Body Weights, Liver Weights, and Peroxisome Data for F344/N Rats Exposed to Di(2-ethylhexyl) Phthalate in the Lactational Exposure Study (continued)

	0 ppm	420 ppm	1,400 ppm	4,200 ppm	14,000 ppm	42,000 ppm
PUP DATA (continued)						
Female						
n	6	6	6	6	6	6
Body weight (g)						
Day 7	11.03 ± 0.22	11.17 ± 0.22	11.08 ± 0.23	11.38 ± 0.31	9.30 ± 0.28**	5.32 ± 0.16**
Day 14	23.64 ± 0.38	22.05 ± 0.44*	21.68 ± 0.70*	21.72 ± 0.58*	16.41 ± 0.57**	)
Day 21	35.94 ± 0.54	33.75 ± 0.49	31.64 ± 1.46**	32.43 ± 0.89**	22.98 ± 1.03**	)
Liver weight (g)						
Day 7	0.283 ± 0.014	0.232 ± 0.007	0.250 ± 0.013	0.312 ± 0.015	0.209 ± 0.008**	0.152 ± 0.011**
Day 14	0.687 ± 0.027	0.577 ± 0.007**	0.593 ± 0.016**	0.725 ± 0.010	0.444 ± 0.019**	)
Day 21	1.444 ± 0.026	1.256 ± 0.042	1.315 ± 0.058	1.371 ± 0.071	0.957 ± 0.039**	)
Palmitoyl-CoA oxidase activity (nmol/minute per mg protein)						
Day 7						
Group 1	0.9 ± 0.1	1.1 ± 0.1				1.7 ± 0.2**
Group 2	1.4 ± 0.2[3]		0.8 ± 0.1	0.7 ± 0.1	2.3 ± 0.2	
Day 14						
Group 1	1.0 ± 0.1	1.6 ± 0.1*				)
Group 2	0.8 ± 0.1		0.7 ± 0.1	0.8 ± 0.1	2.0 ± 0.2*	
Day 21						
Group 1	1.8 ± 0.3	1.4 ± 0.2				)
Group 2	2.4 ± 0.1		2.4 ± 0.1	2.6 ± 0.1	2.4 ± 0.1	

[1] Data are given as mean ± standard error. Statistical tests were performed on unrounded data. Dams were evaluated on Lactation Day 22. Because the day of parturition varied between the exposure groups, the day of lactation also varied; therefore, two sets of analyses were performed for each day of evaluation.

[2] n=0.

[3] n=5.

* Significantly different ($P \leq 0.05$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.

** Significantly different ($P \leq 0.01$) from the control group by Williams', Dunnett's, Dunn's, or Shirley's test.

APPENDIX G

Genetic Toxicology

Table G1 Mutagenicity of Dibutyl Phthalate in *Salmonella typhimurium* G-2

Table G2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Dibutyl Phthalate G-3

Table G3 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Dibutyl Phthalate in Feed for 13 Weeks G-5

TABLE G1 Mutagenicity of Dibutyl Phthalate in *Salmonella typhimurium*[1]

Strain	Dose (µg/plate)	Revertants/plate[2] -S9	+10% hamster S9	+10% rat S9
TA100	0	139 ± 9.2	125 ± 8.5	123 ± 12.2
	100	141 ± 4.3	136 ± 8.4	119 ± 11.3
	333	138 ± 1.8	130 ± 11.7	112 ± 6.7
	1,000	143 ± 6.1	119 ± 2.1	124 ± 10.7
	3,333	134 ± 9.2	124 ± 3.8	117 ± 4.6
	10,000	142 ± 6.4	102 ± 10.9	81 ± 3.8
Trial summary		Negative	Negative	Negative
Positive control[3]		1,190 ± 32.3	1,105 ± 45.5	850 ± 85.5
TA1535	0	26 ± 1.8	12 ± 1.9	16 ± 2.6
	100	26 ± 3.5	12 ± 3.2	12 ± 2.7
	333	24 ± 1.9	15 ± 1.7	10 ± 1.5
	1,000	22 ± 0.0	13 ± 0.0	13 ± 2.9
	3,333	21 ± 1.5	13 ± 0.9	13 ± 0.3
	10,000	26 ± 3.2	8 ± 1.7	9 ± 1.2
Trial summary		Negative	Negative	Negative
Positive control		953 ± 29.7	80 ± 4.5	78 ± 0.7
TA1537	0	6 ± 1.0	6 ± 1.5	7 ± 1.5
	100	5 ± 0.6	7 ± 1.5	9 ± 2.2
	333	6 ± 0.9	7 ± 3.4	4 ± 0.7
	1,000	7 ± 1.5	10 ± 2.3	7 ± 0.7
	3,333	8 ± 0.6	3 ± 0.3	5 ± 2.6
	10,000	6 ± 2.1	3 ± 1.5	2 ± 0.3
Trial summary		Negative	Negative	Negative
Positive control		393 ± 18.0	64 ± 3.0	70 ± 4.9
TA98	0	16 ± 1.8	22 ± 3.7	30 ± 3.6
	100	17 ± 0.6	28 ± 2.9	24 ± 3.5
	333	14 ± 0.3	26 ± 2.9	23 ± 2.7
	1,000	20 ± 3.5	33 ± 2.5	28 ± 3.2
	3,333	19 ± 2.3	25 ± 1.2	14 ± 2.0
	10,000	14 ± 2.9	15 ± 1.2	14 ± 0.7
Trial summary		Negative	Negative	Negative
Positive control		1,624 ± 51.9	943 ± 22.7	713 ± 28.9

[1] Study performed at EG&G Mason Research Institute. The detailed protocol and the complete data set are presented in Zeiger *et al.* (1985). All trials were repeated; only one trial per strain/activation combination is presented here. 0 µg/plate is the solvent control.

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

[3] The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9-aminoacridine (TA1537), and 4-nitro-*o*-phenylenediamine (TA98). The positive control for trials with metabolic activation with both strains was 2-aminoanthracene.

TABLE G2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Dibutyl Phthalate[1]

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction[2]	Average Mutant Fraction
-S9						
Trial 1						
Dimethylsulfoxide						
		71	99	81	38	
		72	111	65	30	
		77	88	51	22	
		62	102	69	37	32
Methyl methanesulfonate						
	15	37	28	198	178	
		34	27	217	212	195*
Dibutyl phthalate						
	12	71	79	50	24	
		78	86	58	25	24
	24	61	68	67	37	
		62	77	45	24	31
	36	68	63	80	39	
		61	61	47	26	32
	48	38	11	82	72	
		48	16	73	51	61*
	60	Lethal				
		Lethal				
Trial 2						
Dimethylsulfoxide						
		87	89	34	13	
		75	94	36	16	
		93	89	30	11	
		77	128	19	8	12
Methyl methanesulfonate						
	15	29	17	81	92	
		41	22	133	109	101*
Dibutyl phthalate						
	30	88	47	50	19	
		71	44	43	20	20
	38	73	36	32	14	
		64	36	41	21	18
	46	71	37	77	36	
		62	25	51	28	32*
	54	66	23	161	82	
		69	19	117	56	69*
	62	66	5	640	323	
		78	8	374	161	242*
	70	Lethal				
		Lethal				

TABLE G2 Induction of Trifluorothymidine Resistance in L5178Y Mouse Lymphoma Cells by Dibutyl Phthalate (continued)

Compound	Concentration (µg/mL)	Cloning Efficiency (%)	Relative Total Growth (%)	Mutant Count	Mutant Fraction	Average Mutant Fraction
-S9 (continued)						
Trial 3						
Dimethylsulfoxide						
		103	101	90	29	
		113	101	84	25	
		88	98	47	18	
		94	101	63	22	23
Methyl methanesulfonate						
	15	36	22	229	213	
		39	23	227	197	205*
Dibutyl phthalate						
	38	75	62	73	32	
		89	67	70	26	29
	46	69	41	81	39	
		63	34	71	37	38
	54	55	19	78	47	
		58	11	130	74	61*
	62	29	4	110	127	
		42	4	157	124	126*
	70	17	1	98	198	
		18	1	164	307	252*

[1] Study performed at Inveresk Research International. The experimental protocol is presented in detail in Myhr *et al.* (1985). All doses were tested in triplicate; the average of the three tests is presented in the table.

[2] Mutant fraction (frequency) is a ratio of the mutant count to the cloning efficiency, divided by 3 (to arrive at MF/1 x 10^6 cells treated); MF=mutant fraction.

* Significant positive response ($P \leq 0.05$).

TABLE G3 Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Treatment with Dibutyl Phthalate in Feed for 13 Weeks[1]

Dose (ppm)	Micronucleated NCEs/1,000 NCEs[2]
MALE	
0	2.90 ± 0.37
1,250	3.00 ± 0.42
2,500	1.80 ± 0.34
5,000	2.40 ± 0.33
10,000	1.90 ± 0.29
20,000	2.80 ± 0.60
	P=0.516[3]
FEMALE	
0	3.10 ± 0.64
1,250	3.80 ± 0.54
2,500	3.40 ± 0.60
5,000	3.70 ± 0.46
10,000	3.40 ± 0.43
20,000	3.80 ± 0.44
	P=0.314

[1] A detailed description of the protocol is found in MacGregor *et al.* (1990). NCEs = normochromatic erythrocytes. Data are presented as mean ± standard error. Differences from the control group were not significant by a *t*-test.
[2] Two thousand normochromatic erythrocytes were scored per animal.
[3] Significance of micronucleated NCEs/1,000 NCEs tested by a one-tailed trend test.