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**BIOASSAY OF
PIPERONYL BUTOXIDE
FOR POSSIBLE CARCINOGENICITY**

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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BIOASSAY OF
PIPERONYL BUTOXIDE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
^{U.S.} National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

Technical report series

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DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

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REPORT ON BIOASSAY OF PARATHION FOR POSSIBLE CARCINOGENICITY

Availability

Parathion (CAS 56-38-2) has been tested for cancer-causing activity with rats and mice in the Bioassay Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay for possible carcinogenicity of technical-grade parathion was conducted by administering the test chemical in the diet to Osborne-Mendel rats and B6C3F1 mice. Applications of the chemical include use as a pesticide.

It is concluded that under the conditions of this bioassay, parathion was not carcinogenic to B6C3F1 mice. In the male and female Osborne-Mendel rats receiving parathion in their diet, there was a higher incidence of cortical tumors of the adrenal than in pooled or historical controls, suggesting that parathion is carcinogenic to this strain of rat.

Single copies of the report are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated:
November 16, 1978

Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)



BIOASSAY OF
PIPERONYL BUTOXIDE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

FOREWORD: This report presents the results of the bioassay of piperonyl butoxide conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of piperonyl butoxide was conducted by Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc., Kensington, Maryland (3).

The manager of the bioassay at FCRC was Dr. D. Creasia. The program manager was Dr. B. Ulland, and the toxicologist was Dr. E. Gordon. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for the management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats were performed by Dr. R. A. Renne (4,5), and the histopathologic evaluations for mice were performed by Dr. C. E. Gilmore (4). The diagnoses included in this report represent the interpretations of Drs. Renne and Gilmore.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (6). Statistical analyses were performed by Dr. J. R. Joiner (7) and Ms. P. L. Yong (7), using methods selected for the bioassay program by Dr. J. J. Gart (8).

The chemicals used in this bioassay were analyzed at Frederick Cancer Research Center by Dr. W. Zielinsky (1). The results of these analyses were reviewed by Dr. C. W. Jameson (7) and Ms. P. M. Wagner (7).

This report was prepared at Tracor Jitco (7) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. M. S. King, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI (2) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Dawn G. Goodman (9), Dr. Richard A. Griesemer, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire (10), Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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[The text in this section is extremely faint and illegible. It appears to be a list of items or a table with multiple columns and rows, but the specific content cannot be discerned.]

SUMMARY

A bioassay of technical-grade piperonyl butoxide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats of each sex were administered piperonyl butoxide in the diet at one of two doses, either 5,000 or 10,000 ppm, for 107 weeks. Matched controls consisted of 20 untreated rats of each sex. All surviving rats were killed at the end of the period of administration of the test chemical.

Groups of 50 mice of each sex were initially administered piperonyl butoxide at one of two doses, either 2,500 or 5,000 ppm. After week 30, the doses for the mice were reduced to 500 and 2,000 ppm, respectively, and administration of the test chemical at the lowered doses was continued for 82 weeks. The time-weighted average doses for the mice were either 1,036 or 2,804 ppm. Matched controls consisted of 20 untreated mice of each sex. All surviving mice were killed at the end of the period of administration of the test chemical.

Mean body weights of dosed groups of rats and mice of each sex were lower than those of corresponding control groups, and the depressions in body weights were dose related. Survival of the rats and mice was unaffected by the piperonyl butoxide and was 80% or greater in all groups at week 90 of the bioassay; thus, sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the female rats, lymphomas occurred at incidences that were dose related ($P = 0.007$); in a direct comparison, the incidence of the tumor in the high-dose group was higher ($P = 0.020$) than that in the control group (controls 1/20, low-dose 7/50, high-dose 15/50). However, the incidence of lymphomas, leukemias, and reticuloses in historical-control female Fischer 344 rats at the same laboratory was 19/191 (10%). These historical-control groups include one with an incidence of animals with lymphoma or leukemia of 7/20 (35%) and another with an incidence of 6/20 (30%). Thus, the incidence of lymphomas in the control female rats of the present bioassay may have been abnormally low, and the occurrence of the higher incidence in the dosed groups cannot be clearly related to administration of piperonyl butoxide.

In the male mice, adenomas of the lacrimal gland occurred at incidences that were dose related ($P = 0.023$), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 0/49, high-dose 4/50); thus, the occurrence of this tumor in the male mice was not clearly related to administration of the test chemical.

It is concluded that under the conditions of this bioassay, piperonyl butoxide was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

TABLE OF CONTENTS

	<u>Page</u>
I. Introduction	1
II. Materials and Methods	3
A. Chemical	3
B. Dietary Preparation	4
C. Animals	4
D. Animal Maintenance	5
E. Subchronic Studies	7
F. Chronic Studies	10
G. Clinical and Pathologic Examinations	13
H. Data Recording and Statistical Analyses	14
III. Results - Rats	21
A. Body Weights and Clinical Signs (Rats)	21
B. Survival (Rats)	21
C. Pathology (Rats)	24
D. Statistical Analyses of Results (Rats)	31
IV. Results - Mice	33
A. Body Weights and Clinical Signs (Mice)	33
B. Survival (Mice)	33
C. Pathology (Mice)	36
D. Statistical Analyses of Results (Mice)	38
V. Discussion	41
VI. Bibliography	45

APPENDIXES

Appendix A	Summary of the Incidence of Neoplasms in Rats Administered Piperonyl Butoxide in the Diet	49
Table A1	Summary of the Incidence of Neoplasms in Male Rats Administered Piperonyl Butoxide in the Diet	51

		<u>Page</u>
Table A2	Summary of the Incidence of Neoplasms in Female Rats Administered Piperonyl Butoxide in the Diet	55
Appendix B	Summary of the Incidence of Neoplasms in Mice Administered Piperonyl Butoxide in the Diet	59
Table B1	Summary of the Incidence of Neoplasms in Male Mice Administered Piperonyl Butoxide in the Diet	61
Table B2	Summary of the Incidence of Neoplasms in Female Mice Administered Piperonyl Butoxide in the Diet	65
Appendix C	Summary of the Incidence of Nonneoplastic Lesions in Rats Administered Piperonyl Butoxide in the Diet	69
Table C1	Summary of the Incidence of Nonneoplastic Lesions in Male Rats Administered Piperonyl Butoxide in the Diet	71
Table C2	Summary of the Incidence of Nonneoplastic Lesions in Female Rats Administered Piperonyl Butoxide in the Diet	75
Appendix D	Summary of the Incidence of Nonneoplastic Lesions in Mice Administered Piperonyl Butoxide in the Diet	79
Table D1	Summary of the Incidence of Nonneoplastic Lesions in Male Mice Administered Piperonyl Butoxide in the Diet	81
Table D2	Summary of the Incidence of Nonneoplastic Lesions in Female Mice Administered Piperonyl Butoxide in the Diet	85
Appendix E	Analyses of the Incidence of Primary Tumors in Rats Administered Piperonyl Butoxide in the Diet	89

	<u>Page</u>
Table E1	Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet 91
Table E2	Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Butoxide in the Diet 97
Appendix F	Analyses of the Incidence of Primary Tumors in Mice Administered Piperonyl Butoxide in the Diet 101
Table F1	Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet 103
Table F2	Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Butoxide in the Diet 108

TABLES

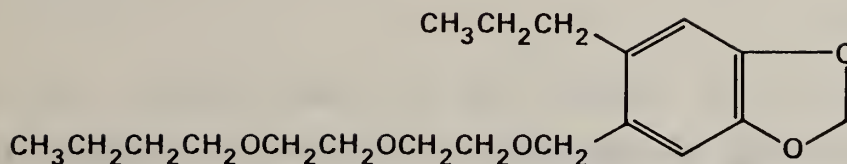
Table 1	Piperonyl Butoxide Subchronic Feeding Studies in Rats and Mice 9
Table 2	Piperonyl Butoxide Chronic Feeding Studies in Rats 11
Table 3	Piperonyl Butoxide Chronic Feeding Studies in Mice 12

FIGURES

Figure 1	Growth Curves for Rats Administered Piperonyl Butoxide in the Diet 22
Figure 2	Survival Curves for Rats Administered Piperonyl Butoxide in the Diet 23
Figure 3	Growth Curves for Mice Administered Piperonyl Butoxide in the Diet 34
Figure 4	Survival Curves for Mice Administered Piperonyl Butoxide in the Diet 35

1	Introduction	1
2	Chapter I	2
3	Chapter II	3
4	Chapter III	4
5	Chapter IV	5
6	Chapter V	6
7	Chapter VI	7
8	Chapter VII	8
9	Chapter VIII	9
10	Chapter IX	10
11	Chapter X	11
12	Chapter XI	12
13	Chapter XII	13
14	Chapter XIII	14
15	Chapter XIV	15
16	Chapter XV	16
17	Chapter XVI	17
18	Chapter XVII	18
19	Chapter XVIII	19
20	Chapter XIX	20
21	Chapter XX	21
22	Chapter XXI	22
23	Chapter XXII	23
24	Chapter XXIII	24
25	Chapter XXIV	25
26	Chapter XXV	26
27	Chapter XXVI	27
28	Chapter XXVII	28
29	Chapter XXVIII	29
30	Chapter XXIX	30
31	Chapter XXX	31
32	Chapter XXXI	32
33	Chapter XXXII	33
34	Chapter XXXIII	34
35	Chapter XXXIV	35
36	Chapter XXXV	36
37	Chapter XXXVI	37
38	Chapter XXXVII	38
39	Chapter XXXVIII	39
40	Chapter XXXIX	40
41	Chapter XL	41
42	Chapter XLI	42
43	Chapter XLII	43
44	Chapter XLIII	44
45	Chapter XLIV	45
46	Chapter XLV	46
47	Chapter XLVI	47
48	Chapter XLVII	48
49	Chapter XLVIII	49
50	Chapter XLIX	50
51	Chapter L	51
52	Chapter LI	52
53	Chapter LII	53
54	Chapter LIII	54
55	Chapter LIV	55
56	Chapter LV	56
57	Chapter LVI	57
58	Chapter LVII	58
59	Chapter LVIII	59
60	Chapter LIX	60
61	Chapter LX	61
62	Chapter LXI	62
63	Chapter LXII	63
64	Chapter LXIII	64
65	Chapter LXIV	65
66	Chapter LXV	66
67	Chapter LXVI	67
68	Chapter LXVII	68
69	Chapter LXVIII	69
70	Chapter LXIX	70
71	Chapter LXX	71
72	Chapter LXXI	72
73	Chapter LXXII	73
74	Chapter LXXIII	74
75	Chapter LXXIV	75
76	Chapter LXXV	76
77	Chapter LXXVI	77
78	Chapter LXXVII	78
79	Chapter LXXVIII	79
80	Chapter LXXIX	80
81	Chapter LXXX	81
82	Chapter LXXXI	82
83	Chapter LXXXII	83
84	Chapter LXXXIII	84
85	Chapter LXXXIV	85
86	Chapter LXXXV	86
87	Chapter LXXXVI	87
88	Chapter LXXXVII	88
89	Chapter LXXXVIII	89
90	Chapter LXXXIX	90
91	Chapter LXXXX	91
92	Chapter LXXXXI	92
93	Chapter LXXXXII	93
94	Chapter LXXXXIII	94
95	Chapter LXXXXIV	95
96	Chapter LXXXXV	96
97	Chapter LXXXXVI	97
98	Chapter LXXXXVII	98
99	Chapter LXXXXVIII	99
100	Chapter LXXXXIX	100
101	Chapter LXXXXX	101

I. INTRODUCTION



Piperonyl butoxide

Piperonyl butoxide (CAS 51-03-6; NCI C02813), 5-((2-(2-butoxyethoxy)ethoxy)-methyl-6-propyl-1,3-benzodioxole), is used to enhance the insecticidal properties of the pyrethrins, by blocking the pyrethrin detoxification enzymes in the insect (Metcalf, 1966). Pyrethrins alone produce a very rapid knockdown of insects, followed by substantial recovery, whereas addition of a synergist such as piperonyl butoxide decreases the insecticidal dose of pyrethrin (Metcalf, 1966). Piperonyl butoxide is also formulated with synthetic pyrethrin analogues, such as allethrin and tetramethrin (Stanford Research Institute, 1976).

Piperonyl butoxide has commercial importance as a synergist for

insecticides. Approximately 1 million pounds of this chemical were used in the United States in 1974, of which 80% was used in commercial, domestic (house and garden), and industrial establishments, and 20% on livestock and poultry (Ayers and Johnson, 1976).

The acute oral LD₅₀ of piperonyl butoxide has been reported as 11.5 ml/kg for rats of unspecified strain (Draize et al., 1948) and as between 7.5 and 10.0 gm/kg for Wistar rats (Sarles et al., 1949); that for mice (strains not specified) has been reported as 8.3 ml/kg (Draize et al., 1948) and as 3,800 mg/kg (Kenaga and Allison, 1969).

The long-term toxicity of piperonyl butoxide was investigated by Innes et al. (1969) as a part of a large-scale test of industrial and agricultural chemicals. These investigators did not obtain a significant incidence of tumors in either the (C57BL/6 x C₃H/Anf)F1 or the (C57BL/6 x AKR)F1 hybrid mice, the only species tested, and they categorized this chemical among those that probably required further testing. On the basis of these preliminary results, piperonyl butoxide was selected for study in the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

The test chemical used in the bioassay was obtained as a 91-kilogram batch of Lot No. 5 technical-grade piperonyl butoxide from Niagara Chemical Company, FMC Corporation, Middleport, New York. Gas chromatographic (gc) analysis performed at Frederick Cancer Research Center, Frederick, Maryland, showed three major components and some minor ones totaling less than 1% each. As identified by the manufacturer, the three major components and their respective percentages of the total gc peak area were piperonyl butoxide, 88.4%; butyl carbitol, 2.1%; and an isomer of piperonyl butoxide, 2.4%. The infrared absorption spectra were identical with that of an authentic standard. The technical-grade piperonyl butoxide as described above will hereinafter be referred to in this report as piperonyl butoxide.

The piperonyl butoxide was stored at 7°C until used.

B. Dietary Preparation

Test diets containing piperonyl butoxide were prepared fresh each week in 6- to 12-kilogram batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The mixing was repeated with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender.

The diets were stored at 7°C in plastic bags during the 1 to 1-1/2 weeks it was used.

C. Animals

Male and female Fischer 344 rats and B6C3F1 mice were obtained from the Frederick Cancer Research Center (Frederick, Md.) as 4-week-old weanlings, all within 3 days of the same age. The animals were housed within the test facility for 2 weeks and then were assigned four rats to a cage and five mice to a cage by a system that averaged the weights per cage for a given species and sex. For use in the chronic study, the male rats were required

to weigh 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri[®] hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed supplied was autoclaved Wayne[®] Sterilizable Lab Meal with 4% fat, provided ad libitum in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied ad libitum from glass bottles and sipper tubes (Lab Products, Inc.) suspended through the tops of the cages.

Contaminated bedding was disposed of through an enclosed vacuum

line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized at 82-88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N. J.) twice per week, using the detergents, Clout® (Pharmaceutical Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The feed hoppers were sanitized twice per month in the same equipment. The glass bottles and sipper tubes were sanitized at 82-88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The air in the animal rooms was regulated automatically at a temperature of 22-24°C and a relative humidity of 45-55%. Nonrecirculated air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and through a "Z"-type roughing filter of 30% efficiency and a bag system of 90-95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). The rate of movement allowed 15 changes of room air per hour. The

air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided on a 12-hour-per-day cycle.

All control and dosed rats were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 999-81-5) chlorocholine chloride
(CAS 88-06-2) 2,4,6-trichlorophenol

All control and dosed mice were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 128-66-5) C. I. vat yellow 4 (amanthrene)
(CAS 103-33-3) azobenzene
(CAS 20941-65-5) ethyl tellurac
(CAS 88-06-2) 2,4,6-trichlorophenol
(CAS 72-56-0) p,p'-ethyl-DDD
(CAS 85-44-9) phthalic anhydride

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTDs) of piperonyl butoxide, on the basis of which two concentrations (hereinafter referred to as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats of each sex and five mice of each sex were administered feed containing piperonyl butoxide at one

of several doses, and groups of five control animals of each species and sex were administered a basal diet only. The doses used for the rats in the subchronic studies were 14,700, 21,500, 31,500, 46,000, and 68,000 ppm; the doses used for the mice in the subchronic studies were 4,600, 6,800, 10,000, 14,700, 21,500, 31,500, 46,000, and 68,000 ppm. The period of administration of the test chemical was 7 weeks, followed by 1 week of additional observation. Each animal was weighed twice per week. Table 1 shows the number of animals dying at each dose and the number of weeks on study when the deaths occurred; the table also shows the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of controls at week 7.

At the end of the subchronic studies, all animals were killed by CO₂ inhalation and necropsied. The lowest doses at which clinical and histopathologic findings were observed were 21,500 ppm in the male and female rats and 14,700 ppm in the male and female mice. In the rats, interstitial pneumonia, perivascular lymphoplasmacytosis, and peribronchiolar lymphocytosis were observed, but were not considered to be related to administration of the test chemical. In the mice, extramedullary hematopoiesis was observed in one male and five females, polypoid nuclei were observed in livers of two males and one female, and slight vacuolization of hepatocytes was observed in four females.

Table 1

Piperonyl Butoxide Subchronic Feeding Studies in Rats and Mice

(ppm)	Male			Female		
	<u>Mortality</u> Number <u>Dead</u>	<u>Week on</u> <u>Study</u>	<u>Mean Weight</u> <u>at Week 7</u> <u>as % of</u> <u>Control</u>	<u>Mortality</u> Number <u>Dead</u>	<u>Week on</u> <u>Study</u>	<u>Mean Weight</u> <u>at Week 7</u> <u>as % of</u> <u>Control</u>
<u>RATS</u>						
14,700			94			95
21,500			73			76
31,500			60			68
46,000	4	8	60	3	8	38
68,000	5	1		5	1	
<u>MICE</u>						
4,600			91			94
6,800			87			93
10,000			76	1	8	76
14,700	1	8	85			72
21,500	4	8	76	4	8	87
31,000	5	2		4	8	
46,000	5	2		5	2	
68,000	5	2		5	2	

Ten percent depression in body weight was taken as the major criterion for estimation of MTDs. The doses required to produce this response were determined by the following procedure: first, least squares regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of dosed groups at day 49 relative to weights of corresponding control groups were plotted against logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

When these data were evaluated in conjunction with the clinical and histopathologic findings, the low and high doses for chronic studies using rats were set at 5,000 and 10,000 ppm, and those for chronic studies using mice were set at 2,500 and 5,000 ppm.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 2 and 3. Doses administered to the mice were reduced after week 30 as indicated.

Table 2. Piperonyl Butoxide Chronic Feeding Studies in Rats

<u>Sex and Test Group</u>	<u>Initial No. of Animals (a)</u>	<u>Piperonyl Butoxide in Diet (b) (ppm)</u>	<u>Time on Study (weeks)</u>
<u>Male</u>			
Matched-Control	20	0	107
Low-Dose	50	5,000	107
High-Dose	50	10,000	107
<u>Female</u>			
Matched-Control	20	0	107
Low-Dose	50	5,000	107
High-Dose	50	10,000	107

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum.

Table 3. Piperonyl Butoxide Chronic Feeding Studies in Mice

<u>Sex and Test Group</u>	<u>Initial No. of Animals (a)</u>	<u>Piperonyl Butoxide in Diet (b) (ppm)</u>	<u>Time on Study (weeks)</u>	<u>Time-Weighted Average Dose (c) (ppm)</u>
<u>Male</u>				
Matched-Control	20	0	112	
Low-Dose	50	2,500	30	1,036
		500	82	
High-Dose	50	5,000	30	2,804
		2,000	82	
<u>Female</u>				
Matched-Control	20	0	112	
Low-Dose	50	2,500	30	1,036
		500	82	
High-Dose	50	5,000	30	2,804
		2,000	82	

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided ad libitum.

(c) Time-weighted average dose =
$$\frac{\Sigma(\text{dose (ppm)} \times \text{no. of weeks at that dose})}{\Sigma(\text{no. of weeks receiving each dose})}$$

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and animals that survived to the end of the bioassay were killed by asphyxiation using CO₂ and necropsied. Necropsies were also performed on all animals found dead, unless precluded by autolysis or severe cannibalization.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

A few tissues from some animals were not examined, particularly from those animals that may have died early, been missing, or been in advanced states of cannibalization or autolysis. 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.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, 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). Data tables were generated for verification of data transcription and for statistical review. These data were analyzed using the appropriate statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative section.

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

The incidence of neoplastic or nonneoplastic lesions has been given the ratio as 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 a number of dosed groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to $0.05/k$. In cases where 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.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, 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 sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship.

Significant departures from linearity (P less than 0.05, two-tailed test) were also noted.

The approximate 95 percent 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 relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

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 (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. 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.

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III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

A dose-related depression in the mean body weights of both dosed male and female rats, when compared with corresponding matched controls, was observed throughout the bioassay (figure 1). However, the depressions in the mean body weights of the low- and high-dose groups were slight during the first 50 weeks. Wasting among the rats and redness of the skin and mucous membranes occurred at low incidences in some of the dosed groups and may have been related to administration of the test chemical. Other clinical signs, including corneal opacity and tissue masses, occurred at comparable incidences in dosed and control groups.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered piperonyl butoxide in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

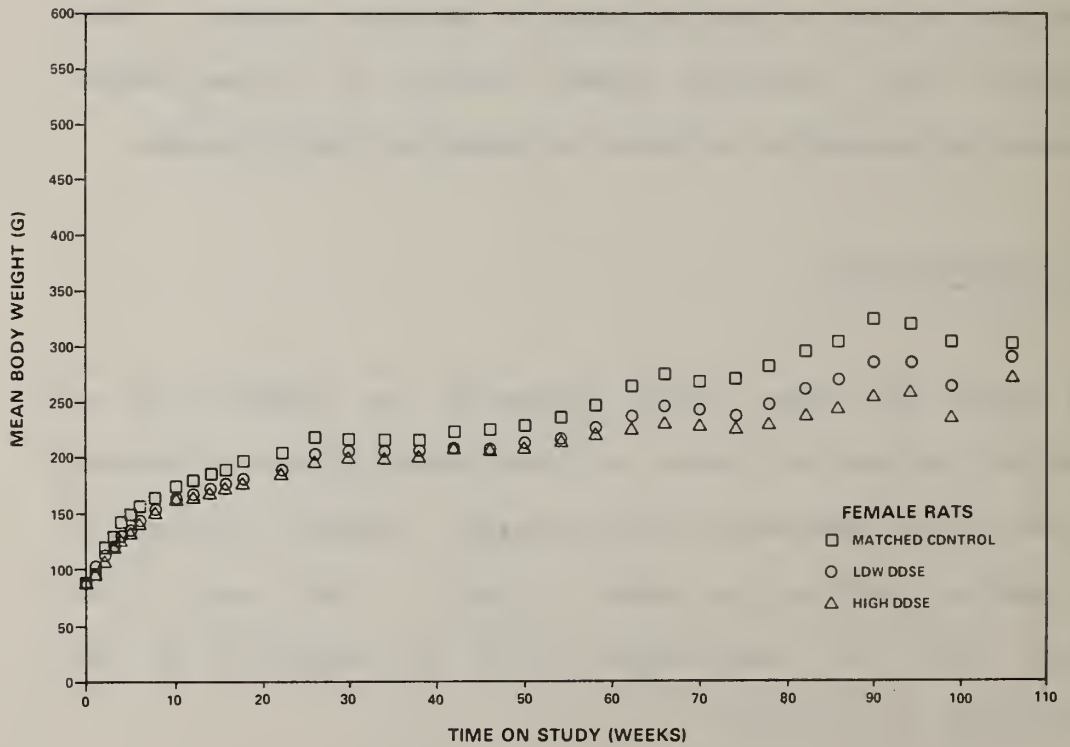
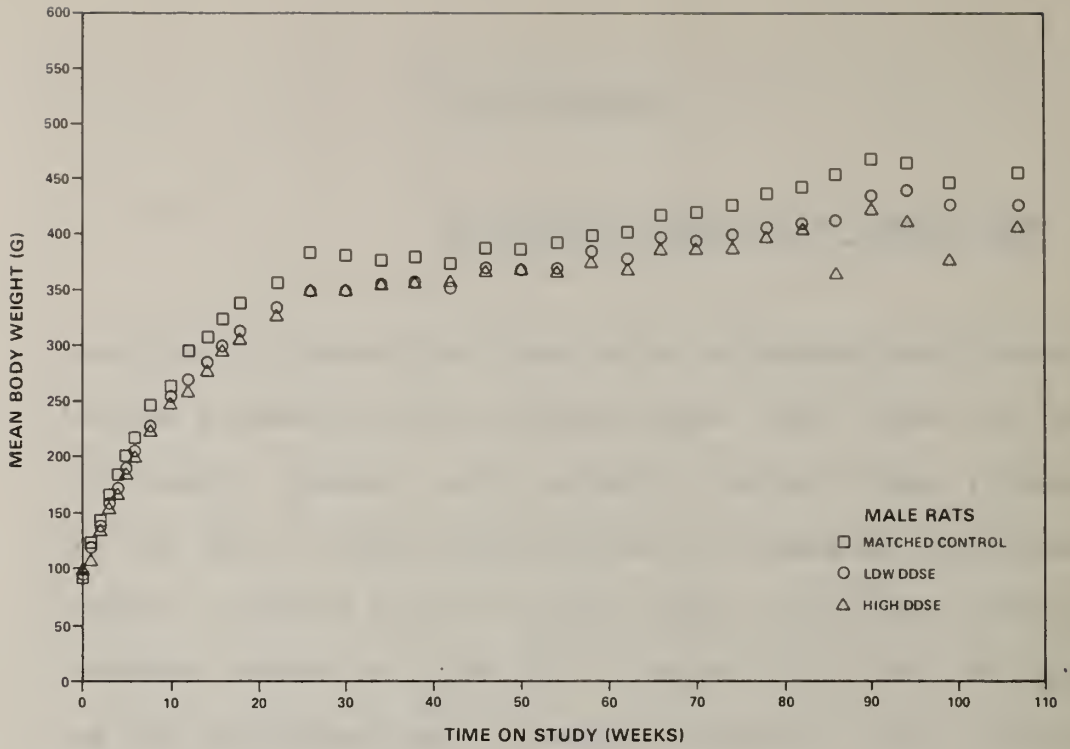


Figure 1. Growth Curves for Rats Administered Piperonyl Butoxide in the Diet

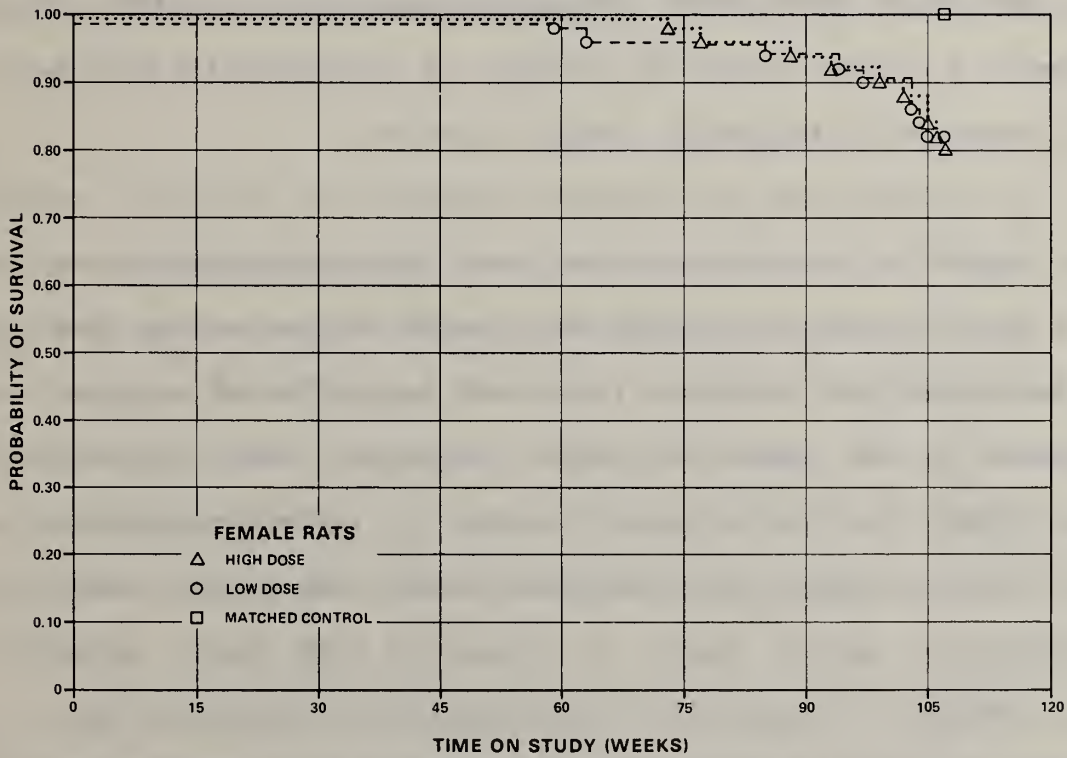
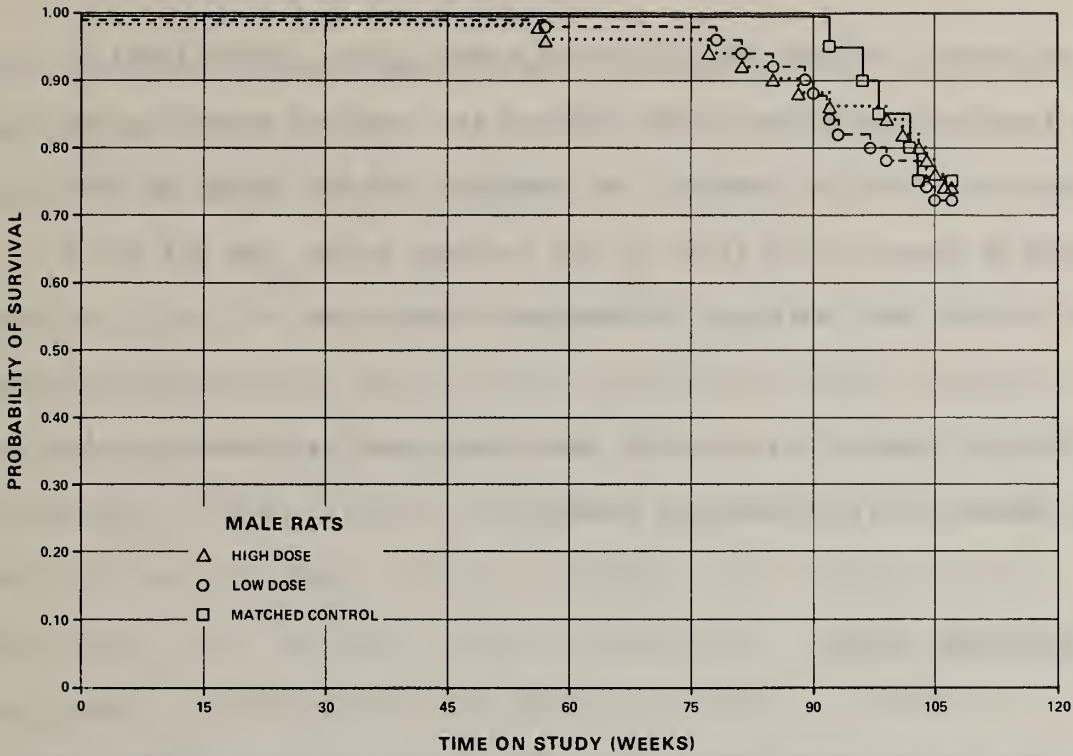


Figure 2. Survival Curves For Rats Administered Piperonyl Butoxide in the Diet

In male rats, 37/50 (74%) of the high-dose group, 36/50 (72%) of the low-dose group, and 15/20 (75%) of the controls were alive at termination of the study. In females, 40/50 (80%) of the high-dose group, 41/50 (82%) of the low-dose group, and all 20 of the controls were alive at termination of the study.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

With regard to liver lesions, the term "focal hyperplasia" was used in this study to indicate the presence of one or more foci of hepatocytes with increased cytoplasmic basophilia and a slight increase in the amount of nuclear chromatin. Many of these hepatocytes also had a slight increase in nuclear:cytoplasmic ratio when compared with adjacent normal hepatocytes, and, infrequently, mitotic figures or hepatocytes with double nuclei were observed. These foci of hyperbasophilic hepatocytes were thought to represent areas of hyperplasia and were diagnosed as

such in this study. They did not compress adjacent hepatic parenchyma. These lesions are similar morphologically to those described by Squire and Levitt (1975) as "basophilic foci."

Lesions classified as "hepatocytomegaly" consisted of foci of enlarged hepatocytes, many of which contained large, vesicular nuclei and numerous fine cytoplasmic vacuoles, which gave the cytoplasm a "ground glass" appearance. Distortion of lobular architecture in these foci was minimal, and trabeculae were continuous with adjacent normal hepatocytes. These lesions correspond morphologically to those described by Squire and Levitt (1975) as "eosinophilic foci," "ground glass foci," or "clear cell foci."

Lesions classified as "neoplastic nodules" had many similarities to the previously described proliferative hepatocytic lesions. However, the lesions classified as neoplastic nodules were larger and contained more distinct abnormality of lobular architecture; liver cords at the periphery of the neoplastic nodules were oriented perpendicular to cords of adjacent normal hepatic parenchyma, and distinct compression of adjacent normal liver was evident.

Endocrine tissues were the most frequent sites of neoplasms in

both dosed and control rats in this study. Interstitial-cell tumors of the testis were observed in nearly all male rats in all groups; a high spontaneous incidence of this tumor is characteristic of aged Fischer 344 rats. Adenomas of the pituitary were also found at a high incidence in all groups, especially females. Other endocrine neoplasms observed included follicular-cell and C-cell tumors of the thyroid, islet-cell tumors of the pancreas, and pheochromocytomas and cortical carcinomas of the adrenal.

In some proliferative endocrine lesions, differentiation between benign and malignant neoplasms was difficult. C-cell lesions of the thyroid were classified as adenomas when the proliferating C cells were present in nodular masses that widely separated the thyroid follicles and distorted the follicular architecture. In some of the larger adenomas, the C cells were present in interlacing bundles of elongated, spindling cells, rather than the polyhedral to spherical shape characteristic of normal C cells. When invasion of thyroid capsule, adjacent tissues, or vessels was present, or when metastasis was detected, the lesion was classified as a C-cell carcinoma.

Follicular-cell neoplasms occurred less frequently than C-cell neoplasms. The follicular-cell adenomas appeared microscopically

as well-circumscribed masses composed of enlarged follicles lined by hyperbasophilic follicular cells which were increased in number per unit area by papillary infolding of simple cuboidal or columnar epithelium into the follicular lumen and by stratification of follicular cells surrounding the lumen. Distinct compression of adjacent normal thyroid parenchyma, with some evidence of fibrous encapsulation, was present. Follicular-cell lesions were classified as carcinomas based upon the presence of anaplasia and histologic arrangement in disorderly nests and/or sheets. Areas with papillary patterns were also present. Fibrous stroma often intermingled with, but did not encapsulate, follicular-cell carcinomas.

The diagnosis of pheochromocytoma was made when the adrenal medullary lesion was present as a discrete mass that compressed adjacent normal adrenal parenchyma. These neoplasms were composed of sheets, nests, and/or cords of polyhedral to spherical cells with abundant, slightly basophilic cytoplasm and large nuclei with abundant chromatin. Islet-cell adenomas appeared as discrete, encapsulated nodules of islet cells that compressed the adjacent normal pancreas; diagnosis of islet-cell carcinoma in a high-dose male rat was based on invasion of the capsule surrounding the neoplasm.

Two neoplasms were observed in the intestinal tract. A malignant spindle-cell tumor was present in the wall of the cecum of a high-dose male rat. This was a large neoplasm that extended into the mucosa; the diagnosis of leiomyosarcoma was based on the location in the wall and the cellular morphology. A hemangiosarcoma was present in the wall of the large intestine of a low-dose female rat.

Malignant lymphomas occurred rather frequently. Most of these neoplasms were composed of relatively undifferentiated lymphoreticular cells and involved numerous organs and tissues throughout the body. The organ most frequently affected was the spleen; also frequently affected were the liver, lymph nodes, thymus, and lungs. There was only one malignant lymphoma in the control group of female rats in this study; this is a relatively common tumor in Fischer 344 rats. Evidence of leukemia (masses of neoplastic lymphoreticular cells in vessel lumens) was seen in some cases.

Proliferative pulmonary lesions were observed rather infrequently in both dosed and control groups. Differentiation between adenomas and carcinomas was based on degree of anaplasia, mitotic index, size of the neoplasm, and presence of apparent invasion of adjacent pulmonary parenchyma in carcinomas, as opposed to mere

compression of adjacent parenchyma, and thus, a more discrete lesion in adenomas.

The most frequently occurring neoplasm of the reproductive tract, other than the previously mentioned interstitial-cell tumor of the testis, was the endometrial stromal polyp of the uterus. This lesion was present as a discrete mass protruding into the lumen of the uterus, lined by endometrium, and sometimes associated with suppurative endometritis and/or cystic endometrial hyperplasia. The stroma was usually proliferating in a rather loosely woven pattern, with numerous small vessels interspersed among stromal cells.

Mesothelioma of the tunica vaginalis was observed in sections of testis from four male rats, all of which were from dosed groups. One generalized peritoneal mesothelioma was present in a low-dose male rat.

Squamous-cell carcinomas were observed in the preputial gland of a high-dose male rat and in the clitoral gland of a high-dose female.

The most common neoplasms of the mammary gland were

fibroadenomas; these occurred only in females, were often multiple, and were seen in both dosed and control groups.

Various other types of malignant and benign neoplasms were observed at low incidences in sections of skin and subcutis, and in other organs and tissues throughout the body. No apparent difference in incidence of these neoplasms between dosed and control groups was present.

Numerous inflammatory, degenerative, and proliferative lesions commonly observed in aged Fischer 344 rats occurred with approximately equal frequency in dosed and control rats. These lesions included chronic tracheitis; multifocal alveolar macrophage aggregates in lung parenchyma; chronic nephritis with scarring, tubular dilatation, and tubular regeneration; ectasia of lymph sinuses in the mesenteric lymph nodes; testicular atrophy; and C-cell hyperplasia of the thyroid.

Other nonneoplastic proliferative lesions included hyperplasia of the follicular cells of the thyroid, adrenal medulla and cortex, parathyroid, endometrium, mammary epithelium, and hepatocytes.

Based on the histopathologic examination, there were instances in this study in which neoplastic or hyperplastic lesions occurred

only in dosed animals, or with increased frequency in dosed animals when compared with control groups. However, the nature, incidence, and severity of the lesions observed provide no clear evidence of carcinogenic effect of piperonyl butoxide in Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In female rats, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of lymphomas is significant ($P = 0.007$). The results of the Fisher exact test show that the incidence in the high-dose group is significantly higher ($P = 0.020$) than that in the control group. The historical record of the incidence of lymphomas and leukemias in female rats at this laboratory to date is 19/191(10%). These historical-control groups include one with an incidence of animals with lymphoma or leukemia of 7/20 (35%) and another with an incidence of 6/20 (30%). The statistical conclusion suggests

that the incidence of lymphomas in female rats may be associated with the administration of piperonyl butoxide; however, this conclusion may be due to the lower than usual incidence in the control group compared with the historical data.

Significant results in the negative direction are observed in the incidences of neoplastic nodules of the liver and of adenomas or carcinomas of the pituitary in the male rats; the incidences of these tumors in the control group exceed those in the dosed groups.

In each of the 95% confidence intervals for relative risk shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

A dose-related depression in the mean body weights of both dosed male and female mice, when compared with corresponding matched controls, was observed throughout the bioassay (figure 3). Alopecia occurred at low incidences in the dosed groups of females and may have been related to administration of the test chemical. Corneal opacity and tissue masses occurred at comparable incidences in dosed and control groups.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered piperonyl butoxide in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 4. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male mice, 41/50 (82%) of the high-dose group, 42/50 (84%) of

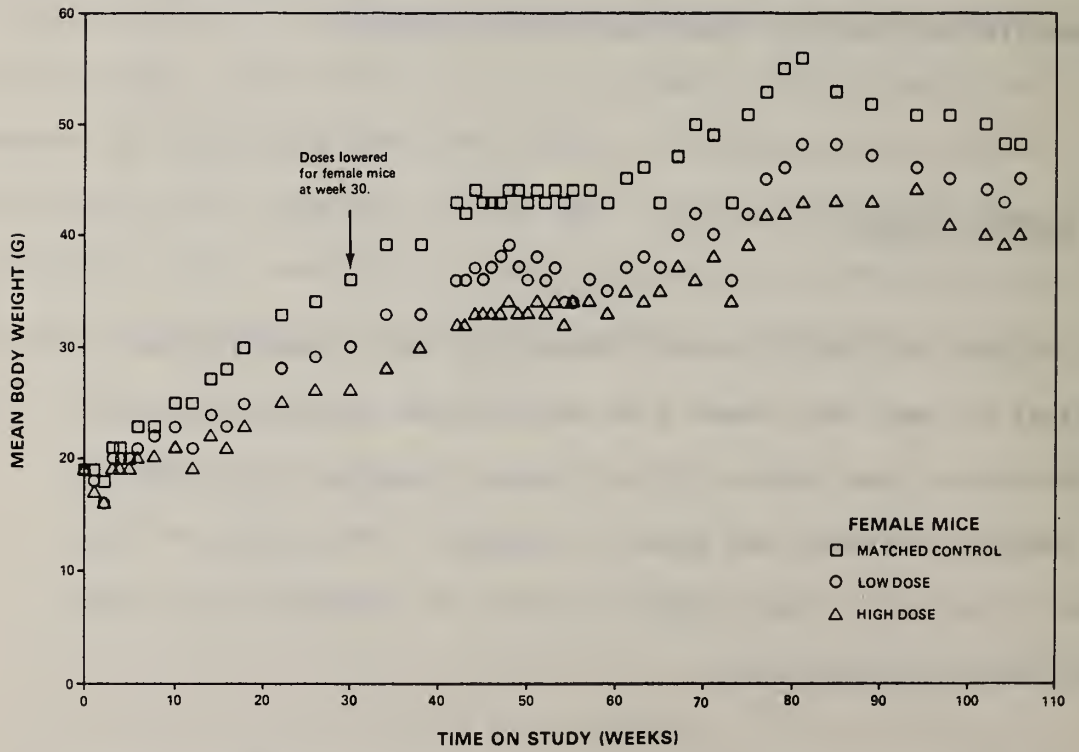
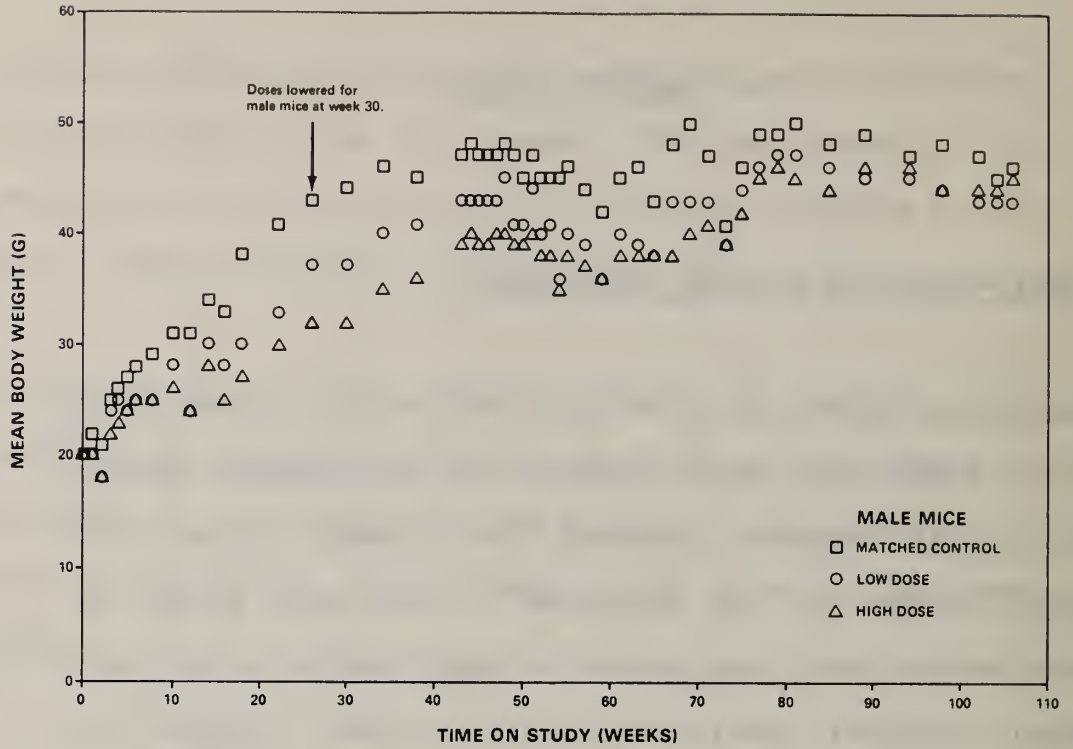


Figure 3. Growth Curves for Mice Administered Piperonyl Butoxide in the Diet

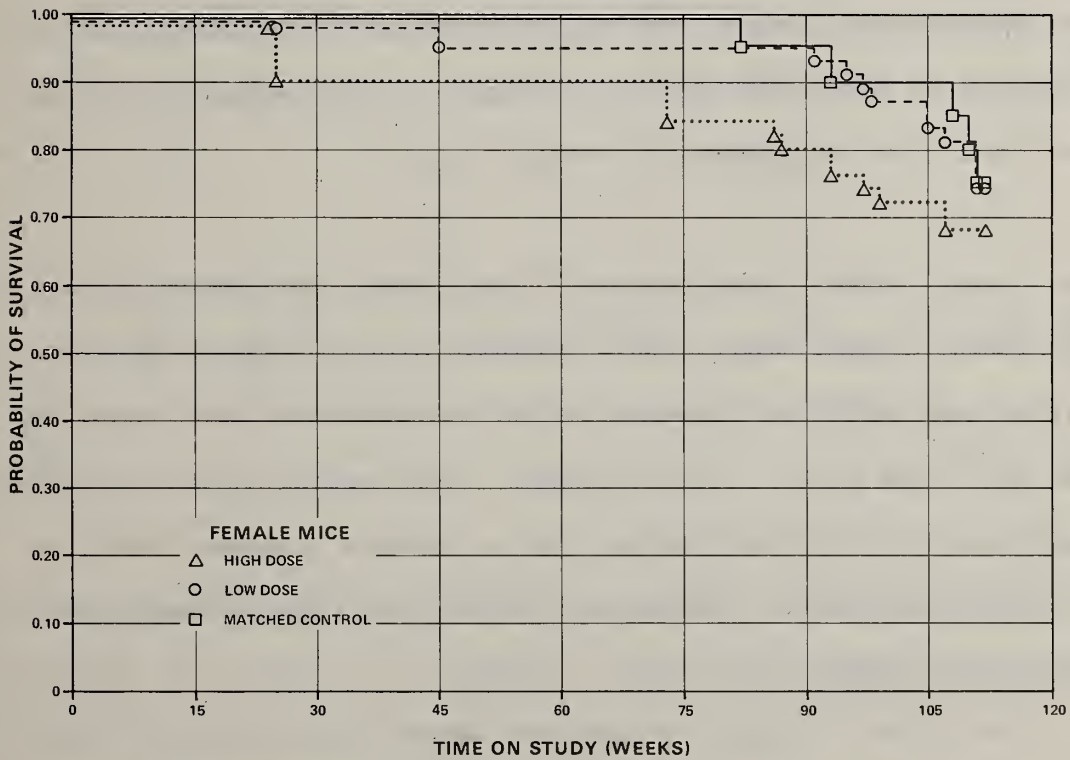
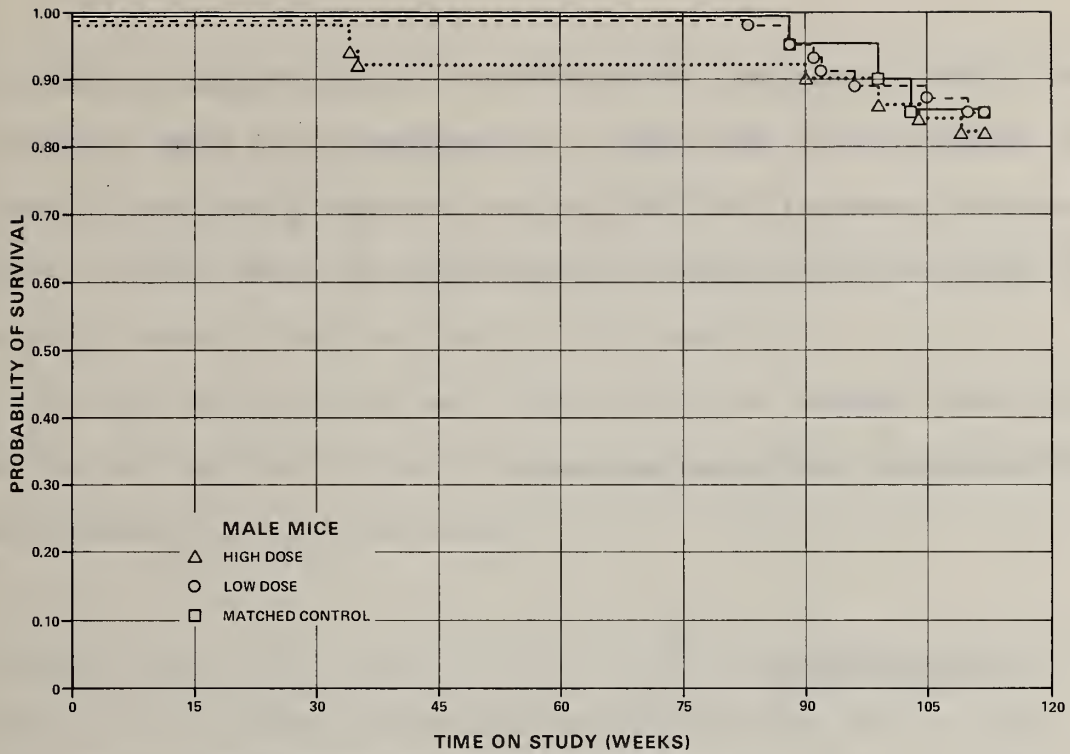


Figure 4. Survival Curves For Mice Administered Piperonyl Butoxide in the Diet

the low-dose group, and 17/20 (85%) of the control group survived to termination of the study. In females, 34/50 (68%) of the high-dose group, 35/50 (70%) of the low-dose group, and 15/20 (75%) of the control group lived to termination of the study.

Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

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.

The most common neoplasm in male mice was hepatocellular carcinoma. There were 10/20 in control males, 17/50 in low-dose males, and 20/50 in high-dose males. Metastases were found in two mice from each of these groups. There was one hepatocellular carcinoma in a control female, two in low-dose females, and five in high-dose females. Metastases to the lungs were found in one high-dose female.

The next most common tumors were of lymphoreticular origin

(malignant lymphoma and reticulum-cell sarcoma). Their incidences were as follows: males — controls 4/20, low-dose 11/49, high-dose 6/50; females — controls 8/20, low-dose 14/47, high-dose 9/50. Many of these tumors were widely disseminated, involving several organs and forming tissue masses.

One low-dose male had a widely disseminated round-cell tumor that was diagnosed as a mast-cell tumor.

Hemangiosarcomas were found in one control male, one control female, three low-dose males, two low-dose females, and in one male and one female in the high-dose groups. Both of the tumors in the low-dose females and the one in a high-dose male were in multiple organs.

There were occasional alveolar-cell adenomas and adenocarcinomas of the lung. They were distributed with approximately equal frequency between males and females and between dosed and control groups.

Most other proliferative or neoplastic lesions were of single occurrence or very low incidence with approximately equal distribution between the dosed and control groups. There were

four adenomas of the lacrimal gland in high-dose males; none were found in any of the female mice.

In addition to the proliferative lesions, there was a scattering of inflammatory and degenerative changes in some mice in each group. These included focal mineralization of the brain, myocarditis, nephritis, focal hepatic necrosis, pancreatic atrophy, cystic endometrium, and ovarian cysts.

Based on the histopathologic examination, there were no differences in the incidences of neoplastic or nonneoplastic lesions between dosed and control mice; it is concluded that the dietary administration of piperonyl butoxide was not carcinogenic in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of adenomas of the lacrimal gland is significant ($P = 0.022$), but the results of the Fisher exact test are not significant.

A significant dose-related trend in the negative direction is observed for the incidence of lymphomas in female mice; the incidence of this tumor in the control group exceeds the incidences in the dosed groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by piperonyl butoxide, which could not be detected under the conditions of this test.

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V. DISCUSSION

Dose-related depressions in mean body weight occurred during most or all of the bioassay in both rats and mice administered piperonyl butoxide. Redness of the skin and mucous membranes and wasting among the rats and alopecia among the mice occurred at low incidences in some of the dosed groups and may have been related to administration of the test chemical. Survival of the rats and mice was unaffected by the piperonyl butoxide and was 80% or greater in all groups at week 90 of the bioassay; thus, sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the female rats, lymphomas occurred at incidences that were dose-related ($P = 0.007$); in a direct comparison, the incidence of the tumor in the high-dose group was higher ($P = 0.020$) than that in the control group (controls 1/20, low-dose 7/50, high-dose 15/50). However, the incidence of lymphomas, leukemias, and reticuloses in historical-control female Fischer 344 rats at the same laboratory was 19/191 (10%). These historical-control groups include one with an incidence of animals with lymphoma or leukemia of 7/20 (35%) and another with incidence of 6/20 (30%). Thus, the incidence of lymphomas in the

control female rats of the present bioassay may have been abnormally low, and the occurrence of the higher incidence in the dosed groups cannot be clearly related to administration of the piperonyl butoxide.

In the male mice, adenomas of the eye or lacrimal gland occurred at incidences that were dose related ($P = 0.023$), but in direct comparisons the incidences in the individual dosed groups were not significantly higher than that in the control group (controls 0/20, low-dose 0/49, high-dose 4/50); thus, the occurrence of this tumor in the male mice was not clearly related to administration of the test chemical.

When piperonyl butoxide was administered in the diet for 2 years to Wistar albino rats at doses as high as 25,000 ppm (Sarles and Vandegrift, 1952), no evidence was found for carcinogenicity. When the chemical was administered at 464 mg/kg by stomach tube for 3 weeks, then in the diet at 1,112 ppm for 18 months, to hybrid mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR), an elevated incidence of reticulum-cell sarcoma was observed ($P = 0.05$) but was not significant at the selected criterion of $P = 0.01$ (NTIS, 1968; Innes et al., 1969). No evidence of carcinogenicity was observed when neonatal Swiss mice were given subcutaneous injections of 0.1 ml of a 5% solution of the chemical in

redistilled tricaprylin at days 1 and 7 and 0.2 ml at days 14 and 21; however, when the chemical was injected subcutaneously into the neonatal mice in combination with Freon 112, hepatomas developed at a statistically significant incidence (Epstein et al., 1967). Certain structural congeners of piperonyl butoxide (safrole, isosafrole, and dihydrosafrole) have been reported carcinogenic for rats (Osborne-Mandel) and mice, (C57BL/6 x C₃H/Anf and C57BL/6 x AKR) inducing tumors of the liver, esophagus, or lung, depending on species and sex (Long et al., 1963; Hagan et al., 1965; Innes et al., 1969).

Piperonyl butoxide is used commercially with pyrethins. This bioassay, however, tests the carcinogenicity of technical-grade piperonyl butoxide alone, and no conclusions can be drawn from the data in this report as to the possible carcinogenic effects of the combination of the two chemicals.

It is concluded that under the conditions of this bioassay, there was no convincing evidence that piperonyl butoxide was carcinogenic for Fischer 344 rats or B6C3F1 mice.

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APPENDIX A

**SUMMARY OF THE INCIDENCE OF NEOPLASMS IN
RATS ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET**

TABLE A1.

**SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET**

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
KERATOACANTHOMA	1 (5%)	1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		2 (4%)	3 (6%)
FIBROSARCOMA		1 (2%)	1 (2%)
LIPOMA		1 (2%)	
LIPOSARCOMA			1 (2%)
NEUPILEMOMA, MALIGNANT			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	3 (6%)	3 (6%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	7 (35%)	12 (24%)	9 (18%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (5%)		
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(49)
NEOPLASTIC NODULE	2 (10%)		
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#CECUM LEIOMYOSARCOMA	(20)	(49)	(48) 1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(49)	(48)
CARCINOMA, NOS			1 (2%)
ADENOMA, NOS	4 (21%)		
CHROMOPHOBE ADENOMA	1 (5%)	7 (14%)	6 (13%)
#ADRENAL	(20)	(50)	(50)
CORTICAL ADENOMA	1 (5%)		
PHEOCHROMOCYTOMA	2 (10%)	4 (8%)	4 (8%)
#THYROID	(20)	(49)	(50)
FOLLICULAR-CELL ADENOMA			2 (4%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	1 (2%)
C-CELL ADENOMA	1 (5%)	5 (10%)	4 (8%)
C-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(18)	(44)	(48)
ISLET-CELL ADENOMA	1 (6%)	4 (9%)	2 (4%)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
#TESTIS	(20)	(49)	(50)
INTERSTITIAL-CELL TUMOR	19 (95%)	48 (98%)	46 (92%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (5%)		
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS		2 (4%)	2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	11	8
MORIBUND SACRIFICE	1	3	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	36	37
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	50	49
TOTAL PRIMARY TUMORS	42	94	92
TOTAL ANIMALS WITH BENIGN TUMORS	19	49	47
TOTAL BENIGN TUMORS	30	72	69
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	17	19
TOTAL MALIGNANT TUMORS	10	19	21
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	2	3	2
TOTAL UNCERTAIN TUMORS	2	3	2
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
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET**

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
* SUBCUT TISSUE	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROMA			1 (2%)
NEURILEMOMA			1 (2%)
RESPIRATORY SYSTEM			
# LUNG	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
# BRAIN	(20)	(50)	(50)
MALIGNANT RETICULOSIS			1 (2%)
* MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	6 (12%)
MALIG. LYMPHOMA, UNDIFFER-TYPE	1 (5%)	5 (10%)	9 (18%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	
# SPLEEN	(18)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
CIRCULATORY SYSTEM			
# HEART	(20)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
DIGESTIVE SYSTEM			
# CECUM	(19)	(50)	(48)
HEMANGIOSARCOMA		1 (2%)	
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*PITUITARY	(19)	(48)	(49)
CARCINOMA, NOS	1 (5%)	1 (2%)	
CHROMOPHOBE ADENOMA	8 (42%)	11 (23%)	14 (29%)
*ADRENAL	(20)	(50)	(50)
CORTICAL CARCINOMA	1 (5%)		2 (4%)
PHEOCHROMOCYTOMA		1 (2%)	
*THYROID	(20)	(50)	(49)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	4 (20%)	4 (8%)	2 (4%)
C-CELL CARCINOMA		2 (4%)	1 (2%)
*PANCREATIC ISLETS	(20)	(49)	(46)
ISLET-CELL ADENOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CARCINOMA, NOS		1 (2%)	
FIBROMA		1 (2%)	1 (2%)
FIBROADENOMA	3 (15%)	8 (16%)	2 (4%)
*CLITORAL GLAND	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
*UTERUS	(20)	(49)	(47)
ENDOMETRIAL STROMAL POLYP	4 (20%)	7 (14%)	3 (6%)
NEPVOUS SYSTEM			
*BRAIN	(20)	(50)	(50)
EPENDYMOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
* NUMBFR OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE SARCOMA, NOS	(20)	(50)	(50) 1 (2%)
*MUSCLE OF NECK RHABDOMYOSARCOMA	(20)	(50) 1 (2%)	(50)
BODY CAVITIES			
*MEDIASTINUM SQUAMOUS CELL CARCINOMA, INVASIV	(20)	(50)	(50) 1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		5	8
MORIBUND SACRIFICE		4	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	20	41	40
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LDW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	33	33
TOTAL PRIMARY TUMORS	23	47	48
TOTAL ANIMALS WITH BENIGN TUMORS	14	21	21
TOTAL BENIGN TUMORS	20	32	24
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	14	21
TOTAL MALIGNANT TUMORS	3	15	24
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN
MICE ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

[The text in this section is extremely faint and illegible, appearing to be a list or table of contents.]

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(49)	(50)
LIPOMA	2 (10%)	3 (6%)	2 (4%)
HEMANGIOSARCOMA		1 (2%)	
NEUROFIBROSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
HEPATOCELLULAR CARCINOMA, METAST	2 (10%)	2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	3 (6%)	6 (13%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (15%)	3 (6%)	2 (4%)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		3 (6%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	1 (2%)
MAST-CELL SARCOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(49)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	1 (2%)	
#SPLEEN	(20)	(49)	(49)
HEMANGIOSARCOMA		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
*MESPENTERIC L. NODE	(20)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*SMALL INTESTINE	(20)	(50)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	3 (15%)	4 (8%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA	10 (50%)	17 (34%)	20 (40%)
HEMANGIOSARCOMA	1 (5%)	1 (2%)	1 (2%)
*ESOPHAGUS	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (5%)		
URINARY SYSTEM			
*KIDNEY	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ENDOCRINE SYSTEM			
*THYROID	(20)	(49)	(50)
ADENOCARCINOMA, NOS			2 (4%)
FOLLICULAR-CELL ADENOMA	1 (5%)	3 (6%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(49)	(50)
ADENOMA, NOS			4 (8%)
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	7	9
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	42	41
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DDSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	32	31
TOTAL PRIMARY TUMORS	26	44	43
TOTAL ANIMALS WITH BENIGN TUMORS	4	9	10
TOTAL BENIGN TUMORS	5	9	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	27	27
TOTAL MALIGNANT TUMORS	21	35	31
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	3
TOTAL SECONDARY TUMORS	4	2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	
ANIMALS NECROPSIED	20	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(47)	(50)
FIBROSARCOMA	1 (5%)		1 (2%)
LIPOMA	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(47)	(48)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	4 (9%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	
FIBROSARCOMA, METASTATIC			2 (4%)
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	3 (15%)	2 (4%)	5 (10%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		6 (13%)	2 (4%)
*SUBCUT TISSUE	(20)	(47)	(50)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	2 (4%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)		
*MESENTERIC L. NODE	(20)	(46)	(44)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
*SMALL INTESTINE	(19)	(46)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
*THYMUS	(18)	(44)	(42)
FIBROSARCOMA			1 (2%)
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	1 (6%)	1 (2%)	
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
*HEART	(19)	(47)	(48)
FIBROSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(47)	(48)
HEPATOCELLULAR CARCINOMA	1 (5%)	2 (4%)	5 (10%)
HEMANGIOSARCOMA	1 (5%)		1 (2%)
*PANCREAS	(17)	(46)	(46)
ADENOCARCINOMA, NOS			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
*PITUITARY	(19)	(45)	(45)
ADENOMA, NOS		1 (2%)	
*ADRENAL	(20)	(45)	(44)
CORTICAL ADENOMA		1 (2%)	
CORTICAL CARCINOMA			1 (2%)
*THYROID	(20)	(44)	(47)
FOLLICULAR-CELL ADENOMA	1 (5%)		2 (4%)
*PANCREATIC ISLETS	(17)	(46)	(46)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(47)	(50)
ADENOCARCINOMA, NOS	1 (5%)	1 (2%)	

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#OVARY THECOMA	(19)	(47) 1 (2%)	(39)
NERVOUS SYSTEM			
NONF			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE OSTEOSARCOMA	(20)	(47)	(50) 1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS SARCOMA, NOS HEMANGIOSARCOMA	(20)	(47) 2 (4%)	(50) 1 (2%)
LOWER LEG OSTEOSARCOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	5	8	15
MORIBUND SACRIFICE		4	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	35	34
ANIMAL MISSING		3	
<u>@ INCLUDES AUTOLYZED ANIMALS</u>			
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	15	22	20
TOTAL PRIMARY TUMORS	16	30	27
TOTAL ANIMALS WITH BENIGN TUMORS	4	7	5
TOTAL BENIGN TUMORS	4	8	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	20	17
TOTAL MALIGNANT TUMORS	12	22	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			3
TOTAL SECONDARY TUMORS			4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS
IN RATS ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

[Faint, illegible text, possibly bleed-through from the reverse side of the page]

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
DERMAL INCLUSION CYST	1 (5%)		
INFLAMMATION, SUPPURATIVE	1 (5%)		
GRANULOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(50)
ALVEOLAR MACROPHAGES		1 (2%)	1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (5%)	5 (10%)	5 (10%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(49)	(49)
ECTOPIA	1 (5%)		
INFARCT, FOCAL		1 (2%)	
#PANCREATIC L.NODE	(20)	(50)	(50)
DILATATION, NOS	1 (5%)		
#MESENTERIC L. NODE	(20)	(50)	(50)
DILATATION, NOS		12 (24%)	32 (64%)
CIRCULATORY SYSTEM			
#HEART/ATRIUM	(20)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(50)	(50)
FIBROSIS	8 (40%)	2 (4%)	
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
*LIVER	(20)	(50)	(49)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY		2 (4%)	1 (2%)
HEPATOCYTOMEGALY	5 (25%)	10 (20%)	10 (20%)
HYPERPLASIA, FOCAL	1 (5%)	1 (2%)	
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	14 (70%)	2 (4%)	
HYPERPLASIA, FOCAL		1 (2%)	
*PANCREAS	(18)	(44)	(48)
FIBROSIS, FOCAL	1 (6%)		
PERIARTERITIS	3 (17%)	1 (2%)	1 (2%)
*PANCREATIC ACINUS	(18)	(44)	(48)
ATROPHY, NOS	3 (17%)	3 (7%)	4 (8%)
*STOMACH	(19)	(50)	(50)
ULCER, NOS			1 (2%)
ULCER, FOCAL		1 (2%)	2 (4%)
NECROSIS, FOCAL			1 (2%)
*LARGE INTESTINE	(20)	(49)	(48)
NEMATODIASIS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		
URINARY SYSTEM			
*KIDNEY	(20)	(50)	(50)
CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC	18 (90%)	41 (82%)	45 (90%)
PERIARTERITIS			1 (2%)
HYPERPLASIA, TUBULAR CELL			1 (2%)
*KIDNEY/CORTEX	(20)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
*KIDNEY/PELVIS	(20)	(50)	(50)
DILATATION, NOS			1 (2%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER HYPERPLASIA, EPITHELIAL	(18)	(46)	(50) 2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY HEMORRHAGE	(19) 1 (5%)	(49) 3 (6%)	(48) 2 (4%)
#ADRENAL METAPLASIA, OSSEOUS	(20)	(50) 1 (2%)	(50)
#ADRENAL CORTEX HYPERPLASIA, FOCAL	(20)	(50)	(50) 1 (2%)
#ADRENAL MEDULLA HYPERPLASIA, FOCAL	(20)	(50) 3 (6%)	(50) 5 (10%)
#THYROID HYPERPLASIA, C-CELL	(20) 8 (40%)	(49) 10 (20%)	(50) 12 (24%)
#PARATHYROID HYPERPLASIA, NOS	(18)	(46)	(42) 1 (2%)
#PANCREATIC ISLETS HYPERPLASIA, NOS	(18) 1 (6%)	(44)	(48)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND CYST, NOS INFLAMMATION, GRANULOMATOUS HYPERPLASIA, NOS	(20)	(50) 1 (2%) 2 (4%)	(50) 1 (2%)
*PROSTATE INFLAMMATION, SUPPURATIVE HYPERPLASIA, EPITHELIAL	(18) 2 (11%)	(47) 8 (17%)	(49) 17 (35%) 1 (2%)
*TESTIS PERIARTERITIS	(20)	(49)	(50) 1 (2%)
*EPIDIDYMIS GRANULOMA, SPERMATIC	(20)	(50) 1 (2%)	(50)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
# BRAIN	(20)	(49)	(49)
HEMORRHAGE	2 (10%)	3 (6%)	
CORPORA AMYLACEA			1 (2%)
SPECIAL SENSE ORGANS			
* EYE	(20)	(50)	(50)
CATARACT	3 (15%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
* MESENTERY	(20)	(50)	(50)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
INFLAMMATION, FOCAL		3	2
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
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR MACROPHAGES	1 (5%)	6 (12%)	3 (6%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		3 (6%)	6 (12%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(49)	(49)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
#SPLEEN	(18)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
HEMATOPOIESIS		3 (6%)	1 (2%)
#MESENTERIC L. NODE	(20)	(50)	(50)
DILATATION, NOS		5 (10%)	18 (36%)
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
NECROSIS, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(49)
NECROSIS, FOCAL		2 (4%)	
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATY	2 (10%)	2 (4%)	
HEPATOCYTOMEGALY	3 (15%)	5 (10%)	10 (20%)
HYPERPLASIA, FOCAL	13 (65%)	28 (56%)	12 (24%)
HEMATOPOIESIS		1 (2%)	
*BILE DUCT HYPERPLASIA, NOS	(20)	(50) 1 (2%)	(50) 1 (2%)
#PANCREATIC ACINUS ATROPHY, NOS	(20) 1 (5%)	(49) 5 (10%)	(46) 3 (7%)
#LARGE INTESTINE NEMATODIASIS	(19)	(50) 1 (2%)	(48) 1 (2%)
URINARY SYSTEM			
*KIDNEY PYELONEPHRITIS SUPPURATIVE INFLAMMATION, CHRONIC PERIARTERITIS	(20) 8 (40%) 1 (5%)	(50) 1 (2%) 25 (50%)	(50) 26 (52%)
*KIDNEY/PELVIS HYPERPLASIA, EPITHELIAL	(20)	(50)	(50) 1 (2%)
*URINARY BLADDER HYPERPLASIA, EPITHELIAL	(19)	(48) 1 (2%)	(46)
ENDOCRINE SYSTEM			
*PITUITARY CYST, NOS	(19)	(48) 1 (2%)	(49)
*ADRENAL COPTX NECROSIS, NOS NECROSIS, FOCAL CYTOMEGALY	(20)	(50) 1 (2%) 1 (2%) 1 (2%)	(50)
*ADRENAL MEDULLA EOSINOPHILIC INFILTRATE HYPERPLASIA, FOCAL	(20)	(50) 1 (2%) 1 (2%)	(50)
*THYROID CYSTIC FOLLICLES	(20)	(50)	(49) 1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, C-CELL	11 (55%)	22 (44%)	22 (45%)
HYPERPLASIA, FOLLICULAR-CELL			2 (4%)
#PARATHYROID	(18)	(45)	(43)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY LOBULE	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(49)	(47)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC	1 (5%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(50)	(50)
CATARACT		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
BACTERIAL SEPTICEMIA		1 (2%)	
ADIPOSE TISSUE			
INFLAMMATION, FOCAL		2	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LDW DDSE	HIGH DDSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS
IN MICE ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

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TABLE D1.

**SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET**

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
ALOPECIA		2 (4%)	2 (4%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*SPLEEN	(20)	(49)	(49)
HYPERPLASIA, LYMPHOID	2 (10%)	1 (2%)	3 (6%)
HEMATOPOIESIS	6 (30%)	5 (10%)	1 (2%)
*MANDIBULAR L. NODE	(20)	(49)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
*MESENTERIC L. NODE	(20)	(49)	(49)
HYPERPLASIA, RETICULUM CELL		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	4 (20%)		1 (2%)
*THYMUS	(20)	(47)	(48)
ATROPHY, NOS			2 (4%)
HYPERPLASIA, LYMPHOID	1 (5%)	1 (2%)	3 (6%)
CIRCULATORY SYSTEM			
*MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	2 (4%)
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (5%)		2 (4%)
HYPERPLASIA, NODULAR	1 (5%)	3 (6%)	5 (10%)
ANGIECTASIS	1 (5%)		
HEMATOPOIESIS	1 (5%)		
*GALLBLADDER	(20)	(49)	(50)
CALCULUS, NOS	1 (5%)		
*BILE DUCT	(20)	(49)	(50)
INFLAMMATION, NOS	2 (10%)		3 (6%)
#PANCREAS	(20)	(47)	(49)
CYSTIC DUCTS			1 (2%)
ATROPHY, NOS		1 (2%)	
#STOMACH	(20)	(49)	(50)
INFLAMMATION, NOS	2 (10%)		
#PEYERS PATCH	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
HYDRONEPHROSIS			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (5%)	2 (4%)	
INFLAMMATION, CHRONIC			1 (2%)
PERIVASCULITIS			1 (2%)
INFARCT, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#THYROID	(20)	(49)	(50)
CYSTIC FOLLICLES	1 (5%)		
FOLLICULAR CYST, NOS		1 (2%)	2 (4%)
#PARATHYROID	(15)	(33)	(34)
CYST, NOS			1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PANCREATIC ISLETS HYPERPLASIA, NOS	(20) 3 (15%)	(47) 3 (6%)	(49) 2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND HYPERPLASIA, CYSTIC	(20) 1 (5%)	(49)	(50)
*PREPUTIAL GLAND CYST, NOS	(20)	(49) 2 (4%)	(50) 2 (4%)
*SEMINAL VESICLE CAST, NOS	(20) 6 (30%)	(49) 11 (22%)	(50) 10 (20%)
*TESTIS ATROPHY, NOS	(20)	(48)	(50) 1 (2%)
NERVOUS SYSTEM			
*BRAIN MINERALIZATION	(20)	(50) 4 (8%)	(49) 8 (16%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA INFLAMMATION, NOS	(20) 1 (5%)	(49)	(50)
ALL OTHER SYSTEMS			
NONE			
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	10	7
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	
ANIMALS NECROPSIED	20	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(47)	(50)
ALOPECIA		3 (6%)	3 (6%)
RESPIRATORY SYSTEM			
*LUNG	(20)	(47)	(48)
ATELECTASIS	1 (5%)		2 (4%)
CONGESTION, NOS		3 (6%)	
HEMATOPOIETIC SYSTEM			
*SPLEEN	(19)	(47)	(46)
HYPERPLASIA, LYMPHOID		4 (9%)	4 (9%)
HEMATOPOIESIS	8 (42%)	8 (17%)	3 (7%)
*LYMPH NODE	(20)	(46)	(44)
CYST, NOS			1 (2%)
*MESENTERIC L. NODE	(20)	(46)	(44)
CYST, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID	1 (5%)		3 (7%)
*THYMUS	(18)	(44)	(42)
HYPERPLASIA, LYMPHOID	2 (11%)		1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
*LIVER	(20)	(47)	(48)
NECROSIS, FOCAL		2 (4%)	2 (4%)
* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR HEMATOPOIESIS	3 (15%) 1 (5%)	7 (15%)	4 (8%)
*HEPATIC CAPSULE INFLAMMATION, NOS	(20) 1 (5%)	(47)	(48)
*GALLBLADDER HYPERPLASIA, NOS	(20) 1 (5%)	(47)	(50)
*BILE DUCT INFLAMMATION, NOS	(20) 2 (10%)	(47) 5 (11%)	(50) 1 (2%)
*PANCREAS CYSTIC DUCTS ATPOPHY, NOS	(17) 1 (6%)	(46) 1 (2%)	(46)
*STOMACH INFLAMMATION, NOS	(20) 3 (15%)	(47) 2 (4%)	(48) 1 (2%)
*SMALL INTESTINE POLYP	(19) 1 (5%)	(46)	(48)
*PEYERS PATCH HYPERPLASIA, NOS	(19)	(46)	(48) 1 (2%)
URINARY SYSTEM			
*KIDNEY PERIVASCULITIS HYPERPLASIA, LYMPHOID	(20) 1 (5%)	(47) 1 (2%)	(48)
ENDOCRINE SYSTEM			
*PITUITARY ANGIECTASIS	(19) 1 (5%)	(45)	(45)
*THYROID CYSTIC FOLLICLES	(20)	(44) 1 (2%)	(47) 5 (11%)
*PANCREATIC ISLETS HYPERPLASIA, NOS	(17)	(46) 1 (2%)	(46)
REPRODUCTIVE SYSTEM			
*UTERUS INFLAMMATION, NOS	(20)	(47)	(44) 1 (2%)

* NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY

* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
POLYPOID HYPERPLASIA	2 (10%)		
#UTERUS/ENDOMETRIUM	(20)	(47)	(44)
CYST, NOS	9 (45%)	21 (45%)	22 (50%)
HYPERPLASIA, NOS			1 (2%)
#OVARY	(19)	(47)	(39)
CYST, NOS	5 (26%)	11 (23%)	5 (13%)
NERVOUS SYSTEM			
#BRAIN	(20)	(47)	(48)
MINIPALIZATION	2 (10%)	9 (19%)	6 (13%)
INFLAMMATION, FOCAL		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(47)	(50)
CYST, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	6	8
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/HISTO PERF			1
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			

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APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS
IN RATS ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

1. 1911

1911 1912 1913 1914 1915 1916 1917 1918 1919 1920 1921 1922 1923 1924 1925 1926 1927 1928 1929 1930 1931 1932 1933 1934 1935 1936 1937 1938 1939 1940 1941 1942 1943 1944 1945 1946 1947 1948 1949 1950 1951 1952 1953 1954 1955 1956 1957 1958 1959 1960 1961 1962 1963 1964 1965 1966 1967 1968 1969 1970 1971 1972 1973 1974 1975 1976 1977 1978 1979 1980 1981 1982 1983 1984 1985 1986 1987 1988 1989 1990 1991 1992 1993 1994 1995 1996 1997 1998 1999 2000 2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021 2022 2023 2024 2025 2026 2027 2028 2029 2030 2031 2032 2033 2034 2035 2036 2037 2038 2039 2040 2041 2042 2043 2044 2045 2046 2047 2048 2049 2050 2051 2052 2053 2054 2055 2056 2057 2058 2059 2060 2061 2062 2063 2064 2065 2066 2067 2068 2069 2070 2071 2072 2073 2074 2075 2076 2077 2078 2079 2080 2081 2082 2083 2084 2085 2086 2087 2088 2089 2090 2091 2092 2093 2094 2095 2096 2097 2098 2099 2100

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Integumentary System: Fibroma of the Subcutaneous Tissue (b)	0/20 (0)	2/50 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		Infinite	Infinite
Upper Limit		0.123	0.250
Weeks to First Observed Tumor	---	Infinite	Infinite
		104	107
<hr/>			
Hematopoietic System: Lymphoma (b)	9/20 (45)	15/50 (30)	13/50 (26)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.667	0.578
Upper Limit		0.346	0.289
Weeks to First Observed Tumor	91	81	77

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Liver: Neoplastic Nodule (b)	2/20 (10)	0/50 (0)	0/49 (0)
P Values (c,d)	P = 0.025(N)	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.043		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.345	1.372
Weeks to First Observed Tumor	98	--	--
Pituitary: Adenoma or Carcinoma, NOS (b)	4/19 (21)	0/49 (0)	1/48 (2)
P Values (c,d)	P = 0.009(N)	P = 0.005(N)	P = 0.020(N)
Departure from Linear Trend (e)	P = 0.004		
Relative Risk (f)		0.000	0.099
Lower Limit		0.000	0.002
Upper Limit		0.413	0.932
Weeks to First Observed Tumor	102	--	103

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Pituitary: Chromophobe Adenoma (b)	1/19 (5)	7/49 (14)	6/48 (13)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.714	2.375
Lower Limit		0.393	0.324
Upper Limit		119.544	106.788
Weeks to First Observed Tumor	107	104	107
Adrenal: Pheochromocytoma (b)	2/20 (10)	4/50 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.800	0.800
Lower Limit		0.128	0.128
Upper Limit		8.436	8.436
Weeks to First Observed Tumor	107	57	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Pituitary: Chromophobe Adenoma, Adenoma, NOS or Carcinoma (b)	5/19 (26)	7/49 (14)	7/48 (15)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.543	0.554
Upper Limit		0.176	0.180
		1.959	1.997
Weeks to First Observed Tumor	102	104	103
Thyroid: Follicular-cell Adenoma or Carcinoma (b)	0/20 (0)	1/49 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		Infinite	Infinite
Upper Limit		0.023	0.250
		Infinite	Infinite
Weeks to First Observed Tumor	--	107	81

(continued)

Table E1 Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Thyroid: C-cell Adenoma or Carcinoma (b)	1/20 (5)	6/49 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		2.449	1.600
Upper Limit		0.332	0.175
		110.166	77.169
Weeks to First Observed Tumor	107	104	107
<hr/>			
Pancreatic Islets: Islet-cell Adenoma or Carcinoma (b)	1/18 (6)	4/44 (9)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.636	1.125
Upper Limit		0.181	0.100
		78.690	57.811
Weeks to First Observed Tumor	107	107	107

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Butoxide in the Diet (a)

(continued)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Testis: Interstitial-cell Tumor (b)	19/20 (95)	48/49 (98)	46/50 (92)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.031	0.968
Upper Limit		0.961	0.902
		1.109	1.174
Weeks to First Observed Tumor	96	78	77

(a) Dosed groups received 5,000 or 10,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P 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 matched-control group when P 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 less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Hematopoietic System: Lymphoma (b)	1/20 (5)	7/50 (14)	15/50 (30)
P Values (c,d)	P = 0.007	N.S.	P = 0.020
Relative Risk (f)		2.800	6.000
Lower Limit		0.403	1.048
Upper Limit		123.407	245.704
Weeks to First Observed Tumor	107	94	73
<hr/>			
Pituitary: Chromophobe Adenoma (b)	8/19 (42)	11/48 (23)	14/49 (29)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.544	0.679
Lower Limit		0.251	0.336
Upper Limit		1.348	1.606
Weeks to First Observed Tumor	107	107	105

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Thyroid: C-cell Adenoma or Carcinoma (b)	4/20 (20)	6/50 (12)	3/49 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.600	0.306
Upper Limit		0.164	0.050
		2.659	1.675
Weeks to First Observed Tumor	107	107	105
Mammary Gland: Fibroadenoma (b)	3/20 (15)	8/50 (16)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.067	0.267
Upper Limit		0.295	0.024
		5.813	2.190
Weeks to First Observed Tumor	107	107	105

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Butoxide in the Diet (a)

(continued)

<u>Topography:</u>	<u>Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Uterus:	Endometrial Stromal Polyp (b)	4/20 (20)	7/49 (14)	3/47 (6)
P Values (c,d)		N.S.	N.S.	N.S.
Relative Risk (f)				
Lower Limit			0.714	0.319
Upper Limit			0.211	0.052
			3.052	1.743
Weeks to First Observed Tumor		107	107	107

(a) Dosed groups received 5,000 or 10,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P 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 matched-control group when P 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 less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

The first part of the document discusses the importance of maintaining accurate records of all transactions. It emphasizes that every entry should be supported by a valid receipt or invoice. This not only helps in tracking expenses but also ensures compliance with tax regulations.

In the second section, the author provides a detailed breakdown of the monthly budget. It lists various categories such as housing, utilities, food, and transportation, along with their respective costs. This helps in understanding where the money is being spent and allows for better financial planning.

The third part of the document focuses on investment strategies. It discusses the benefits of diversifying one's portfolio and the importance of long-term investing. The author mentions several options, including stocks, bonds, and real estate, and provides some insights into how to choose the right investment based on individual goals and risk tolerance.

Finally, the document concludes with some general advice on financial health. It stresses the importance of staying informed about market trends and seeking professional advice when needed. The author also encourages readers to regularly review their financial situation to ensure they are on track to meet their long-term objectives.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS
IN MICE ADMINISTERED PIPERONYL BUTOXIDE IN THE DIET

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Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Integumentary System: Lipoma of the Subcutaneous Tissue (b)	2/20 (10)	3/49 (6)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.612	0.400
Upper Limit		0.078	0.032
		6.996	5.277
Weeks to First Observed Tumor	112	112	112
Lung: Alveolar/Bronchiolar Carcinoma (b)	3/20 (15)	3/50 (6)	2/48 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.400	0.278
Upper Limit		0.060	0.025
		2.802	2.278
Weeks to First Observed Tumor	103	105	112

Table Fl. Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	5/20 (25)	6/50 (12)	8/48 (17)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.480	0.667
Lower Limit		0.143	0.227
Upper Limit		1.807	2.338
Weeks to First Observed Tumor	103	105	112
<hr/>			
Hematopoietic System: Lymphoma (b)	4/20 (20)	10/49 (20)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.020	0.600
Lower Limit		0.346	0.164
Upper Limit		4.068	2.659
Weeks to First Observed Tumor	112	91	90

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
All Sites: Hemangiosarcoma (b)	1/20 (5)	3/49 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.224	0.400
Upper Limit		0.108	0.005
		62.958	30.802
Weeks to First Observed Tumor	112	105	112
<hr/>			
Liver: Hepatocellular Carcinoma (b)	10/20 (50)	17/50 (34)	20/50 (40)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.680	0.800
Upper Limit		0.379	0.462
		1.403	1.604
Weeks to First Observed Tumor	88	83	99

Table Fl. Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Thyroid: Follicular-cell Adenoma (b)	1/20 (5)	3/49 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		1.224	0.000
Upper Limit		0.108	0.000
		62.958	7.475
Weeks to First Observed Tumor	112	112	--
<hr/>			
Lacrimal Gland: Adenoma, NOS (b)	0/20 (0)	0/49 (0)	4/50 (8)
P Values (c,d)	P = 0.022	N.S.	N.S.
Relative Risk (f)			
Lower Limit		--	Infinite
Upper Limit		--	0.386
		--	Infinite
Weeks to First Observed Tumor	--	--	112

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Butoxide in the Diet (a)

(continued)

- (a) Dosed groups received time-weighted average doses of 1,036 or 2,804 ppm.
- (b) Number of tumor-bearing animals/number of animals examined at site (percent).
- (c) Beneath the incidence of tumors in the matched-control group is the probability level for the Cochran-Armitage test when P 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 matched-control group when P 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 less than 0.05 for any comparison.
- (f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	2/20 (10)	6/47 (13)	2/48 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.277	0.417
Lower Limit		0.258	0.033
Upper Limit		12.285	5.490
Weeks to First Observed Tumor	111	98	112
<hr/>			
Hematopoietic System: Lymphoma (b)	8/20 (40)	14/47 (30)	9/50 (18)
P Values (c,d)	P = 0.030(N)	N.S.	N.S.
Relative Risk (f)		0.745	0.450
Lower Limit		0.364	0.190
Upper Limit		1.765	1.174
Weeks to First Observed Tumor	82	45	73

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Butoxide in the Diet (a)

<u>Topography: Morphology</u>	<u>Matched Control</u>	<u>Low Dose</u>	<u>High Dose</u>
Liver: Hepatocellular Carcinoma (b)	1/20 (5)	2/47 (4)	5/48 (10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)			
Lower Limit		0.851	2.083
Upper Limit		0.048	0.259
		49.164	96.358
Weeks to First Observed Tumor	112	111	107

109

- (a) Dosed groups received time-weighted average doses of 1,036 or 2,804 ppm.
- (b) Number of tumor-bearing animals/number of animals examined at site (percent).
- (c) Beneath the incidence of tumors in the matched-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 matched-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 than 0.05 for any comparison.
- (f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of Piperonyl Butoxide* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

August 31, 1978

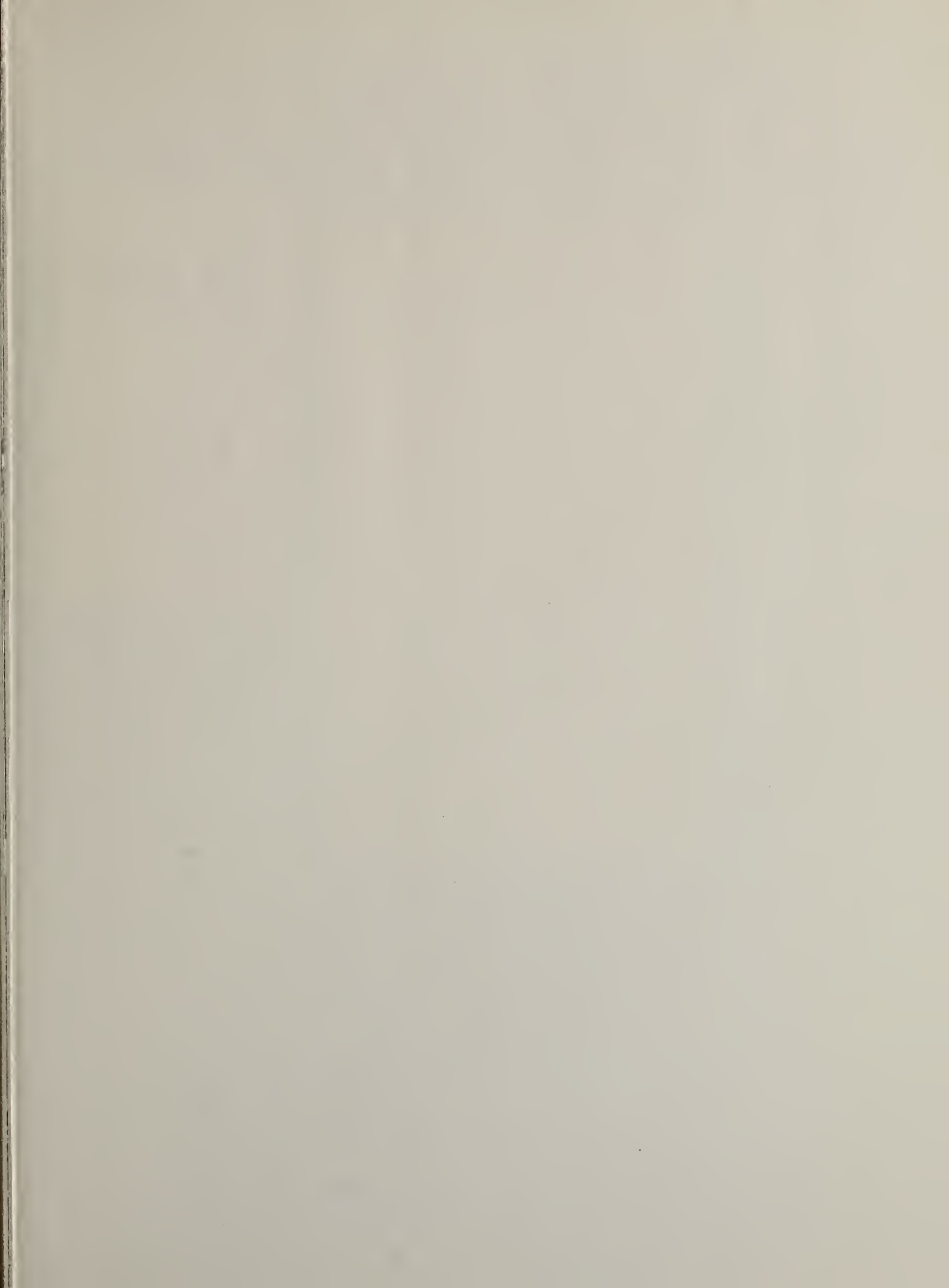
The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Piperonyl Butoxide for carcinogenicity.

The primary reviewer said that the staff concluded that Piperonyl Butoxide was not carcinogenic in rats or mice, under the conditions of test. He briefly described the experimental design and noted the increased incidence of lymphomas observed among treated female rats. He said that the neoplasms were not regarded as significant when compared to historical controls. The primary reviewer concluded that the study was adequate and his recommendation was approved unanimously that the report be accepted as written.

Members present were:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.





DHEW Publication No. (NIH) 79-1375