NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 212

CARCINOGENESIS BIOASSAY OF DI(2-ETHYLHEXYL)ADIPATE

(CAS NO. 103-23-1)

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.

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NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

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(CAS No. 103-23-1)

IN F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

National Cancer Institute
NATIONAL TOXICOLOGY PROGRAM
P. O. Box 12233
Research Triangle Park
North Carolina 27709
and
Bethesda, Maryland 20205

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CARCINOGENESIS
BIOASSAY OF
DI(2-ETHYLHEXYL)ADIPATE
(CAS NO. 103-23-1)

Carcinogenesis Testing Program
National Cancer Institute/National Toxicology Program

FOREWORD

This report presents the results of the bioassay of di(2-ethylhexyl)adipate conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI)/National Toxicology Program (NTP). This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does not necessarily mean that a test chemical is not a carcinogen inasmuch as the experiments are conducted under a limited set of circumstances. A positive result demonstrates that a test chemical is carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical may pose a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the preview of this study.

CONTRIBUTORS

The bioassay of di(2-ethylhexyl)adipate was conducted from April 1977 to May 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 supervision of Drs. H. Lilja (1) and E. Massaro (1,2), principal investigators, and Mr. G. Wade (1). Doses of the test chemical were selected by Drs. O. G. Fitzhugh (3,4), J. F. Robens (3,5), and C. Cueto (6,7). 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. A. S. Krishna Murthy (1) and Dr. D. S. Wyand (1), pathologists, 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 (8). The statistical analyses were performed by Dr. J. R. Joiner (3) and Ms. S. Vatsan (3), using methods selected for the bioassay program by Dr. J. J. Gart (9).

Chemicals used in this bioassay were analyzed at Midwest Research Institute (10), and dosed feed mixtures were analyzed by Dr. M. Hagopian (1).

This report was prepared at Tracor Jitco (3) and reviewed by NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI (6) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles K. Grieshaber, Dr. Thomas E. Hamm, Dr. Larry Hart, Dr. William V. Hartwell, Dr. Joseph Haseman, Dr. James E. Huff, Dr. C. W. Jameson, Dr. Mary R. Kornreich, Dr. Ernest E. McConnell, Dr. John A. Moore, Dr. Marcelina B. Powers (Chemical Manager), Dr. Sherman F. Stinson, Dr. Raymond Tennant, and Dr. Jerrold M. Ward.

On June 27, 1980, this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Room 1331, Switzer Building, 330 C Street, S.W., Washington, D.C. Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, Thomas Shepard, and Alice Whittemore. Members of the Panel are: Drs. Norman Breslow, Joseph Highland, Charles Irving, Frank Mirer, Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams. Drs. Highland, Schwetz, and Swenberg were unable to attend the review.

Dr. Murphy, the primary reviewer for the report on the bioassay of di(2-ethylhexyl)adipate, agreed with the conclusion in the report that, under the conditions of the test, di(2-ethylhexyl)adipate was not carcinogenic to F344 rats of either sex and that the compound was carcinogenic for female B6C3F1 mice, causing increased incidences of hepatocellular adenomas or carcinomas. Although there is less certain evidence for carcinogenicity in male mice, he thought that the compound is "probably" rather than "possibly" carcinogenic for male mice because there were higher incidences of liver tumors in both dosage groups, albeit only the findings in the high-dose group were statistically significant, and because there was a reduced latent period for appearance of hepatocellular carcinomas. Dr. Murphy noted there were significant negative trends for tumors of the hematopoietic system in mice and there were no "toxic" lesions seen in the livers of dosed mice. However, there was a small dose-related increase in colon nematodiasis in both sexes of both species.

As the secondary reviewer, Dr. Mirer agreed with the conclusions in the report, including Dr. Murphy's modification. He stated a concern relating to the fact that test animals shared rooms with animals being fed other phthalate esters, which raised the question of cross contamination.

Dr. Murphy moved that the report on the bioassay of di(2-ethylhexyl)adipate be accepted with the proviso that NTP staff incorporate clarifications regarding the conclusions on the findings in male mice. Dr. Mirer seconded the motion and it was approved unanimously.

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(1) EG&G Mason Research Institute, 57 Union Street, Worcester, Massachusetts 01608.
(2) Now with Pennsylvania State University, 226 Fenske Laboratory, University Park, Pennsylvania 16802.
(3) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland 20852.
(4) Now at 4208 Dresden Street, Kensington, Maryland 20795.
(5) Now with Bureau of Veterinary Medicine, Food and Drug Administration, 5600 Fishers Lane, Rockville, Maryland 20851.
(6) Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205; National Toxicology Program, Research Triangle Park, Box 12233, North Carolina 27709.
(7) Now with Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland 20852.
(8) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland 20852.
(9) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20205.
(10) Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri 64110.

SUMMARY

Di(2-ethylhexyl)adipate is a plasticizer used to give flexibility to vinyl plastics. A carcinogenesis bioassay was conducted by feeding diets containing 12,000 or 25,000 ppm of di(2-ethylhexyl)adipate to groups of 50 male and 50 female F344 rats and 50 male and 50 female B6C3F1 mice for 103 weeks. Groups of 50 undosed rats and mice of each sex served as controls. All surviving animals were killed at 104 to 107 weeks.

Mean body weights of high-dose rats and mice of either sex were lower than those of the controls throughout the study.

Compound administration was not associated with tumor formation in F344 rats of either sex.

Hepatocellular carcinomas or adenomas occurred in mice of both sexes in a dose-related fashion at incidences that were significantly higher for high-dose males and for low- and high-dose females than those in the controls. When compared with the incidence in historical laboratory control mice, however, the liver tumors in male mice could not be clearly related to compound administration.

Under the conditions of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic for F344 rats. Di(2-ethylhexyl)adipate was carcinogenic for female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male B6C3F1 mice, causing hepatocellular adenomas.

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Di(2-ethylhexyl)adipate was detected (limit < 0.5 μg/ml) in one of the five tested tubings -- di(2-ethylhexyl)phthalate was found in all five. Hourly concentrations of di(2-ethylhexyl)adipate in the plasma perfusate were 2.7, 3.7, 7.3, 8.5, and 9.7 μg/ml. After 5 hours, 4.2 mg di(2-ethylhexyl)adipate were present in the plasma.

Forty-four million pounds of di(2-ethylhexyl)adipate were produced in 1978 (U.S. Int. Trade Commission, 1979).

The following LD_{50} values were reported (Blaisdell, 1954 and Patty, 1981) for rats and mice:

Species	Sex	Route	LD_{50}
Rats	Unspecified	Intravenous	0.90 ml/kg
"	"	Oral	5.6 g/kg
"	"	Intraperitoneal	47 ml/kg
Rats (F344)	Male	Gavage	45 g/kg*
"	Female	"	25 g/kg*
Mice (Harlan/ICR Swiss)	Male/Female	Intraperitoneal	47 ml/kg
Mice (B6C3F1)	Male	Gavage	15 g/kg*
"	Female	"	25 g/kg*

* This NTP bioassay study.

When rats (unspecified sex and strain) were fed diets containing di(2-ethylhexyl)adipate at doses equivalent to 0.16 g/kg/day for 90 days, no effects on growth or on liver or kidney weights or histopathologic effects were observed (Smyth et al., 1951). However, reduced growth and altered liver or kidney weights were observed in the rats receiving 2.9-4.7 g/kg/day for 90 days.

Di(2-ethylhexyl)adipate was not mutagenic for Salmonella typhimurium TA 1535, TA 1537, TA 1538, TA 98, and TA 100, with and without activation, or for Saccharomyces cerevisiae (Simmon et al., 1977). Di(2-ethylhexyl)adipate is teratogenic for Sprague-Dawley rats (Singh et al., 1973) and causes dominant lethal mutations in ICR mice (Singh et al., 1975).

Di(2-ethylhexyl)adipate was tested by the Carcinogenesis Testing Program as a representative of the adipate class of plasticizers and because human exposure is widespread.

II. MATERIALS AND METHODS

A. Chemical

Di(2-ethylhexyl)adipate (CAS No. 103-23-1), a clear colorless liquid, was obtained from W. R. Grace Co. (Fords, NJ) in two batches: Lot. No. GC-2-27-76 was used for the subchronic studies and for the first 57 weeks of the chronic studies; and Lot No. 0-62-494 was used for the final 46 weeks of the chronic studies. Identity and purity analyses of di(2-ethylhexyl)adipate (elemental analysis, boiling point, thin-layer and vapor-phase chromatography; and spectral analyses including infrared, ultraviolet, and nuclear magnetic resonance) performed at Midwest Research Institute confirmed the identity of di(2-ethylhexyl)adipate (Appendixes E and F).

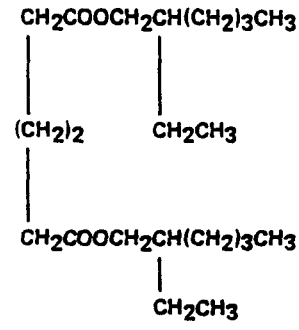
Results of thin-layer chromatography indicated only one component for both Lot No. GC-2-27-76 and Lot No. 0-62-494. Results of vapor-phase chromatography of Lot No. GC-2-27-76 indicated seven impurities with a 1.9% total area relative to the major peak. Results of vapor-phase chromatography of Lot No. 0-62-494 by one system indicated two impurities which totaled 0.11% of the major peak area. Results of vapor-phase chromatography of Lot No. 0-62-494 by a second system indicated five impurities with areas totaling 0.27% of the major peak area. Chromatography systems are described in Appendixes E and F.

Ester titration indicated a purity of 100.5% for Lot No. 0-62-494 and 101.4% for Lot No. GC-2-27-76. Di(2-ethylhexyl)adipate was stored at 4°C in its original container.

B. Dietary Preparation

Test diets were prepared by mixing the chemical with an aliquot of powdered Wayne® Lab Blox animal feed (Allied Mills, Chicago, IL), placing the mixture in a Patterson-Kelly® twin-shell intensifier bar V-blender with the remainder of the feed, and mixing for 10 minutes. Test diets were sealed in labelled plastic bags and stored at 4°C for no longer than 14 days.

I. INTRODUCTION



Di(2-ethylhexyl)adipate

Molecular Formula: $C_{22}H_{42}O_4$

Percentage Composition: C - 71.3%

H - 11.4%

O - 17.3%

Di(2-ethylhexyl)adipate (CAS No. 103-23-1) -- synonyms, bis(2-ethylhexyl)adipate, DEHA, octyl adipate, diocytl adipate, DOA -- is a plasticizer added to vinyl plastics to give low temperature flexibility (Grace Co., 1976). Di(2-ethylhexyl)adipate is not chemically bound to the vinyl plastic, but it is dispersed in the matrix of polymer chains (Autian, 1973).

Di(2-ethylhexyl)adipate is approved by the U.S. Food and Drug Administration for use in plastics that are in contact with non-fatty, non-alcoholic foods, provided that the level of di(2-ethylhexyl)adipate does not exceed 24% by weight of the plastic polymers (CFR, 1976). It is widely used in vinyl packaging film for refrigerated and frozen food products. An aerosol of di(2-ethylhexyl)adipate is formed during the hot-wire cutting of polyvinyl chloride meat-wrapping film in supermarkets and butcher shops. Concentrations of 0.14 mg/m^3 have been found in air directly above the wire (Vandervort and Brooks, 1977).

Other products containing di(2-ethylhexyl)adipate include electric wire insulation, garden hoses, vinyl coated fabrics for automotive and upholstery use, synthetic rubber, base oils for hydraulic fluids, and polyvinyl tubing for hemodialysis (Grace Co., 1976; Easterling et al., 1974). Easterling et al. (1974) perfused *in vitro* 500-700 ml of human plasma using commercially available medical grade polyvinyl tubing designed for hemodialysis.

The stability of di(2-ethylhexyl)adipate in feed was determined at Midwest Research Institute by assaying sample diet mixtures containing 100,000 ppm di(2-ethylhexyl)adipate that had been stored at -20°, 5°, 25°, or 45°C for 2 weeks. The amounts of the test chemical found to be present by vapor-phase chromatography (Appendix G) indicate that the compound was stable in feed for 2 weeks at temperatures as high as 45°C.

The amounts of di(2-ethylhexyl)adipate in selected batches of feed were measured by vapor-phase chromatography of 50-ml methanol extracts of 2-g samples. At each dietary concentration, the mean of the analytical concentration was usually within ±10% of the theoretical (Appendix H).

C. Animals

Three-week old F344 rats and 4-week old B6C3F1 mice obtained from the NCI Frederick Cancer Research Center, Frederick, Maryland, were observed for 2 weeks, examined for the absence of parasites or other diseases, and then assigned to control or dosed groups so that average cage weights were approximately equal for all animals of the same sex and species.

D. Animal Maintenance

Rats and mice were each housed five per cage in solid bottom suspended polycarbonate cages (Lab Products, Inc., Garfield, NJ) equipped with disposable nonwoven fiber filter sheets (Lab Products). Aspen-bed® hardwood chips (American Excelsior, Baltimore, MD) were used as bedding. Clean bedding and cages were provided twice weekly, and cage racks were changed every 2 weeks.

Water, available via an Edstrom automatic watering system (Waterford, WI), and powdered Wayne® Lab Blox diet in stainless-steel, gang-style hoppers (Scientific Cages, Inc., Bryan, TX), were available ad libitum.

Temperature in the animal rooms was 18°-31°C and relative humidity was 10%-88%. Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters, with 10 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 also being conducted on the following chemicals:

Butyl benzyl phthalate (CAS No. 85-68-7)
Di(2-ethylhexyl)phthalate (CAS No. 117-81-7)
Guar gum (CAS No. 9000-30-0)

E. Acute-Toxicity and 14-Day Repeated Dose Studies

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

In the acute-toxicity test, groups of five males and five females of each species were administered a single dose of the test substance in corn oil by gavage. Rats were administered doses of 0.08, 0.16, 0.31, 0.63, 1.25, 2.5, 5.0, 10, or 20 g/kg body weight and mice were administered doses of 1.25, 2.5, 5.0, 10, or 20 g/kg (Table 1). All surviving animals were killed after 14 days. The estimated LD_{50} was 45.0 g/kg for male rats and 24.6 g/kg for females. The estimated LD_{50} was 15.0 g/kg for male mice and 24.6 g/kg for females.

In the repeated dose study, groups of five males and five females of each species were tested for 14 days with five dose levels of the test substance in feed, followed by 1 day of observation with control diet. Groups of five males and five females of each species were maintained as untreated controls (Tables 2 and 3). All surviving animals were killed after 15 days.

One female rat receiving 100,000 ppm died. Weight gain was depressed 25% or more in male rats fed 50,000 ppm and in females fed 25,000 ppm or more. Female rats fed 100,000 ppm lost weight. Feed consumption was reduced in rats fed 50,000 ppm or more.

All female mice fed 100,000 ppm died. Weight loss occurred among male mice fed 50,000 ppm and females fed 25,000 ppm or more. Feed consumption was reduced in females fed 100,000 ppm.

Table 1. Dosage and Survival of Rats and Mice Administered a Single Dose of Di(2-ethylhexyl)adipate by Gavage

Dose (g/kg)	Survival (a) Male	Female
Rats		
0.08	5/5	5/5
0.16	5/5	5/5
0.31	5/5	5/5
0.63	5/5	5/5
1.25	5/5	5/5
1.25 (b)	5/5	5/5
2.50 (b)	5/5	5/5
5.00 (b)	5/5	5/5
10.00 (b)	3/5	5/5
20.00 (b)	4/5	4/5
Mice		
1.25	4/5 (c)	5/5
2.5	5/5	5/5
5.0	5/5	5/5
10.0	3/5	5/5
20.0	2/5	4/5

(a) Number surviving/number per group
(b) The single dose acute toxicity study at these higher doses was initiated 1 week after the single dose acute toxicity study at lower doses.
(c) Accidental death

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

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (%) (b)
MALE					
0	5/5	132.5	150.0	17.5	
3,100	5/5	132.5	190.0	57.5	+229
6,300	5/5	132.5	182.6	49.5	+183
12,500	5/5	132.5	177.8	45.3	+159
25,000	5/5	132.5	159.6	27.1	+55
50,000 (c)	5/5	132.5	145.6	13.1	-25
FEMALE					
0	5/5	103.5	124.4	20.9	
6,300	5/5	103.5	121.4	17.5	-16
12,500	5/5	103.5	125.0	21.5	+2.9
25,000	5/5	103.5	119.0	15.5	-26
50,000 (c)	5/5	103.5	109.6	6.1	-71
100,000 (c)	4/5	103.5	82.0	-21.5	-203

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

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

(c) Feed consumption was reduced in these groups of animals compared with the controls

Table 3. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing Di(2-ethylhexyl)adipate for 14 Days

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (%) (b)
MALE					
0	5/5	23.0	25.2	2.2	
3,100	5/5	23.0	25.2	2.2	0
6,300	5/5	23.0	25.6	2.6	+18
12,500	5/5	23.0	24.8	1.8	-18
25,000	5/5	23.0	23.6	0.6	-73
50,000	5/5	23.0	20.6	-2.4	-218
FEMALE					
0	5/5	18.0	18.8	0.8	
6,300	5/5	18.0	20.0	2.0	+150
12,500	5/5	18.0	18.2	0.2	-75
25,000	5/5	18.0	17.4	-0.6	-175
50,000	5/5	18.0	15.2	-2.8	-450
100,000	0/5 (c)	18.0	11.5	-6.5	-913

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

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

(c) Feed consumption was reduced in this group compared with the controls

F. Subchronic Studies

Subchronic studies were conducted to determine the two concentrations 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)adipate were fed for 13 weeks to groups of 10 rats and mice of each sex (Tables 4 and 5). Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day study, survivors were killed, necropsies were performed on all animals, and tissues were taken for histopathologic analysis.

Rats: One female rat receiving 1,600 ppm died, but its death was not considered to be compound-related. Weight gain depression was 11% or more for male rats fed 12,500 or 25,000 ppm. No compound-related histopathologic effects or reduction in feed consumption were observed.

Based on depression in mean weight gain, high and low doses selected for the chronic study with rats were 12,000 ppm and 25,000 ppm di(2-ethylhexyl)-adipate in feed.

Mice: One female mouse died as a result of an accident. Weight gain depression was 10% or more for male mice fed 3,100 ppm or more. Weight gain depression was 10% or more for females fed 6,000 or 25,000 ppm. No compound-related histopathologic effects or reduction in feed consumption were observed.

High and low doses selected for the chronic study with mice were 12,000 ppm and 25,000 ppm di(2-ethylhexyl)adipate in feed.

G. Chronic Studies

The number of animals in test groups, doses administered, and times on study of the chronic studies in rats and mice are shown in Table 6.

H. Clinical Examinations and Pathology

Animals were inspected twice daily, and body weights were recorded every 4 weeks. Animals that were moribund and those that survived to the end of the study were killed using CO_2 inhalation and necropsied.

Table 4. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing Di(2-ethylhexyl)adipate for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (%) (b)
MALE					
0	10/10	80.0	342	262	
1,600	10/10	80.0	320	240	-8.4
3,100	10/10	80.0	330	250	-4.6
6,300	10/10	80.0	325	245	-6.5
12,500	10/10	80.0	312	232	-11.5
25,000	10/10	80.0	296	216	-17.6
FEMALE					
0	10/10	71.0	193	122	
1,600	9/10	71.0	197	126	+3.3
3,100	10/10	71.0	191	120	-1.6
6,300	10/10	71.0	195	124	+1.6
12,500	10/10	71.0	186	115	-5.7
25,000	10/10	71.0	183	112	-8.2

(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)adipate for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (%) (b)
MALE					
0	10/10	20.2	33.3	13.1	
1,600	10/10	20.7	33.5	13.5	+3.1
3,100	10/10	20.7	30.7	10.0	-24
6,300	10/10	20.7	32.4	11.7	-10.7
12,500	10/10	20.7	31.8	11.1	-15.3
25,000	10/10	20.7	30.5	9.8	-25.2
FEMALE					
0	10/10	16.8	25.3	8.5	
1,600	10/10	16.8	25.0	8.2	-3.5
3,100	10/10	16.8	25.6	8.8	+3.5
6,300	10/10	16.8	21.5	4.7	-45
12,500	9/10 (c)	16.8	25.8	9.0	+5.6
25,000	10/10	16.8	24.2	7.4	-13

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

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

(c) Accidental death

Table 6. Experimental Design of Chronic Feeding Studies with Di(2-ethylhexyl)adipate in Rats and Mice

Test Group	Initial No. of Animals	Di(2-ethylhexyl) adipate (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male Rats				
Control	50	0	0	106-107
Low-Dose	50	12,000	103	3
High-Dose	50	25,000	103	1-2
Female Rats				
Control	50	0	0	107
Low-Dose	50	12,000	103	1-3
High-Dose	50	25,000	103	2
Male Mice				
Control	50	0	0	106
Low-Dose	50	12,000	103	2-3
High-Dose	50	25,000	103	1-2
Female Mice				
Control	50	0	0	106
Low-Dose	50	12,000	103	2-3
High-Dose	50	25,000	103	2

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) 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 dose level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The 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, however, 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.

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 ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ 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 that 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 and Clinical Signs (Rats)

Mean body weights of high-dose rats of either sex were lower than those of the controls throughout the study (Figure 1). No other compound-related clinical signs were observed.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered di(2-ethylhexyl)adipate in feed at the doses of this bioassay, and those of the controls, are shown by the Kaplan and Meier curves in Figure 2. Survival in the female control group declined relative to the dosed groups after 80 weeks on study. The survival between the dosed groups in females and among all three groups in males were comparable.

In male rats, 34/50 (68%) of the control and low-dose groups and 40/50 (80%) of the high-dose group lived to the end of the study at 105-107 weeks. In females, 29/50 (58%) of the controls, 39/50 (78%) of the low-dose group, and 44/50 (88%) of the high-dose group lived to the end of the study at 105-107 weeks.

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.

A variety of neoplasms were seen in both control and dosed rats. Tumors noted were those seen routinely in this strain of rat, and they occurred in comparable numbers in control and dosed rats.

Several nonneoplastic lesions were seen in control and dosed rats. None appeared to be related to chemical administration.

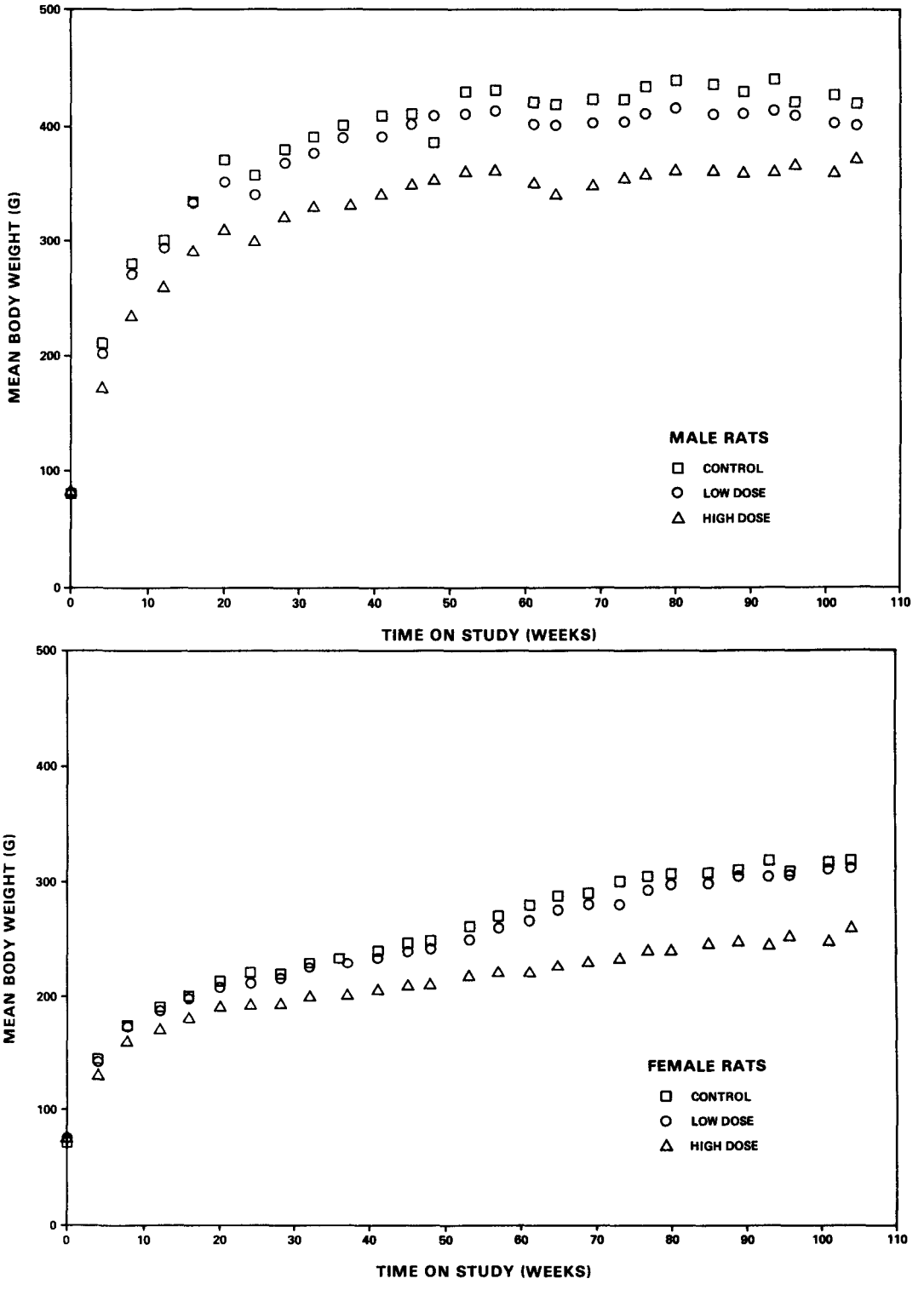


Figure 1. Growth Curves for Rats Fed Diets Containing Di(2-ethylhexyl)adipate

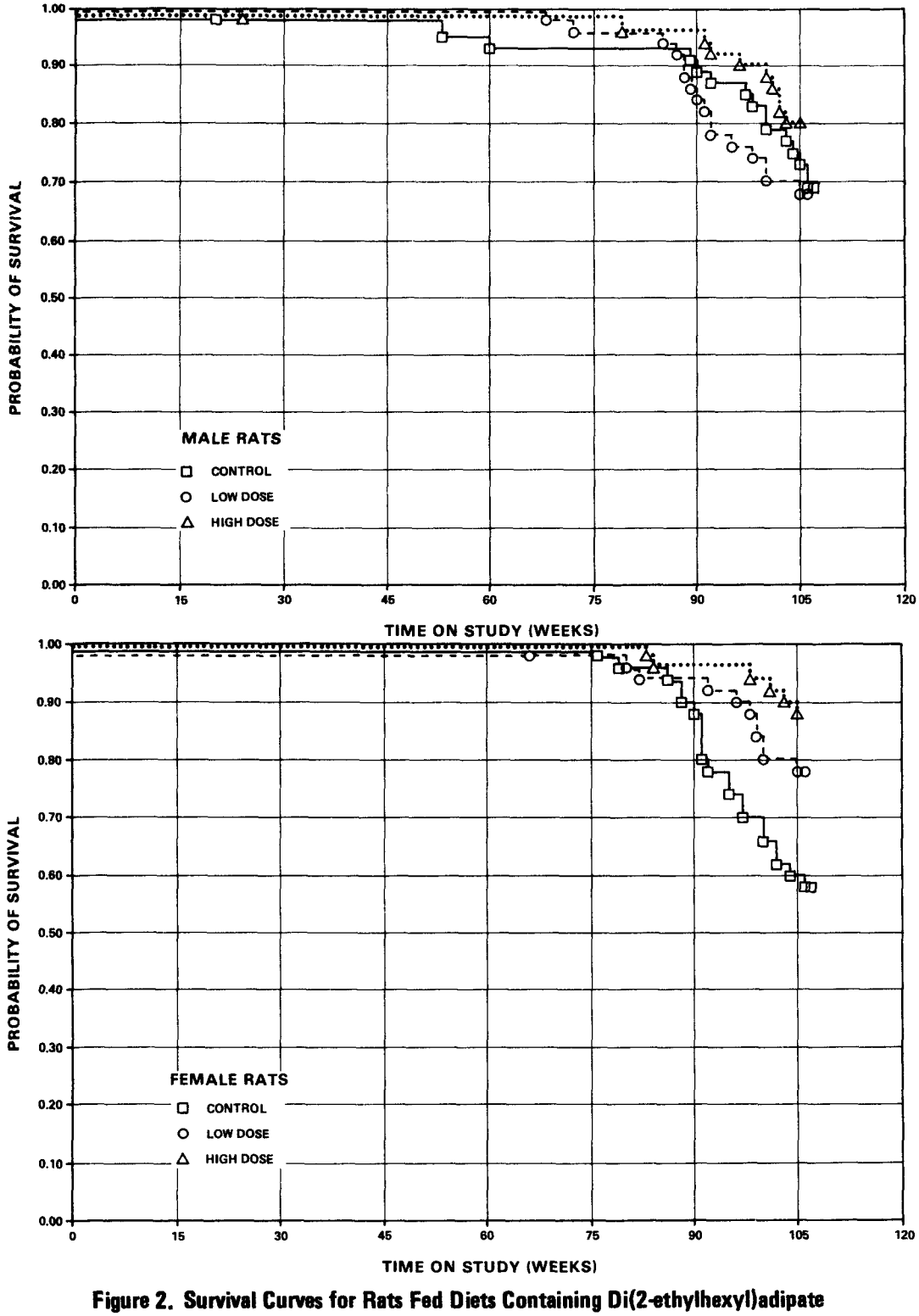


Figure 2. Survival Curves for Rats Fed Diets Containing Di(2-ethylhexyl)adipate

The results of histopathologic examination indicated that, under conditions of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic in F344 rats.

D. Statistical Analysis of Results (Rats)

Tables 7 and 8 contain the statistical analysis of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

The incidence of interstitial tumors in the testes was dose-related (P=0.010) and significantly higher (P=0.013) in the high-dose group of male rats, but this type of lesion occurs at a very high incidence in aging F344 rats. Historical records of the Mason control male rats indicate that incidences are usually over 80%. The test for differences in time to observation of these tumors was not significant.

In female rats the Cochran-Armitage test was not significant for the incidences of adenomas in the pituitary gland, but a departure from linear trend (P=0.037) was due to higher incidence in the low-dose group than in the other two groups. The result of the Fisher exact test is not significant when the low-dose group is compared with the control group. When the incidence of animals with either adenomas or carcinomas is considered, no significant results are obtained.

Pheochromocytomas in the adrenal glands of male rats occurred with a negative trend (P=0.031) and the incidence in the high-dose group was significantly lower than that in the controls. A negative trend (P=0.040) is also indicated in the incidence of cortical adenomas in the adrenal of female rats.

Fibroadenomas in the mammary gland occurred with a negative dose-related trend (P=0.001), and the incidence in the low- and high-dose groups of female rats was significantly lower (P=0.033 and P=0.002, respectively) than that in the controls.

The statistical analysis indicates no significant increase in tumor incidence that was associated with the administration of the chemical at any site. In each of the 95% confidence intervals for relative risk shown in the tables, except for the incidence of interstitial-cell tumors in the testis of high-dose group males, the value of one or less than one is

included, suggesting the absence of significant positive results. It should also be noted that each of the intervals, except for the incidence of fibroadenomas in the mammary gland of high-dose group females, has an upper limit greater than one, indicating the theoretical possibility of tumor induction by di(2-ethylhexyl)adipate which could not be detected under the conditions of this test.

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a)

Topography: Morphology	Control	Low Dose	High Dose
Skin: Squamous Cell			
Papilloma (b)	1/49(2)	4/50(8)	1/50(2)
P Values (c),(d)	N.S	N.S.	N.S.
Relative Risk (Control) (e)		3.920	0.980
Lower Limit		0.407	0.013
Upper Limit		188.989	75.404
Weeks to First Observed Tumor	90	106	105
Subcutaneous Tissue:			
Fibroma (b)	4/49(8)	1/50(2)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.245	0.490
Lower Limit		0.005	0.046
Upper Limit		2.362	3.251
Weeks to First Observed Tumor	100	106	104
Subcutaneous Tissue:			
Fibroma or Fibrosarcoma (b)	4/49(8)	2/50(4)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.490	0.490
Lower Limit		0.046	0.046
Upper Limit		3.251	3.251
Weeks to First Observed Tumor	100	106	104

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Myelomonocytic Leukemia (b)	9/49(18)	11/50(22)	8/50(16)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.198	0.871
Lower Limit		0.497	0.319
Upper Limit		2.979	2.333
Weeks to First Observed Tumor	20	89	79
Hematopoietic System:			
All Leukemias (b)	9/49(18)	11/50(22)	8/50(16)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.198	0.871
Lower Limit		0.497	0.319
Upper Limit		2.979	2.333
Weeks to First Observed Tumor	20	89	79
Pituitary:			
Adenoma, NOS (b)	3/44(7)	2/47(4)	3/44(7)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.624	1.000
Lower Limit		0.054	0.141
Upper Limit		5.195	7.086
Weeks to First Observed Tumor	106	106	101

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS, or Carcinoma, NOS (b)	4/44(9)	3/47(6)	3/44(7)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.702	0.750
Lower Limit		0.108	0.116
Upper Limit		3.919	4.174
Weeks to First Observed Tumor	90	106	101
Adrenal: Pheochromocytoma (b)	3/48(6)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.960	0.320
Lower Limit		0.135	0.006
Upper Limit		6.844	3.822
Weeks to First Observed Tumor	106	106	105
Adrenal: Pheochromocytoma, Malignant (b)	3/48(6)	0/50(0)	0/50(0)
P Values (c),(d)	P=0.036(N)	N.S.	N.S.
Relative Risk (Control) (e)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.596	1.596
Weeks to First Observed Tumor	106	--	--

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	6/48(13)	3/50(6)	1/50(2)
P Values (c),(d)	P=0.031(N)	N.S.	P=0.050(N)
Relative Risk (Control) (e)		0.480	0.160
Lower Limit		0.082	0.004
Upper Limit		2.111	1.249
Weeks to First Observed Tumor	106	106	105
Thyroid: C-Cell Carcinoma (b)	2/49(4)	3/49(6)	0/46(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	0.000
Lower Limit		0.180	0.000
Upper Limit		17.316	3.593
Weeks to First Observed Tumor	97	106	--
Thyroid: C-Cell Carcinoma or Adenoma (b)	3/49(6)	4/49(8)	1/46(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.333	0.355
Lower Limit		0.238	0.007
Upper Limit		8.675	4.230
Weeks to First Observed Tumor	97	100	105

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Preputial Gland: Carcinoma, NOS (b)	1/49(2)	4/50(8)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.920	0.980
Lower Limit		0.407	0.013
Upper Limit		188.989	75.404
Weeks to First Observed Tumor	98	88	104
Preputial Gland: Carcinoma, NOS, or Adenoma, NOS (b)	1/49(2)	4/50(8)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.920	1.960
Lower Limit		0.407	0.106
Upper Limit		188.989	113.312
Weeks to First Observed Tumor	98	88	104
Testis: Interstitial-Cell Tumor (b)	43/49(88)	47/50(94)	49/49(100)
P Values (c),(d)	P=0.010	N.S.	P=0.013
Relative Risk (Control) (e)		1.071	1.140
Lower Limit		0.930	1.014
Upper Limit		1.187	1.140
Weeks to First Observed Tumor	89	72	79

Table 7. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
All Sites: Mesothelioma, NOS, or Mesothelioma, Malignant (b)	1/49(2)	1/50(2)	3/50(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.980	2.940
Lower Limit		0.013	0.246
Upper Limit		75.404	151.180
Weeks to First Observed Tumor	106	85	102

(a) Dosed groups received doses of 12,000 or 25,000 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 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Myelomonocytic Leukemia (b)	12/50(24)	5/50(10)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.417	0.750
Lower Limit		0.124	0.307
Upper Limit		1.167	1.760
Weeks to First Observed Tumor	88	100	84
Hematopoietic System: All Leukemias (b)	12/50(24)	5/50(10)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.417	0.833
Lower Limit		0.124	0.356
Upper Limit		1.167	1.905
Weeks to First Observed Tumor	88	100	83
Hematopoietic System: Leukemia or Lymphoma (b)	12/50(24)	6/50(12)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.500	0.833
Lower Limit		0.167	0.356
Upper Limit		1.318	1.905
Weeks to First Observed Tumor	88	100	83

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule or Hepatocellular Carcinoma (b)	0/49(0)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		Infinite	Infinite
Lower Limit		0.590	0.053
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	99	105
Pituitary: Adenoma, NOS (b)	14/47(30)	23/48(48)	15/49(31)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P=0.037		
Relative Risk (Control) (f)		1.609	1.028
Lower Limit		0.914	0.523
Upper Limit		2.912	2.035
Weeks to First Observed Tumor	95	80	84
Pituitary: Carcinoma, NOS (b)	7/47(15)	2/48(4)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.280	0.411
Lower Limit		0.030	0.072
Upper Limit		1.378	1.683
Weeks to First Observed Tumor	86	98	105

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS, or Carcinoma, NOS (b)	21/47(45)	25/48(52)	18/49(37)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		1.166	0.822
Lower Limit		0.739	0.481
Upper Limit		1.845	1.401
Weeks to First Observed Tumor	86	80	84
Adrenal: Cortical Adenoma (b)	3/50(6)	0/50(0)	0/48(0)
P Values (c),(d)	P=0.040(N)	N.S.	N.S.
Relative Risk (Control) (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.663	1.730
Weeks to First Observed Tumor	91	--	--
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	1/50(2)	2/50(4)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		2.000	3.125
Lower Limit		0.108	0.262
Upper Limit		115.621	160.536
Weeks to First Observed Tumor	107	98	101

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Carcinoma (b)	3/50(6)	0/50(0)	2/47(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.000	0.709
Lower Limit		0.000	0.061
Upper Limit		1.663	5.913
Weeks to First Observed Tumor	79	--	105
Thyroid: C-Cell Carcinoma or Adenoma (b)	4/50(8)	0/50(0)	3/47(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.000	0.798
Lower Limit		0.000	0.123
Upper Limit		1.079	4.463
Weeks to First Observed Tumor	79	--	105
Mammary Gland: Fibroadenoma (b)	13/50(26)	5/50(10)	2/50(4)
P Values (c),(d)	P=0.001(N)	P=0.033(N)	P=0.002(N)
Relative Risk (Control) (f)		0.385	0.154
Lower Limit		0.116	0.018
Upper Limit		1.054	0.632
Weeks to First Observed Tumor	91	106	105

Table 8. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	11/50(22)	10/50(20)	13/50(26)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.909	1.182
Lower Limit		0.381	0.542
Upper Limit		2.140	2.626
Weeks to First Observed Tumor	76	96	98

(a) Dosed groups received doses of 12,000 or 25,000 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 probability level for departure from linear trend is given when P is less then 0.05 for any comparison.
(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed mice of either sex were lower than those of the corresponding controls throughout the bioassay, and the decrease in weight gain was dose related (Figure 3). No other compound-related clinical signs were observed.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered di(2-ethylhexyl)adipate in feed at the doses of this bioassay, and those of the controls, are shown by the Kaplan and Meier curves in Figure 4. The Cox test indicates significantly less (P=0.040) survival in the low-dose group than in the high-dose group of male mice, and survival in this group was less than that in the control from week 15 to the end of the study. The survival among all three groups of females was comparable.

In male mice, 36/50 (72%) of the control group, 32/50 (64%) of the low-dose group, and 41/50 (82%) of the high-dose group lived to the end of the study at 105-106 weeks. In females, 42/50 (84%) of the control group, 39/50 (78%) of the low-dose group, and 36/49 (73%) of the high-dose group were alive at the end of the study at 105-106 weeks.

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.

In mice, the incidence of neoplasms of the liver appeared to be related to the feeding of di(2-ethylhexyl)adipate. The numbers of mice with hepatocellular neoplasms and the sites of metastases are summarized in Table 9.

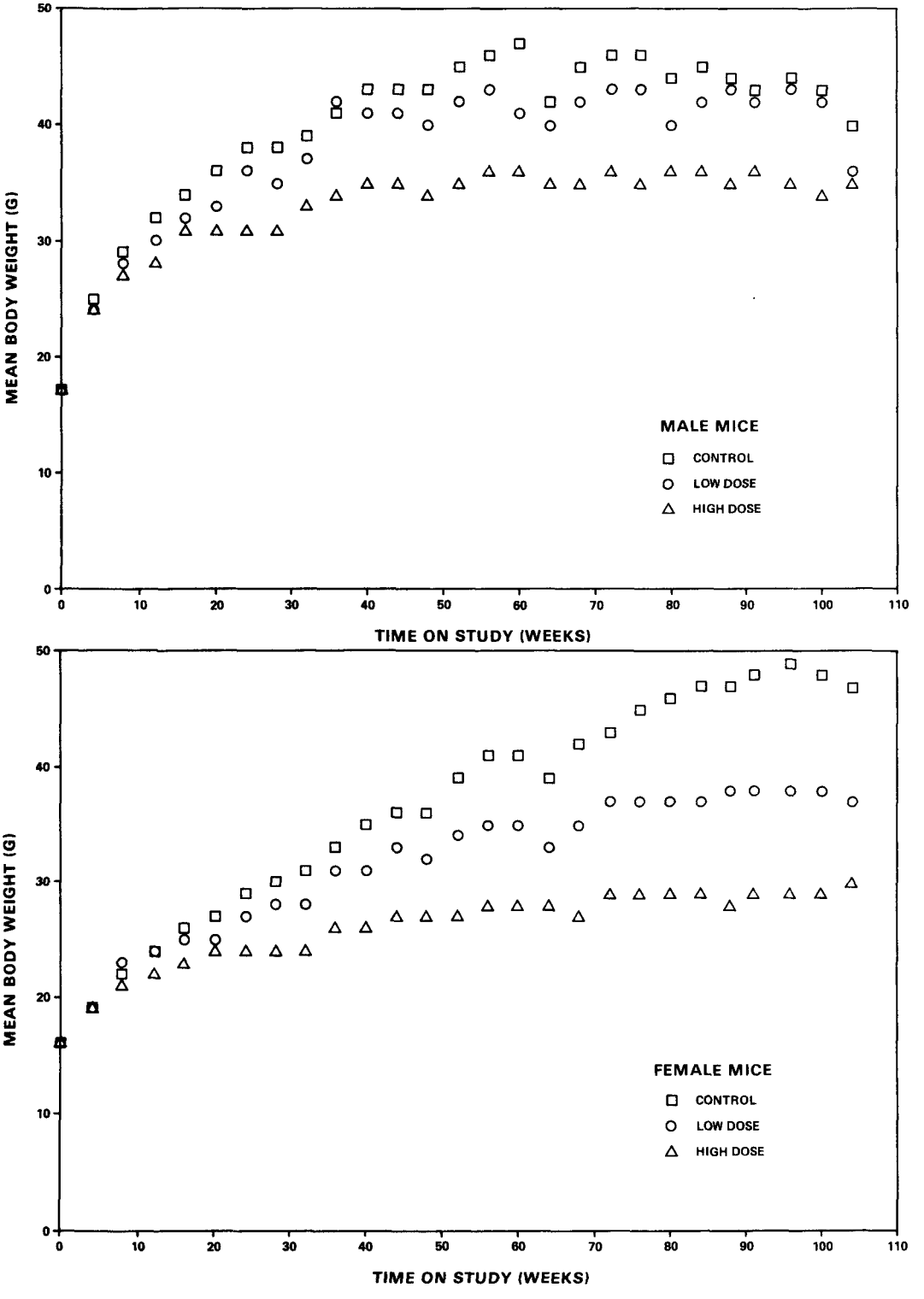


Figure 3. Growth Curves for Mice Fed Diets Containing Di(2-ethylhexyl)adipate

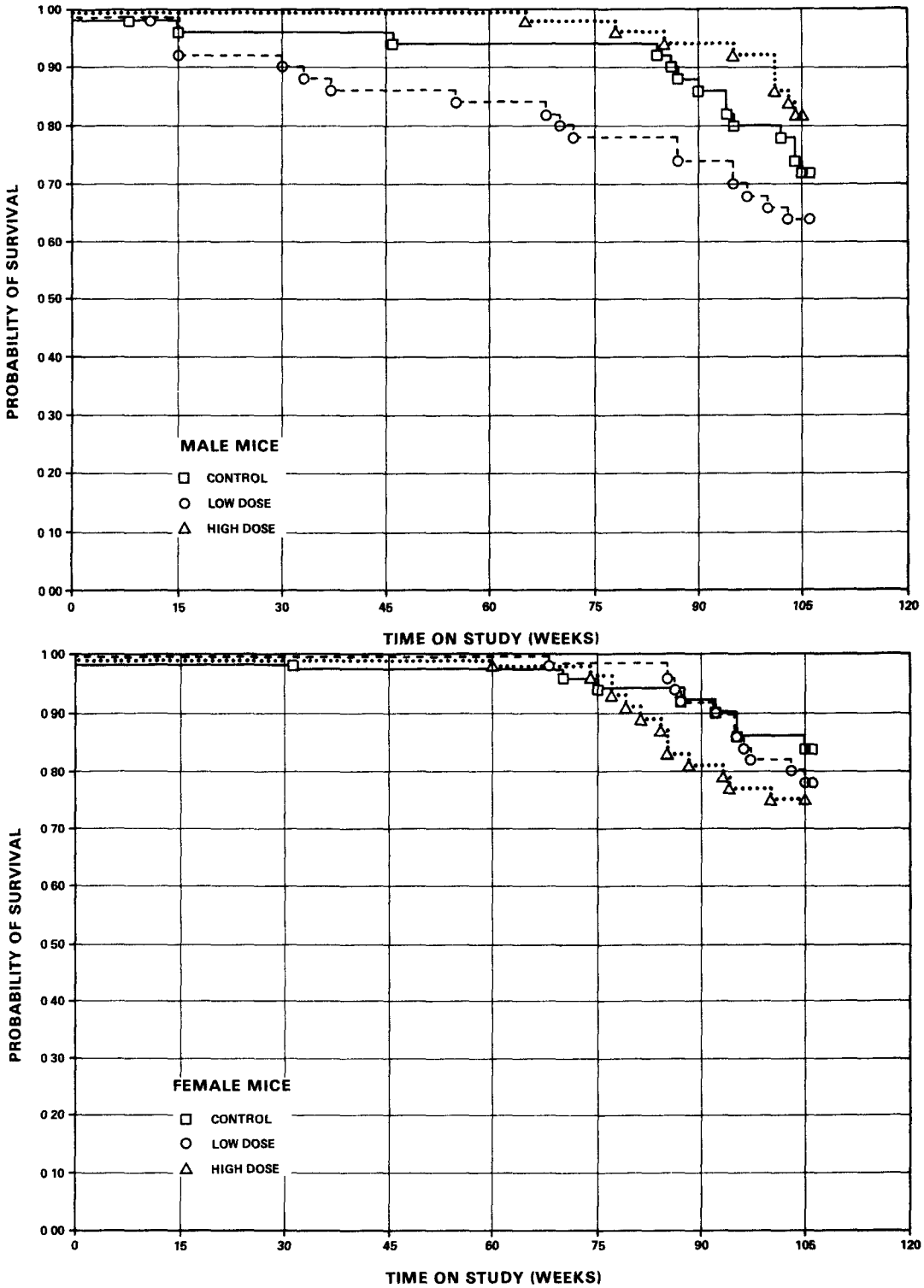


Figure 4. Survival Curves for Mice Fed Diets Containing Di(2-ethylhexyl)adipate

Table 9. Hepatocellular Neoplasms and Sites of Metastases in Mice Fed Diets Containing Di(2-ethylhexyl)adipate

	Male			Female		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of livers evaluated	50	49	49	50	50	49
Hepatocellular:						
Adenoma	6	8	15 (a)	2	5	6
Carcinoma	7	12	12	1	14 (b)	12(c)
Neoplasm, NOS	-	-	1	-	-	-
Percent Mice with liver tumors	26%	41%	56%	6%	38%	37%
Metastasis:						
Lung	5	4	5	-	6	5
Kidney	-	-	-	-	1	-
Adrenal	-	-	-	-	-	1
Lymph Node	-	-	-	-	1	-

(a) $P < 0.025$
(b) $P < 0.001$
(c) $P = 0.001$

Hepatocellular adenoma compressed the adjacent liver tissue. Cells in the adenoma were large. Cytoplasm of the cells was acidophilic or vacuolated and nuclei were hyperchromatic. Hepatocellular carcinoma involved a part or an entire lobe of the liver. The lobular architecture was distorted and cell plates were two or more cells thick, forming trabeculae. A pleomorphism in the size of cells was apparent. The nuclei had coarse chromatin, and the nucleoli were prominent. Both normal and abnormal mitotic figures were numerous. Areas of necrosis and mineralization were common in the large tumors.

The hepatocellular carcinoma metastasized to the lung in 14 male mice (control - 5; low-dose - 4; high-dose - 5) and in 11 female mice (low-dose - 6; high-dose - 5). In all cases, the primary liver tumors were of the trabecular type.

The other sites of metastases were the kidney, adrenal, and lymph nodes in dosed female mice.

A variety of nonneoplastic lesions were seen in control and dosed mice. None appeared to be related to chemical administration. No toxic lesions were seen in livers of dosed mice.

The results of histopathologic examination indicated that di(2-ethylhexyl)adipate was carcinogenic in B6C3F1 mice, inducing liver tumors under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables 10 and 11 contain the statistical analysis of the incidence of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

The incidence of hepatocellular adenomas in male mice was dose related (P=0.013) and statistically significant in the high-dose group (P=0.021). The incidence of hepatocellular carcinomas in male mice was higher in dosed groups than in controls, but the increase was not statistically significant. The incidence of male mice with either hepatocellular adenomas or carcinomas was dose related (P=0.002), and the high-dose group incidence is significantly higher (P=0.003) than that in the controls. The historical incidence in male B6C3F1 mice at this laboratory is: adenomas, 35/398 (9%, range 0%-16%); carcinomas, 86/398 (22%, range 14%-30%); combined tumors, 16/398 (29%, range 18%-36%). In female mice, there is a significant (P=0.001) dose-related trend and significantly higher (P less than 0.001 in

each instance) incidence of animals with hepatocellular adenomas or carcinomas in each of the dosed groups than in the control group. The historical incidence in female B6C3F1 mice at this laboratory is: adenomas, 18/397 (5%, range 0%-18%); carcinomas, 14/397 (4%, range 0%-8%); combined tumors, 31/397 (8%, range 2%-20%). A departure (P=0.024) from a linear trend has been indicated in females due to a sharp increase in the dosed groups' incidences. The test for the time to observation of this tumor in female mice indicates that there is a significantly shorter (P=0.002) time to the observation of these tumors in the dosed groups compared with the control group. In male mice, the result of this test was not significant.

Negative trends (P=0.010 in males and P=0.001 in females) and significantly lower incidences of animals with lymphomas or leukemias in the hematopoietic system in each of the dosed groups were observed (P=0.028 and P=0.016 in males and P=0.048 and P=0.001, respectively, in females). Incidences that are higher in the control groups (16/50 or 32% in males and 23/50 or 46% in females) than their respective incidence in the pooled historical controls were found in studies conducted at this laboratory for 100 weeks or more (165/3,543 or 5% in males and 331/3,617 or 9% in females).

A negative trend (P=0.005) and significantly lower (P=0.003) incidence of adenomas in the pituitary of high-dose group female mice were observed. The pooled historical control incidence for this tumor type in female B6C3F1 mice at this laboratory is 18/253 (7%) as compared with 8/39 (21%) in the control group.

Statistical analysis indicates that the incidence of liver tumors in mice of both sexes in this study is related to the administration of di(2-ethylhexyl)adipate in feed.

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma or Fibrosarcoma (b)	3/50(6)	2/50(4)	0/49(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	0.000
Lower Limit		0.058	0.000
Upper Limit		5.570	1.696
Weeks to First Observed Tumor	84	55	--
Lung: Alveolar/Bronchiolar Adenoma (b)	8/50(16)	9/49(18)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.148	0.383
Lower Limit		0.429	0.069
Upper Limit		3.138	1.488
Weeks to First Observed Tumor	84	105	104
Hematopoietic System: Malignant Lymphoma, NOS (b)	16/50(32)	5/50(10)	4/49(8)
P Values (c),(d)	P=0.002(N)	P=0.006(N)	P=0.003(N)
Relative Risk (Control) (e)		0.313	0.255
Lower Limit		0.097	0.067
Upper Limit		0.814	0.724
Weeks to First Observed Tumor	94	105	104

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: All Lymphomas (b)	16/50(32)	7/50(14)	6/49(12)
P Values (c),(d)	P=0.010(N)	P=0.028(N)	P=0.016(N)
Relative Risk (Control) (e)		0.438	0.383
Lower Limit		0.167	0.134
Upper Limit		1.018	0.934
Weeks to First Observed Tumor	94	72	104
Hematopoietic System: Lymphoma or Leukemia (b)	16/50(32)	7/50(14)	6/49(12)
P Values (c),(d)	P=0.010(N)	P=0.028(N)	P=0.016(N)
Relative Risk (Control) (e)		0.438	0.383
Lower Limit		0.167	0.134
Upper Limit		1.018	0.934
Weeks to First Observed Tumor	94	72	104
Liver: Hepatocellular Adenoma (b)	6/50(12)	8/49(16)	15/49(31)
P Values (c),(d)	P=0.013	N.S.	P=0.021
Relative Risk (Control) (e)		1.361	2.551
Lower Limit		0.448	1.029
Upper Limit		4.414	7.344
Weeks to First Observed Tumor	46	37	101

Table 10. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	7/50(14)	12/49(24)	12/49(24)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.749	1.749
Lower Limit		0.696	0.696
Upper Limit		4.802	4.802
Weeks to First Observed Tumor	86	68	65
Liver: Hepatocellular Adenoma or Carcinoma (b)	13/50(26)	20/49(41)	27/49(55)
P Values (c),(d)	P=0.002	N.S.	P=0.003
Relative Risk (Control) (e)		1.570	2.119
Lower Limit		0.843	1.216
Upper Limit		3.013	3.821
Weeks to First Observed Tumor	46	37	65

(a) Dosed groups received doses of 12,000 or 25,000 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 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	5/49(10)	1/49(2)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.200	0.613
Lower Limit		0.004	0.100
Upper Limit		1.698	2.965
Weeks to First Observed Tumor	106	106	79
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	6/49(12)	1/49(2)	3/48(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.167	0.510
Lower Limit		0.004	0.087
Upper Limit		1.301	2.241
Weeks to First Observed Tumor	106	106	79
Hematopoietic System: Malignant Lymphoma, NOS (b)	23/50(46)	14/50(28)	7/49(14)
P Values (c),(d)	P=0.001(N)	P=0.048(N)	P=0.001(N)
Relative Risk (Control) (f)		0.609	0.311
Lower Limit		0.333	0.126
Upper Limit		1.081	0.667
Weeks to First Observed Tumor	70	87	79

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: All Lymphomas (b)	23/50(46)	14/50(28)	7/49(14)
P Values (c),(d)	P=0.001(N)	P=0.048(N)	P=0.001(N)
Relative Risk (Control) (f)		0.609	0.311
Lower Limit		0.333	0.126
Upper Limit		1.081	0.667
Weeks to First Observed Tumor	70	87	79
Hematopoietic System: Lymphoma or Leukemia (b)	23/50(46)	14/50(28)	7/49(14)
P Values (c),(d)	P=0.001(N)	P=0.048(N)	P=0.001(N)
Relative Risk (Control) (f)		0.609	0.311
Lower Limit		0.333	0.126
Upper Limit		1.081	0.667
Weeks to First Observed Tumor	70	87	79
Circulatory System: Angiosarcoma (b)	3/50(6)	1/50(2)	1/49(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.333	0.340
Lower Limit		0.006	0.007
Upper Limit		3.983	4.062
Weeks to First Observed Tumor	105	106	105

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma (b)	2/50(4)	5/50(10)	6/49(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		2.500	3.061
Lower Limit		0.432	0.581
Upper Limit		25.286	29.826
Weeks to First Observed Tumor	106	103	84
Liver: Hepatocellular Carcinoma (b)	1/50(2)	14/50(28)	12/49(24)
P Values (c),(d)	P=0.003	P<0.001	P=0.001
Departure from Linear Trend (e)	P=0.022		
Relative Risk (Control) (f)		14.000	12.245
Lower Limit		2.274	1.931
Upper Limit		575.964	509.639
Weeks to First Observed Tumor	106	85	79
Liver: Hepatocellular Adenoma or Carcinoma (b)	3/50(6)	19/50(38)	18/49(37)
P Values (c),(d)	P=0.001	P<0.001	P<0.001
Departure from Linear Trend (e)	P=0.024		
Relative Risk (Control) (f)		6.333	6.122
Lower Limit		2.034	1.949
Upper Limit		31.235	30.333
Weeks to First Observed Tumor	106	85	79

Table 11. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Di(2-ethylhexyl)adipate (a) (Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	8/39(21)	6/37(16)	0/39(0)
P Values (c),(d)	P=0.005(N)	N.S.	P=0.003(N)
Relative Risk (Control) (f)		0.791	0.000
Lower Limit		0.250	0.000
Upper Limit		2.339	0.433
Weeks to First Observed Tumor	106	105	--

(a) Dosed groups received doses of 12,000 or 25,000 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 probability level for departure from linear trend is given when P is less then 0.05 for any comparison.
(f) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

V. DISCUSSION

Mean body weights of high-dose rats and mice of either sex were lower than those of the controls throughout the study. No other clinical signs were observed.

Interstitial cell tumors in the testes occurred in high-dose male rats at an incidence significantly higher (P=0.013) than that in the controls; however, since this type of lesion normally occurs at incidences approaching 100 per cent in aging F344 male rats (Goodman et al., 1979), the increased incidence is probably not compound related.

Hepatocellular adenomas or carcinomas occurred in high-dose mice of either sex and in low-dose female mice at incidences that were dose related and significantly higher than those in the controls. The time to observation of hepatocellular adenomas or carcinomas in the dosed female mice, but not in dosed male mice, was significantly shorter than the time to observation of these tumors in the controls. Because the incidence of hepatocellular adenomas or carcinomas in the male high-dose group is not greatly increased over that in the male B6C3F1 historical control mice in the same laboratory and because the time to observation of tumors in dosed groups as compared with the control group was not significantly different, the association of liver tumors in the males with administration of di(2-ethylhexyl)adipate is not considered conclusive.

Di(2-ethylhexyl)adipate was tested in the same room with three other chemicals--butyl benzyl phthalate, di(2-ethylhexyl)phthalate, and guar gum -- undergoing carcinogenesis bioassays. Butyl benzyl phthalate (NCI/NTP, in press) was probably carcinogenic for female F344 rats, causing myelomonocytic leukemia; di(2-ethylhexyl)phthalate (NCI/NTP, in press-a) was carcinogenic for female F344 rats, causing hepatocellular carcinomas and neoplastic nodules and for male and female B6C3F1 mice, causing hepatocellular carcinomas; guar gum (NCI/NTP, in press-b) was not carcinogenic for male or female F344 rats or B6C3F1 mice.

Although chemical cross-contamination among groups cannot be excluded completely, the responses in the separate testing experiments persuade that any adjacent chemical effect was absent or minimal. The results of these other studies support the conclusion that di(2-ethylhexyl)adipate caused

carcinomas of the liver in female mice and adenomas of the liver in male mice. These data stand independently because the guar gum exposed groups did not show any compound-related tumor development, because butyl benzyl phthalate exposed animals did not exhibit any liver tumor induction, and because di(2-ethylhexyl)phthalate induced, in addition to liver carcinomas in female mice, these same lesions in female rats and in male mice.

VI. CONCLUSIONS

Under the conditions of this bioassay, di(2-ethylhexyl)adipate was not carcinogenic for F344 rats. Di(2-ethylhexyl)adipate was carcinogenic for female B6C3F1 mice, causing increased incidences of hepatocellular carcinomas, and was probably carcinogenic for male B6C3F1 mice, causing hepatocellular adenomas.

VII. BIBLIOGRAPHY

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

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

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

Berenblum, I., ed., Carcinogenicity Testing: A Report of the Panel on Carcinogenicity of the Cancer Research Commission of UICC, Vol. 2, International Union Against Cancer, Geneva, 1969.

Blaisdell, C., Toxicity of certain engine oils, hydraulic fluids, and base oils for the compounding of both hydraulic fluids and engine oils. U.S. Army Chemical Corps Medical Laboratories Research Reports No. 256, 1954.

CFR, U.S. Code of Federal Regulations 21:121.2511 and 21:121.2550, 1976.

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

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

Easterling, R.A., Johnson, E., and Napier, E.A., Plasma extraction of plasticizers from "medical grade" polyvinylchloride tubing. Proc. Soc. Exp. Biol. Med. 147:572-574, 1974.

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

Goodman, D., Ward, J., Squire, R., Chu, K., and Linhart, M., Neoplastic and nonneoplastic lesions in aging F344 rats. Toxicol. Appl. Pharmacol. 48:237-248, 1979.

Grace, W. R. and Co., Technical information: Hatcol-DOA®, di-2-ethylhexyl adipate, 1976.

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

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

Miller, R. G., Jr., Simultaneous Statistical Inference, McGraw-Hill Book Co., New York, 1966, pp. 6-10.

Patty's Industrial Hygiene and Toxicology, 3rd rev. ed., Vol. 2, John Wiley & Sons, New York, 1981, p. 2330.

NCI/NTP, National Cancer Institute/National Toxicology Program, NTP Technical Report on the Carcinogenesis Bioassay of Butyl Benzyl Phthalate (CAS No. 85-68-7), DHHS Publication No. (NIH) 81-1769, Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, MD, in press.

NCI/NTP, National Cancer Institute/National Toxicology Program, NTP Technical Report on the Carcinogenesis Bioassay of Di(2-ethylhexyl)phthalate (CAS No. 117-81-7), DHHS Publication No. (NIH) 81-1773, Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, MD, in press-a.

NCI/NTP, National Cancer Institute/National Toxicology Program, NTP Technical Report on the Carcinogenesis Bioassay of Guar Gum (CAS No. 9000-30-0), DHHS Publication No. (NIH) 81-1775, Carcinogenesis Testing Program, National Cancer Institute, National Institutes of Health, Bethesda, MD, in press-b.

Sadtler Standard Spectra, Sadtler Research Laboratories, Philadelphia, Pennsylvania, IR No. 24, NMR No. 2913 M.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F. and Kaufman, D. G., Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo(a)pyrene and ferric oxide. Cancer Res. 32:1073-81, 1972.

Simmon, V., Kauhanen, K., and Tardiff, R., Mutagenic activity of chemicals identified in drinking water. Dev. Toxicol. Environ. Sci. 2:249-258, 1977.

Singh, A., Lawrence, W., and Autian, J., Embryonic-fetal toxicity and teratogenic effects of adipic acid esters in rats. J. Pharm. Sci. 62:1596-1600, 1973.

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

Smyth, H., Carpenter, C., and Weil, C., Range-finding toxicity data: List IV. Arch. Ind. Hyg. 4:119,1951.

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

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

Vandervort, R. and Brooks, S., Polyvinyl chloride film thermal decomposition products as an occupational illness. J. Occup. Med. 19:188-191, 1977.

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

APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)	4 (8%)	1 (2%)
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(49)	(50)	(50)
UNDIFFERENTIATED CARCINOMA		1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	1 (2%)
SARCOMA, NOS			1 (2%)
FIBROMA	4 (8%)	1 (2%)	2 (4%)
FIBROSARCOMA		1 (2%)	
LIPOMA	1 (2%)		
LIPOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(49)	(50)	(50)
UNDIFFERENTIATED CARCINOMA, INVA		1 (2%)	
#LUNG	(49)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
LIPOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA	9 (18%)	11 (22%)	8 (16%)
#SPLEEN	(49)	(50)	(50)
SARCOMA, NOS		1 (2%)	
#MEDIASTINAL L.NODE	(48)	(49)	(44)
SQUAMOUS CELL CARCINOMA, METASTA		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)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE	2 (4%)	1 (2%)	
HEPATOCELLULAR CARCINOMA		1 (2%)	2 (4%)
#JEJUNUM	(48)	(50)	(49)
FIBROSARCOMA	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
#URINARY BLADDER	(47)	(50)	(49)
LEIOMYOSARCOMA			1 (2%)
*PROSTATIC URETHRA	(49)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(47)	(44)
CARCINOMA,NOS	1 (2%)	1 (2%)	
ADENOMA, NOS	3 (7%)	2 (4%)	3 (7%)
#ANTERIOR PITUITARY	(44)	(47)	(44)
CARCINOMA,NOS	1 (2%)		
#ADRENAL	(48)	(50)	(50)
CORTICAL ADENOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA	3 (6%)	3 (6%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT	3 (6%)		
#THYROID	(49)	(49)	(46)
C-CELL ADENOMA	1 (2%)	1 (2%)	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 CARCINOMA	2 (4%)	3 (6%)	
#THYROID FOLLICLE	(49)	(49)	(46)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
#PANCREATIC ISLETS	(47)	(50)	(49)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROADENOMA	1 (2%)	1 (2%)	
*PREPUTIAL GLAND	(49)	(50)	(50)
CARCINOMA,NOS	1 (2%)	4 (8%)	1 (2%)
ADENOMA, NOS			1 (2%)
#TESTIS	(49)	(50)	(49)
INTERSTITIAL-CELL TUMOR	43 (88%)	47 (94%)	49 (100%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*HARDERIAN GLAND	(49)	(50)	(50)
ADENOMA, NOS		1 (2%)	
*EAR CANAL	(49)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA	2 (4%)	2 (4%)	
MUSCULOSKELETAL SYSTEM			
*SKULL	(49)	(50)	(50)
OSTEOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*MANDIBLE	(49)	(50)	(50)
FIBROSARCOMA	1 (2%)		
ODONTOMA	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
SARCOMA, NOS			1 (2%)
*TUNICA VAGINALIS	(49)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
MESOTHELIOMA, MALIGNANT		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	8	8	9
MORIBUND SACRIFICE	7	8	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	34	40
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	48	48	50
TOTAL PRIMARY TUMORS	84	95	81
TOTAL ANIMALS WITH BENIGN TUMORS	43	47	49
TOTAL BENIGN TUMORS	58	63	61
TOTAL ANIMALS WITH MALIGNANT TUMORS	20	28	17
TOTAL MALIGNANT TUMORS	22	31	17
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	1
TOTAL SECONDARY TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	4	1	3
TOTAL UNCERTAIN TUMORS	4	1	3
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)ADIPATE

	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)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SARCOMA, NOS	1 (2%)		
FIBROMA		1 (2%)	
FIBROSARCOMA	1 (2%)		
FIBROADENOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
OSTEOSARCOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
LEUKEMIA,NOS			1 (2%)
MYELOMONOCYTIC LEUKEMIA	12 (24%)	5 (10%)	9 (18%)
#MANDIBULAR L. NODE	(48)	(49)	(47)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
#MEDIASTINAL L.NODE	(48)	(49)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#UTERUS	(50)	(50)	(50)
HEMANGIOMA	1 (2%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE		2 (4%)	1 (2%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
URINARY SYSTEM			
#URINARY BLADDER	(49)	(47)	(48)
TRANSITIONAL-CELL PAPILLOMA		1 (2%)	
TRANSITIONAL-CELL CARCINOMA	1 (2%)	1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(48)	(49)
CARCINOMA,NOS	7 (15%)	2 (4%)	3 (6%)
ADENOMA, NOS	14 (30%)	23 (48%)	15 (31%)
#ADRENAL	(50)	(50)	(48)
CORTICAL ADENOMA	3 (6%)		
PHEOCHROMOCYTOMA		1 (2%)	2 (4%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	1 (2%)	1 (2%)
GANGLIONEUROMA	1 (2%)		
#THYROID	(50)	(50)	(47)
C-CELL ADENOMA	1 (2%)		1 (2%)
C-CELL CARCINOMA	3 (6%)		2 (4%)
#THYROID FOLLICLE	(50)	(50)	(47)
PAPILLARY CYSTADENOCARCINOMA,NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)	2 (4%)	
PAPILLARY ADENOCARCINOMA			1 (2%)
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
FIBROADENOMA	13 (26%)	5 (10%)	2 (4%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	2 (4%)	1 (2%)	1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*VAGINA	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)		
#UTERUS	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
LEIOMYOSARCOMA		2 (4%)	
ENDOMETRIAL STROMAL POLYP	11 (22%)	10 (20%)	13 (26%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	2 (4%)	
#OVARY	(49)	(49)	(49)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
#CEREBRUM	(50)	(50)	(50)
OLIGODENDROGLIOMA	1 (2%)		
#BRAIN	(50)	(50)	(50)
GLIOMA, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	2 (4%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
LEIOMYOSARCOMA	1 (2%)		
CRANIAL CAVITY			
SQUAMOUS CELL CARCINOMA, INVASIV	1		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	9	6	3
MORIBUND SACRIFICE	12	5	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	29	39	44
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	44	41	34
TOTAL PRIMARY TUMORS	83	64	53
TOTAL ANIMALS WITH BENIGN TUMORS	32	33	27
TOTAL BENIGN TUMORS	45	41	34
TOTAL ANIMALS WITH MALIGNANT TUMORS	28	18	16
TOTAL MALIGNANT TUMORS	38	20	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	
TOTAL SECONDARY TUMORS	2	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	1
TOTAL UNCERTAIN TUMORS		3	1
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)ADIPATE

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
SARCOMA, NOS	2 (4%)	1 (2%)	
FIBROMA	1 (2%)		
FIBROSARCOMA	2 (4%)	2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST	5 (10%)	4 (8%)	5 (10%)
ALVEOLAR/BRONCHIOLAR ADENOMA	8 (16%)	9 (18%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	13 (26%)	3 (6%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
*HEMATOPOIETIC SYSTEM	(50)	(50)	(49)
NEOPLASM, NOS		1 (2%)	
#SPLEEN	(50)	(46)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#LYMPH NODE	(43)	(37)	(45)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#MESENTERIC L. NODE	(43)	(37)	(45)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#LIVER	(50)	(49)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PEYER'S PATCH	(45)	(40)	(45)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (3%)	
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			1 (2%)
#SPLEEN	(50)	(46)	(48)
ANGIOSARCOMA	1 (2%)		
#LYMPH NODE	(43)	(37)	(45)
HEMANGIOSARCOMA	1 (2%)		
#LIVER	(50)	(49)	(49)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
NEOPLASM, NOS			1 (2%)
HEPATOCELLULAR ADENOMA	6 (12%)	8 (16%)	15 (31%)
HEPATOCELLULAR CARCINOMA	7 (14%)	12 (24%)	12 (24%)
#ILEUM	(45)	(40)	(45)
ADENOCARCINOMA, NOS	2 (4%)		
ADENOMATOUS POLYP, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
TUBULAR-CELL ADENOMA			1 (2%)
#URINARY BLADDER	(50)	(44)	(47)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(46)	(44)	(48)
ADENOMA, NOS	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CORTICAL ADENOMA	1 (2%)		
#ADRENAL CORTEX	(46)	(44)	(48)
ADENOMA, NOS	1 (2%)		
#THYROID	(42)	(44)	(46)
FOLLICULAR-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(49)
ADENOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
ADENOMA, NOS		2 (4%)	
*EXTERNAL EAR	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	15	8
MORIBUND SACRIFICE	4	3	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	32	41
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	33	32	34
TOTAL PRIMARY TUMORS	50	46	41
TOTAL ANIMALS WITH BENIGN TUMORS	16	17	17
TOTAL BENIGN TUMORS	18	21	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	25	22	19
TOTAL MALIGNANT TUMORS	32	24	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	5	4	5
TOTAL SECONDARY TUMORS	5	4	5
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

TABLE B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(49)
NEOPLASM, NOS	1 (2%)		
SARCOMA, NOS	1 (2%)		1 (2%)
FIBROSARCOMA	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		6 (12%)	5 (10%)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	1 (2%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	22 (44%)	9 (18%)	5 (10%)
#SPLEEN	(50)	(50)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#LYMPH NODE	(43)	(45)	(40)
NEOPLASM, NOS			1 (3%)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
MALIGNANT LYMPHOMA, NOS	1 (2%)	3 (7%)	
#LIVER	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS		2 (4%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
HEMANGIOSARCOMA		1 (2%)	1 (2%)
ANGIOSARCOMA	1 (2%)	1 (2%)	
#SPLEEN	(50)	(50)	(48)
HEMANGIOSARCOMA		1 (2%)	
ANGIOSARCOMA	2 (4%)	1 (2%)	
#LIVER	(50)	(50)	(49)
HEMANGIOMA		1 (2%)	1 (2%)
#UTERUS	(50)	(48)	(49)
HEMANGIOMA			1 (2%)
ANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
HEPATOCELLULAR ADENOMA	2 (4%)	5 (10%)	6 (12%)
HEPATOCELLULAR CARCINOMA	1 (2%)	14 (28%)	12 (24%)
#STOMACH	(49)	(49)	(48)
SQUAMOUS CELL PAPILLOMA	1 (2%)	1 (2%)	
#DUODENUM	(50)	(46)	(47)
ADENOMATOUS POLYP, NOS		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(49)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(37)	(39)
ADENOMA, NOS	8 (21%)	6 (16%)	
#ADRENAL	(45)	(48)	(45)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PHEOCHROMOCYTOMA			1 (2%)
#ADRENAL CORTEX	(45)	(48)	(45)
ADENOMA, NOS		2 (4%)	1 (2%)
#THYROID	(43)	(46)	(46)
FOLLICULAR-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOCARCINOMA, NOS	1 (2%)		
#UTERUS	(50)	(48)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
SARCOMA, NOS			1 (2%)
#OVARY	(38)	(40)	(44)
TUBULAR ADENOMA	1 (3%)	1 (3%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
ADENOMA, NOS	1 (2%)	1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
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@	8	9	10
MORIBUND SACRIFICE		2	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	39	36
ANIMAL MISSING			1
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	37	33	30
TOTAL PRIMARY TUMORS	51	53	40
TOTAL ANIMALS WITH BENIGN TUMORS	16	13	9
TOTAL BENIGN TUMORS	19	20	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	26	28	23
TOTAL MALIGNANT TUMORS	31	33	25
TOTAL ANIMALS WITH SECONDARY TUMORS#		7	5
TOTAL SECONDARY TUMORS		11	6
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)ADIPATE

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
ULCER, FOCAL	1 (2%)		
HYPERKERATOSIS	1 (2%)		1 (2%)
ACANTHOSIS	1 (2%)		1 (2%)
*SUBCUT TISSUE	(49)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(49)	(50)	(49)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG	(49)	(50)	(50)
INFLAMMATION, INTERSTITIAL	1 (2%)		
PNEUMONIA, CHRONIC MURINE			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(50)	(50)
HEMATOPOIESIS		3 (6%)	
#LYMPH NODE	(48)	(49)	(44)
HYPERPLASIA, NOS			1 (2%)
#MANDIBULAR L. NODE	(48)	(49)	(44)
INFLAMMATION, CHRONIC	1 (2%)		
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, PLASMA CELL	4 (8%)		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, LYMPHOID		1 (2%)	
#MEDIASTINAL L.NODE	(48)	(49)	(44)
INFLAMMATION, ACUTE	1 (2%)		
#MESENTERIC L. NODE	(48)	(49)	(44)
HYPERPLASIA, PLASMA CELL			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(49)	(50)	(50)
THROMBUS, MURAL		2 (4%)	
#HEART/ATRIUM	(49)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
THROMBUS, MURAL	1 (2%)	1 (2%)	1 (2%)
#MYOCARDIUM	(49)	(50)	(50)
FIBROSIS	2 (4%)		2 (4%)
CALCIFICATION, NOS	1 (2%)		
*AORTA	(49)	(50)	(50)
MEDIAL CALCIFICATION	1 (2%)		
*CORONARY ARTERY	(49)	(50)	(50)
MEDIAL CALCIFICATION	1 (2%)		
#PANCREAS	(47)	(50)	(49)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(50)	(48)
INFLAMMATION, ACUTE	2 (4%)		
INFLAMMATION, CHRONIC	9 (19%)		
METAPLASIA, SQUAMOUS	6 (13%)	1 (2%)	1 (2%)
#LIVER	(49)	(50)	(50)
INFLAMMATION, GRANULOMATOUS	1 (2%)		
NECROSIS, NOS	1 (2%)		1 (2%)
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY		2 (4%)	
BASOPHILIC CYTO CHANGE	15 (31%)	4 (8%)	8 (16%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
GROUND-GLASS CYTO CHANGE	1 (2%)		
CLEAR-CELL CHANGE	2 (4%)	1 (2%)	2 (4%)
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
CONGESTION, NOS	1 (2%)		1 (2%)
NECROSIS, NOS		2 (4%)	
#BILE DUCT	(49)	(50)	(50)
HYPERPLASIA, NOS	40 (82%)	32 (64%)	5 (10%)
HYPERPLASIA, FOCAL		1 (2%)	6 (12%)
#PANCREAS	(47)	(50)	(49)
STEATITIS	1 (2%)		
INFLAMMATION, CHRONIC FOCAL			1 (2%)
NECROSIS, FAT	1 (2%)		
#PANCREATIC ACINUS	(47)	(50)	(49)
ATROPHY, FOCAL	1 (2%)	1 (2%)	
#FORESTOMACH	(49)	(50)	(50)
ULCER, FOCAL			1 (2%)
HYPERPLASIA, BASAL CELL	1 (2%)	1 (2%)	
#CARDIAC STOMACH	(49)	(50)	(50)
ULCER, ACUTE	1 (2%)		
#ILEUM	(48)	(50)	(49)
NEMATODIASIS			1 (2%)
#COLON	(46)	(47)	(47)
NEMATODIASIS	1 (2%)	4 (9%)	5 (11%)
#CECUM	(46)	(47)	(47)
NECROSIS, FAT	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
NEPHROSIS, NOS	45 (92%)	42 (84%)	41 (82%)
CALCIFICATION, NOS	1 (2%)		
HEMOSIDEROSIS			1 (2%)
#KIDNEY/CORTEX	(49)	(50)	(50)
HAMARTOMA		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
CYST, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(47)	(44)
CYST, NOS	3 (7%)		
MULTIPLE CYSTS			1 (2%)
HEMORRHAGIC CYST			1 (2%)
ANGIECTASIS			2 (5%)
#ADRENAL	(48)	(50)	(50)
CYST, NOS		1 (2%)	
HEMORRHAGIC CYST		1 (2%)	
ATROPHY, NOS		1 (2%)	1 (2%)
#ADRENAL CORTEX	(48)	(50)	(50)
HEMORRHAGIC CYST			1 (2%)
#ADRENAL MEDULLA	(48)	(50)	(50)
HEMORRHAGE	1 (2%)		
#THYROID	(49)	(49)	(46)
CYSTIC FOLLICLES		1 (2%)	1 (2%)
HYPERPLASIA, C-CELL	2 (4%)		2 (4%)
#PARATHYROID	(19)	(23)	(12)
HYPERPLASIA, FOCAL			1 (8%)
#PANCREATIC ISLETS	(47)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(50)	(50)
DILATATION, NOS	1 (2%)		
CYSTIC DUCTS	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
LACTATION	1 (2%)		
*PREPUTIAL GLAND	(49)	(50)	(50)
DILATATION, NOS			1 (2%)
PUS	2 (4%)		
INFLAMMATION, SUPPURATIVE	1 (2%)	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
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, NOS	2 (4%)	1 (2%)	
HYPERPLASIA, EPITHELIAL	1 (2%)		
HYPERPLASIA, FOCAL		1 (2%)	
#PROSTATE	(45)	(49)	(48)
CYST, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE	4 (9%)	4 (8%)	2 (4%)
*SEMINAL VESICLE	(49)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*COAGULATING GLAND	(49)	(50)	(50)
PUS		1 (2%)	
NECROSIS, NOS		1 (2%)	
#TESTIS	(49)	(50)	(49)
HYPERPLASIA, INTERSTITIAL CELL	1 (2%)	2 (4%)	
#TESTIS/TUBULE	(49)	(50)	(49)
DEGENERATION, NOS	2 (4%)	3 (6%)	
NERVOUS SYSTEM			
#CEREBRAL CORTEX	(48)	(50)	(50)
HEMORRHAGE			1 (2%)
#PONS	(48)	(50)	(50)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
*EYE	(49)	(50)	(50)
HEMORRHAGE			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*INGUINAL REGION	(49)	(50)	(50)
STEATITIS	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
*MESENTERY	(49)	(50)	(50)
STEATITIS	1 (2%)		
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
TAIL			
KERATIN-PEARL FORMATION			1
ADIPOSE TISSUE			
CONGESTION, NOS	1		
HEMORRHAGE	2		
NECROSIS, FAT		1	
OMENTUM			
STEATITIS			1
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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)ADIPATE

	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)
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEAL SUBMUCOSA	(50)	(50)	(48)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#LUNG	(50)	(50)	(50)
INFLAMMATION, INTERSTITIAL		2 (4%)	1 (2%)
BRONCHOPNEUMONIA, ACUTE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(49)	(46)
HYPOPLASIA, NOS			1 (2%)
#SPLEEN	(49)	(50)	(50)
HEMOSIDEROSIS	1 (2%)	1 (2%)	
HEMATOPOIESIS	2 (4%)	2 (4%)	1 (2%)
ERYTHROPOIESIS	1 (2%)		
#MANDIBULAR L. NODE	(48)	(49)	(47)
HYPERPLASIA, PLASMA CELL	1 (2%)	1 (2%)	
#MEDIASTINAL L.NODE	(48)	(49)	(47)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, PLASMA CELL	1 (2%)		
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(50)	(50)	(50)
THROMBUS, MURAL	1 (2%)	1 (2%)	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
#RIGHT VENTRICLE	(50)	(50)	(50)
SCAR		1 (2%)	
#MYOCARDIUM	(50)	(50)	(50)
CALCIFICATION, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(50)	(48)
INFLAMMATION, ACUTE	1 (2%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		1 (2%)
METAPLASIA, SQUAMOUS	3 (6%)	1 (2%)	
#LIVER	(49)	(50)	(50)
FIBROSIS, FOCAL	1 (2%)		
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY	2 (4%)	3 (6%)	
CYTOPLASMIC VACUOLIZATION	1 (2%)	1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE	28 (57%)	38 (76%)	39 (78%)
CLEAR-CELL CHANGE	2 (4%)	2 (4%)	2 (4%)
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
CONGESTION, NOS			1 (2%)
NECROSIS, NOS		1 (2%)	
#BILE DUCT	(49)	(50)	(50)
HYPERPLASIA, NOS	12 (24%)	16 (32%)	17 (34%)
HYPERPLASIA, FOCAL		2 (4%)	2 (4%)
#PANCREAS	(49)	(50)	(48)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#GASTRIC MUCOSA	(50)	(50)	(48)
INFLAMMATION, CHRONIC			1 (2%)
ULCER, CHRONIC	1 (2%)		
#FORESTOMACH	(50)	(50)	(48)
ULCER, ACUTE		1 (2%)	
HYPERPLASIA, BASAL CELL	1 (2%)		
#COLON	(48)	(49)	(47)
ULCER, ACUTE		1 (2%)	
NEMATODIASIS	1 (2%)	5 (10%)	4 (9%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
NEPHROSIS, NOS	29 (58%)	31 (62%)	20 (40%)
HEMOSIDEROSIS	2 (4%)	2 (4%)	
#KIDNEY/CORTEX	(50)	(50)	(50)
FIBROSIS	1 (2%)		
NEPHROSIS, NOS			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(48)	(49)
CYST, NOS	2 (4%)	1 (2%)	3 (6%)
MULTIPLE CYSTS	7 (15%)	2 (4%)	5 (10%)
HEMORRHAGE		1 (2%)	
HEMORRHAGIC CYST	2 (4%)	3 (6%)	
ANGIECTASIS	1 (2%)		
#ADRENAL CORTEX	(50)	(50)	(48)
HEMORRHAGE	1 (2%)		
HEMORRHAGIC CYST		1 (2%)	
NEPHROSIS, NOS	1 (2%)		
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(50)	(50)	(47)
CYSTIC FOLLICLES		1 (2%)	1 (2%)
HYPERPLASIA, C-CELL	1 (2%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NOS	1 (2%)		1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	
FIBROCYSTIC DISEASE		1 (2%)	
LACTATION	2 (4%)	1 (2%)	1 (2%)
*CLITORAL GLAND	(50)	(50)	(50)
PUS	1 (2%)		
INFLAMMATION, SUPPURATIVE		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
#UTERUS	(50)	(50)	(50)
HYDROMETRA	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		
HYPERPLASIA, CYSTIC	2 (4%)	1 (2%)	3 (6%)
#OVARY	(49)	(49)	(49)
CYST, NOS	1 (2%)	1 (2%)	1 (2%)
CYSTIC FOLLICLES			2 (4%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		
#PONS	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

APPENDIX D

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

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
INFLAMMATION, NOS			3 (6%)
ULCER, NOS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(49)
MINERALIZATION		1 (2%)	
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS	1 (2%)		4 (8%)
NECROSIS, NOS	2 (4%)	1 (2%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(49)
MINERALIZATION			1 (2%)
HEMORRHAGE	1 (2%)	1 (2%)	1 (2%)
INFLAMMATION, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(43)	(44)	(36)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
#SPLEEN	(50)	(46)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	3 (6%)
HEMATOPOIESIS	7 (14%)	6 (13%)	5 (10%)
#LYMPH NODE	(43)	(37)	(45)
HEMORRHAGIC CYST	1 (2%)	2 (5%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	6 (14%)	2 (5%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(50)	(49)	(49)
HEMATOPOIESIS		1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
PERIVASCULITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
NECROSIS, FOCAL	4 (8%)	1 (2%)	2 (4%)
METAMORPHOSIS FATTY	2 (4%)	2 (4%)	
BASOPHILIC CYTO CHANGE			2 (4%)
EOSINOPHILIC CYTO CHANGE		1 (2%)	
CLEAR-CELL CHANGE		3 (6%)	
#BILE DUCT	(50)	(49)	(49)
HYPERPLASIA, NOS	1 (2%)		
#PANCREAS	(45)	(45)	(47)
INFLAMMATION, NOS		1 (2%)	
#PANCREATIC ACINUS	(45)	(45)	(47)
ATROPHY, FOCAL	1 (2%)	1 (2%)	
#STOMACH	(49)	(47)	(49)
MINERALIZATION	1 (2%)		
INFLAMMATION, NOS	2 (4%)	1 (2%)	1 (2%)
HYPERPLASIA, BASAL CELL	1 (2%)		
HYPERKERATOSIS	3 (6%)		
#PEYER'S PATCH	(45)	(40)	(45)
HYPERPLASIA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(49)
MINERALIZATION	3 (6%)	1 (2%)	1 (2%)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
NEPHROPATHY	3 (6%)	2 (4%)	4 (8%)
#URINARY BLADDER	(50)	(44)	(47)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(46)	(44)	(48)
HYPERPLASIA, NOS	7 (15%)	2 (5%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
NECROSIS, NOS		2 (4%)	
#TESTIS	(50)	(44)	(48)
MINERALIZATION			1 (2%)
ATROPHY, NOS		1 (2%)	1 (2%)
#TESTIS/TUBULE	(50)	(44)	(48)
MINERALIZATION			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
INFLAMMATION, NOS	1 (2%)	1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*KNEE JOINT	(50)	(50)	(49)
OSTEOARTHRITIS	1 (2%)		
BODY CAVITIES			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
OMENTUM			
MINERALIZATION	1		
NECROSIS, FAT	3	2	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	10	6
AUTO/NECROPSY/HISTO PERF	1		
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
NECROSIS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(48)
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS		2 (4%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	5 (10%)		1 (2%)
HEMATOPOIESIS	5 (10%)	10 (20%)	12 (25%)
#LYMPH NODE	(43)	(45)	(40)
HEMORRHAGE	1 (2%)		
HEMATOPOIESIS		1 (2%)	1 (3%)
#LIVER	(50)	(50)	(49)
HEMATOPOIESIS	2 (4%)	2 (4%)	1 (2%)
#ADRENAL	(45)	(48)	(45)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
#THYROID	(43)	(46)	(46)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(49)	(47)
ENDOCARDITIS, BACTERIAL		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
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	3 (6%)	3 (6%)	
METAMORPHOSIS FATTY	2 (4%)		
BASOPHILIC CYTO CHANGE		1 (2%)	
EOSINOPHILIC CYTO CHANGE			1 (2%)
#PANCREATIC ACINUS	(48)	(47)	(45)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL		1 (2%)	1 (2%)
#STOMACH	(49)	(49)	(48)
NECROSIS, NOS		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, BASAL CELL	1 (2%)		
HYPERKERATOSIS	1 (2%)	1 (2%)	1 (2%)
ACANTHOSIS		1 (2%)	1 (2%)
#PEYER'S PATCH	(50)	(46)	(47)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(49)
INFLAMMATION, NOS	1 (2%)	1 (2%)	1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
NEPHROPATHY			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(37)	(39)
DILATATION, NOS			1 (3%)
HEMORRHAGE	1 (3%)		
#ADRENAL CORTEX	(45)	(48)	(45)
METAMORPHOSIS FATTY	1 (2%)		
HYPERPLASIA, NOS	18 (40%)	11 (23%)	15 (33%)
#THYROID	(43)	(46)	(46)
HYPERPLASIA, FOLLICULAR-CELL	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
REPRODUCTIVE SYSTEM			
#UTERUS	(50)	(48)	(49)
HYDROMETRA	11 (22%)	8 (17%)	4 (8%)
INFLAMMATION, NOS	3 (6%)	5 (10%)	3 (6%)
#UTERUS/ENDOMETRIUM	(50)	(48)	(49)
HYPERPLASIA, NOS	4 (8%)	12 (25%)	4 (8%)
HYPERPLASIA, CYSTIC	9 (18%)	6 (13%)	11 (22%)
#OVARY	(38)	(40)	(44)
MINERALIZATION		2 (5%)	
CYST, NOS	5 (13%)	2 (5%)	1 (2%)
INFLAMMATION, NOS		1 (3%)	
INFLAMMATION, NECROTIZING	1 (3%)	2 (5%)	
DEGENERATION, CYSTIC	1 (3%)	3 (8%)	3 (7%)
NECROSIS, NOS		1 (3%)	
METAPLASIA, GLANDULAR			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT	1	3	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	3	4
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF			1

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

APPENDIX E

ANALYSIS OF DI(2-ETHYLHEXYL)ADIPATE
LOT NO. 0-62-494
MIDWEST RESEARCH INSTITUTE

APPENDIX E

Analysis of Di(2-ethylhexyl)adipate Lot No. 0-62-494

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	71.30	11.43
Determined	72.19	11.70
	72.40	11.54

B. ESTER TITRATION (Annual Book of ASTM Standards, 1974)

Samples were hydrolyzed at room temperature with 1.0N potassium hydroxide. A time study was done to assure complete hydrolysis. Hydrolysis was complete at 5 hours. After hydrolysis, samples were back-titrated with standardized H_2SO_4. Unhydrolyzed samples were also titrated to quantitate any titratable free acidity (assumed to be adipic acid) which could affect the ester titration. 100.5% $\pm$0.5(δ)% (No titratable acidity).

C. THIN-LAYER CHROMATOGRAPHY

Plates: Silica gel 60 F254

Amount spotted: 100 and 300 μg (10 mg/ml methanol)

System 1: Toluene (100%)

R_f: 0.21 (major)
R_{st}: 0.46

Ref. Standard: Di(2-ethylhexyl)phthalate

Visualization: Iodine vapor

System 2: Methylene chloride (100%)

R_f: 0.44 (major)
R_{st}: 0.58

D. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Varian 3740
Detector: Flame ionization
Inlet temperature: 200°C
Detector temperature: 260°C
Carrier gas: Nitrogen
Carrier flow rate: 70 cc/min

VAPOR-PHASE CHROMATOGRAPHY (continued)

(1) System 1

Column: 3% OV-225 on 80/100 Supelcoport 1.8 M x 4 mm ID, glass.
Oven temperature program: 100° to 200°C at 10°C/min
Sample injected: A solution (6 µl) of 60% di(2-ethylhexyl)adipate in chloroform and 1.0% and 0.5% in chloroform to quantitate the major peak and check for detector overload.
Results: Major peak and two impurities which totaled 0.11% of the major peak area.

Peak	Retention Time (min)	Retention Time (Relative to Di(2-ethylhexyl)adipate)	Area Percent of Di(2-ethyl-hexyl)adipate
1	12.7	0.64	0.06
2	13.2	0.67	0.05
3	19.7	1.00	100

(2) System 2

Column: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm ID, glass
Oven temperature program: 150°-250°C at 10°/min
Sample injected: A solution (6 µl) of 1.0% of di(2-ethylhexyl)-adipate in methanol and 0.5% in methanol to check for detector overload.
Results: Major peak and five impurities which totaled 0.27% of the major peak area.

Peak	Retention Time (min)	Retention Time (Relative to Di(2-ethylhexyl)adipate	Area Percent of Di(2-ethyl-hexyl)adipate
1	6.8	0.64	0.01
2	7.3	0.68	0.05
3	8.5	0.79	0.12
4	10.4	0.97	0.07
5	10.7	1.00	100
6	12.3	1.14	0.02

E. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12
Cell: Neat, thin film between sodium chloride plates
Results: See Figure 5

Consistent with literature spectrum (Sadtler Standard Spectra)

(2) Ultraviolet/Visible

Instrument: Cary 118
No absorbance was found at a concentration of 1 mg/ml in the visible region (800-350 nm). In the ultraviolet region (350-215 nm), no maxima were observed at a concentration of 1 mg/ml; however, a gradually increasing absorbance was noted between 280 nm and the solvent cut-off at 215 nm.
Solvent: 95% methanol

No literature spectrum found

(3) Nuclear Magnetic Resonance

Instrument: Varian EM360-A
Solvent: Chloroform-d with internal tetramethylsilane
Assignments: See Figure 6

Consistent with literature spectrum (Sadtler Standard Spectra)

(a) t, δ 0.90 ppm, J_{a-b} = 6 Hz
(b) m, δ 1.07 to 1.90 ppm, J_{b-c} = 6 Hz, J_{b-d} = 5 Hz
(c) m, δ 2.30 ppm
(d) d, δ 4.00 ppm

Integration Ratios:
(a) 12.84
(b) 21.79
(c) 3.89
(d) 3.50

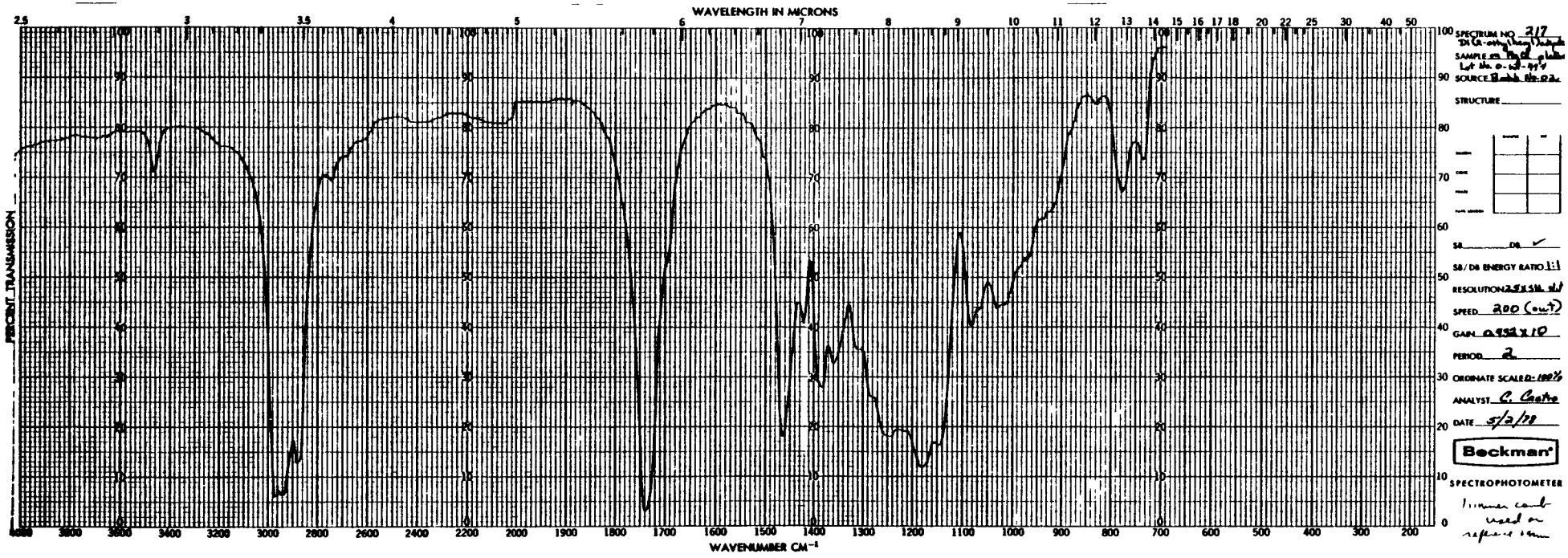


Figure 5. Infrared Absorption Spectrum Di(2-ethylhexyl)adipate Lot No. 0-62-494

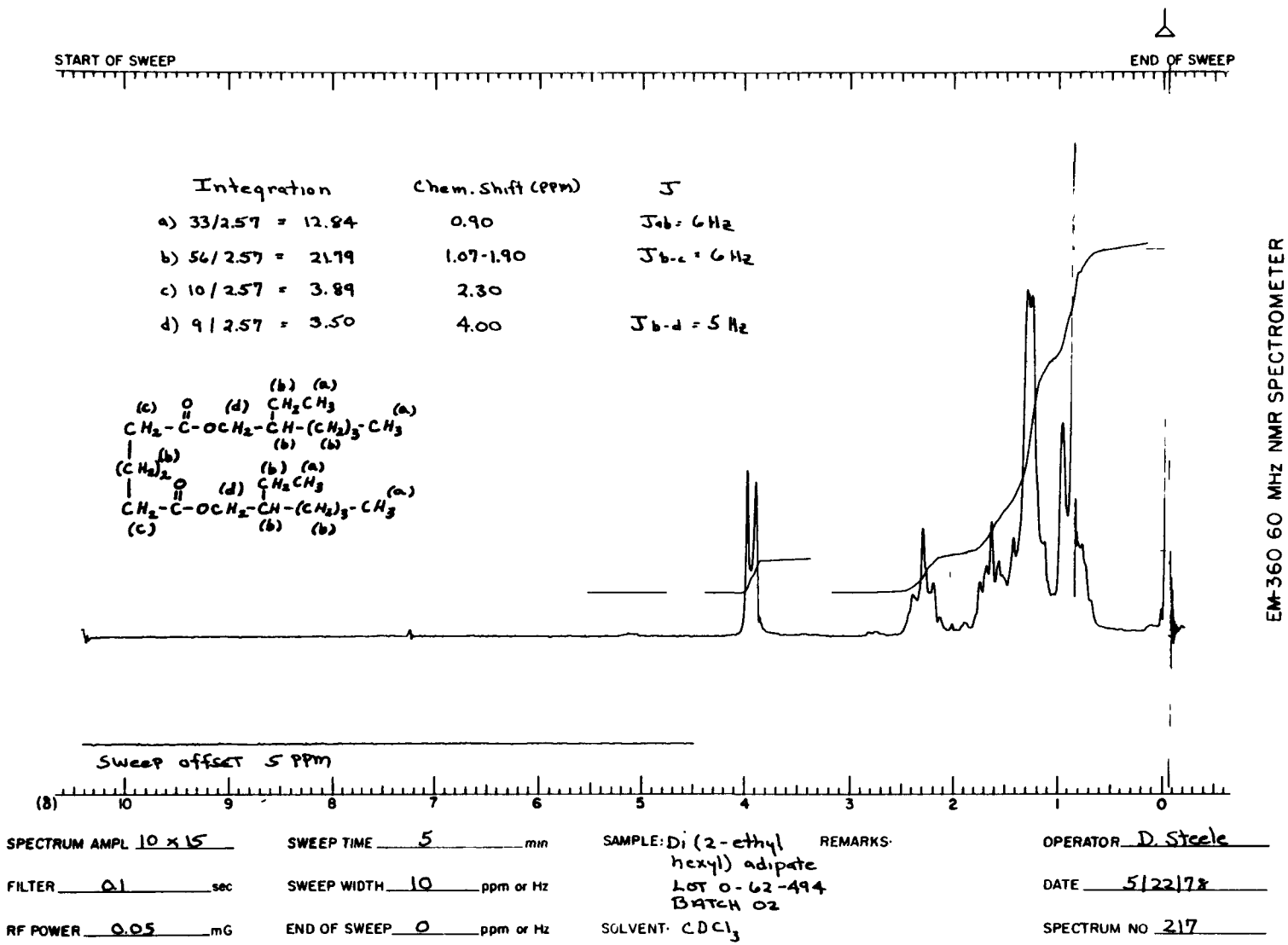


Figure 6. Nuclear Magnetic Resonance Spectrum Di(2-ethylhexyl)adipate Lot No. 0-62-494

APPENDIX F

ANALYSIS OF DI(2-ETHYLHEXYL)ADIPATE
LOT NO. GC-2-27-76
MIDWEST RESEARCH INSTITUTE

APPENDIX F

Analysis of Di(2-ethylhexyl)adipate Lot No. GC-2-27-76

A. ELEMENTAL ANALYSIS

Element	C	H
Theory	71.31	11.42
Determined	71.69 71.62	11.50 11.55

B. BOILING POINT

Determined	Literature Values
b.p. endotherm observed at 363° to 373°C (corr) at 744 torr (DuPont 900 DTA)	210° at 5 torr (Grace and Co., 1976)

C. DENSITY

Determined	Literature Values
$d_4^{24.5}$ 0.918±0.000	d_{25}^{25} 0.925±0.003 (Grace and Co., 1976)

D. REFRACTIVE INDEX

Determined	Literature Values
n_D^{20} 1.4460/n_D^{25}1.4451	n_D^{25} 1.4470 (Grace and Co., 1976)

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica Gel 60 F254
Amount spotted: 100 and 300 µg
Ref. Standard: Di(2-ethylhexyl)phthalate
Visualization: Potassium Dichromate

System 1: Benzene
R_f 0.24 (major)
R_{st}: 0.50

System 2: Methylene chloride
R_f: 0.33 (major)
R_{st}: 0.52

F. VAPOR-PHASE CHROMATOGRAPHY

Instrument: Tracor MT-220
Detector: Flame ionization
Oven temperature program: 150° to 250°C, 10°/min
Inlet temperature: 200°C
Detector temperature: 260°C
Column 1: 3% OV-1 on 80/100 Supelcoport, 1.8 m x 4 mm ID, glass
Results: Major peak and two impurities

Peak	Retention Time (min)	Relative Retention Time	Relative Area
1	4.88	0.65	0.07
2	7.24	0.96	0.19 (shoulder)
3 (major)	7.56	1.00	100.00

Column 2: 3% OV-225 on 80/100 Chromosorb W (HP), 1.8 m x 4 mm ID, glass
Results: Major peak and seven impurities which totalled 1.9% of the major peak area.

Peak	Retention Time (min)	Relative Retention Time	Relative Area
1	3.6	0.59	< 0.01
2	4.4	0.72	0.13
3	5.7	0.92	0.38
4 (major)	6.1	1.00	100.00
5	7.4	1.21	0.23
6	7.9	1.28	< 0.01
7	8.2	1.33	1.12
8	8.8	1.44	0.01

G. SPECTRAL DATA

(1) Infrared

Instrument: Beckmann IR-12
Cell: Neat, thin film between sodium chloride plates
Results: See Figure 7.

Consistent with literature spectrum (Sadtler Standard Spectra)

(2) Ultraviolet/Visible

Instrument: Cary 118
Compound exhibits no visible absorption. Ultraviolet absorbance is detectable in the 230 to 215 nm region, but no maximum was observed before the low-wavelength solvent cut-off (220 nm).

Solvent: 95% Ethanol

Literature Values

No literature spectrum found

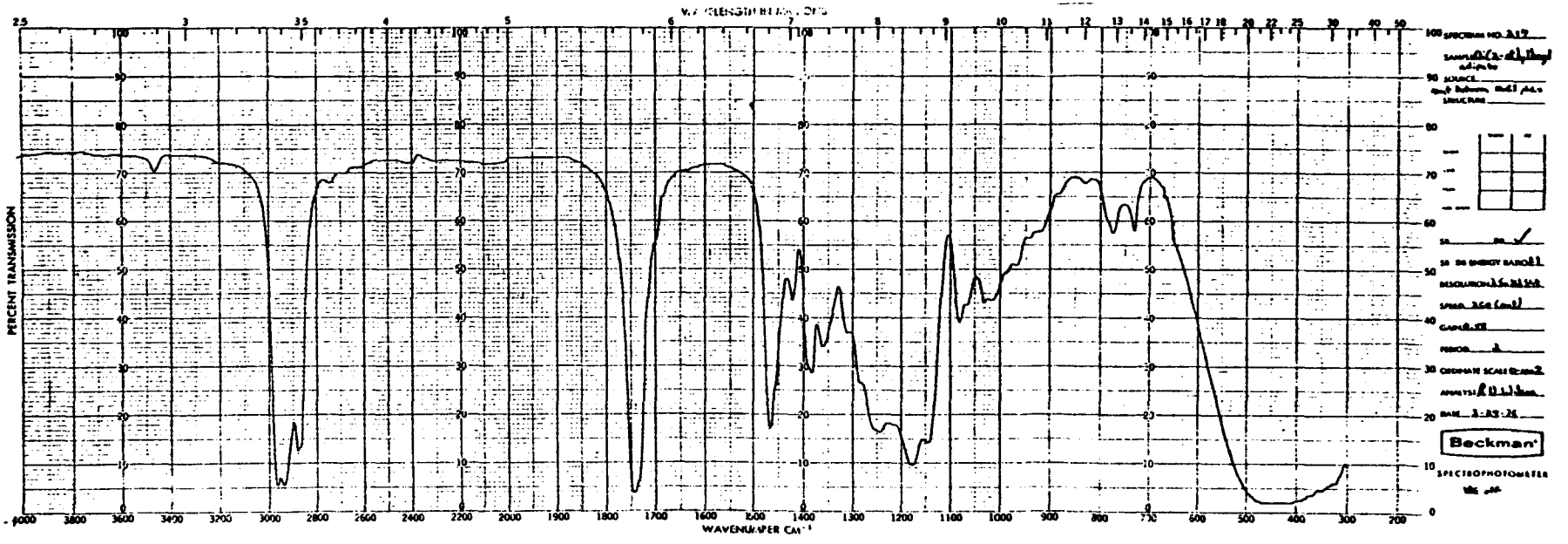


Figure 7. Infrared Absorption Spectrum Di(2-ethylhexyl)adipate Lot No. GC-2-27-76

(3) Nuclear Magnetic Resonance

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

Consistent with literature spectrum (Sadtler Standard Spectra)

Assignments (See Figure 8).
a = t, δ 0.88 ppm
(J_{ab} = 7 Hz)
b = m, δ 1.15 to 1.90 ppm
c = m, δ 2.31 ppm
d = d, δ 4.00 ppm
(J_{bd} = 5 Hz)

Integration Ratios:

a = 11.54
b = 22.19
c = 3.85
d = 4.48

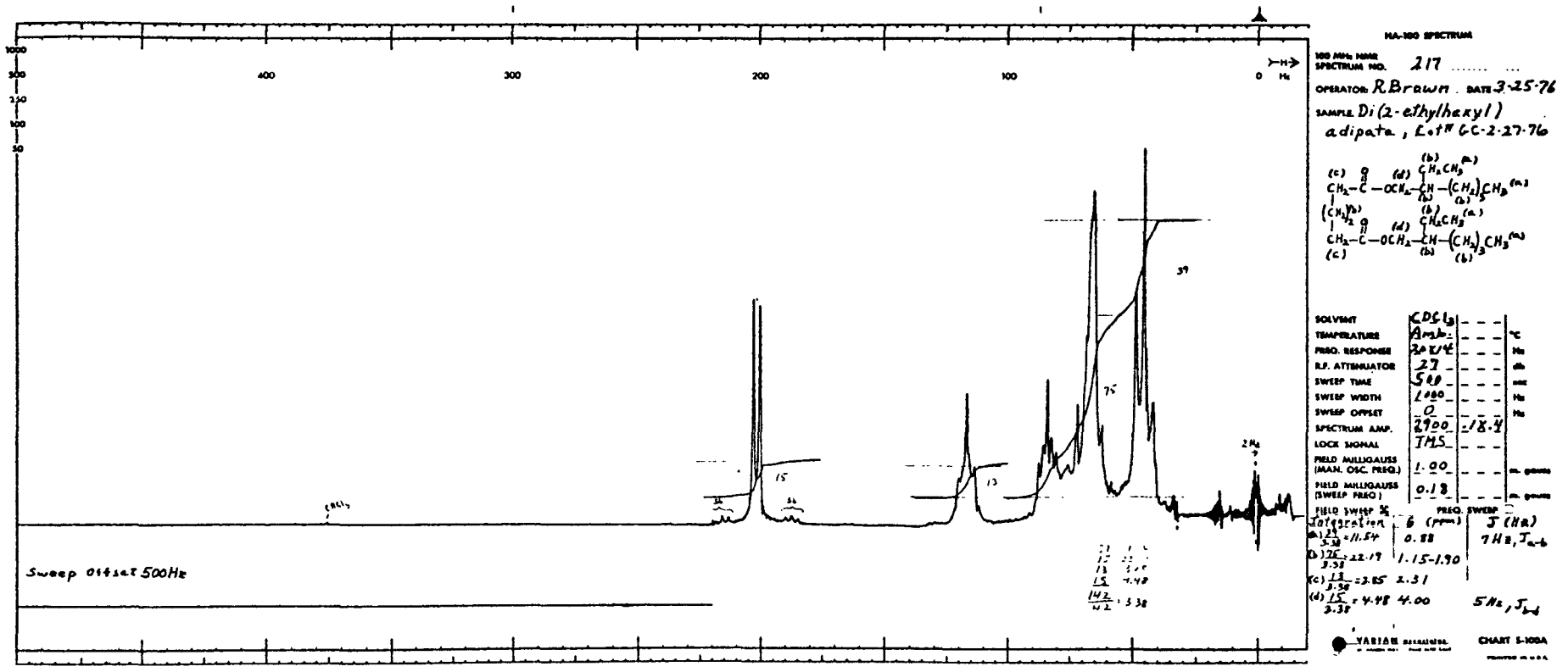


Figure 8. Nuclear Magnetic Resonance Spectrum Di(2-ethylhexyl)adipate Lot No. GC-2-27-76

APPENDIX G

STABILITY ANALYSIS OF DI(2-ETHYLHEXYL)ADIPATE
IN FORMULATED DIETS
MIDWEST RESEARCH INSTITUTE

APPENDIX G

Stability Analysis of Di(2-Ethylhexyl)Adipate in Formulated Diets

Midwest Research Institute

HEAT STABILITY

(1) MIXING AND STORAGE: Di(2-ethylhexyl)adipate (2.6071 g) and Wayne® Lab-Blox Rodent Feed (22.5353 g) were mixed in a mortar. Samples of the mixture were then removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively. These samples were analyzed by vapor-phase chromatography as described below.

(2) EXTRACTION AND ANALYSIS: One-gram samples of each of the above stability mixtures were triturated twice with 50-ml portions of methanol. The supernatant solutions were combined and diluted to 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, glass, 1.8 m x 4 mm ID
Oven temperature: 250°C
Retention time of test compound: 1.9 min
Inlet temperature: 235°C
Detector temperature: 250°C

RESULTS

Sample (oC)	Average % Compound Recovered (a)
-20	10.1±0.2
5	10.1±0.2
25	10.0±0.2
45	9.8±0.2

(a) Corrected for a spike recovery value of 100%±0.6%.

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

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

APPENDIX H

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF DI(2-ETHYLHEXYL)ADIPATE

APPENDIX H

Analyses of Formulated Diets for Concentrations of Di(2-ethylhexyl)adipate

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 80/100 Supelcoport glass column at 240°C, isothermal.

Theoretical Concentration (ppm)	Number of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
12,000	13	11,885	4.0	11,100-12,600
25,000	13	25,492	5.9	23,100-28,600

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