NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 217

CARCINOGENESIS BIOASSAY OF DI(2-ETHYLHEXYL)PHTHALATE

(CAS NO. 117-81-7)

IN F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

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

NATIONAL TOXICOLOGY PROGRAM

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

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

NTP Technical Report

on the

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

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NOTE TO THE READER

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

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

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650).

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

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

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CONTRIBUTORS

The bioassay of di(2-ethylhexyl)phthalate was conducted between March 1977 and June 1979 at EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program.

The bioassay was conducted under the supervison of Drs. H. Lilja (1) and E. Massaro (1,2), principal investigators. The program manager was Ms. R. Monson (1). Ms. A. Good (1) supervised the technicians in charge of animal care, and Ms. E. Zepp (1) supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Ms. D. Bouthot (1) kept all daily records of the test. Dr. D. S. Wyand (1), pathologist, directed the necropsies and performed the histopathologic evaluations. The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group with final approval by the NCI Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland (3). The statistical analyses were performed by Dr. J. R. Joiner (4) and Ms. S. Vatsan (4), using methods selected for the bioassay program by Dr. J. J. Gart (5). Chemicals used in this bioassay were analyzed at Midwest Research Institute (6), and dosed feed mixtures were analyzed by Dr. M. Hagopian (1).

This report was prepared at Tracor Jitco (4) and reviewed by NTP. Those responsible for the report at Tracor Jitco were Dr. Cipriano Cueto, Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. W. D. Theriault, reports manager; Dr. A. C. Jacobs, bioscience writer; and Ms. M. Glasser, technical editor.

The following scientists at NTP (7) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas (Chemical Manager), Dr. Charles K. Grieshaber, Dr. Larry Hart, Dr. William V. Hartwell, Dr. Joseph Haseman, Dr. James Huff, Dr. William Kluwe, Dr. Mary Kornreich, Dr. Ernest E. McConnell, Dr. John A. Moore, Dr. Sherman F. Stinson, Dr. R. Tennant, and Dr. Jerrold M. Ward.

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REVIEWERS

On October 15, 1980, this carcinogenesis bioassay report on di(2-ethylhexyl)phthalate underwent peer review and was approved by the National Toxicology Program Board of Scientific Counselor's Technical Report Review Subcommittee and associated Panel of Experts at an open meeting held in Conference Room 6, Building 31C, National Institutes of Health, Bethesda, Maryland.

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ABSTRACT

A bioassay of di(2-ethylhexyl)phthalate, the most commonly used plasticizer for polyvinylchloride polymers, for possible carcinogenicity was conducted by feeding diets containing 6,000 or 12,000 ppm of the test chemical to groups of 50 male and 50 female F344 rats and 3,000 or 6,000 ppm to groups of 50 male and 50 female B6C3F1 mice for 103 weeks. Controls consisted of 50 untreated rats and 50 untreated mice of either sex.

Mean body weights of dosed male rats (high- and low-dose), high-dose female rats, and dosed female mice (high- and low-dose) were marginally-to-moderately lower than those of the corresponding controls at the end of the chronic study, reflecting a decrease in body weight gain. Food consumption was reduced slightly in rats of either sex, whereas there was no apparent difference among the mouse groups.

Female rats and male and female mice administered di(2-ethylhexyl) phthalate had significantly higher incidences of hepatocellular carcinomas than those observed in the controls (rats -- males: 1/50, 2%; 1/49, 2%; 5/49, 10%; females -- 0/50, 0%; 2/49, 4%; 8/50, 16%, P=0.003; mice -- males: 9/50, 18%; 14/48, 29%; 19/50, 38%, P=0.022; females: 0/50, 0%; 7/50, 14%; P=0.006, 17/50, 34%, $P < 0.001$). Further, a statistically significant positive trend for hepatocellular carcinomas occurred in female rats (P=0.002) and in male (P=0.018) and female ($P < 0.001$) mice.

In addition, di(2-ethylhexyl)phthalate caused a statistically significant increased incidence of male rats with either hepatocellular carcinomas or neoplastic nodules (3/50, 6%; 6/49, 12%; 12/49, 24%; P=0.010).

Degeneration of the seminiferous tubules was observed in the high-dose male rats (1/49, 2%; 2/44, 5%; 43/48, 90%) and in the high-dose male mice (1/49, 2%; 2/48, 4%; 7/49, 14%). Hypertrophy of cells in the anterior pituitary was also found at increased incidences in the high-dose male rats (1/46, 2%; 0/43, 0%; 22/49, 45%).

Under the conditions of this bioassay, di(2-ethylhexyl)phthalate was carcinogenic for F344 rats and B6C3F1 mice, causing increased incidences of female rats and male and female mice with hepatocellular carcinomas, and inducing an increased incidence of male rats with either hepatocellular carcinomas or neoplastic nodules.

SUMMARY OF PEER REVIEW COMMENTS ON THE BIOASSAY OF DI(2-ETHYLHEXYL)PHTHALATE

Dr. Nielsen, as the primary reviewer for the report on the bioassay of di(2-ethylhexyl)phthalate (DEHP), agreed with the conclusion that, under the conditions of the bioassay, DEHP was carcinogenic for F344 rats and B6C3F1 mice of either sex, causing increased incidence of hepatocellular carcinomas. He also noted that clear cell cytoplasmic changes of the liver occurred in multiple foci more frequently in low- and high-dose male rats than in controls.

As a secondary reviewer, Dr. Breslow agreed with the conclusion of the report. He pointed out that 21 of 57 hepatocellular carcinomas in dosed mice (both sexes) gave rise to pulmonary metastases, while there were no metastases in control mice or dosed rats. Tubular degeneration and atrophy of the testes was noted in 90% of high-dose male rats, against 2% and 5% of the control and low-dose animals. The high-dose male also had an elevated incidence of hypertrophy of the pituitary but decreased rates of pituitary carcinomas/adenomas, thyroid carcinomas/adenomas, and testicular interstitial-cell tumors compared with controls. The incidence of testicular or tubular degeneration in high-dose male mice (14%) was also elevated in comparison with the other groups. Possible shortcomings included the fact that there was unexplained early mortality in the low-dose female mice, leaving only 50% for terminal kill, and probably reducing the numbers of tumors appearing at necropsy.

As another secondary reviewer, Dr. Hitchcock also agreed with the conclusions of the report. She pointed out that statistically significant negative trends were observed in male rats for the incidence of carcinomas/adenomas of the pituitary, C-cell carcinomas/adenomas of the thyroid, and interstitial-cell tumors of the testes. She commented on the failure of the study to detect hepatomegaly, even though others have demonstrated this effect in subacute studies at concentrations below the lowest dose used in this study. She emphasized that hepatomegaly is an important toxicologic effect and may be an early indication for the development of hepatic lesions.

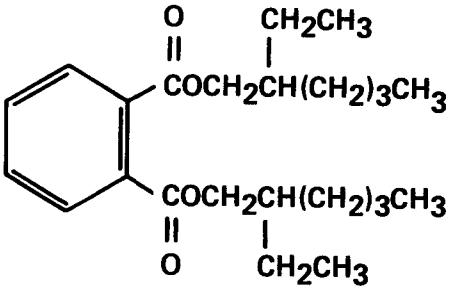
Dr. John Thomas (not a member of peer review panel) stated that these were the first reported positive studies with DEHP. He questioned why the F344 rat was used in view of the high spontaneous incidence of interstitial-cell tumors, approaching 100% at 24 months of age. Dr. Thomas also emphasized the mortality in control animals of about 33% in mice and 40% in male rats. Finally, he stated that the reviewers should address the significance of hepatocellular carcinomas and neoplastic nodules.

Dr. Swenberg initiated a discussion about combining hepatocellular carcinomas and neoplastic nodules for making a determination of carcinogenicity in male rats. Dr. Breslow raised the same question on combining hepatocellular carcinomas and adenomas in mice. Dr. Ward stated that the bioassay program for the last several years combined carcinomas and nodules or adenomas. He emphasized that neoplastic nodules and adenomas are tumors

which ordinarily progress. Dr. Swenberg contended that, since one cannot predict how many of the nodules progress to malignancy, one should do the statistical analyses for each type of liver lesion. Dr. David Rall (Director, NTP) and Dr. Norton Nelson (NTP Board Chairman) said that there were two separate issues: the description and analysis of the experimental findings, and the biological significance of these DEHP-induced tumors. A distinction should be made in the report. The NTP diagnoses these liver tumors as adenomas, as neoplastic nodules, or as carcinomas; the incidences of these tumors, as compared among dosed and control groups, are analyzed both separately and in combination. Dr. Nelson and Dr. Rall indicated that this is scientifically sound, and that the NTP would continue this practice. The Peer Review Panel in general seemed to agree that this was appropriate.

Dr. Nielsen moved that the report on the bioassay of di(2-ethylhexyl) phthalate be accepted after minor changes. Dr. Breslow added an amendment to the motion that the summary should distinguish statistically between hepatocellular adenomas and neoplastic nodules and carcinomas, identifying each specific type of neoplasm and calculating the statistical significance of each type.

I. INTRODUCTION



DI(2-ETHYLHEXYL) PHTHALATE

Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) is the most commonly used plasticizer added to polymers such as polyvinylchloride for flexibility (Autian, 1973; and Tanaka et al., 1975). This phthalate ester is also used in nitrocellulose lacquers, cellulose acetate-butyrate, ethyl cellulose, natural and synthetic rubbers (W.R. Grace and Co., 1976) and as a pump fluid for oil diffusion pumps (Kirk-Othmer, 1970). Di(2-ethylhexyl)phthalate is present in vinyl tubing used for delivery of blood and intravenous fluids, and for milk processing (W. R. Grace and Co., 1976) and was approved by the U.S. Food and Drug Administration for use in polymers used in food contact articles (CFR, 1976). Three hundred and eighty-nine million pounds of di(2-ethylhexyl)phthalate were produced in the United States in 1977 (USITC, 1978). The recommended 8-hour time-weighted average for exposure of workers to di(2-ethylhexyl)phthalate in air is 5 mg/m^3 (Fed. Reg., 1974).

Although di(2-ethylhexyl)phthalate is insoluble in water, this viscous liquid is soluble in materials containing lipoproteins (Jaeger and Rubin, 1972; Gesler, 1973; and Thomas et al., 1978) and is found in concentrations as great as 66 mg/liter in blood that has been stored in vinyl bags and transferred through vinyl tubings (Baker, 1978). Concentrations of di(2-ethylhexyl)phthalate may reach 250 mg/liter in bagged plasma (Jacobson et al., 1974). Di(2-ethylhexyl)phthalate has been isolated from tissues of patients transfused with blood or blood products stored in flexible polyvinyl chloride containers, and was found in neonatal tissues after umbilical catheterization (Jaeger and Rubin, 1972; Hillman et al., 1975). It has been

estimated that a multi-transfused pediatric patient being treated for aplastic anemia, leukemia, or hemophilia might receive as much as 1,500 mg di(2-ethylhexyl)phthalate per year (28 mg/kg) (Jacobson et al., 1977).

A number of studies have been carried out on the disposition, toxic effects, and metabolic fate of di(2-ethylhexyl)phthalate in experimental animals. The LD_{50} values reported for rats and mice administered di(2-ethylhexyl)phthalate are presented in Table 1. For the most part, the reported oral and intraperitoneal LD_{50} values exceed 30 g/kg. The LD_{50} values after intravenous administration ranged from 1.0 to 4.2 g/kg.

Steady state concentrations of 120 and 80 ppm di(2-ethylhexyl)phthalate were observed in the liver and fat of rats fed diets containing 5,000 ppm di(2-ethylhexyl)phthalate for 14 days (Daniel and Bratt, 1974). When the ester was administered by gavage to rats, it was first metabolized to mono(2-ethylhexyl)phthalate, which then underwent ω and ω-1 oxidation as in endogenous fatty acid metabolism (Albro et al., 1973). These authors identified four mono(2-ethylhexyl)phthalate derivatives and phthalic acid in rat urine. The four mono(2-ethylhexyl)phthalate derivatives were: 5-keto-2-ethylhexyl ester, 5-hydroxy-2-ethylhexyl ester, 5-carboxyl-2-ethylpentyl ester, and 2-carboxylmethylbutyl ester. Free phthalic acid comprised less than 3% of the urinary metabolites. Furthermore, no evidence of conjugation was detected. Daniel and Bratt (1974) confirmed these findings of Albro et al. (1973). Di(2-ethylhexyl)phthalate was also degraded to mono(2-ethyl-hexyl)phthalate by the contents of the small intestine of Sprague-Dawley rats (Rowland, 1974).

Hepatomegaly was observed in male F344 rats fed diets containing 5,000 ppm di(2-ethylhexyl)phthalate for 1 week or administered 2,000 mg/kg by gavage for 21 days (Table 2), and similar effects were seen in Wistar rats administered the metabolite mono(2-ethylhexyl)phthalate by gavage (Lake et al., 1975).

Carcinogenic effects were not reported in 2-year bioassays with Sherman or Wistar rats of either sex fed diets containing up to 5,000 ppm di(2-ethylhexyl)phthalate (Carpenter et al., 1953; Harris et al., 1956). The design and reporting of these studies, however, were insufficient by

Table 1. LD_{50} Values for Rats and Mice Administered Di(2-ethylhexyl)phthalate

Species	Route of Administration	LD_{50} (mg/kg)	Reference
Rat	Oral	26,000	Patty, 1967
Rat (Wistar, male)	Oral	greater than 34,000	Hodge, 1943
Rat (Wistar, male)	Oral	30,600	Shaffer et al., 1945
Rat	Intravenous (sonicated in rat serum)	2,080	Petersen et al., 1974
Rat	Intraperitoneal	49,000	Singh et al., 1972
Rat (Wistar, male)	Intraperitoneal	30,600	Shaffer et al., 1945
Mouse	Oral	49,000	Yamada, 1974
Mouse	Oral	26,000	Patty, 1967
Mouse	Intravenous	1,060	Petersen et al., 1974
Mouse	Intraperitoneal	4,200	Calley et al., 1966
Mouse (ICR, male)	Intraperitoneal	38,000	Lawrence et al., 1975

Table 2. Effects of Di(2-ethylhexyl)phthalate Administered to Rats and Mice

Species/Sex	Route of Administration	Dose/Duration	Effects Observed	Reference
Rat (Wistar, male)	Dosed Feed	900-1,900 mg/kg for 90 days (a)	Tubular atrophy and degeneration of the testes	Shaffer et al.,1945
Rat (F344, male)	Dosed Feed	5,000 ppm for 1 week	Hepatomegaly	Reddy et al., 1976
Rat (CD, male)	Dosed Feed	10,000 ppm for 17 weeks	Seminiferous tubular atrophy and cessation of spermatogenesis; increased kidney weight	Gray et al., 1977
Rat (Wistar, male)	Gavage	2,000 mg/kg/day for 21 days	Hepatomegaly	Lake et al., 1975
Rat (male)	Intravenous	Single dose 200 mg/kg (b)	Lung edema	Rubin and Chang, 1978
Mouse (Swiss-Webster male)	Dosed feed	20,000 ppm for 4 weeks	Hepatomegaly	Reddy et al., 1976
Mouse	Dosed feed	5g/kg/day for 3 months	Renal cysts and hepatocellular necrosis	Ota et al., 1974

(a) 1.5%-3.0% in feed
(b) Solubilized in rat plasma

present-day standards for assessing carcinogenic potential. The test substance was found to be fetotoxic in Wistar rats (Onda et al., 1976) and fetotoxic and teratogenic in ddY-SLC mice (Yagi et al., 1976).

Abnormal liver histopathology was observed in rhesus monkeys receiving repeated transfusions of plasma containing low concentrations of the chemical (Jacobson et al., 1977). Changes consisted of vacuolated Kupffer cells, foci of parenchymal necrosis, chronic inflammatory cell infiltrates, and prominence or hyperplasia of Kupffer cells. Total doses of di(2-ethylhexyl)phthalate ranged from 7 to 33 mg over the 1-year administration period. Detectable concentrations of di(2-ethylhexyl)phthalate were present in the liver as long as 5 months following cessation of the transfusions. Decreased sulfobromophthalein clearance was observed, accompanied by abnormal liver histopathology, indicating a hepatotoxic potential in monkeys.

Results of chromosome aberration tests in Chinese hamster cells were negative (Ishidate and Odashima, 1977; and Abe and Sasaki, 1977), but positive results were obtained in a dominant lethal test in ICR mice (Singh et al., 1974). Di(2-ethylhexyl)phthalate was not mutagenic in Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100 -- both with and without metabolic activation (Simmon et al., 1977); however, mono(2-ethylhexyl)phthalate was mutagenic in E. Coli (Yagi et al., 1976).

Di(2-ethylhexyl)phthalate was tested by the Carcinogenesis Testing Program because of the large amount produced, occupational exposure, and the widespread exposure of the general population to products containing this substance.

II. MATERIALS AND METHODS

A. Chemical

Di(2-ethylhexyl)phthalate (CAS No. 117-81-7) was obtained from W.R. Grace and Company (Fords, NJ) in one batch (Lot No. GC-2-26-76). Results of analyses of di(2-ethylhexyl)phthalate at Midwest Research Institute, Kansas City, Missouri, were consistent with the structure (Appendix E). Results of thin-layer chromatography indicated one homogeneous spot, and those of vapor-phase chromatography indicated two minor impurities having a total area less than 0.5% of the major peak. Integration values for aromatic protons in the nuclear magnetic resonance spectrum were slightly high.

B. Dietary Preparation

Test diets were prepared by mixing the chemical with an aliquot of powdered Wayne Lab Blox® animal feed (Table 3) and placing this mixture with the remainder of the feed in a Patterson-Kelly blender with an intensifier bar and mixing for 15 minutes. Test diets were sealed in labeled plastic bags and stored at 4°C for no longer than 14 days.

Sample diets formulated with 100,000 ppm di(2-ethylhexyl)phthalate were stored at -20°, 5°, 25°, or 45°C for 2 weeks. Di(2-ethylhexyl)phthalate was found to be stable at 45°C (Appendix F). The concentrations of the test substance in randomly selected batches of formulated diets were within ±10% of the target concentrations (Appendix G).

C. Animals

Four- to five-week old F344 rats and 5-week old B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center (Frederick, MD), isolated for 9 days (mice) or 7 days (rats), examined for the presence of parasites or other diseases, and assigned to control or dosed groups so that average cage weights were approximately equal for all animals of the same sex and species.

Table 3. Sources and Descriptions of Materials Used for Animal Maintenance

Item	Description	Source
Animal Feed	Wayne® Lab Blox Meal	Allied Mills (Chicago, IL)
Feed Hoppers	Stainless steel, gang style	Scientific Cages, Inc. (Bryan, TX)
Cages	Polycarbonate	Lab Products, Inc. (Rochelle Park, NJ)
Filter Sheets	Disposable, nonwoven fiber	Lab Products, Inc. (Rochelle Park, NJ)
Bedding	Hardwood chips: Aspen bed	American Excelsior (Baltimore, MD.)
	Betta Chips®	Agway Corp. (Syracuse, NY)

D. Animal Maintenance

Rats and mice were housed five per cage in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets (Table 3). Hardwood chip bedding and cages were changed twice weekly, and cage racks were changed every 2 weeks. Water, supplied by an Edstrom automatic watering system, and powdered Wayne Lab Blox® meal in stainless-steel, gang-style hoppers that were changed once per week were available ad libitum.

The temperature of animal rooms was 17°-31°C (average 23°C) and relative humidity was 10%-88%. Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters, with 10 room air changes per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice were housed by species in rooms in which chronic feeding studies were being conducted on guar gum (CAS 9000-30-0), butyl benzyl phthalate (CAS 85-68-7), and di(2-ethylhexyl)adipate (CAS 103-23-1).

E. Single-Dose, Acute, and 14-Day Repeated-Dose Studies

Single-dose and 14-day repeated-dose feed studies using F344 rats and B6C3F1 mice were conducted to determine the concentrations of di(2-ethylhexyl)phthalate to be used in the subchronic studies.

In the acute toxicity test, groups of five males and five females of each species were treated once with various doses (0.8-20 g/kg, rats; 1.25-20 g/kg, mice) of the test substance in corn oil by gavage. All animals survived the 14-day observation period.

In the repeated-dose study, groups of five males and five females of each species were tested for 2 weeks with five concentrations of the test substance in feed, followed by 1 day of observation (control diet); similar groups were maintained as untreated controls (Tables 4 and 5). All surviving animals were killed on day 16.

Two of 5 male rats and 4/5 female rats fed 100,000 ppm di(2-ethylhexyl) phthalate died during the study. Weight gain (when compared with controls) was depressed by more than 25% in males receiving 25,000 ppm or more and

Table 4. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing Di(2-ethylhexyl)phthalate for 14 Days

Dose (ppm)	Survival(a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (Percent)
Male					
0	5/5	122.8	168.4	45.0	
6,300	5/5	123.4	174.0	50.6	+12
12,500	5/5	123.4	175.6	52.2	+16
25,000	5/5	123.4	155.2	31.8	-29
50,000	5/5	123.4	126.2	2.8	-94
100,000	3/5	123.4	79.6	-43.8	-197
Female					
0	5/5	101.2	116.8	15.6	
6,300	5/5	101.0	133.4	32.4	+108
12,500	5/5	101.0	121.0	20.0	+28
25,000	5/5	101.0	117.6	16.6	+6
50,000	5/5	101.0	90.8	-10.2	-165
100,000	1/5	101.0	90.0	-11.0	-171

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

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

Table 5. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing Di(2-ethylhexyl)phthalate for 14 Days

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (Percent)
Male					
0	5/5	25.4	28.0	2.6	
6,300	5/5	25.4	26.2	0.8	-69
12,500	5/5	25.4	26.0	0.6	-77
25,000	5/5	25.4	23.0	-2.4	-192
50,000	4/5	25.4	20.0	-5.4	-308
100,000	0/5	25.4	19.8	-5.6	-315
Female					
0	5/5	18.6	19.4	0.8	
6,300	5/5	18.6	19.0	0.4	-50
12,500	5/5	18.6	19.8	1.2	+50
25,000	5/5	18.6	19.8	1.2	+50
50,000	1/5	18.6	14.7	-3.9	-588
100,000	0/5	18.6	14.0	-4.6	-675

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

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

in females receiving 50,000 ppm or more. Males receiving 100,000 ppm and females receiving 50,000 or 100,000 ppm lost weight. Rats receiving 100,000 ppm maintained hunched positions and were lethargic.

One of five male mice receiving 50,000 ppm and all (5/5) males receiving 100,000 ppm died. Four of five female mice receiving 50,000 ppm and all (5/5) females receiving 100,000 ppm died. A dose-related decrease in mean body weight gain occurred in male mice. Male mice receiving 25,000 ppm or more and females receiving 50,000 ppm or more lost weight.

F. Subchronic Studies

Subchronic studies were conducted to determine the high and low doses to be used in the chronic studies. Diets containing 0, 1,600, 3,100, 6,300, 12,500, or 25,000 ppm di(2-ethylhexyl)phthalate were fed for 13 weeks to groups of male and female rats. Similar groups of male and female mice received diets containing 0, 800, 1,600, 3,100, 6,300, or 12,500 ppm (Tables 6 and 7). Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day study, survivors were killed, necropsies were performed on all animals, and tissues were taken for histopathologic analysis (see Section H).

Rats: Five female controls died due to accidents. One male rat fed 6,300 ppm died. Depression of mean body weight gain of male and female rats fed 25,000 ppm was 29% and 53%, respectively, relative to controls. Testicular atrophy, not considered to be life threatening, was observed in all 10 male rats fed 25,000 ppm and was present, but less pronounced, in rats fed 12,500 ppm. No other compound-related histopathologic effects were observed. Doses selected for rats for the chronic study were 6,000 and 12,000 ppm di(2-ethylhexyl)phthalate in feed.

Mice: Six of the seven deaths in the males fed 12,500 ppm were accidental, and one female died in each of the control, 6,300- and 12,500-ppm groups. Two females died in the 3,100 ppm group. A mean body weight gain depression of 10% or more was observed in males fed 3,100, 6,300 or 12,500 ppm and in all groups of dosed females except for those fed 1,600 ppm. No

Table 6. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing Di(2-ethylhexyl)phthalate for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weight (grams) Initial	Final	Change (b)	Weight Change Relative to Controls (c) (%)
Male					
0	10/10	79.9 ± 3.3	291.7 ± 15.5	+211.8 ± 16.5	
1,600	10/10	79.9 ± 4.2	325.9 ± 9.0	+246.0 ± 6.3	+16.1
3,100	10/10	79.6 ± 4.2	315.2 ± 6.4	+235.6 ± 3.6	+11.2
6,300	9/10	81.3 ± 4.3	290.8 ± 12.4	+209.5 ± 9.0	- 1.1
12,500	10/10	79.7 ± 3.2	271.5 ± 7.4	+191.8 ± 6.5	- 9.4
25,000	10/10	79.8 ± 3.1	231.2 ± 10.3	+151.4 ± 7.7	-28.5
Female					
0	5/10	74.8 ± 3.1	181.4 ± 7.1	+106.6 ± 8.2	
1,600	10/10	76.2 ± 5.4	197.5 ± 5.5	+121.3 ± 4.6	+13.8
3,100	10/10	76.2 ± 4.0	196.2 ± 3.7	+120.0 ± 5.1	+12.6
6,300	10/10	76.2 ± 2.7	192.1 ± 3.3	+115.9 ± 2.8	+ 8.7
12,500	10/10	76.2 ± 2.1	179.0 ± 4.7	+102.8 ± 3.7	- 3.6
25,000	10/10	76.2 ± 1.6	126.4 ± 3.6	+ 50.2 ± 3.4	-52.9

(a) Number surviving/number initially in the group. All calculations are based on those animals surviving to the end of the study.

(b) Mean weight change of the survivors of the group ± Standard error of the mean

(c) Weight change of the dosed survivors relative to the survivors of the controls =

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

Table 7. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing Di(2-ethylhexyl)phthalate for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weight (grams) Initial	Final	Change (b)	Weight Change Relative to Controls (c) (%)
Male					
0	10/10	20.0 ± 0.4	34.4 ± 1.0	+14.4 ± 1.1	
800	10/10	20.0 ± 0.4	33.9 ± 0.6	+13.9 ± 0.5	- 3.5
1,600	10/10	20.0 ± 0.4	34.4 ± 0.9	+14.4 ± 1.1	0.0
3,100	10/10	20.0 ± 0.5	32.8 ± 0.8	+12.8 ± 0.8	-11.1
6,300	10/10	20.0 ± 0.5	31.3 ± 0.5	+11.3 ± 0.4	-21.5
12,500	3/10	19.0 ± 0.6	29.4 ± 0.8	+10.4 ± 0.4	-27.8
Female					
0	9/10	16.7 ± 0.4	26.3 ± 0.5	+9.6 ± 0.3	
800	10/10	16.6 ± 0.3	24.5 ± 0.3	+7.9 ± 0.2	-17.7
1,600	10/10	16.6 ± 0.3	25.4 ± 0.5	+8.8 ± 0.4	- 8.3
3,100	8/10	16.8 ± 0.4	25.2 ± 0.8	+8.4 ± 0.7	-12.5
6,300	9/10	16.6 ± 0.4	23.7 ± 0.7	+7.1 ± 0.5	-26.0
12,500	9/10	16.6 ± 0.4	23.1 ± 0.5	+6.5 ± 0.4	-32.3

(a) Number surviving/number initially in the group. All calculations are based on those animals surviving to the end of the study.

(b) Mean weight change of the survivors of the group ± Standard error of the mean

(c) Weight change of the dosed survivors relative to the survivors of the controls =

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

other compound-related effects were observed. Doses selected for mice for the chronic study were 3,000 and 6,000 ppm di(2-ethylhexyl)phthalate in feed.

G. Chronic Studies

The test groups, doses administered, and durations of the chronic studies are shown in Table 8.

H. Clinical Examinations and Pathology

Animals were inspected twice daily. Individual animal body weights were recorded monthly. Animals that were moribund and those that survived to the end of the study were killed using CO_2 inhalation and necropsied.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

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

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations,

Table 8. Numbers of Animals, Dosage, and Weeks on Test for Rats and Mice on Chronic Feeding Studies of Di(2-ethylhexyl)phthalate

Test Group	Initial No. of Animals	Di(2-ethylhexyl)-phthalate (ppm)	Weeks on Study	
			Dosed	Observed
Male Rats				
Control	50	0	0	105
Low-Dose	50	6,000	103	2
High-Dose	50	12,000	103	1
Female Rats				
Control	50	0	0	105
Low-Dose	50	6,000	103	2
High-Dose	50	12,000	103	2
Male Mice				
Control	50	0	0	105
Low-Dose	50	3,000	103	2
High-Dose	50	6,000	103	1
Female Mice				
Control	50	0	0	105
Low-Dose	50	3,000	103	2
High-Dose	50	6,000	103	2

survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extension of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is reported only when its two-tailed P value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni inequality criterion (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.025. When this correction was used, it is discussed in the narrative section. It is not presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve

is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was also applied. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived 52 weeks, unless a tumor was found at an anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for the analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage Tests, etc.) were followed.

Life table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The approximate 95% confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that, in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment.

When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero).

When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights, Food Consumption, and Clinical Signs (Rats)

A dose-related decrease in mean body weight gain was observed throughout the study for male rats (Figure 1). Body weight gain in female rats was also reduced by the high (12,000 ppm) dose of di(2-ethylhexyl)phthalate. Daily mean food consumptions were reduced slightly in the dosed groups relative to controls (Tables 9 and 10). The average daily feed consumption per rat was 86% and 85% that of controls for low-dose males and females, respectively, and 86% and 75% for high-dose males and females, respectively. Mean daily doses of di(2-ethylhexyl)phthalate (g per kg body weight) were 0.322 and 0.674 for low- and high-dose males, respectively, and 0.394 and 0.774 for low- and high-dose females, respectively. No other compound-related clinical signs of toxicity were reported.

B. Survival (Rats)

Estimates of the probabilities of survival of male and female rats administered di(2-ethylhexyl)phthalate in feed, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 2. No significant trends in mortality were observed.

In male rats, 30/50 (60%) of the control, 28/50 (56%) of the low-dose, and 33/50 (66%) of the high-dose group lived to the end of the study at 104-105 weeks. In female rats, 36/50 (72%) of the control, 34/50 (68%) of the low-dose, and 38/50 (76%) of the high-dose group lived to the end of the study at 105 weeks.

A sufficient number of rats were at risk for the development of late appearing tumors.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2.

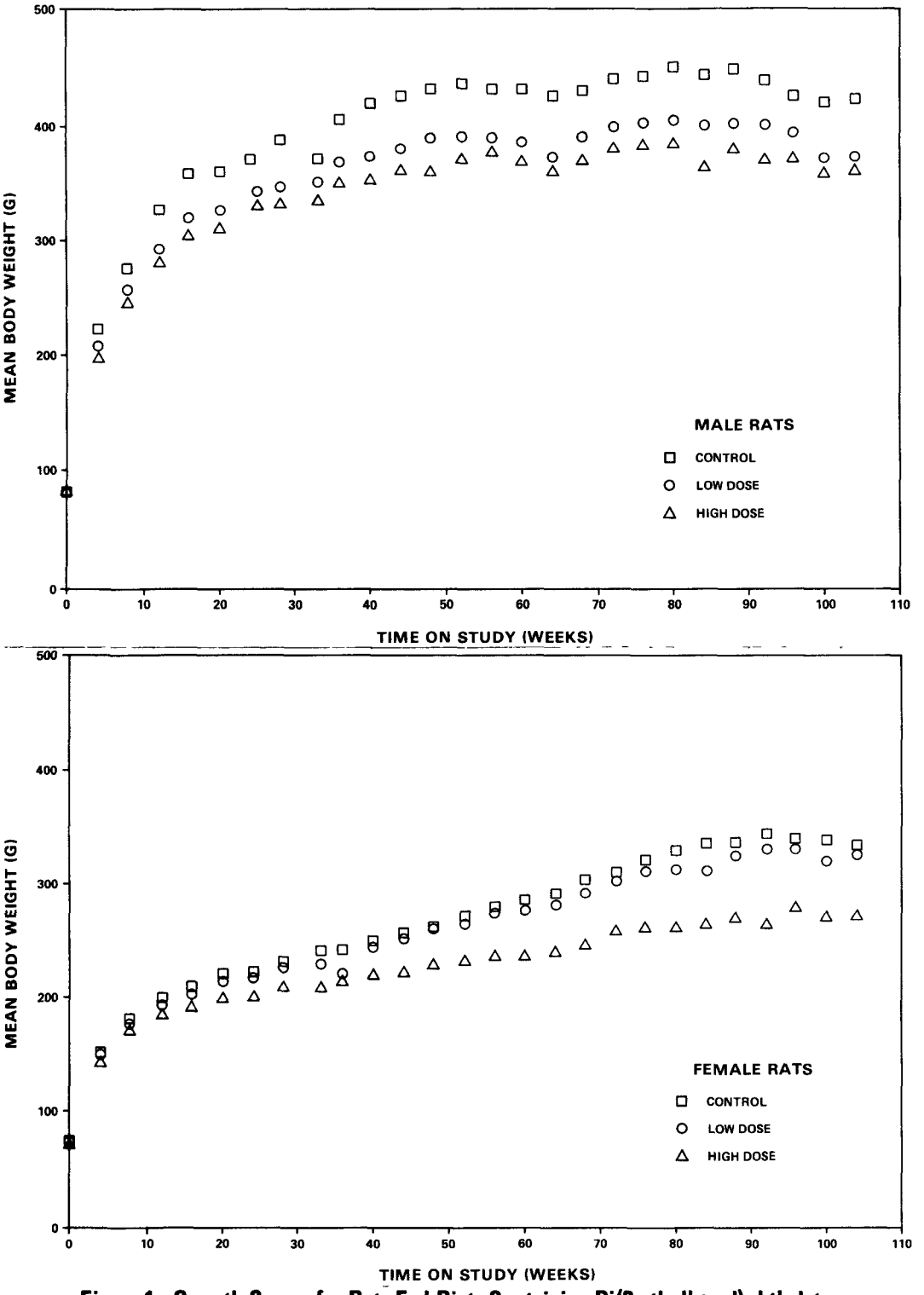


Figure 1. Growth Curves for Rats Fed Diets Containing Di(2-ethylhexyl)phthalate

Table 9. Feed and Compound Consumption in Male F344 Rats Receiving Di(2-ethylhexyl)phthalate

	Control		Low				High			
Week	Grams Feed/ Day(a)	Body Weight (grams)	Grams Feed/ Day(a)	Body Weight (grams)	Low Control (b)	Dose/ Day (c)	Grams Feed/ Day(a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
Male										
4	29.7	223	26.6	207	0.9	0.770	25.6	197	0.9	1.558
8	24.4	275	21.0	256	0.9	0.492	19.4	245	0.8	0.952
12	23.7	326	18.3	293	0.8	0.374	18.4	281	0.8	0.787
16	24.6	357	19.6	318	0.8	0.369	20.1	305	0.8	0.793
20	21.1	359	18.1	326	0.9	0.334	18.1	311	0.9	0.700
25	14.1	370	11.9	344	0.8	0.207	12.1	330	0.9	0.442
28	21.9	387	17.1	346	0.8	0.297	18.6	332	0.8	0.671
33	19.6	371	20.0	352	1.0	0.341	17.7	335	0.9	0.635
36	19.6	406	17.9	367	0.9	0.292	18.4	351	0.9	0.630
40	23.6	419	18.9	373	0.8	0.303	19.1	354	0.8	0.649
44	21.7	426	18.4	380	0.8	0.291	18.9	363	0.9	0.623
48	23.0	433	19.6	389	0.9	0.302	20.4	361	0.9	0.679
52	22.9	436	19.9	391	0.9	0.305	20.1	373	0.9	0.648
56	20.1	433	18.1	389	0.9	0.280	19.7	377	1.0	0.628
60	24.1	433	20.1	386	0.8	0.313	17.6	368	0.7	0.573
64	24.6	426	16.1	373	0.7	0.260	15.1	360	0.6	0.505
68	22.4	430	18.9	390	0.8	0.290	14.9	369	0.7	0.483
72	23.7	440	17.0	398	0.7	0.256	20.6	380	0.9	0.650
78	26.4	443	23.4	403	0.9	0.349	22.6	384	0.9	0.705
80	19.0	450	16.0	405	0.8	0.237	16.1	385	0.8	0.503
84	19.0	444	20.0	400	1.1	0.300	18.0	364	0.9	0.593
88	13.0	448	12.3	402	0.9	0.183	13.1	379	1.0	0.416
92	20.4	438	19.1	401	0.9	0.286	18.9	372	0.9	0.608
96	21.9	426	20.7	395	0.9	0.315	22.6	373	1.0	0.726
100	19.6	421	19.1	373	1.0	0.308	20.4	358	1.0	0.685
Mean	21.8	401	18.7	362	0.9	0.322	18.7	344	0.9	0.674
SD (d)	3.5	56.9	3.0	49.5	0.1	0.110	2.9	45.9	0.1	0.217
CV (e)	16.1	14.2	16.0	13.7	11.1	34.2	15.5	13.3	11.1	32.2

(a) Grams of feed consumed per animal per day.
(b) Grams of feed per day for the dosed group divided by the same value for the controls.
(c) Grams of compound consumed per day per kg of body weight.
(d) Standard Deviation
(e) Coefficient of Variation (standard deviation/mean x 100).

Table 10. Feed and Compound Consumption in Female F344 Rats Receiving Di(2-ethylhexyl)phthalate

Week	Control		Low				High			
	Grams Feed/ Day(a)	Body Weight (grams)	Grams Feed/ Day(a)	Body Weight (grams)	Low Control (b)	Dose/ Day (c)	Grams Feed/ Day(a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
Female										
4	26.6	152	23.9	149	0.9	0.961	21.0	143	0.8	1.762
8	22.0	181	16.9	176	0.8	0.575	14.3	168	0.6	1.020
12	16.4	199	14.9	193	0.9	0.462	13.0	185	0.8	0.843
16	21.3	209	16.1	203	0.8	0.477	13.6	191	0.6	0.853
20	20.1	220	16.1	214	0.8	0.453	12.6	199	0.6	0.758
24	12.1	222	11.6	216	1.0	0.321	8.7	200	0.7	0.523
28	20.4	232	16.9	226	0.8	0.448	13.6	209	0.7	0.779
33	19.0	240	17.0	228	0.9	0.447	13.3	208	0.7	0.766
36	18.0	241	12.6	220	0.7	0.343	12.9	215	0.7	0.718
40	20.9	249	12.3	244	0.6	0.302	13.1	219	0.6	0.720
44	20.4	256	18.0	252	0.9	0.429	14.9	223	0.7	0.799
48	21.1	262	17.7	258	0.8	0.412	14.9	228	0.7	0.782
52	19.9	272	16.9	264	0.8	0.383	14.6	233	0.7	0.750
56	18.4	279	16.0	274	0.9	0.350	13.6	236	0.7	0.690
60	20.1	286	17.0	276	0.8	0.370	14.9	236	0.7	0.755
64	18.1	292	13.1	281	0.7	0.281	12.4	239	0.7	0.624
68	19.9	304	16.1	293	0.8	0.331	9.7	246	0.5	0.474
72	20.9	312	15.6	303	0.7	0.308	15.7	257	0.8	0.734
76	23.7	322	21.0	312	0.9	0.404	19.4	261	0.8	0.893
80	16.6	328	14.3	314	0.9	0.273	13.9	261	0.8	0.637
84	20.0	336	18.1	313	0.9	0.348	17.0	264	0.8	0.773
88	12.6	337	11.6	325	0.9	0.214	10.9	269	0.9	0.484
92	17.1	345	17.7	331	1.0	0.321	15.1	264	0.9	0.688
96	16.7	340	18.9	331	1.1	0.342	18.9	278	1.1	0.814
100	16.3	338	16.0	320	1.0	0.300	16.1	271	1.0	0.715
Mean	19.1	270	16.2	261	0.9	0.394	14.3	228	0.8	0.774
SD (d)	3.1	55.2	2.8	52.2	0.1	0.142	2.8	34.7	0.1	0.239
CV (e)	16.2	20.4	17.3	20.0	11.1	36.0	19.6	15.2	12.5	30.9

(a) Grams of feed consumed per animal per day.
(b) Grams of feed per day for the dosed group divided by the same value for the controls.
(c) Grams of compound consumed per day per kg of body weight.
(d) Standard Deviation
(e) Coefficient of Variation (standard deviation/mean x 100).

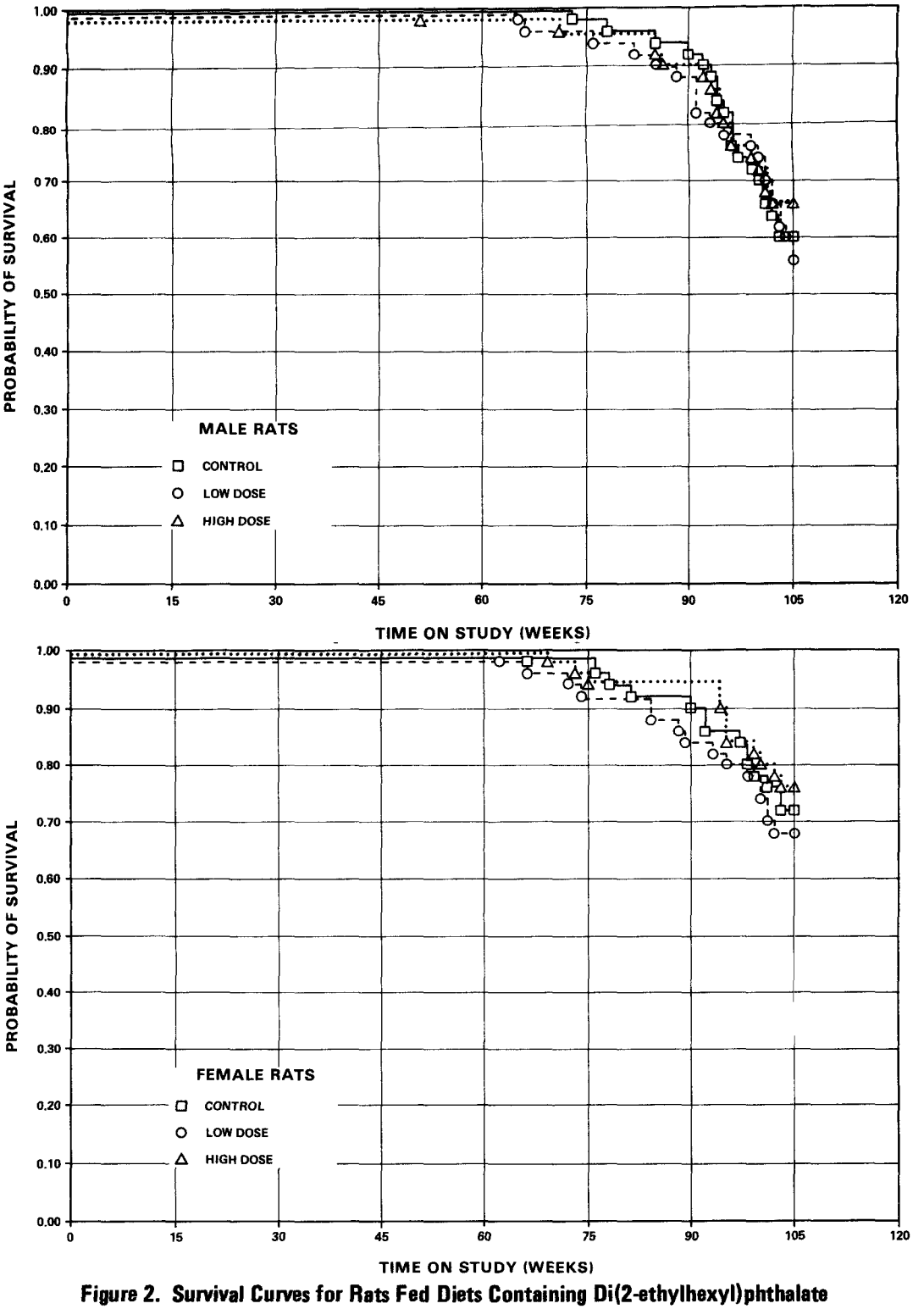


Figure 2. Survival Curves for Rats Fed Diets Containing Di(2-ethylhexyl)phthalate

Neoplastic nodules of the liver were observed at increased incidences in low- (5/49, 10%) and high-dose (7/49, 14%) males compared with controls (2/50, 4%) and in high-dose (5/50, 10%) and low-dose (4/49, 8%) females compared with controls (0/50,0%). The combined incidences of neoplastic nodules and hepatocellular carcinoma were elevated in high-dose (12/49, 24%) males and in both low (6/49, 12%) and high-dose (13/50, 26%) females. Control males had a combined incidence of 3/50 (6%), while the incidence in control female rats was zero.

Neoplastic nodules were generally spherical and caused compression of adjacent liver plates. The hepatocytes were well differentiated, and the general architecture of hepatic cords remained intact. Nodules were comprised of eosinophilic, vacuolated, and basophilic cells; however, the predominant neoplastic hepatocyte had basophilic cytoplasm. Some of the smaller nodules were composed of clear and basophilic hepatocytes, suggesting that clear cell foci progressed to these nodules.

Hepatocellular carcinomas varied from well to poorly differentiated. Well-differentiated tumors had trabecular and solid patterns with sinusoidal ectasia. The majority of carcinomas were composed of hepatocytes with basophilic cytoplasm. A few tumors had poorly differentiated areas containing solid, papillary, and trabecular foci with considerably more pleomorphism. Sinusoidal ectasia was often extensive. Large hyperchromatic nuclei, numerous mitoses, hyaline droplets, and fatty vacuoles were seen. Invasive growth at the tumor margins was greater in poorly differentiated tumors. There were no metastatic hepatocellular carcinomas in either male or female rats.

Clear cell cytoplasmic change, usually multiple, occurred in the livers of both low-dose (10/49, 20%) and high-dose (11/49, 22%) male rats. The change was present in only 4/50 (8%) of male controls.

Bilateral tubular degeneration and atrophy of the testes were seen in 90% of high-dose males. This lesion was noted in only 2% of control rats. Microscopically, the seminiferous tubules were devoid of germinal epithelium and spermatocytes. Only Sertoli cells were seen on tubular basement membranes. Interstitial cells were somewhat prominent.

Hypertrophy of cells in the anterior pituitary occurred in 45% of high-dose male rats compared with 2% of controls. These cells had cytoplasmic enlargement with the nucleus pushed to one side of the cell. The cytoplasm had a ground glass appearance, and clear cytoplasmic vacuoles were found in some hypertrophied cells.

No other toxic or preneoplastic lesions were associated with compound administration. The remaining nonneoplastic lesions were those commonly found in aging rats of this strain and were not considered to be compound related.

Histopathologic examination indicated that, under the conditions of this bioassay, the administration of di(2-ethylhexyl)phthalate was associated with an increased incidence of liver tumors in F344 rats.

D. Statistical Analyses of Results (Rats)

Tables 11 and 12 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Hepatocellular carcinomas or neoplastic nodules of the liver in male rats were observed in a statistically significant positive relation. The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.007). The Fisher exact test between the high-dose group and the control group was significant (P=0.01). No significant incidence was observed in the low-dose group; however, these tumors occurred in increased incidences in the low-dose group compared with the control group. In female rats, hepatocellular carcinomas or neoplastic nodules were observed in increased incidence in the dosed groups compared with the control group, the incidences being 0/50 (0%) in the controls, 6/49 (12%) in the low-dose, and 13/50 (26%) in the high-dose groups. The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P less than 0.001) and the Fisher exact test between the control group and either of the dosed groups was significant (P=0.012 in the low-dose and P less than 0.001 in the high-dose groups). The incidence of hepatocellular carcinomas alone was significantly (P=0.003) increased in the high-dose female rats in comparison to controls.

Carcinomas or adenomas of the pituitary in male rats were observed in a statistically significant negative relation to dose. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.012) and the Fisher exact test between the high-dose group and the control group was significant in the negative direction (P=0.012).

In female rats, carcinomas or adenomas of the pituitary gland were observed in decreased proportion in the low-dose group compared with the other two groups. While the Fisher exact test between the low-dose group and the control group was significant (P=0.017), no significant difference from the control group was observed in the high-dose group.

C-cell carcinomas or adenomas of the thyroid in male rats were observed in decreased incidence in the dosed groups compared with the control group. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.019), and the Fisher exact test between the high-dose group and the control group indicated a value of P=0.031, which is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. In female rats, this tumor was not observed in statistically significant proportions.

Interstitial-cell tumors of the testis were observed in a statistically significant negative relation to dose. The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P less than 0.001). The Fisher exact test between the high-dose group and the control group was significant (P less than 0.001). No significant difference from the control group was observed in the low-dose group. It should be noted that the pathologic findings include a statistically significant increase in degeneration in the seminiferous tubule (testis) in male rats. The incidences of this lesion are: 1/49 (2.0%) in the control, 2/44 (5%) in the low-dose, and 43/48 (90%) in the high-dose groups.

Fibroadenomas of the mammary gland in female rats were observed in decreased incidence in the high-dose group. The Fisher exact test between the high-dose group and the control group indicated a value of P=0.036, but

this value is above the P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group.

Since only one rat in the entire study died before week 52, time-adjusted analysis eliminating the rat that died before 52 weeks produced essentially no change in the statistical analysis. Similarly, the analysis of tumor incidence by life table methods did not materially alter the significance of differences reported in Tables 11 and 12.

Statistically, the incidences of liver tumors in male and female F344 rats were increased by the administration of di(2-ethylhexyl)phthalate.

Table 11. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Myelomonocytic Leukemia (b)	13/50(26)	20/50(40)	17/50(34)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.538	1.308
Lower Limit		0.825	0.674
Upper Limit		2.960	2.597
Weeks to First Observed Tumor	92	82	85
Hematopoietic System:			
Leukemia or Lymphoma (e)	14/50(26)	21/50(42)	17/50(34)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	1.214
Lower Limit		0.828	0.636
Upper Limit		2.789	2.354
Weeks to First Observed Tumor	95	76	85
Liver: Hepatocellular			
Carcinoma (b)	1/50(2)	1/49(2)	5/49(10)
P Values (c),(d)	P=0.047	N.S.	N.S.
Relative Risk (Control) (e)		1.020	5.102
Lower Limit		0.013	0.601
Upper Limit		78.488	236.025
Weeks to First Observed Tumor	105	105	101

Table 11. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule (b)	2/50(4)	5/49(10)	7/49(14)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.551	3.571
Lower Limit		0.441	0.723
Upper Limit		25.786	33.856
Weeks to First Observed Tumor	103	88	104
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	3/50(6)	6/49(12)	12/49(24)
P Values (c),(d)	P=0.007	N.S.	P=0.010
Relative Risk (Control) (e)		2.041	4.082
Lower Limit		0.464	1.190
Upper Limit		11.991	21.269
Weeks to First Observed Tumor	103	88	101
Pituitary: Carcinoma, NOS (b)	4/46(9)	1/43(2)	0/49(0)
P Values (c),(d)	P=0.024(N)	N.S.	N.S.
Relative Risk (Control) (e)		0.267	0.000
Lower Limit		0.006	0.000
Upper Limit		2.562	1.012
Weeks to First Observed Tumor	78	105	--

Table 11. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Carcinoma or Adenoma, NOS (b)	8/46(17)	6/43(14)	1/49(2)
P Values (c),(d)	P=0.012(N)	N.S.	P=0.012(N)
Relative Risk (Control) (e)		0.802	0.117
Lower Limit		0.249	0.003
Upper Limit		2.412	0.824
Weeks to First Observed Tumor	78	93	92
Thyroid: C-cell Carcinoma (b)	4/48(8)	1/47(2)	0/46(0)
P Values (c),(d)	P=0.028(N)	N.S.	N.S.
Relative Risk (Control) (e)		0.255	0.000
Lower Limit		0.005	0.000
Upper Limit		2.456	1.123
Weeks to First Observed Tumor	78	100	--
Thyroid: C-cell Carcinoma or Adenoma (b)	5/48(10)	2/47(4)	0/46(0)
P Values (c),(d)	P=0.019(N)	N.S.	P=0.031(N)
Relative Risk (Control) (e)		0.409	0.000
Lower Limit		0.040	0.000
Upper Limit		2.355	0.825
Weeks to First Observed Tumor	78	100	--

Table 11. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial Cell Tumor (b)	47/49(96)	42/44(95)	11/48(23)
P Values (c),(d)	P < 0.001(N)	N.S.	P < 0.001(N)
Departure from Linear Trend (f)	P < 0.001		
Relative Risk (Control) (e)		0.995	0.239
Lower Limit		0.921	0.194
Upper Limit		1.075	0.358
Weeks to First Observed Tumor	85	66	95

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

Table 12. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Myelomonocytic Leukemia (b)	10/50(20)	14/50(28)	17/50(34)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.400	1.700
Lower Limit		0.642	0.821
Upper Limit		3.177	3.719
Weeks to First Observed Tumor	90	66	94
Liver: Hepatocellular Carcinoma (b)	0/50(0)	2/49(4)	8/50(16)
P Values (c),(d)	P=0.002	N.S.	P=0.003
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.302	2.284
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	---	93	95
Liver: Neoplastic Nodule (b)	0/50(0)	4/49(8)	5/50(10)
P Values (c),(d)	P=0.030	N.S.	P=0.028
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.946	1.261
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	---	105	105

Table 12. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Neoplastic Nodule (b)	0/50(0)	6/49(12)	13/50(26)
P Values (c),(d)	P < 0.001	P=0.012	P < 0.001
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		1.633	4.014
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	93	95
Pituitary: Carcinoma, NOS	4/47(9)	1/47(2)	2/48(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.250	0.490
Lower Limit		0.005	0.046
Upper Limit		2.404	3.241
Weeks to First Observed Tumor	105	84	105
Pituitary: Carcinoma, NOS or Adenoma, NOS (b)	24/47(51)	13/47(28)	20/48(42)
P Values (c),(d)	N.S.	P=0.017(N)	N.S.
Departure From Linear Trend (f)	P=0.032		
Relative Risk (Control) (e)		0.542	0.816
Lower Limit		0.296	0.506
Upper Limit		0.960	1.314
Weeks to First Observed Tumor	76	93	69

Table 12. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	10/50(20)	16/50(32)	3/50(6)
P Values (c),(d)	P=0.050(N)	N.S.	P=0.036(N)
Departure From Linear Trend (f)	P=0.006		
Relative Risk (Control) (e)		1.600	0.300
Lower Limit		0.761	0.056
Upper Limit		3.540	1.083
Weeks to First Observed Tumor	101	74	75
Clitoral Gland: Carcinoma, NOS (b)	4/50(8)	1/50(2)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.250	0.250
Lower Limit		0.005	0.005
Upper Limit		2.411	2.411
Weeks to First Observed Tumor	105	105	105
Uterus: Endometrial Stromal Polyp (b)	7/49(14)	13/50(26)	13/50(26)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.820	1.820
Lower Limit		0.742	0.742
Upper Limit		4.929	4.929
Weeks to First Observed Tumor	101	62	69

Table 12. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)phthalate (*a*)

(Continued)

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

IV. RESULTS - MICE

A. Body Weights, Food Consumption, and Clinical Signs (Mice)

A dose-related decrease in mean body weight gain in female mice was observed from week 25 to the end of the study (Figure 3). Daily mean food consumptions were similar among control and dosed groups throughout the study (Tables 13 and 14). The average daily food consumption per mouse was 100% and 96% that of controls for low-dose males and females, respectively, and 96% and 100% for high-dose males and females, respectively. Mean daily doses of di(2-ethylhexyl)phthalate as g per kg body weight were 0.672 and 1.325 for low- and high-dose males, respectively, and 0.799 and 1.821 for low- and high-dose females, respectively. No other compound-related clinical signs of toxicity were reported.

B. Survival (Mice)

Estimates of the probabilities of survival of male and female mice administered di(2-ethylhexyl)phthalate in feed at the concentrations of this bioassay, together with those of control group, are shown by the Kaplan and Meier curves in Figure 4. No positive trends in mortality were observed. Several deaths in the male control group at 19-21 weeks resulted in shortened survival in that group when compared with the dosed groups. The low-dose female mice had significantly shortened survival compared with that in the control group (P=0.006), but no positive trend was observed since there was somewhat longer survival in the high-dose group than in the low-dose group.

In male mice, 34/50 (68%) of the control, 38/50 (76%) of the low-dose and 35/50 (70%) of the high-dose groups lived to the end of the study at 104 weeks. In female mice, 39/50 (78%) of the control, 25/50 (50%) of the low-dose, and 33/50 (66%) of the high-dose groups lived to the end of the study at 104 weeks.

A sufficient number of mice were at risk for the development of late-appearing tumors.

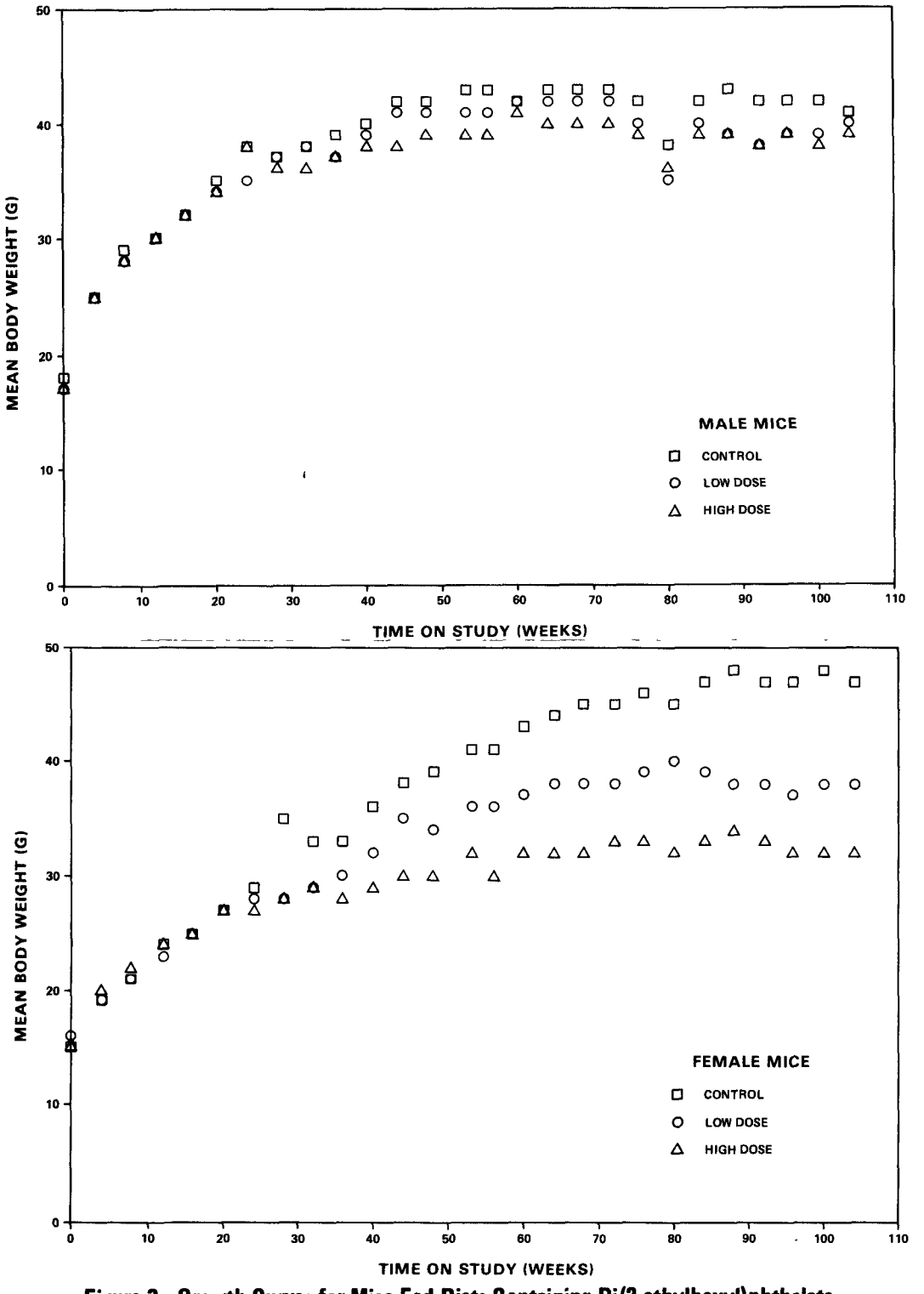


Figure 3. Growth Curves for Mice Fed Diets Containing Di(2-ethylhexyl)phthalate

Table 13. Feed and Compound Consumption in Male B6C3F1 Mice Receiving Di(2-ethylhexyl)phthalate

Week	Control		Low				High			
	Grams Feed/ Day(a)	Body Weight (grams)	Grams Feed/ Day(a)	Body Weight (grams)	Low Control (b)	Dose/ Day (c)	Grams Feed/ Day(a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
Male										
8	8.0	29	8.6	28	1.1	0.918	7.9	28	1.0	1.684
12	8.0	30	8.6	30	1.1	0.857	7.9	30	1.0	1.571
16	8.6	32	8.6	32	1.0	0.804	9.1	32	1.1	1.714
20	8.6	35	7.9	34	0.9	0.693	7.7	34	0.9	1.361
24	9.1	38	8.1	35	0.9	0.698	7.7	35	0.8	1.322
28	8.0	37	8.4	37	1.1	0.683	7.3	36	0.9	1.214
32	8.1	38	7.1	38	0.9	0.564	7.1	36	0.9	1.190
36	7.7	39	8.3	37	1.1	0.672	10.1	37	1.3	1.645
40	7.9	40	7.6	39	1.0	0.582	6.9	38	0.9	1.083
44	6.4	42	3.4	41	0.5	0.251	6.6	38	1.0	1.038
48	4.9	42	5.1	41	1.1	0.376	5.0	39	1.0	0.769
53	7.1	43	8.4	41	1.2	0.617	7.3	39	1.0	1.121
56	7.9	43	8.1	41	1.0	0.596	6.1	39	0.8	0.945
60	8.0	42	8.0	42	1.0	0.571	7.6	41	0.9	1.108
64	7.9	43	8.1	42	1.0	0.582	6.9	40	0.9	1.029
68	8.9	43	7.6	42	0.9	0.541	7.9	40	0.9	1.179
72	9.4	43	9.0	42	1.0	0.643	7.9	40	0.8	1.179
76	9.6	42	9.9	40	1.0	0.739	8.7	39	0.9	1.341
80	8.9	38	8.9	35	1.0	0.759	7.9	36	0.9	1.310
84	10.6	42	9.3	40	0.9	0.696	8.6	39	0.8	1.319
88	10.9	43	9.3	39	0.9	0.714	9.6	39	0.9	1.473
92	11.0	42	10.1	38	0.9	0.801	10.1	38	0.9	1.602
96	9.0	42	13.0	39	1.4	1.000	11.0	39	1.2	1.692
100	7.7	42	10.0	39	1.3	0.769	12.1	38	1.6	1.917
Mean	8.4	40	8.4	38	1.0	0.672	8.1	37	1.0	1.325
SD (d)	1.3	4.2	1.7	3.9	0.2	0.160	1.6	3.3	0.2	0.285
CV (e)	15.5	10.5	20.2	10.3	20.0	23.8	19.8	8.9	20.0	21.5

(a) Grams of feed consumed per animal per day.
(b) Grams of feed per day for the dosed group divided by the same value for the controls.
(c) Grams of compound consumed per day per kg of body weight.
(d) Standard Deviation
(e) Coefficient of Variation (standard deviation/mean x 100).

Table 14. Feed and Compound Consumption in Female B6C3F1 Mice Receiving Di(2-ethylhexyl)phthalate

	Control		Low				High			
Week	Grams Feed/ Day(a)	Body Weight (grams)	Grams Feed/ Day(a)	Body Weight (grams)	Low Control (b)	Dose/ Day (c)	Grams Feed/ Day(a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
Female										
8	9.0	21	9.1	21	1.0	1.306	9.4	22	1.0	2.571
12	9.9	24	9.9	23	1.0	1.286	9.4	24	1.0	2.357
16	10.7	25	9.7	25	0.9	1.166	11.0	25	1.0	2.640
20	9.6	27	8.6	27	0.9	0.952	8.7	27	0.9	1.937
24	10.1	29	9.6	28	0.9	1.026	9.7	27	1.0	2.159
28	9.1	35	8.1	28	0.9	0.872	9.1	28	1.0	1.959
32	9.0	33	7.3	29	0.8	0.754	7.9	29	0.9	1.626
36	8.4	33	8.1	30	1.0	0.814	8.6	28	1.0	1.837
40	8.6	36	7.9	32	0.9	0.737	7.9	29	0.9	1.626
44	5.9	38	3.3	35	0.6	0.282	5.6	30	1.0	1.114
48	5.6	39	4.1	34	0.7	0.366	4.9	30	0.9	0.971
53	8.6	41	7.3	36	0.8	0.607	8.6	32	1.0	1.607
56	8.6	41	8.3	36	1.0	0.690	9.1	30	1.1	1.829
60	7.3	43	7.6	37	1.0	0.614	9.1	32	1.3	1.714
64	8.0	44	7.9	38	1.0	0.620	9.0	32	1.1	1.688
68	8.7	45	8.6	38	1.0	0.677	5.1	32	0.6	0.964
72	9.1	45	9.1	38	1.0	0.722	10.1	33	1.1	1.844
76	9.7	46	10.6	39	1.1	0.813	9.3	33	1.0	1.688
80	9.4	45	9.6	40	1.0	0.718	8.4	32	0.9	1.580
84	9.4	47	9.1	39	1.0	0.703	7.6	33	0.8	1.377
88	9.7	48	10.9	38	1.1	0.857	9.6	34	1.0	1.689
92	10.6	47	10.6	38	1.0	0.835	11.7	33	1.1	2.130
96	10.7	47	11.0	37	1.0	0.892	12.0	32	1.1	2.250
100	9.6	48	11.0	38	1.1	0.868	13.6	32	1.4	2.545
Mean	9.0	39	8.6	34	1.0	0.799	9.0	30	1.0	1.821
SD (d)	1.3	8.5	1.9	5.7	0.1	0.241	2.0	3.2	0.2	0.460
CV (e)	14.4	21.8	22.1	16.8	10.0	30.2	22.2	10.7	20.0	25.3

(a) Grams of feed consumed per animal per day.
(b) Grams of feed per day for the dosed group divided by the same value for the controls.
(c) Grams of compound consumed per day per kg of body weight.
(d) Standard Deviation
(e) Coefficient of Variation (standard deviation/mean x 100).

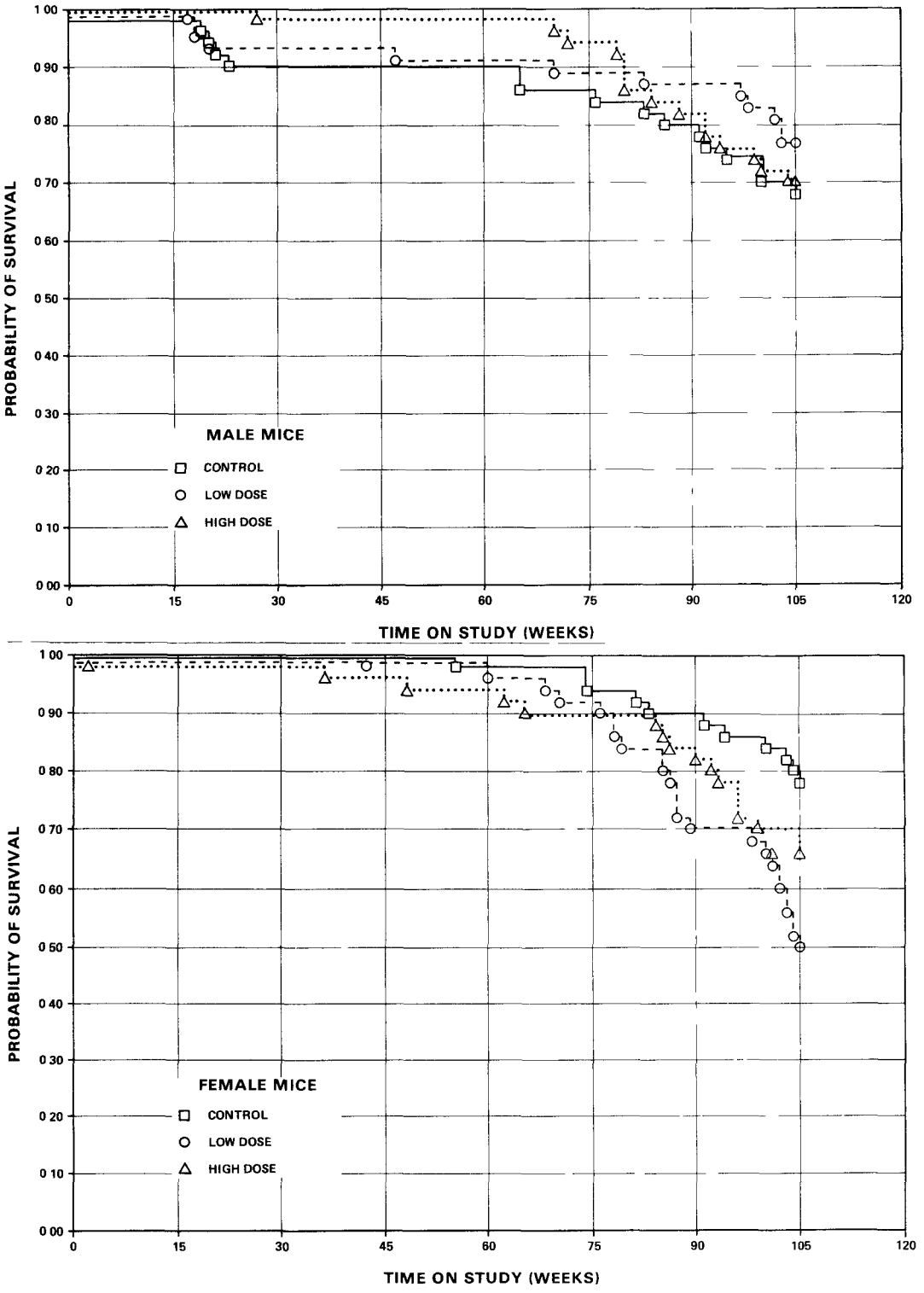


Figure 4. Survival Curves for Mice Fed Diets Containing Di(2-ethylhexyl)phthalate

C. Pathology (Mice)

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

Hepatocellular carcinomas were observed at an increased incidence in high-dose males (19/50, 38%) and in both low- (7/50, 14%) and high-dose females (17/50, 34%). The incidence was 9/50 (18%) in control males and 0/50 in control females. The combined incidence of hepatocellular carcinoma and hepatocellular adenoma was elevated in low- (25/48, 52%) and high-dose (29/50, 58%) males and low- (12/50, 24%) and high-dose (18/50, 36%) females. Male controls had a combined incidence of 14/50 (28%), while female control values were 1/50 (2%). Multiple liver tumors occurred more frequently in dosed mice than in controls.

Hepatocellular adenomas were fairly large, expanding, well-differentiated tumors that caused compression of the adjacent hepatic parenchyma. Tumor cells were larger than normal hepatocytes, and many were composed of hepatocytes with basophilic cytoplasm. Some adenomas had hepatocytes with eosinophilic, vacuolated, or clear cytoplasm. Clear cell foci also occurred within adenomas.

Hepatocellular carcinomas had solid areas, trabecular and papillary formations, sinusoidal ectasia, necrosis, hemorrhage, thrombosis, and calcification. There was considerable pleomorphism characterized by many large hyperchromatic bizarre nuclei and by variation in cell size.

Cytoplasm was usually basophilic, while hyaline droplets (inclusions) and fatty vacuoles were common. Dosed mice of both sexes had pulmonary metastases. Metastatic hepatocellular carcinoma was found in seven low-dose and five high-dose males and in two low-dose and seven high-dose females. The primary liver tumors of these mice were always of the trabecular type. No metastatic hepatocellular carcinoma occurred in the lungs of control mice of either sex. Toxic hepatic lesions were not observed in dosed mice.

Bilateral tubular degeneration of the testes was seen in 14% of the high-dose males. This lesion was observed in one control male and in two low-dose males.

The usual inflammatory and other degenerative lesions commonly found in this strain of mouse were seen in comparable numbers in dosed groups and controls. None were judged to be compound related.

Histopathologic examination indicated that, under the conditions of this bioassay, administration of di(2-ethylhexyl)phthalate was associated with an increased incidence of liver tumors in B6C3F1 mice.

D. Statistical Analyses of Results (Mice)

Tables 15 and 16 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

A statistically significant positive dose-related trend was observed for hepatocellular carcinomas in male mice. The Cochran-Armitage test indicated a probability level of P=0.018 for linear trend in males (9/50, 18% in the control; 14/48, 29% in the low-dose; and 19/50, 38% in the high-dose group). The Fisher exact test between the high-dose group and the control group was significant (P=0.02). A statistically significant positive dose-related trend was also observed for hepatocellular carcinomas alone in female mice. The Cochran-Armitage test indicated a probability level of less than 0.001 for linear trend in females (0/50 in the control; 7/50, 14% in the low-dose; and 17/50, 34% in the high-dose group). The Fisher exact tests were also significant (P=0.006 between low-dose and control groups and P less than 0.001 between high-dose and control groups).

Hepatocellular carcinomas or adenomas of the liver in either sex of mice were observed in a statistically significant positive relation to dose in that results of the Cochran-Armitage test values indicated a probability level of P=0.002 for linear trend in males (14/50, 28% in the control; 25/48, 52% in the low-dose; and 29/50, 58% in the high-dose groups). The Fisher exact test between the high-dose group and the control group was significant (P=0.002), and also a significant incidence was observed in the low-dose male group (P=0.013). The incidences of hepatocellular carcinomas or adenomas in the other male control groups concurrently on test in the room used for the di(2-ethylhexyl)phthalate study were: guar gum (16/50, 32%), butyl benzyl

phthalate (13/50, 26%), and di(2-ethylhexyl)adipate (13/50, 26%), compared with 14/50 (28%) in the control for the present study, indicating that the incidence of these tumors in the controls of this study was within a normal range. Hepatocellular carcinomas or adenomas of the liver in female mice were observed in a statistically significant positive relation to dose in the dosed groups compared with the control group: 1/50 (2%) in the controls, 12/50 (24%) in the low-dose, and 18/50 (36%) in the high-dose groups. The Cochran-Armitage test for linear trend was significant (P less than 0.001) and the Fisher exact tests were significant (P less than or equal to 0.001) in either group.

For liver tumors in mice, the estimated probabilities of remaining tumor-free in the absence of competing risk are shown in Figure 5 and 6. There is a significant (P less than 0.001) trend in the incidence of the tumors in female mice and (P=0.009) in male mice as determined by life-table methods. A time-adjusted analysis was made eliminating those animals that died before 52 weeks on study. The new incidences of male mice with liver tumors were 14/45(31%) in the controls, 25/45(56%) in the low-dose group, and 29/49(59%) in the high-dose group. This analysis indicated a P value for trend of P=0.007, and for the Fisher exact tests the probability levels were P=0.016 (low-dose) and P=0.007 (high-dose). Therefore, this time adjusted analysis does not materially affect the results reported in the previous paragraphs.

Statistically, the incidences of liver tumors in male and female B6C3F1 mice were increased by the administration of di(2-ethylhexyl)phthalate.

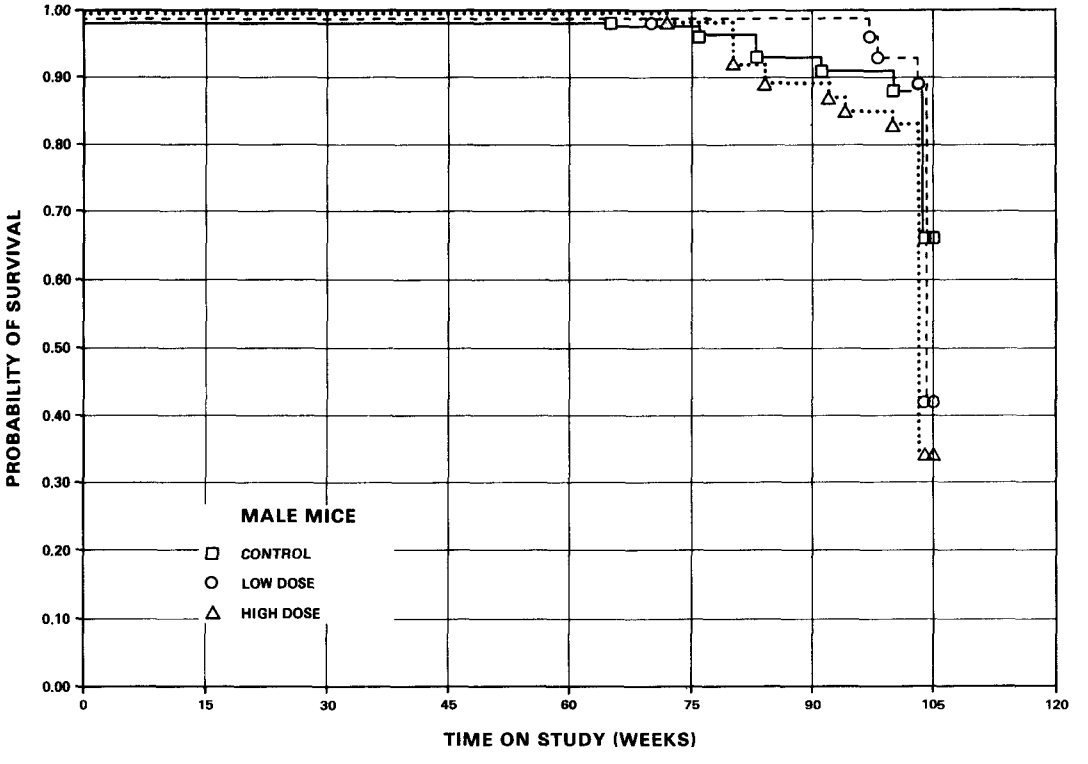


Figure 5. Life Table for Male Mice Fed Diets Containing Di(2-ethylhexyl)phthalate: Liver Tumors

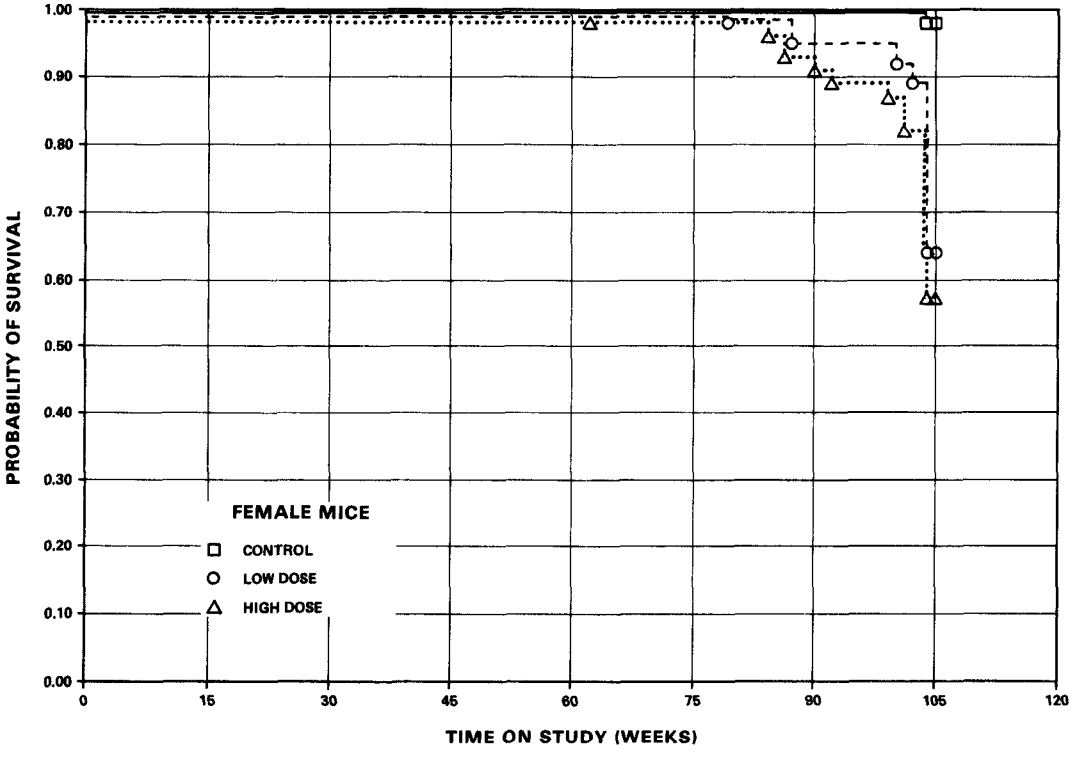


Figure 6. Life Table for Female Mice Fed Diets Containing Di(2-ethylhexyl)phthalate: Liver Tumors

Table 15. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	4/50(8)	3/49(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.765	0.500
Lower Limit		0.118	0.047
Upper Limit		4.288	3.318
Weeks to First Observed Tumor	105	70	104
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	10/50(20)	9/49(18)	7/50(14)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.918	0.700
Lower Limit		0.362	0.246
Upper Limit		2.292	1.869
Weeks to First Observed Tumor	83	70	104
Hematopoietic System: Lymphoma (b)	8/50(16)	8/49(16)	8/50(16)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.020	1.000
Lower Limit		0.363	0.355
Upper Limit		2.869	2.815
Weeks to First Observed Tumor	100	88	87

Table 15. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	9/50(18)	14/48(29)	19/50(38)
P Values (c),(d)	P=0.018	N.S.	P=0.022
Relative Risk (Control) (e)		1.620	2.111
Lower Limit		0.724	1.017
Upper Limit		3.828	4.737
Weeks to First Observed Tumor	65	70	72
Liver: Hepatocellular Adenoma (b)	6/50(12)	11/48(23)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.910	1.667
Lower Limit		0.706	0.597
Upper Limit		5.793	5.164
Weeks to First Observed Tumor	105	105	104
Liver: Hepatocellular Carcinoma or Adenoma	14/50(28)	25/48(52)	29/50(58)
P Values (c),(d)	P=0.002	P=0.013	P=0.002
Relative Risk (Control) (e)		1.860	2.071
Lower Limit		1.070	1.226
Upper Limit		3.315	3.597
Weeks to First Observed Tumor	65	70	72

Table 15. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

(a) Dosed groups received doses of 3,000 or 6,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 16. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Lymphomas (b)	10/50(20)	18/50(36)	15/50(30)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.800	1.500
Lower Limit		0.882	0.701
Upper Limit		3.897	3.359
Weeks to First Observed Tumor	83	85	48
Circulatory System:			
Hemangioma (b)	3/50(6)	1/50(2)	0/50(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	0.000
Lower Limit		0.006	0.000
Upper Limit		3.983	1.663
Weeks to First Observed Tumor	103	104	--
Liver: Hepatocellular Carcinoma (b)	0/50(0)	7/50(14)	17/50(34)
P Values (c),(d)	P < 0.001	P=0.006	P < 0.001
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		1.941	5.408
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	79	62

Table 16. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma (b)	1/50(2)	5/50(10)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure From Linear Trend (f)	P=0.028		
Relative Risk (Control) (e)		5.000	1.000
Lower Limit		0.588	0.013
Upper Limit		231.346	76.970
Weeks to First Observed Tumor	105	105	105
Liver: Hepatocellular Carcinoma or Adenoma (b)	1/50(2)	12/50(24)	18/50(36)
P Values (c),(d)	P < 0.001	P=0.001	P < 0.001
Relative Risk (Control) (e)		12.000	18.000
Lower Limit		1.891	3.047
Upper Limit		499.771	726.973
Weeks to First Observed Tumor	105	79	62
Mammary Gland: Adenocarcinoma, NOS (b)	2/50(4)	3/50(6)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	1.500
Lower Limit		0.180	0.180
Upper Limit		17.329	17.329
Weeks to First Observed Tumor	105	87	85

Table 16. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)phthalate (a)

(Continued)

(a) Dosed groups received doses of 3,000 or 6,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

V. DISCUSSION

Minimal-to-moderate decreases in body weight gain occurred in some groups of treated animals, most notably female mice. It is not believed, however, that such a decrease in body weight gain in the treated animals weakens the conclusions of this study, since liver tumors occurred also in groups that did not exhibit excessive decreases in body weight gain, and because di(2-ethylhexyl)phthalate did not decrease lifespan in either male or female rats or mice. According to modern standards, a maximum tolerated dose (MTD) is:

> ...the highest dose of the test agent given during the chronic study that can be predicted not to alter the animal's normal longevity from effects other than carcinogenicity. Since these data may not always be easily interpretable, a degree of judgement is often necessary in estimating the MTD. The MTD should be the highest dose that causes no more than a 10% weight decrement (although a depressed weight gain is a clinical sign of toxicity, this particular effect is acceptable when estimating the MTD), as compared to the appropriate control groups; and does not produce mortality, clinical signs of toxicity or pathological lesions (other than those that may be related to a neoplastic response) that would be predicted to shorten the animal's natural life span. (Sontag et al., 1976).

Because individual body weights were not recorded in this study, it is impossible to discern whether or not decreased weight gain occurred secondary to the development of liver tumors and was, therefore, related to the neoplastic response. Even if tumorigenicity were not the cause of the decreased weight gain, it must be emphasized that the 10% weight differential is only a guideline. Moreover, it is to be assessed during the prechronic phase of the study, not during the chronic phase, unless two lifetime bioassays are to be performed -- the former to set the dose for the latter (Federal Register, 1980). There is, in fact, no precise definition of MTD. The primary reason for not surpassing the theoretical MTD in chronic studies is that early deaths could preclude tumor formation. Alternatively, tumors might be formed secondary to non-specific effects such as irritation (e.g., bladder stones and diethylene glycol), tissue damage (e.g., liver cirrhosis and alcohol), or tissue hyperplasia (e.g., thyroid

hyperplasia and goitrogens) (Federal Register, 1980). The absence of early deaths or significant pathological changes in the liver other than neoplasia in this study indicates that tumor development was directly related to the biological effects of di(2-ethylhexyl)phthalate and that a maximally tolerated dose, for the purpose of carcinogenicity testing, was not exceeded.

Liver tumors were associated with the administration of di(2-ethylhexyl)-phthalate in both rats and mice of either sex. Hepatocellular carcinomas or neoplastic nodules in high-dose rats of either sex and in low-dose female rats, and hepatocellular carcinomas or adenomas in low- and high-dose mice of either sex occurred at incidences significantly higher than those in the controls. Hepatocellular carcinomas alone were observed at statistically significant increased incidences in high-dose female rats, high-dose male mice, and in both high-dose and low-dose female mice in comparison to controls. Twenty-one of the 57 hepatocellular carcinomas in treated mice (both sexes) gave rise to pulmonary metastases. There were no lung metastases in control in mice or in any rats.

Recent evidence indicates species differences in the metabolism of di(2-ethylhexyl)phthalate. While humans and African Green monkeys glucuronidate the hydrolysis product of di(2-ethylhexyl)phthalate, mono(2-ethylhexyl)phthalate, at the free carboxylate group, rats do not and must perform successive oxidation reactions to produce a molecule sufficiently polar for urinary excretion (Albro, et al., 1973; 1981; Peck et al., 1978). The importance of this species difference in di(2-ethylhexyl)phthalate metabolism relative to extrapolation of the carcinogenic potential in rats to that in humans awaits elucidation of a probable mechanism of tumorigenic action. Moreover, it is not clear at the present time whether mice, a species that also develops tumors, metabolize di(2-ethylhexyl)phthalate in a manner similar to rats or to primate species.

Clear cell cytoplasmic change, usually occurring in multiple foci, was observed more frequently in low- and high-dose male rats than in controls. No other compound related non-neoplastic liver lesions were observed in the present study. Hepatomegaly, as reported by Lake et al. (1975) in rats fed

similar concentrations of di(2-ethylhexyl)phthalate in the diet, was not detected grossly or histologically in this study. However, the absolute or relative weights of the liver were not specifically measured.

Carcinogenic effects of di(2-ethylhexyl)phthalate were not reported in previous chronic studies utilizing Sherman or Wistar rats (Carpenter et al., 1953; Harris et al., 1956). As discussed earlier, however, these studies were insufficient, by current standards, for assessing carcinogenic potential due to small group size, poor survival, and incomplete reporting. Thus, the results of the bioassay of di(2-ethylhexyl)phthalate reported herein are not in conflict with previous studies of chronic di(2-ethylhexyl)phthalate toxicity.

Hypertrophy of cells in the anterior pituitary, seminiferous degeneration, and atrophy were seen in male rats in the subchronic study as well as in rats in other feeding studies with di(2-ethylhexyl)phthalate (Gray et al., 1977). The hypertrophy of cells in the anterior pituitary is descriptively similar to the "castration cells" in the pars distalis reported by Gray et al. (1977) in rats fed diets containing 1% or 2% di(2-ethylhexyl)-phthalate for 17 weeks. The observed testicular atrophy and cellular hypertrophy of the pituitary could be causally related. (Likewise, the decreased incidence of tumors of the thyroid, pituitary, and testis could be correlated with increased endocrine activity of the pituitary gland.)

Two other plasticizers chemically related to di(2-ethylhexyl)phthalate were on test concurrently in the same room as di(2-ethylhexyl)phthalate. Butyl benzyl phthalate was not found to be carcinogenic for male or female B6C3F1 mice, but was associated with an increased incidence of leukemia in female F344 rats (NTP, in press). Di(2-ethylhexyl)adipate was not carcinogenic for F344 rats of either sex. DEHA was carcinogenic for female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male B6C3F1 mice, causing an increased incidence of hepatocellular adenomas (NTP, 1981).

Two other compounds structurally related to di(2-ethylhexyl)phthalate (phthalamide and phthalic anhydride) were tested in the Bioassay Program and were not found to be carcinogenic for male or female F344 rats or for male or female B6C3F1 mice (NCI, 1979b; NCI, 1979c). Administration of dimethylterephthalate to mice resulted in a dose-related trend for increased incidences of lung neoplasia in male and for lymphoma or leukemia in female B6C3F1 mice (NCI, 1979a). These lesions, however, were judged to be of equivocal biological significance. Results of these studies are summarized in Table 17.

Table 17. Comparison of Target Organs Affected in Chronic Bioassays of Some Compounds Structurally Related to Di(2-ethylhexyl)phthalate

Compound	Species	Sex	Dose (ppm)	Duration (Weeks)	Liver	Testes	Pituitary	Lymphoma or Leukemia	Urinary Bladder	Lung
Di(2-ethylhexyl)phthalate	Rats	M	4,000	104	-	-	-	-	-	-
(Carpenter, 1953)	(Sherman)	F	4,000	104	-	-	-	-	-	-
Di(2-ethylhexyl)phthalate	Rats	M	5,000	103	-	-	-	-	-	-
(Harris, 1956)	(Wistar)	F	5,000	103	-	-	-	-	-	-
Di(2-ethylhexyl)phthalate	Rats	M	12,000	103	N (a)	T (b)	T	-	-	-
(Current Study)	(F344)	F	12,000	103	N	-	-	-	-	-
Di(2-ethylhexyl)phthalate	Mice	M	6,000	103	N	-	-	-	-	-
(Current Study)	(B6C3F1)	F	6,000	103	N	-	-	-	-	-
Butyl benzyl phthalate	Rats	M	Not adequately tested		-	-	-	-	-	-
(NTP, in press)	(F344)	F	12,000	103	-	-	-	N	-	-
Butyl benzyl phthalate	Mice	M	12,000	103	-	-	-	-	-	-
(NTP, in press)	(B6C3F1)	F	12,000	103	-	-	-	-	-	-
Phthalamide	Rats	M	30,000	106	T	-	-	-	-	-
(NCI, 1979b)	(F344)	F	10,000	106	T	-	-	-	T	-
Phthalamide	Mice	M	50,000	104	-	-	-	-	-	-
(NCI, 1979b)	(B6C3F1)	F	25,000	104	-	-	-	-	T	-
Phthalic anhydride	Rats	M	15,000	105	-	-	-	-	-	-
(NCI, 1979c)	(F344)	F	15,000	105	-	-	-	-	-	D (c)
Phthalic anhydride	Mice	M	32,000 (d)	104	-	-	-	-	-	-
(NCI, 1979c)	(B6C3F1)	F	24,000 (d)	104	-	-	-	-	-	-

Table 17. Comparison of Target Organs Affected in Chronic Bioassays of Some Compounds Structurally Related to Di(2-ethylhexyl)phthalate

(Continued)

Compound	Species	Sex	Dose (ppm)	Duration (Weeks)	Liver	Testes	Pituitary	Lymphoma or Leukemia	Urinary Bladder	Lung
Dimethyl terephthalate	Rats	M	5,000 (e)	103	-	-	-	-	-	-
(NCI, 1979a)	(F344)	F	5,000 (e)	103	-	-	-	-	-	-
Dimethyl terephthalate	Mice	M	5,000 (e)	103	-	-	-	-	-	D
(NCI, 1979a)	(B6C3F1)	F	5,000 (e)	103	-	-	-	D	-	-
Di(2-ethylhexyl)adipate	Rats	M	25,000	103	-	-	-	-	-	-
(NTP, 1981)	(F344)	F	25,000	103	-	-	-	-	-	-
Di(2-ethylhexyl)adipate	Mice	M	25,000	103	N	-	-	-	-	-
(NTP, 1981)	(B6C3F1)	F	25,000	103	N	-	-	-	-	-

(a) N = Neoplastic lesion
(b) T = Toxic lesion
(c) D = Neoplastic lesion occurred with dose-related trend
(d) Time weighted average
(e) May not be maximum tolerated dose

VI. CONCLUSION

Under the conditions of this bioassay, di(2-ethylhexyl)phthalate was carcinogenic for F344 rats and B6C3F1 mice, causing increased incidences of female rats and male and female mice with hepatocellular carcinomas, and inducing an increased incidence of male rats with either hepatocellular carcinomas or neoplastic nodules.

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APPENDIX A

Summary of the Incidence of Neoplasms in Rats Fed Diets Containing Di(2-ethylhexyl)phthalate

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA			2 (4%)
SEBACEOUS ADENOMA	1 (2%)		
KERATOACANTHOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
FIBROMA	1 (2%)	2 (4%)	1 (2%)
FIBROSARCOMA	1 (2%)		1 (2%)
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
C-CELL CARCINOMA, METASTATIC	1 (2%)		
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	13 (26%)	20 (40%)	17 (34%)
#SALIVARY GLAND	(47)	(46)	(49)
LEUKEMIA,NOS		1 (2%)	
#LIVER	(50)	(49)	(49)
KUPFFER-CELL SARCOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#SPLEEN	(49)	(48)	(47)
HEMANGIOSARCOMA	1 (2%)		
#HEART/ATRIUM	(50)	(50)	(49)
HEMANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
NEOPLASTIC NODULE	2 (4%)	5 (10%)	7 (14%)
HEPATOCELLULAR CARCINOMA	1 (2%)	1 (2%)	5 (10%)
#PANCREAS	(48)	(46)	(46)
ACINAR-CELL ADENOMA			1 (2%)
ACINAR-CELL CARCINOMA		1 (2%)	
#DUODENUM	(48)	(46)	(47)
SARCOMA, NOS			1 (2%)
#JEJUNUM	(48)	(46)	(47)
MUCINOUS ADENOCARCINOMA	1 (2%)		
URINARY SYSTEM			
#URINARY BLADDER	(47)	(45)	(44)
TRANSITIONAL-CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(43)	(49)
CARCINOMA,NOS	4 (9%)	1 (2%)	
ADENOMA, NOS	4 (9%)	5 (12%)	1 (2%)
#ADRENAL	(50)	(49)	(49)
PHEOCHROMOCYTOMA	2 (4%)	2 (4%)	
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	1 (2%)	
#THYROID	(48)	(47)	(46)
FOLLICULAR-CELL CARCINOMA	1 (2%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
C-CELL ADENOMA	1 (2%)	1 (2%)	
C-CELL CARCINOMA	4 (8%)	1 (2%)	
#PANCREATIC ISLETS	(48)	(46)	(46)
ISLET-CELL ADENOMA	2 (4%)	1 (2%)	
ISLET-CELL CARCINOMA	1 (2%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)	2 (4%)	
#TESTIS	(49)	(44)	(48)
INTERSTITIAL-CELL TUMOR	47 (96%)	42 (95%)	11 (23%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
ASTROCYTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*EAR	(50)	(50)	(50)
C-CELL CARCINOMA, METASTATIC	1 (2%)		
*EXTERNAL EAR	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROSARCOMA		1 (2%)	
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
CERUMINOUS CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*BODY CAVITIES	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	12	20	11
MORIBUND SACRIFICE	8	2	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	30	28	33
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	47	38
TOTAL PRIMARY TUMORS	98	93	54
TOTAL ANIMALS WITH BENIGN TUMORS	47	43	15
TOTAL BENIGN TUMORS	61	56	15
TOTAL ANIMALS WITH MALIGNANT TUMORS	30	26	25
TOTAL MALIGNANT TUMORS	34	30	31
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		1
TOTAL SECONDARY TUMORS	3		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	3	7	8
TOTAL UNCERTAIN TUMORS	3	7	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
UNDIFFERENTIATED CARCINOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(50)
UNDIFFERENTIATED CARCINOMA METAS	1 (2%)		
FIBROSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA	10 (20%)	14 (28%)	17 (34%)
GRANULOCYTIC LEUKEMIA	1 (2%)		
#MEDIASTINAL L.NODE	(47)	(48)	(46)
UNDIFFERENTIATED CARCINOMA METAS	1 (2%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
CIRCULATORY SYSTEM			
#UTERUS	(49)	(50)	(50)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(50)
NEOPLASTIC NODULE		4 (8%)	5 (10%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA		2 (4%)	8 (16%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(47)	(48)
CARCINOMA,NOS	4 (9%)	1 (2%)	2 (4%)
ADENOMA, NOS	20 (43%)	12 (26%)	18 (38%)
#ADRENAL	(49)	(48)	(50)
CORTICAL ADENOMA		1 (2%)	2 (4%)
PHEOCHROMOCYTOMA		1 (2%)	
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	1 (2%)	
#THYROID	(48)	(49)	(49)
C-CELL ADENOMA		1 (2%)	
C-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
PAPILLARY CYSTADENOCARCINOMA,NOS	1 (2%)		
FIBROADENOMA	10 (20%)	16 (32%)	3 (6%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	4 (8%)	1 (2%)	1 (2%)
#UTERUS	(49)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
ENDOMETRIAL STROMAL POLYP	7 (14%)	13 (26%)	13 (26%)
#OVARY	(48)	(50)	(49)
GRANULOSA-CELL TUMOR	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(50)
ASTROCYTOMA	2 (4%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MENINGIOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*EAR	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
*EXTERNAL EAR	(50)	(50)	(50)
FIBROSARCOMA			2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, INVASIV		1 (2%)	
SARCOMA, NOS		1 (2%)	1 (2%)
LEIOMYOSARCOMA	1 (2%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	10	6
MORIBUND SACRIFICE	4	6	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	34	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	41	43	49
TOTAL PRIMARY TUMORS	67	72	74
TOTAL ANIMALS WITH BENIGN TUMORS	32	33	31
TOTAL BENIGN TUMORS	38	45	36
TOTAL ANIMALS WITH MALIGNANT TUMORS	24	20	30
TOTAL MALIGNANT TUMORS	28	23	33
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	1
TOTAL SECONDARY TUMORS	2	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	4	5
TOTAL UNCERTAIN TUMORS	1	4	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

Summary of the Incidence of Neoplasms in Mice Fed Diets Containing Di(2-ethylhexyl)phthalate

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*EXTERNAL EAR	(50)	(49)	(50)
FIBROUS HISTIOCYTOMA	1 (2%)		
*SKIN	(50)	(49)	(50)
FIBROMA	1 (2%)		1 (2%)
*SUBCUT TISSUE	(50)	(49)	(50)
FIBROMA	1 (2%)		
FIBROSARCOMA		3 (6%)	1 (2%)
LIPOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST		7 (14%)	5 (10%)
ALVEOLAR/BRONCHIOLAR ADENOMA	6 (12%)	6 (12%)	5 (10%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (8%)	3 (6%)	2 (4%)
SARCOMA, NOS, METASTATIC	1 (2%)		
LIPOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	6 (12%)	3 (6%)	5 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#SPLEEN	(50)	(48)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#MESENTERIC L. NODE	(43)	(40)	(42)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
#PEYER'S PATCH	(44)	(46)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)
#HEART	(50)	(48)	(50)
SARCOMA, NOS, METASTATIC	1 (2%)		
#LIVER	(50)	(48)	(50)
HEMANGIOSARCOMA	1 (2%)		
#U.BLADDER/SUBMUCOSA	(48)	(47)	(50)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(50)
HEPATOCELLULAR ADENOMA	6 (12%)	11 (23%)	10 (20%)
HEPATOCELLULAR CARCINOMA	9 (18%)	14 (29%)	19 (38%)
SARCOMA, NOS, METASTATIC	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
TUBULAR-CELL ADENOMA	1 (2%)		
SARCOMA, NOS, METASTATIC	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(34)	(40)	(36)
ADENOMA, NOS		1 (3%)	
#ADRENAL	(48)	(47)	(48)
CORTICAL ADENOMA	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(50)
CARCINOMA,NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(50)
ADENOMA, NOS	1 (2%)	1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
*RIB	(50)	(49)	(50)
SARCOMA, NOS	1 (2%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHə	16	9	15
MORIBUND SACRIFICE		2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	38	35
ANIMAL MISSING			

ə INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	29	37	38
TOTAL PRIMARY TUMORS	42	48	50
TOTAL ANIMALS WITH BENIGN TUMORS	14	18	14
TOTAL BENIGN TUMORS	18	20	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	23	26
TOTAL MALIGNANT TUMORS	24	28	32
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	8	5
TOTAL SECONDARY TUMORS	5	8	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
NEOPLASM, NOS, MALIGNANT	1 (2%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS		2 (4%)	
FIBROSARCOMA	1 (2%)		
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
NEOPLASM, NOS, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	7 (14%)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	10 (20%)	9 (18%)	13 (26%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (8%)	1 (2%)
*HEMATOPOIETIC SYSTEM	(50)	(50)	(50)
NEOPLASM, NOS	1 (2%)	1 (2%)	
#SPLEEN	(48)	(48)	(50)
MALIGNANT LYMPHOMA, NOS		2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#PEYER'S PATCH	(46)	(45)	(44)
MALIGNANT LYMPHOMA, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#KIDNEY	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#SPLEEN	(48)	(48)	(50)
HEMANGIOSARCOMA		1 (2%)	
#LIVER	(50)	(50)	(50)
HEMANGIOMA	1 (2%)		
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
#UTERUS	(48)	(48)	(50)
HEMANGIOMA	1 (2%)		
#OVARY	(48)	(43)	(41)
HEMANGIOMA	1 (2%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATOCELLULAR ADENOMA	1 (2%)	5 (10%)	1 (2%)
HEPATOCELLULAR CARCINOMA		7 (14%)	17 (34%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(38)	(35)	(35)
CARCINOMA,NOS		1 (3%)	1 (3%)
ADENOMA, NOS	1 (3%)		1 (3%)
#ADRENAL	(47)	(45)	(46)
PHEOCHROMOCYTOMA		1 (2%)	1 (2%)
#THYROID	(44)	(41)	(45)
FOLLICULAR-CELL ADENOMA	1 (2%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	2 (4%)	3 (6%)	3 (6%)
#UTERUS	(48)	(48)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
#CERVIX UTERI	(48)	(48)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
#OVARY	(48)	(43)	(41)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
TUBULAR ADENOMA	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(49)	(48)
GLIOMA, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOMA	1 (2%)		
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	22	16
MORIBUND SACRIFICE	1	3	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	39	25	33
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	35	34
TOTAL PRIMARY TUMORS	24	48	42
TOTAL ANIMALS WITH BENIGN TUMORS	6	10	4
TOTAL BENIGN TUMORS	8	10	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	14	30	32
TOTAL MALIGNANT TUMORS	15	37	38
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	8
TOTAL SECONDARY TUMORS		4	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	1	
TOTAL UNCERTAIN TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

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

APPENDIX C

Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Diets Containing Di(2-ethylhexyl)phthalate

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
HYPERKERATOSIS		1 (2%)	
ACANTHOSIS	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
INFLAMMATION, INTERSTITIAL	1 (2%)		2 (4%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
BRONCHOPNEUMONIA, ACUTE		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(50)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#SPLEEN	(49)	(48)	(47)
HEMATOMA, NOS			1 (2%)
FIBROSIS	1 (2%)		
NECROSIS, FOCAL		1 (2%)	
LYMPHOID DEPLETION	1 (2%)		
ERYTHROPOIESIS	1 (2%)		
HYPOPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(42)	(43)	(47)
HYPERPLASIA, PLASMA CELL		1 (2%)	
#PANCREATIC L.NODE	(42)	(43)	(47)
EDEMA, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, NOS			1 (2%)
#MESENTERIC L. NODE	(42)	(43)	(47)
HEMORRHAGE		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, PLASMA CELL		1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
EMBOLISM, NOS		1 (2%)	
#HEART	(50)	(50)	(49)
FIBROSIS, DIFFUSE			1 (2%)
#HEART/ATRIUM	(50)	(50)	(49)
THROMBUS, MURAL	1 (2%)	1 (2%)	3 (6%)
#HEART/VENTRICLE	(50)	(50)	(49)
FIBROSIS		1 (2%)	
#LEFT VENTRICLE	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#MYOCARDIUM	(50)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
CALCIFICATION, NOS	1 (2%)		
#CARDIAC VALVE	(50)	(50)	(49)
ENDOCARDITIS, BACTERIAL		1 (2%)	
#GASTRIC SEROSA	(49)	(49)	(48)
PERIVASCULITIS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(46)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LIVER	(50)	(49)	(49)
INFLAMMATION, CHRONIC			1 (2%)
NECROSIS, FOCAL			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY	2 (4%)	1 (2%)	2 (4%)
CYTOPLASMIC CHANGE, NOS			1 (2%)
BASOPHILIC CYTO CHANGE	4 (8%)	2 (4%)	1 (2%)
GROUND-GLASS CYTO CHANGE			2 (4%)
CLEAR-CELL CHANGE	4 (8%)	10 (20%)	11 (22%)
HYPERPLASIA, NOS	1 (2%)		
ANGIECTASIS	1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(49)	(49)
CONGESTION, NOS		1 (2%)	
NECROSIS, NOS	2 (4%)	3 (6%)	1 (2%)
METAMORPHOSIS FATTY	1 (2%)		
#BILE DUCT	(50)	(49)	(49)
HYPERPLASIA, NOS	31 (62%)	2 (4%)	
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(48)	(46)	(46)
ATROPHY, FOCAL	1 (2%)		
#GASTRIC MUCOSA	(49)	(49)	(48)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
CALCIFICATION, NOS	1 (2%)		
#FORESTOMACH	(49)	(49)	(48)
ULCER, FOCAL	1 (2%)		
ULCER, ACUTE			2 (4%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, BASAL CELL			2 (4%)
#CARDIAC STOMACH	(49)	(49)	(48)
CALCIFICATION, NOS		1 (2%)	
#COLON	(48)	(46)	(44)
NEMATODIASIS	3 (6%)	1 (2%)	3 (7%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
PYELONEPHRITIS SUPPURATIVE	1 (2%)		
NEPHROSIS, NOS	49 (98%)	45 (92%)	46 (94%)
CALCINOSIS, NOS	1 (2%)		
HEMOSIDEROSIS		4 (8%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#RENAL PAPILLA	(50)	(49)	(49)
CALCIFICATION, NOS			1 (2%)
#KIDNEY/TUBULE	(50)	(49)	(49)
NECROSIS, NOS		1 (2%)	
#URINARY BLADDER	(47)	(45)	(44)
INFLAMMATION, SUPPURATIVE			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(43)	(49)
CYST, NOS	1 (2%)	1 (2%)	1 (2%)
HEMORRHAGIC CYST	1 (2%)		1 (2%)
HYPERTROPHY, NOS	1 (2%)		22 (45%)
#ADRENAL	(50)	(49)	(49)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)		
#ADRENAL MEDULLA	(50)	(49)	(49)
HEMORRHAGE		1 (2%)	
#THYROID	(48)	(47)	(46)
HYPERPLASIA, C-CELL	1 (2%)	1 (2%)	
#PARATHYROID	(16)	(15)	(20)
HYPERPLASIA, NOS		1 (7%)	
#PANCREATIC ISLETS	(48)	(46)	(46)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
LACTATION	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS			1 (2%)
#PROSTATE	(48)	(44)	(46)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	3 (7%)
#TESTIS	(49)	(44)	(48)
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)
#TESTIS/TUBULE	(49)	(44)	(48)
DEGENERATION, NOS	1 (2%)	2 (5%)	43 (90%)
NERVOUS SYSTEM			
#CEREBRAL CORTEX	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
#CEREBELLUM	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
NECROSIS, FAT		1 (2%)	
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, GRANULOMATOUS			1 (2%)
*MESENTERY	(50)	(50)	(50)
STEATITIS	2 (4%)		
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
NECROSIS, FAT	1		1

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
OMENTUM			
STEATITIS			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ULCER, FOCAL			1 (2%)
HYPERKERATOSIS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#LUNG	(49)	(50)	(50)
CONGESTION, ACUTE PASSIVE	1 (2%)		
CONGESTION, CHRONIC PASSIVE		1 (2%)	
INFLAMMATION, INTERSTITIAL			1 (2%)
BRONCHOPNEUMONIA, ACUTE	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(50)
NECROSIS, NOS		1 (2%)	
HEMOSIDEROSIS		2 (4%)	
LYMPHOID DEPLETION		1 (2%)	
HEMATOPOIESIS	1 (2%)	1 (2%)	
ERYTHROPOIESIS		1 (2%)	
HYPOPLASIA, LYMPHOID			1 (2%)
#MEDIASTINAL L.NODE	(47)	(48)	(46)
CONGESTION, NOS		1 (2%)	
#MESENTERIC L. NODE	(47)	(48)	(46)
CONGESTION, NOS		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(49)	(50)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(49)	(49)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
FIBROSIS			1 (2%)
DEGENERATION, NOS	1 (2%)		
*MESENTERY	(50)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(50)
INFLAMMATION ACTIVE CHRONIC			1 (2%)
GRANULOMA, NOS	1 (2%)		
HEPATITIS, TOXIC		1 (2%)	
METAMORPHOSIS FATTY	4 (8%)		1 (2%)
BASOPHILIC CYTO CHANGE	27 (54%)	29 (59%)	17 (34%)
FOCAL CELLULAR CHANGE	1 (2%)		2 (4%)
CLEAR-CELL CHANGE		3 (6%)	3 (6%)
ANGIECTASIS			1 (2%)
#LIVER/CENTRILOBULAR	(50)	(49)	(50)
NECROSIS, NOS	1 (2%)	1 (2%)	
#BILE DUCT	(50)	(49)	(50)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	6 (12%)	1 (2%)	1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	2 (4%)
#PANCREATIC ACINUS	(47)	(47)	(49)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL			1 (2%)
#GASTRIC MUCOSA	(49)	(50)	(50)
ULCER, NOS	1 (2%)		
#FORESTOMACH	(49)	(50)	(50)
INFLAMMATION, NECROTIZING			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ULCER, ACUTE	1 (2%)		
#COLON	(49)	(48)	(48)
NEMATODIASIS	4 (8%)	3 (6%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
NEPHROSIS, NOS	29 (58%)	32 (64%)	33 (66%)
HEMOSIDEROSIS	1 (2%)	4 (8%)	6 (12%)
#KIDNEY/CORTEX	(50)	(50)	(50)
CYST, NOS	1 (2%)		
#RENAL PAPILLA	(50)	(50)	(50)
CALCIFICATION, NOS	1 (2%)		
#KIDNEY/TUBULE	(50)	(50)	(50)
INFECTION, BACTERIAL		1 (2%)	
#URINARY BLADDER	(47)	(49)	(47)
HYPERPLASIA, EPITHELIAL			1 (2%)
#U. BLADDER/MUCOSA	(47)	(49)	(47)
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, DIFFUSE			1 (2%)
#U.BLADDER/SUBMUCOSA	(47)	(49)	(47)
HEMORRHAGE	1 (2%)		
#U.BLADDER/SEROSA	(47)	(49)	(47)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
NECROSIS, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(47)	(48)
CYST, NOS	1 (2%)		4 (8%)
MULTIPLE CYSTS	10 (21%)	10 (21%)	5 (10%)
HEMORRHAGIC CYST		1 (2%)	1 (2%)
HEMOSIDEROSIS		1 (2%)	
#ADRENAL	(49)	(48)	(50)
METAMORPHOSIS FATTY	1 (2%)		2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS	1 (2%)		
#ADRENAL CORTEX	(49)	(48)	(50)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(49)	(48)	(50)
HYPERPLASIA, FOCAL	1 (2%)		
#THYROID	(48)	(49)	(49)
CYSTIC FOLLICLES		1 (2%)	1 (2%)
HYPERPLASIA, C-CELL	1 (2%)	1 (2%)	2 (4%)
#THYROID FOLLICLE	(48)	(49)	(49)
HYPERPLASIA, CYSTIC	1 (2%)		
#PANCREATIC ISLETS	(47)	(47)	(49)
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
DILATATION/DUCTS	2 (4%)		
HYPERPLASIA, CYSTIC	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
ABSCESS, NOS		1 (2%)	
#UTERUS	(49)	(50)	(50)
HYDROMETRA		1 (2%)	
NECROSIS, NOS	1 (2%)		
NECROSIS, HEMORRHAGIC		1 (2%)	
#UTERUS/ENDOMETRIUM	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, CYSTIC		3 (6%)	
#OVARY	(48)	(50)	(49)
FOLLICULAR CYST, NOS			2 (4%)
CORPUS LUTEUM CYST			1 (2%)
NERVOUS SYSTEM			
#PONS	(49)	(50)	(50)
HEMORRHAGE		2 (4%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#CEREBELLUM	(49)	(50)	(50)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
*EXTERNAL EAR	(50)	(50)	(50)
ACANTHOSIS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
NECROSIS, FAT		1 (2%)	2 (4%)
*MESENTERY	(50)	(50)	(50)
STEATITIS		1 (2%)	2 (4%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS		1	
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF	1		

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

APPENDIX D

Summary of the Incidence of Nonneoplastic Lesions in Mice Fed Diets Containing Di(2-ethylhexyl)phthalate

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
ULCER, NOS	1 (2%)		
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
ACANTHOSIS	1 (2%)		
*SUBCUT TISSUE	(50)	(49)	(50)
EDEMA, NOS	1 (2%)		
STEATITIS	1 (2%)		
ABSCESS, NOS	2 (4%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
HEMORRHAGE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(47)	(48)	(48)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)	2 (4%)	
HEMATOPOIESIS		1 (2%)	
#SPLEEN	(50)	(48)	(49)
CONGESTION, NOS			1 (2%)
ANGIECTASIS			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	1 (2%)	1 (2%)	2 (4%)
#LYMPH NODE	(43)	(40)	(42)
HYPERPLASIA, LYMPHOID	1 (2%)		
#MEDIASTINAL L.NODE	(43)	(40)	(42)
HYPERPLASIA, LYMPHOID	1 (2%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC L.NODE	(43)	(40)	(42)
CONGESTION, NOS	2 (5%)		
#AORTIC LYMPH NODE	(43)	(40)	(42)
HYPERPLASIA, LYMPHOID			1 (2%)
#LUMBAR LYMPH NODE	(43)	(40)	(42)
INFLAMMATION, CHRONIC		1 (3%)	
#MESENTERIC L. NODE	(43)	(40)	(42)
CONGESTION, NOS	3 (7%)	2 (5%)	3 (7%)
HEMORRHAGE		1 (3%)	
INFLAMMATION, GRANULOMATOUS		1 (3%)	
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, PLASMA CELL		1 (3%)	
HYPERPLASIA, LYMPHOID	5 (12%)	1 (3%)	1 (2%)
HEMATOPOIESIS			1 (2%)
#PEYER'S PATCH	(44)	(46)	(47)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
PERIARTERITIS			1 (2%)
#RIGHT VENTRICLE	(50)	(48)	(50)
THROMBUS, MURAL			1 (2%)
#MYOCARDIUM	(50)	(48)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
#CARDIAC VALVE	(50)	(48)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(50)
CYST, NOS		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
AMYLOIDOSIS	1 (2%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY	1 (2%)		1 (2%)
MEGALOCYTOSIS	1 (2%)		
ANGIECTASIS		1 (2%)	
#HEPATIC CAPSULE	(50)	(48)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#BILE DUCT	(50)	(48)	(50)
CYST, NOS	1 (2%)	2 (4%)	3 (6%)
CYSTIC DUCTS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
#PANCREAS	(47)	(46)	(46)
CYSTIC DUCTS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
ATROPHY, NOS	1 (2%)		
#PANCREATIC ACINUS	(47)	(46)	(46)
ATROPHY, FOCAL	1 (2%)		
#GASTRIC MUCOSA	(48)	(47)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
#FORESTOMACH	(48)	(47)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, FOCAL		2 (4%)	
#GASTRIC FUNDUS	(48)	(47)	(50)
HYPERPLASIA, FOCAL			1 (2%)
HYPERKERATOSIS			1 (2%)
#SMALL INTESTINE	(44)	(46)	(47)
HYPERPLASIA, ADENOMATOUS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(50)
HYDRONEPHROSIS	1 (2%)		1 (2%)
INFLAMMATION, NOS			1 (2%)
PYELONEPHRITIS SUPPURATIVE			1 (2%)
PYELONEPHRITIS, ACUTE	1 (2%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE FOCAL		2 (4%)	
PYELONEPHRITIS, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)	2 (4%)	10 (20%)
GLOMERULOSCLEROSIS, NOS	2 (4%)		
AMYLOIDOSIS	2 (4%)		
#URINARY BLADDER	(48)	(47)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE	1 (2%)		
ULCER, ACUTE		1 (2%)	
INFLAMMATION ACUTE AND CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
ENDOCRINE SYSTEM			
#THYROID	(48)	(47)	(47)
FOLLICULAR CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
REPRODUCTIVE SYSTEM			
*PENIS	(50)	(49)	(50)
ULCER, NOS	1 (2%)	1 (2%)	
*PREPUTIAL GLAND	(50)	(49)	(50)
CYSTIC DUCTS	1 (2%)	1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#PROSTATE	(47)	(43)	(46)
INFLAMMATION, ACUTE	2 (4%)		
#TESTIS/TUBULE	(49)	(48)	(49)
DEGENERATION, NOS	1 (2%)	2 (4%)	7 (14%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(50)
NECROSIS, FAT	1 (2%)		
*MESENTERY	(50)	(49)	(50)
STEATITIS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
INFLAMMATION, ACUTE		1 (2%)	
OMENTUM			
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	4	4

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING DI(2-ETHYLHEXYL)PHTHALATE

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFECTION, FUNGAL			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(50)	(50)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(48)	(48)
LEUKEMOID REACTION	1 (2%)		
#SPLEEN	(48)	(48)	(50)
HYPERPLASIA, LYMPHOID		2 (4%)	2 (4%)
HEMATOPOIESIS		2 (4%)	2 (4%)
#LYMPH NODE	(42)	(39)	(40)
HYPERPLASIA, NOS		1 (3%)	
#MEDIASTINAL L.NODE	(42)	(39)	(40)
INFLAMMATION ACUTE PUSTULAR		1 (3%)	
HYPERPLASIA, PLASMA CELL	1 (2%)		1 (3%)
#MESENTERIC L. NODE	(42)	(39)	(40)
CYST, NOS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LUNG	(50)	(50)	(50)
LEUKEMOID REACTION	1 (2%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#LIVER	(50)	(50)	(50)
LEUKOCYTOSIS, NOS	1 (2%)		
HEMATOPOIESIS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(49)	(48)
INFLAMMATION, ACUTE		1 (2%)	
*UTERINE ARTERY	(50)	(50)	(50)
NECROSIS, FIBRINOID	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
FIBROSIS		1 (2%)	
NECROSIS, NOS		2 (4%)	
NECROSIS, FOCAL			2 (4%)
METAMORPHOSIS FATTY	3 (6%)		1 (2%)
BASOPHILIC CYTO CHANGE		1 (2%)	
HEPATOCYTOMEGALY	1 (2%)		
ANGIECTASIS		2 (4%)	
#BILE DUCT	(50)	(50)	(50)
CYST, NOS		1 (2%)	
#PANCREAS	(46)	(46)	(45)
DILATATION/DUCTS	1 (2%)	1 (2%)	
CYSTIC DUCTS	3 (7%)	2 (4%)	
ABSCESS, NOS			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
ATROPHY, NOS	1 (2%)		
#PANCREATIC ACINUS	(46)	(46)	(45)
ATROPHY, NOS		1 (2%)	
ATROPHY, FOCAL		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PEYER'S PATCH	(46)	(45)	(44)
HYPERPLASIA, NOS			1 (2%)
#COLON	(38)	(44)	(38)
NEMATODIASIS			1 (3%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
INFLAMMATION, INTERSTITIAL			2 (4%)
INFLAMMATION, CHRONIC	5 (10%)	3 (6%)	3 (6%)
GLOMERULONEPHRITIS, CHRONIC	1 (2%)		
AMYLOIDOSIS	1 (2%)		
#KIDNEY/CORTEX	(50)	(50)	(50)
SCAR	1 (2%)		
#KIDNEY/TUBULE	(50)	(50)	(50)
CYST, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(47)	(45)	(46)
HYPERPLASIA, NODULAR			1 (2%)
#THYROID	(44)	(41)	(45)
CYSTIC FOLLICLES			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(48)	(48)	(50)
HYDROMETRA	1 (2%)		1 (2%)
HEMATOMA, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	1 (2%)		
ATROPHY, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(48)	(48)	(50)
INFLAMMATION, SUPPURATIVE		2 (4%)	6 (12%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, CYSTIC	29 (60%)	28 (58%)	22 (44%)
#ENDOMETRIAL GLAND	(48)	(48)	(50)
CYST, NOS			1 (2%)
#OVARY/PAROVARIAN	(48)	(43)	(41)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
#OVARY	(48)	(43)	(41)
CYST, NOS	4 (8%)	3 (7%)	5 (12%)
FOLLICULAR CYST, NOS	3 (6%)		2 (5%)
HEMATOMA, NOS	1 (2%)	1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		1 (2%)
ABSCESS, NOS		1 (2%)	
AMYLOIDOSIS	1 (2%)		
EOSINOPHILIC GRANULOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(50)	(50)
STEATITIS	1 (2%)		
ABSCESS, NOS	1 (2%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	3	

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

APPENDIX E

Analysis of Di(2-ethylhexyl)phthalate
Midwest Research Institute

APPENDIX E

Analysis of Di(2-ethylhexyl)phthalate

Midwest Research Institute

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	73.80	9.81
Determined	73.77	9.78
	73.89	9.87

B. BOILING POINT

Determined	Literature Values
b.p. 375° to 392°C (corr.) at 744 torr (Dupont 900 DTA)	No literature b.p. found at atmospheric pressure. Decomposition found to occur at 235°C and 20 mm Hg (Korosy, 1966).

C. DENSITY

Determined	Literature Values
$d_4^{24.5}$ 0.9765±0.00055	d_4^{20} 0.9843 (Reith and Eckhardt, 1962)

D. REFRACTIVE INDEX

Determined	Literature Values
n_D^{20} 1.4854 n_D^{25} 1.4845	n_D^{20} 1.4868 (Reith and Eckhardt, 1962)

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel 60 F254	Ref. Standard: Dimethyl terephthalate

Amount spotted: 100 and 300 μg

Visualization: Ultraviolet, (254 nm); zinc chloride plus 20% resorcinol in ethanol, heat, and 4N sulfuric acid, and then heat and 40% aqueous potassium hydroxide

System 1: Benzene, 100%

R_f: 0.45

R_{st}: 2.0

System 2: Ethyl acetate, 100%

R_f: 0.92

R_{st}: 1.0

F. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Tracor MT 220
Detection: Flame ionization
Inlet temperature: 200°C
Detector temperature: 260°C

System 1:
Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm I.D., glass
Oven temperature program: 150° to 250°C at 10°C/min
Results: Major peak and two impurities

Peak	Retention Time (min)	Relative Retention Time	Relative Area
1	3.0	0.34	0.2
2	7.2	0.84	0.2
3	8.7	1.00	100.0

System 2:
Column: 3% OV-225 on 80/100 Chromosorb W (HP), 1.8 m x 4 mm I.D., glass
Oven temperature program: 150° to 250°C at 10°C/min
Results: Major peak and two impurities

Peak	Retention Time (min)	Relative Retention Time	Relative Area
1	2.2	0.30	0.07
2	7.1	0.96	0.20
3	7.4	1.00	100.00

G. SPECTRAL DATA

1. Infrared

Instrument: Beckman IR-12
Cell: Neat, thin film on sodium chloride plates
Results: See Figure 7

Consistent with literature spectrum (Sadtler Standard Spectra).

2. Ultraviolet/Visible

Instrument: Cary 118

No literature values found.

λ max (nm)	$\epsilon \times 10^{-3}$
226	8.7±0.4 (δ)
247	1.29±0.004 (δ)
281 shoulder	1.171±0.002 (δ)

No absorbance between 350 and 800 nm (visible range) at a concentration of 0.2 mg/ml.

Solvent: 95% ethanol

3. Nuclear Magnetic Resonance

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

Assignments: (See Figure 8)
(a) m, δ 0.62-1.13 ppm
(b) m, δ 1.13-1.88 ppm
(c) d, δ 4.25 ppm, J_{bc} = 6Hz
(d) m, δ 7.55 ppm
(e) m, δ 7.77 ppm

Basically consistent with literature spectrum (Sadtler Standard Spectra). Integration for aromatic protons slightly high.

Integration ratios:
(a) 11.6
(b) 17.4
(c) 4.0
(d) 2.52
(e) 2.52

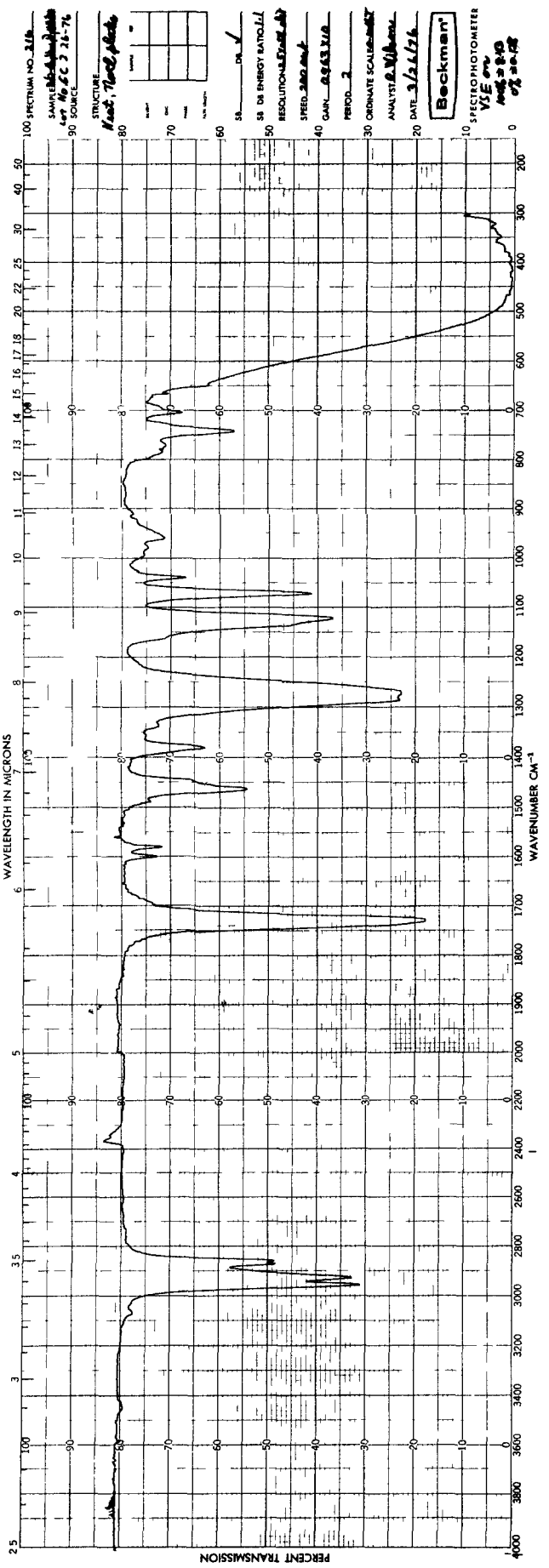

Figure 7. Infrared Absorption Spectrum of Di(2-ethylhexyl)phthalate

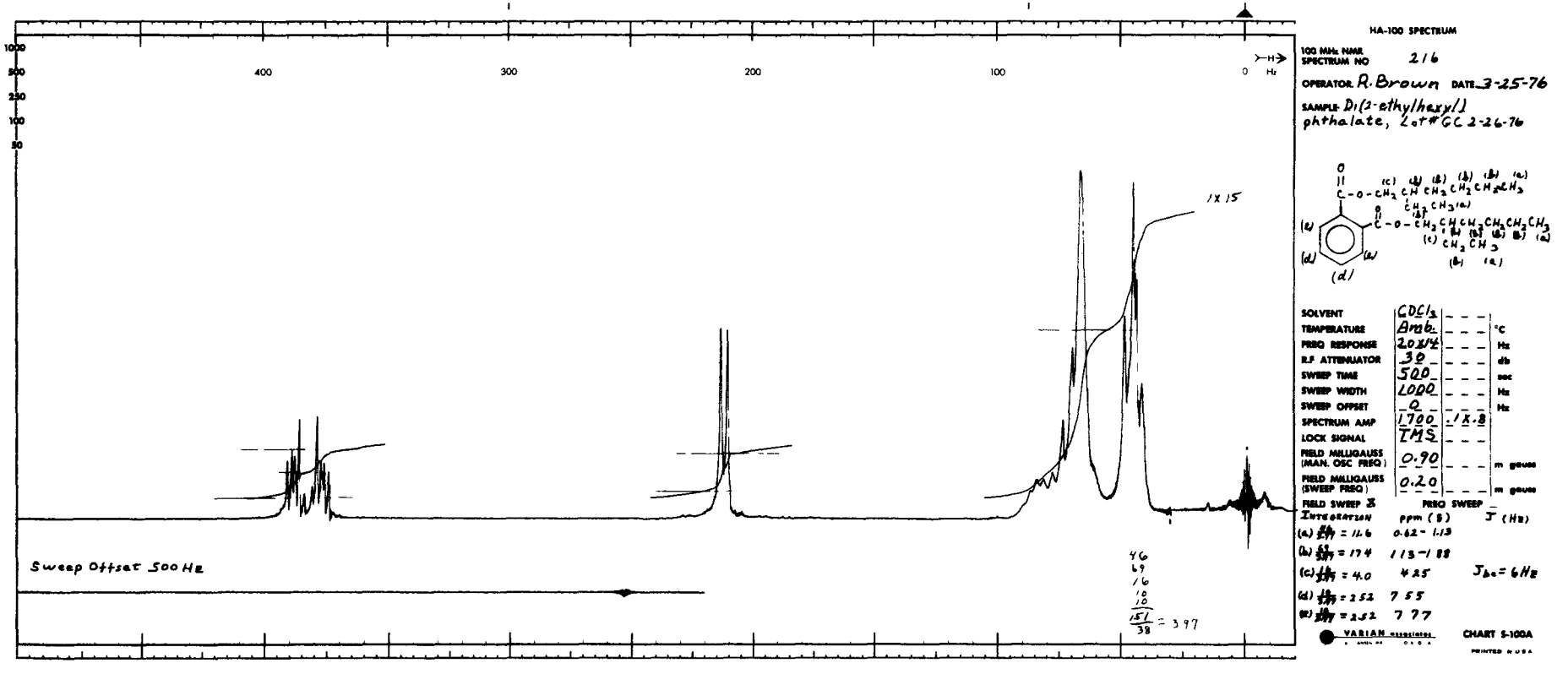

Figure 8. Nuclear Magnetic Resonance Spectrum of Di(2-ethylhexyl)phthalate

APPENDIX F

Stability Analysis of Di(2-ethylhexyl)phthalate
Midwest Research Institute

APPENDIX F

Stability Analysis of Di(2-ethylhexyl)phthalate

Midwest Research Institute

HEAT STABILITY

1. MIXING AND STORAGE: Di(2-ethylhexyl)phthalate (2.41657 g) and Wayne Lab Blox®Rodent Feed (22.49301 g) were mixed in a mortar. Samples of the mixture were then removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively.

2. EXTRACTION AND ANALYSIS: One-gram samples of each of the above stability mixtures were triturated twice with 50-ml portions of methanol. The supernatant solutions were combined and diluted to a volume of 100 ml and analyzed by vapor-phase chromatography using the following system.

Instrument: Bendix 2500
Column: 3% OV-17 on 80/100 Supelcoport, 1.8 m x 4 mm I.D., glass
Detection: Flame ionization
Oven temperature: 250°C, isothermal
Inlet temperature: 240°C
Detector temperature: 280°C
Retention time: 3.79 min

3. RESULTS

Sample (°C)	Average % Compound Recovered[a]
-20	9.7±0.1
5	9.7±0.1
25	9.9±0.1
45	9.7±0.1

[a] Corrected for a spike recovery value of 100%±3%. Theoretical expected value, 9.7%.

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

4. CONCLUSION: Di(2-ethylhexyl)phthalate mixed with feed is stable for 2 weeks at temperatures of up to 45°C.

APPENDIX G

Analysis of Formulated Diets for Concentrations of Di(2-ethylhexyl)phthalate

Appendix G

Analysis of Formulated Diets for Concentrations of Di(2-ethylhexyl)phthalate

Mason Research Institute

Samples of 2 g each were extracted with 50 ml methanol. The supernatant solutions were analyzed by vapor-phase chromatography on a 3% OV-17 glass column at 240°C, isothermal.

Theoretical Concentration (ppm)	Number of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
3,000	11	2,836	3.6	2,700-3,000
6,000	12	5,850	4.0	5,500-6,200
12,000	11	11,881	5.2	10,800-12,700

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