National Cancer Institute
CARCINOGENESIS
Technical Report Series
NO. 124
1979

BIOASSAY OF PIPERONYL SULFOXIDE FOR POSSIBLE CARCINOGENICITY

CAS No. 120-62-7

NCI-CG-TR-124

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

PIPERONYL SULFOXIDE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 79-1379

BIOASSAY OF
PIPERONYL SULFOXIDE
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 sulfoxide 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 sulfoxide 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 management of the facilities. Mr. A. Butler performed the computer services. Necropsies were performed by Drs. B. Ulland, R. Schueler, R. Ball, and R. Cardy. 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).

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). The structural formula was supplied by NCI.

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.

---

(1) Frederick Cancer Research Center, P.O. Box B, Frederick, Maryland.

(2) Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(3) Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.

(4) Experimental Pathology Laboratories, Inc., P.O. Box 474, Herndon, Virginia.

(5) Now with Battelle Pacific Northwest Laboratories, Battelle Boulevard, Richland, Washington.

(6) EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

(7) Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

(8) Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

(9) Now with Clement Associates, Inc., 1010 Wisconsin Avenue, N.W., Suite 660, Washington, D. C.

(10) Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of technical-grade piperonyl sulfoxide 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 sulfoxide in the diet at one of several doses, either 1,500 or 3,000 ppm for the males and either 3,000 or 6,000 ppm for the females, for 105 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 male mice were administered one of two doses, either 350 or 700 ppm, for 104 or 105 weeks. Groups of 50 female mice were initially administered one of two doses, either 700 or 1,400 ppm. Due to excessive weight depression in the dosed female mice, the doses for this sex were reduced after week 20 to 200 and 600 ppm, respectively, and administration of the test chemical at the lower doses was continued for 84 or 85 weeks. The time-weighted average doses for the females were 295 and 754 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 the amount of mean body weight gained were dose related for most or all of the bioassay; the depression in the amount of mean body weight gained was slight, however, in the dosed male rats. Survival of the rats and mice was unaffected by the piperonyl sulfoxide and was 78% or higher 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 male and female rats and in the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, hepatocellular carcinomas occurred at incidences that were dose related (P less than 0.001); in direct comparisons, the incidence of these tumors in the high-dose group was significantly higher (P less than 0.001) than that in the control group (controls 6/18, low-dose 31/50, high-dose 46/50).

It is concluded that under the conditions of this bioassay, technical-grade piperonyl sulfoxide was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice, but was carcinogenic for male B6C3F1 mice, producing an increased incidence of hepatocellular carcinomas.

TABLE OF CONTENTS

		Page
I.	Introduction	1
II.	Materials and Methods	3
	A. Chemical	3
	B. Dietary Preparation	3
	C. Animals	4
	D. Animal Maintenance	5
	E. Subchronic Studies	8
	F. Chronic Studies	11
	G. Clinical and Pathologic Examinations	11
	H. Data Recording and Statistical Analyses	15
III.	Results - Rats	21
	A. Body Weights and Clinical Signs (Rats)	21
	B. Survival (Rats)	21
	C. Pathology (Rats)	23
	D. Statistical Analyses of Results (Rats)	33
IV.	Results - Mice	35
	A. Body Weights and Clinical Signs (Mice)	35
	B. Survival (Mice)	35
	C. Pathology (Mice)	37
	D. Statistical Analyses of Results (Mice)	40
V.	Discussion	43
VI.	Bibliography	47

APPENDIXES

Appendix A	Summary of the Incidence of Neoplasms in Rats Administered Piperonyl Sulfoxide in the Diet	51
Table A1	Summary of the Incidence of Neoplasms in Male Rats Administered Piperonyl Sulfoxide in the Diet	53

Table A2	Summary of the Incidence of Neoplasms in Female Rats Administered Piperonyl Sulfoxide in the Diet	57
Appendix B	Summary of the Incidence of Neoplasms in Mice Administered Piperonyl Sulfoxide in the Diet	61
Table B1	Summary of the Incidence of Neoplasms in Male Mice Administered Piperonyl Sulfoxide in the Diet	63
Table B2	Summary of the Incidence of Neoplasms in Female Mice Administered Piperonyl Sulfoxide in the Diet	66
Appendix C	Summary of the Incidence of Nonneoplastic Lesions in Rats Administered Piperonyl Sulfoxide in the Diet	71
Table C1	Summary of the Incidence of Nonneoplastic Lesions in Male Rats Administered Piperonyl Sulfoxide in the Diet.....	73
Table C2	Summary of the Incidence of Nonneoplastic Lesions in Female Rats Administered Piperonyl Sulfoxide in the Diet	78
Appendix D	Summary of the Incidence of Nonneoplastic Lesions in Mice Administered Piperonyl Sulfoxide in the Diet	83
Table D1	Summary of the Incidence of Nonneoplastic Lesions in Male Mice Administered Piperonyl Sulfoxide in the Diet	85
Table D2	Summary of the Incidence of Nonneoplastic Lesions in Female Mice Administered Piperonyl Sulfoxide in the Diet	88
Appendix E	Analyses of the Incidence of Primary Tumors in Rats Administered Piperonyl Sulfoxide in the Diet	91
Table E1	Analyses of the Incidence of Primary Tumors in Male Rats Administered Piperonyl Sulfoxide in the Diet	93

Table E2 Analyses of the Incidence of Primary Tumors in Female Rats Administered Piperonyl Sulfoxide in the Diet 98

Appendix F Analyses of the Incidence of Primary Tumors in Mice Administered Piperonyl Sulfoxide in the Diet 103

Table F1 Analyses of the Incidence of Primary Tumors in Male Mice Administered Piperonyl Sulfoxide in the Diet 105

Table F2 Analyses of the Incidence of Primary Tumors in Female Mice Administered Piperonyl Sulfoxide in the Diet 109

TABLES

Table 1 Piperonyl Sulfoxide Subchronic Feeding Studies in Rats and Mice 9

Table 2 Piperonyl Sulfoxide Chronic Feeding Studies in Rats 12

Table 3 Piperonyl Sulfoxide Chronic Feeding Studies in Mice 13

FIGURES

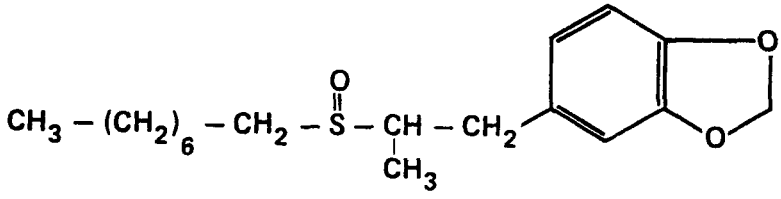
Figure 1 Growth Curves for Rats Administered Piperonyl Sulfoxide in the Diet 22

Figure 2 Survival Curves for Rats Administered Piperonyl Sulfoxide in the Diet 24

Figure 3 Growth Curves for Mice Administered Piperonyl Sulfoxide in the Diet..... 36

Figure 4 Survival Curves for Mice Administered Piperonyl Sulfoxide in the Diet 38

I. INTRODUCTION



Piperonyl sulfoxide

Piperonyl sulfoxide (CAS 120-62-7; NCI C02824), is 1,2-(methylenedioxy)-4-(2-(octylsulfinyl)propyl)benzene. It is used to enhance the insecticidal properties of the pyrethrins by inhibiting pyrethrin detoxification enzymes, probably microsomal oxidases, in the insect (Metcalf, 1966). Pyrethrins alone produce a transient paralysis, whereas pyrethrins combined with a synergist such as piperonyl sulfoxide are insecticidal (Metcalf, 1966).

There are no estimates of the amount of piperonyl sulfoxide produced in the United States, since production figures on this

chemical are grouped with all other cyclic insecticides and rodenticides (United States International Trade Commission, 1977).

The long-term toxicity of piperonyl sulfoxide was investigated by Innes et al. (1969) as a part of a large-scale test of industrial and agricultural chemicals. These investigators, testing mice only, obtained an increased, but not significant, incidence of tumors in mice administered piperonyl sulfoxide, and they categorized this chemical among those that required additional testing. On the basis of these preliminary results, piperonyl sulfoxide was selected for study in the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

The test chemical used in the bioassay is a commercially available product and was obtained as two 20-kilogram batches of Lot No. 291-M00-12 technical-grade piperonyl sulfoxide from S. B. Penick and Company, CPC International, Inc., Lyndhurst, New Jersey. According to the manufacturer's specification and assay, the compound contained at least 88% piperonyl sulfoxide plus 12% related compounds. The test chemical is hereinafter referred to as piperonyl sulfoxide.

The piperonyl sulfoxide was stored at 5°C until used.

B. Dietary Preparation

Test diets containing piperonyl sulfoxide were prepared fresh every 1 to 1-1/2 weeks in 6- to 12-kilogram batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of powdered 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. Uniformity of the mixtures was established by comparative analysis of samples taken from three different locations within the blender. A study of the stability of the test chemical at 6,000 ppm in the diet showed a loss of only 0.25% per day.

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 quarantined 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 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.

The cages were sanitized at 82-88°C in a tunnel-type cagewasher (Industrial Washing Machine Corp., Mataway, N. J.) twice per week, using the detergents, Clout® (Pharmacal Research

Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The 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 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 changed and 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 103-33-3) azobenzene
(CAS 72-56-0) p,p'-ethyl-DDD

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

(CAS 298-00-0) methyl parathion
(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

All procedures involving animals receiving any one of the test chemicals were separated as much as possible from procedures involving animals receiving another test chemical in the same room.

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of piperonyl sulfoxide, 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 sulfoxide 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 4,600, 6,800, 10,000, 14,700, and 21,500 ppm; the doses used for the mice in the subchronic studies were 1,500, 2,200, 3,200, 4,600, 6,800, 10,000, 14,700, and 21,500 ppm. At a later date, a second series of subchronic studies were conducted on female mice, using doses of 50, 100, 200, 300, 600, 1,200, 1,600, and 2,400 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 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 CO_2 inhalation and necropsied. The lowest dose at which histopathologic findings were observed was 21,500 ppm in the male and female rats. At this dose, there were moderate to marked

Table 1. Piperonyl Sulfoxide Subchronic Feeding Studies in Rats and Mice

	Male			Female		
	Mortality		Mean Weight at Week 7 as % of Control	Mortality		Mean Weight at Week 7 as % of Control
(ppm)	Number Dead	Week on Study		Number Dead	Week on Study	
RATS						
4,600			93			104
6,800			83			90
10,000			76			94
14,700	1	3	68			80
21,500	2	5	46			60
MICE						
1,500			74			73
2,200			81			90
3,200			81			83
4,600			76			89
6,800	2	8	73	2	8	84
10,000	5	6		4	8	
14,700	5	2		5	2	
21,500	5	2		5	2	
50						101
100						99
200						97
300						104
600						101
1,200						91
1,600						89
2,400						82

increases in splenic hematopoiesis. No clinical or histopathologic findings were reported for the mice.

Doses required to induce 10% depression in body weight were taken as the major criteria for MTD's. These were estimated as follows: 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 the 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.

Using these calculations, the low and high doses for chronic studies using male rats were set at 1,500 and 3,000 ppm; using female rats, 3,000 and 6,000 ppm; using male mice, 350 and 700 ppm; and using female mice, 700 and 1,400 ppm. The findings at gross or histopathologic examination did not affect the selection of the doses.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic feeding studies are shown in tables 2 and 3. Due to excessive weight depression in the dosed female mice, doses for the low- and high-dose groups were reduced to 200 and 600 ppm, respectively, after week 20.

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. Sick, tumor-bearing, and moribund animals were observed daily. Moribund animals and animals that survived to the end of the bioassay were killed by asphyxiation using CO_2 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,

Table 2. Piperonyl Sulfoxide Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals(a)	Sulfoxide in Diet(b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	105
Low-Dose	50	1,500	105
High-Dose	50	3,000	105
Female			
Matched-Control	20	0	105
Low-Dose	50	3,000	105
High-Dose	50	6,000	105

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

(b) Test and control diets were provided *ad libitum* 7 days per week.

Table 3. Piperonyl Sulfoxide Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals(a)	Sulfoxide in Diet(b) (ppm)	Time on Study (weeks)	Time-Weighted Average Dose(c) (ppm)
Male				
Matched-Control	20	0	105	
Low-Dose	50	350	104-105	
High-Dose	50	700	104	
Female				
Matched-Control	20	0	105	
Low-Dose	50	700	20	
		200	85	295
High-Dose	50	1,400	20	
		600	84	754

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

(b) Test and control diets were provided *ad libitum* 7 days per week.

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

embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes (submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, pancreatic islets, mammary gland, testis, prostate, tunica vaginalis, 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 sections.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of dosed animals at each

dose level. When results for 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 a greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility

of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of the dosed female rats were lower than those of corresponding matched controls, and the depressions in the amount of mean body weight gained were dose related during most of the bioassay (figure 1). The amounts of mean body weight gained by the dosed male rats were equal to or lower than those of the controls from week 15 to the end of the bioassay. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Wasting and alopecia 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

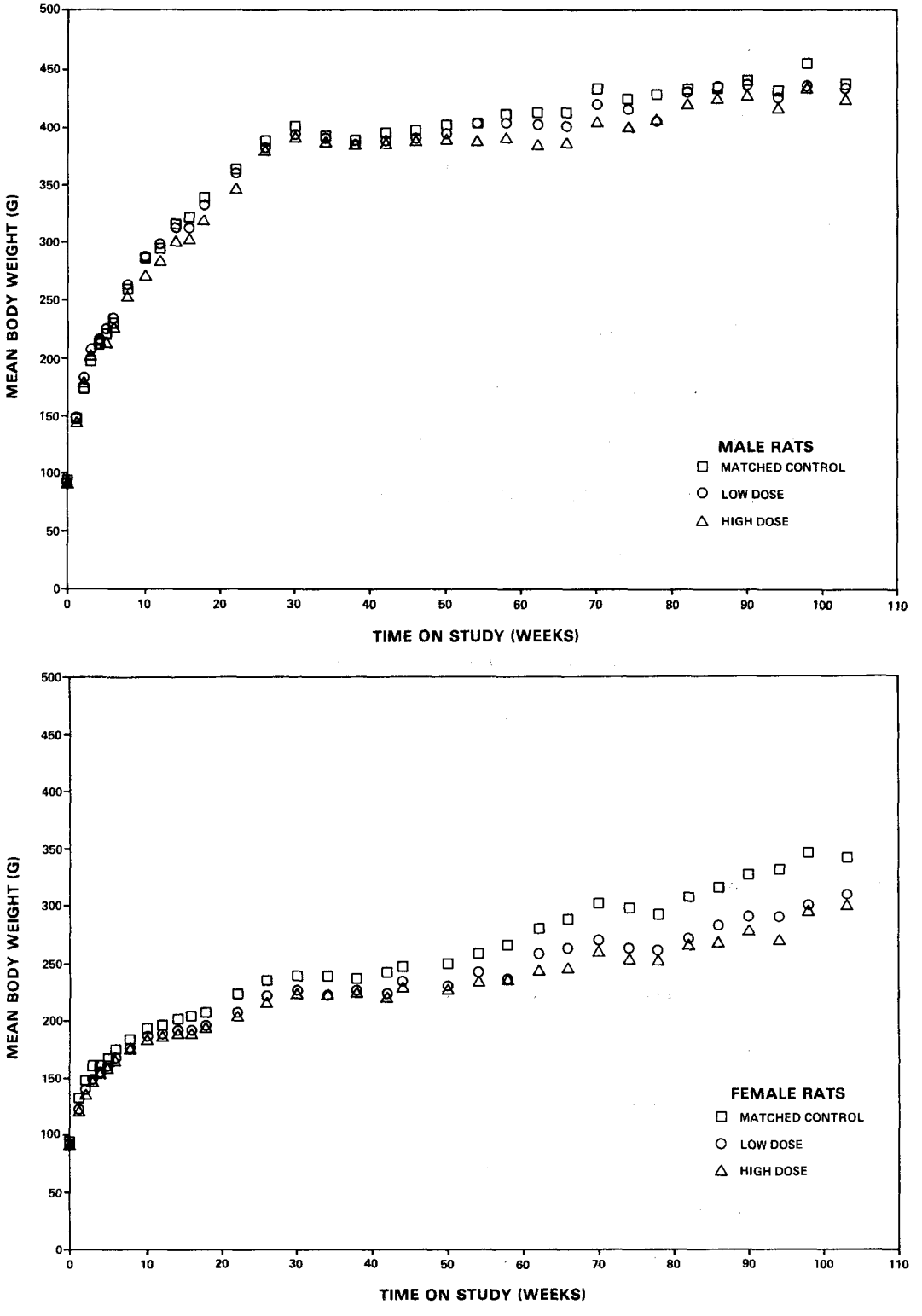


Figure 1. Growth Curves for Rats Administered Piperonyl Sulfoxide in the Diet

sulfoxide 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.

In male rats, 39/50 (78%) of the high-dose group, 36/50 (72%) of the low-dose group, and 13/20 (65%) of the control group were alive at the end of the study. In females, 36/50 (72%) of the high-dose group, 40/50 (80%) of the low-dose group, and 18/20 (90%) of the control group were alive at the end 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.

Several inflammatory, degenerative, and proliferative lesions commonly observed in aged Fischer 344 rats occurred with approximately equal frequency in dosed and control rats. These

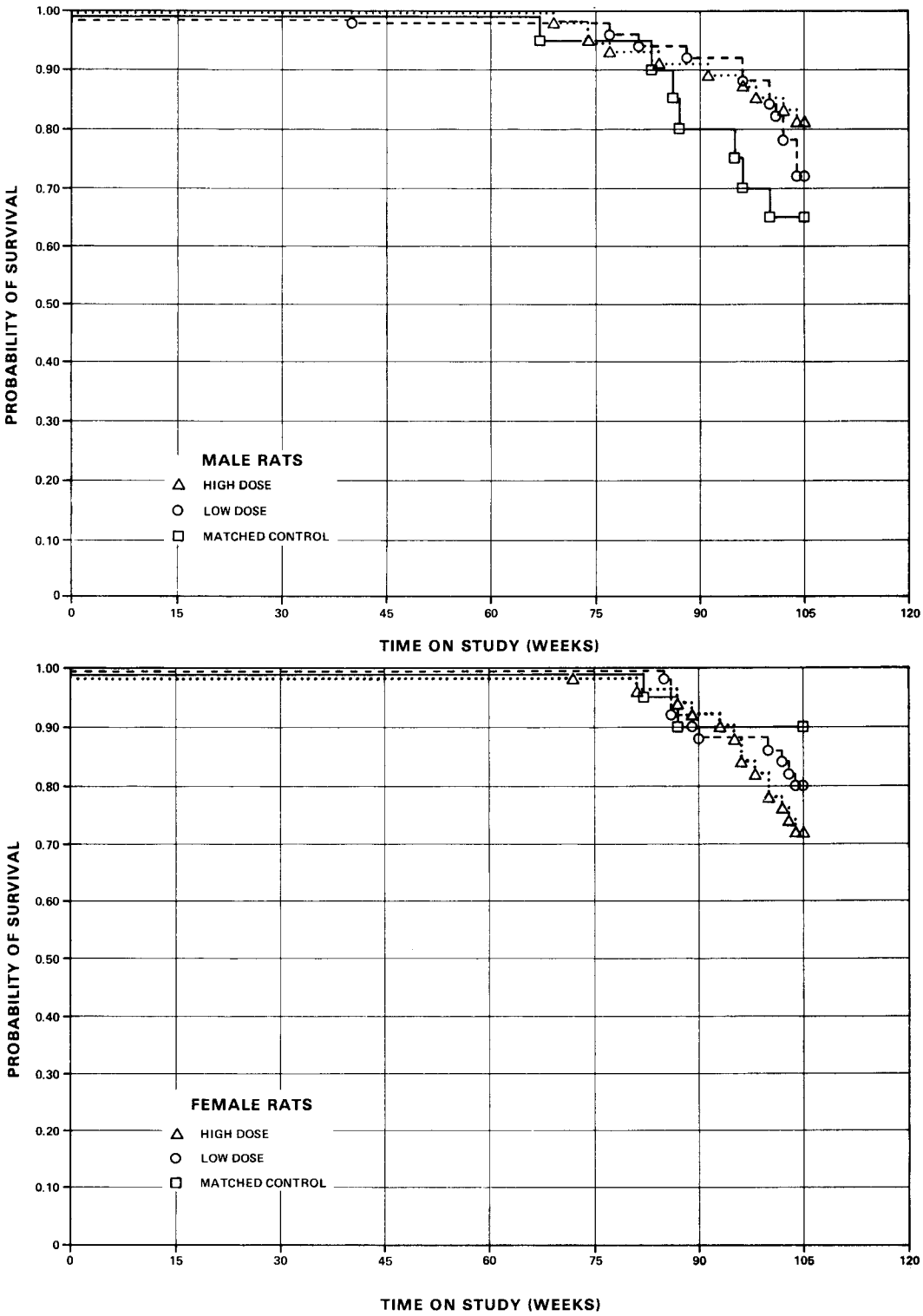


Figure 2. Survival Curves for Rats Administered Piperonyl Sulfoxide in the Diet

lesions included 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; and testicular atrophy.

Other nonneoplastic proliferative lesions included hyperplasia of the follicular cells and C cells of the thyroid, adrenal medulla and cortex, parathyroid, islet cells of the pancreas, and hepatocytes.

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. Compression of adjacent hepatic parenchyma was minimal or absent. 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."

The lesion in one high-dose and that in one low-dose male rat, both classified as hepatocellular carcinomas, were present in livers which were also diffusely infiltrated by neoplastic lymphoreticular cells. In both of these liver sections, most hepatocytes were larger than normal, with increased cytoplasmic basophilia and large, vesicular nuclei. However, in both cases there was a large, compressing nodule in which the hepatocytes were even larger and more basophilic than the surrounding parenchyma, with loss of normal trabecular architecture. Both of these lesions were interpreted as malignancies of hepatocytes, arising in livers that were undergoing a diffuse proliferative process. The relationship of these lesions to the diffusely infiltrating malignant lymphomas involving the same livers is not known.

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, carcinomas of the parathyroid, islet-cell tumors of the pancreas, and medullary and cortical tumors 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 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 carcinoma 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.

Two adrenal tumors were composed of neural tissue and microscopically resembled tumors described in the literature as "ganglioneuromas" (Todd et al., 1970). One of the neoplasms also had a component of small, basophilic cells reminiscent of neuroblastoma. This neoplasm was classified as a ganglioneuroblastoma.

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.

The malignant lymphomas are summarized below. The majority of the malignant lymphomas were composed of undifferentiated lymphoreticular cells. The spleen was the organ most frequently involved and most severely affected by this neoplasm; the red pulp and marginal zones of the white pulp were filled with undifferentiated lymphoreticular cells and splenomegaly was often detected grossly. The liver and lung also were frequently involved with large numbers of neoplastic lymphoreticular cells present in the hepatic sinusoids and alveolar septa. Involvement of peripheral blood, lymph nodes, and other tissues was observed in some cases. There were also animals in which increased numbers of undifferentiated lymphoreticular cells were present in the spleen, liver, and/or blood smear, but the degree of change was not sufficient for diagnosis of malignant lymphoma. These lesions were classified as lymphoid hyperplasia. The incidence of malignant lymphoma and lymphoid hyperplasia is summarized below.

	Males			Females		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of animals necropsied	20	50	48	20	50	50
Malignant Lymphoma	2	12	4	2	5	12
Lymphoid Hyperplasia	3	0	4	2	1	4

The increased incidence of malignant lymphoma in the dosed rats when compared with controls is difficult to evaluate. This is a frequently occurring neoplasm in aged Fischer 344 rats (Davey and Moloney, 1970; Moloney et al., 1970; Sass et al., 1975), and the incidence observed in test animals in this study does not exceed that observed in control groups of Fischer 344 rats from several other bioassay studies evaluated in this laboratory. Further, the average age at which the neoplasm was observed is not strikingly different in dosed and control rats in this study (mean age of rats with malignant lymphoma at necropsy was as follows: control males, 104 weeks; low-dose males, 106 weeks; high-dose males, 92 weeks; control females, 93 weeks; low-dose females, 107 weeks; and high-dose females, 100 weeks). For these reasons, the increased incidence of lymphomas in the dosed rats in this study was not considered to be sufficient evidence for classifying the compound as a carcinogen.

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 (controls 0/20, low-dose 5/50, high-dose 3/47). 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. Although this lesion was observed only in dosed animals in this study, it is a frequently occurring spontaneous tumor in aged female Fischer 344 rats (Davey and Moloney, 1970) and was not considered to have been induced by the test chemical.

In addition to the stromal polyps, two malignant neoplasms were observed in the uterus, one an endometrial stromal sarcoma and the other an adenocarcinoma.

Four carcinomas of the preputial gland were observed in low-dose male rats, and one carcinoma of the clitoral gland was observed in a control female.

The most common neoplasms of the mammary gland were fibroadenomas; these occurred in both males and females, were often multiple, and were seen in both dosed and control groups.

In the transitional epithelium of the urinary tract, one neoplasm was observed, a well-differentiated transitional-cell carcinoma of the urinary bladder in a high-dose male rat. This tumor consisted of a papillary mass lined by thickened transitional epithelium in which some cells had hyperchromatic nuclei. The supporting stroma also contained numerous large cells with large vesicular nuclei, which were interpreted as neoplastic cells. There was no normal bladder tissue attached to the neoplasm; thus it was impossible to determine if invasion of adjacent normal bladder submucosa was present. Hyperplasia of transitional epithelium of the renal pelvis, bladder, and urethra were observed infrequently in both dosed and control rats.

Various other types of malignant and benign neoplasms were observed at low incidences in sections of skin and subcutis, bone marrow, and in other organs and tissues throughout the body. No

apparent difference in incidence of these neoplasms between dosed and control groups was present.

Based on the histopathologic examination, there were instances in this study where 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 sulfoxide 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.

The results of the Cochran-Armitage test for positive dose-related trend in incidences of tumors and those of the Fisher exact test comparing the incidences of tumors in the dosed groups with those in the control groups in the positive direction are not significant in either sex.

Significant results in the negative direction are observed in the incidences of adenoma of the pituitary and of astrocytoma of the brain in male rats and in the incidence of fibroadenoma of the mammary gland in female rats; the incidences of these tumors in the control groups exceed those in the dosed groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals (except that for the incidence of fibroadenoma of the mammary gland in female rats) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by piperonyl sulfoxide, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of both dosed male and dosed female mice were lower than those of corresponding matched controls, and the depressions in the amount of mean body weight gained were dose related throughout the bioassay (figure 3). In comparison with their respective controls, the depressions in the amount of mean body weight gained were greater for the dosed females than for the dosed males. Fluctuation in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Alopecia occurred at low incidences in most of the dosed groups, and may have been related to administration of the test chemical. Corneal opacity occurred only in one low-dose male mouse. 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

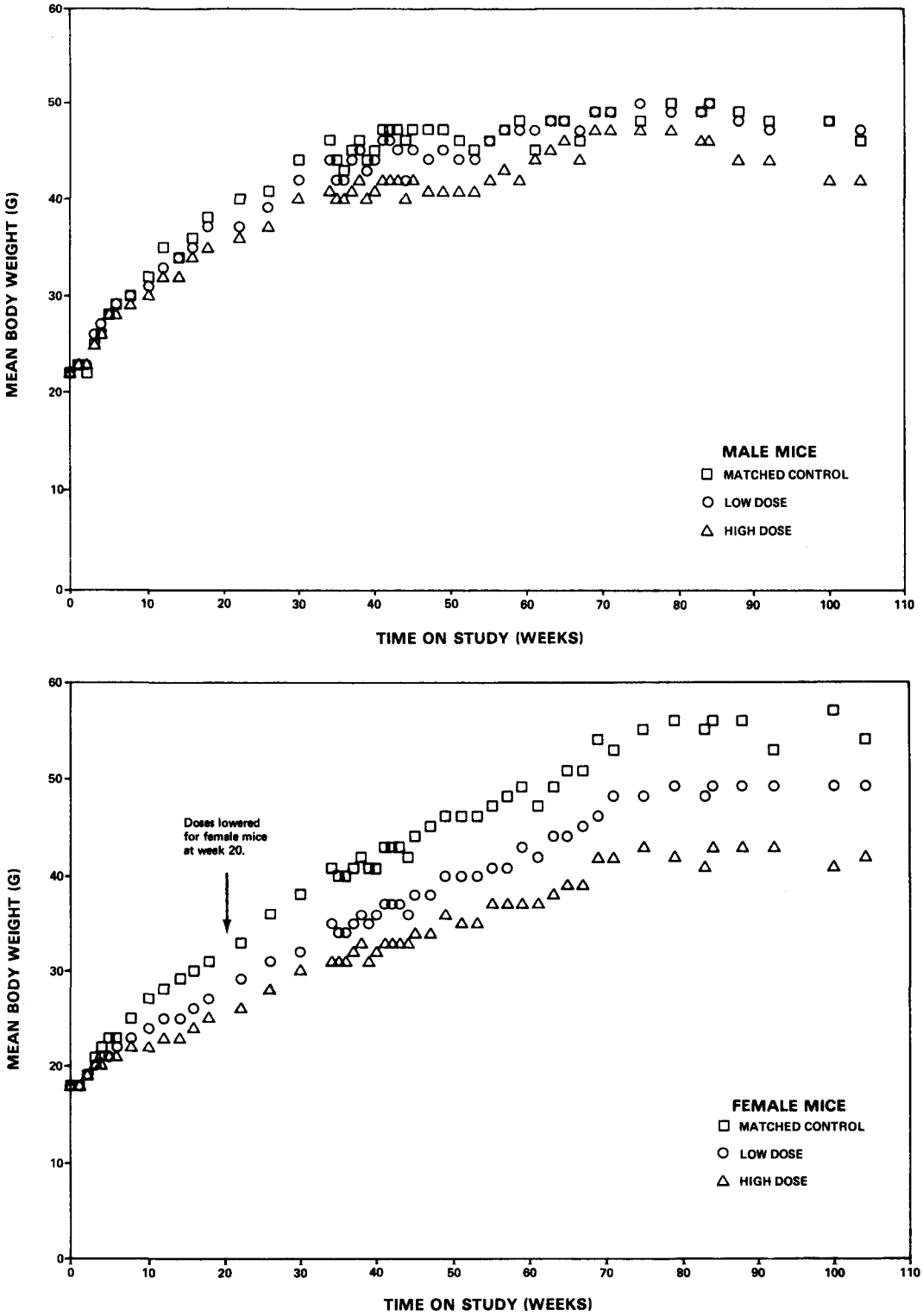


Figure 3. Growth Curves for Mice Administered Piperonyl Sulfoxide in the Diet

sulfoxide 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, 44/50 (88%) of the high-dose group, 43/50 (86%) of the low-dose group, and 13/20 (65%) of the control group were alive at week 104. In females, 41/50 (82%) of the high-dose group, 40/50 (80%) of the low-dose group, and 16/20 (80%) of the control group were alive at week 104.

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; the incidence was increased in relation to the doses of piperonyl sulfoxide:

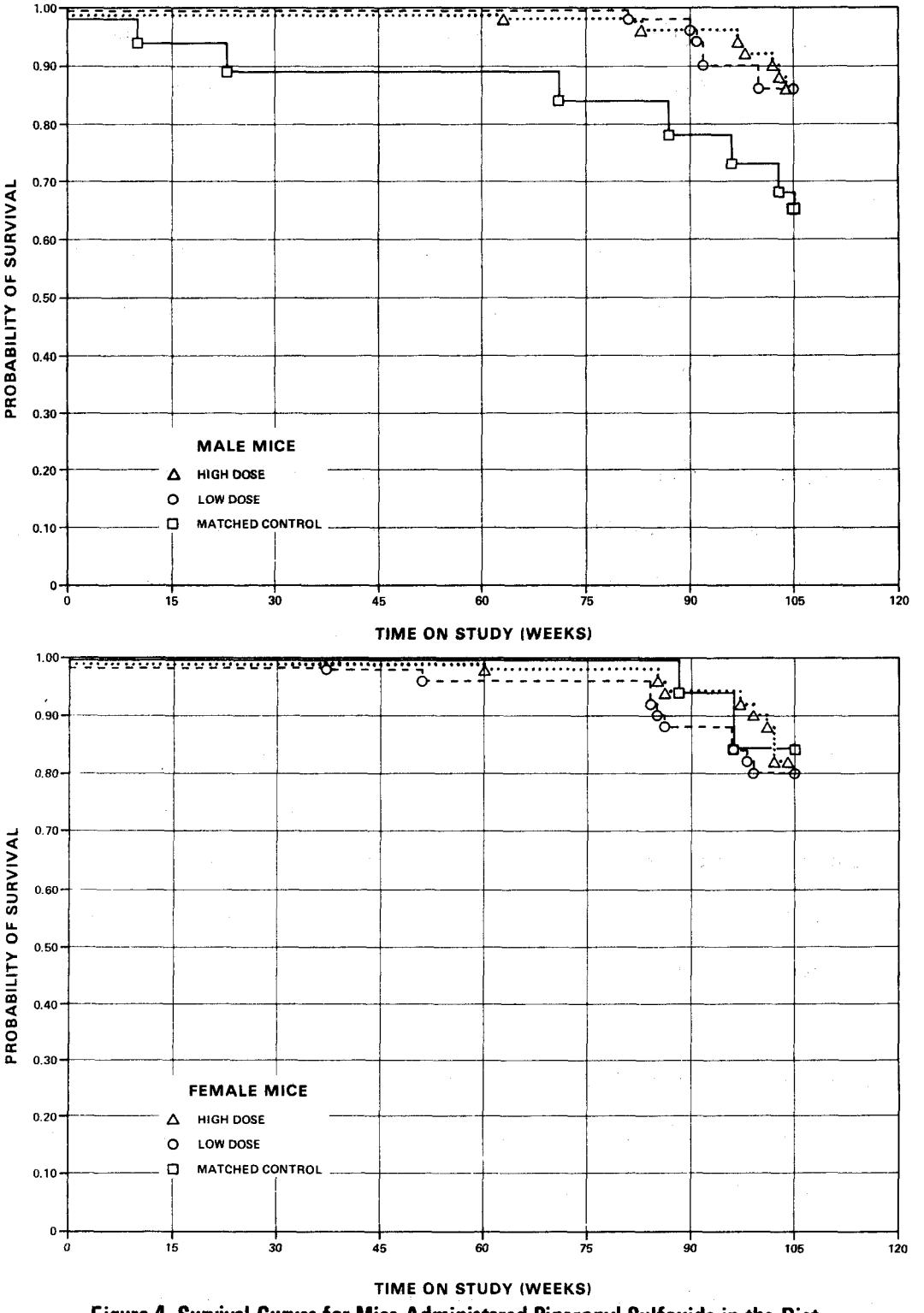


Figure 4. Survival Curves for Mice Administered Piperonyl Sulfoxide in the Diet

Control Males	Low-Dose Males	High-Dose Males
6/18 (33%)	31/50 (62%)	46/50 (92%)

Metastases to the lungs occurred in one control male, four low-dose males, and one high-dose male. Hepatocellular carcinomas were also found in 1/19 (5%) control females, 3/50 (6%) low-dose females, and 6/50 (12%) high-dose females.

The hepatocellular carcinomas had a wide morphologic spectrum, varying from moderately well to very poorly differentiated. Some occurred as relatively small expanding and invasive nodules; others were large masses involving most of the liver.

The next most common tumor was malignant lymphoma. The incidence was approximately equal in males and females and slightly higher in controls than in dosed mice. Many of these tumors were widely disseminated to involve several organs and form tissue masses. They accounted for most of the premature deaths.

Tumors of the lung (adenocarcinomas and alveolar-cell adenomas) were found in 22% of the control mice, 12% of the low-dose mice, and 9% of the high-dose mice.

Most other proliferative or neoplastic lesions were of single

occurrence or very low incidence. Carcinomas of the pancreatic islets were found in a single high-dose male and single high-dose female. There were metastases of the tumor to the lungs and liver in the male. An osteosarcoma of the femur with metastases to the lung was present in a low-dose female.

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, focal hepatic necrosis, cystic pancreatic ducts, ovarian cysts, and cystic endometrium. There was no apparent difference in the incidence of these changes among the three groups.

In conclusion, based on the histopathologic examination, the incidence of hepatocellular carcinomas in male B6C3F1 mice is associated with the administration of the test chemical 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 hepatocellular carcinoma is significant (P less than 0.001) and the result of the Fisher exact test comparing the incidence of this tumor in the high-dose group with that in the control group is also significant (P less than 0.001). The Fisher exact comparison of the incidence in the low-dose group with that in the control group indicates a P value of 0.034, which is above the 0.025 level required for significance when the Bonferroni inequality criterion is used for multiple comparison. The statistical conclusion is that the incidence of hepatocellular carcinoma in male mice is associated with the administration of piperonyl sulfoxide.

In females, the results of the Cochran-Armitage test and of the Fisher exact test are not significant. A significant trend in the negative direction is observed in the incidence of lymphoma in male mice. The incidence of this tumor in the control group exceeds the incidences in the dosed groups.

DISCUSSION

Dose-related depressions in the amount of mean body weight gained occurred in both rats and mice administered technical-grade piperonyl sulfoxide under the conditions of the bioassay; the depression in the amount of mean body weight gained was slight, however, in the male rats. Low incidences of wasting and alopecia among the dosed rats and of alopecia among the dosed mice may have been related to administration of the test chemical. Survival of the rats and mice was unaffected by the piperonyl sulfoxide and was 78% or higher 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 male and female rats and the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, hepatocellular carcinomas occurred at incidences that were dose related (P less than 0.001); in direct comparisons, the incidence of the tumors in the high-dose group was significantly higher (P less than 0.001) than that in the

control group (controls 6/18 or 33%, low-dose 31/50 or 62%, high-dose 46/50 or 92%).

The acute oral LD_{50} of piperonyl sulfoxide for rats (strain not specified) has been reported as 2 g/kg (NIOSH, 1976) and for mice (strain not specified) as 12 g/kg (Suspected Carcinogens, 1976). In tests for tumorigenicity (NTIS, 1968; Innes et al., 1969), it was reported that when the chemical was administered at 46.4 mg/kg by stomach tube for 3 weeks, then in the diet at 111 ppm for 18 months to hybrid mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR), an elevated incidence of reticulum-cell sarcomas (P = 0.01) was observed, but additional evaluation was proposed. Certain structural congeners of piperonyl sulfoxide (safrole, isosafrole, and dihydrosafrole) have been reported to be carcinogenic in rats (Osborne-Mendel) and mice (C57BL/6 x C3H/Anf and C57BL/6 x AKR), inducing tumors of the liver, esophagus, or lung, depending on the species and sex (Long et al., 1963; Hagan et al., 1965; Innes et al., 1969).

Piperonyl sulfoxide is used commercially with pyrethrins. This bioassay, however, tests the carcinogenicity of commercial technical-grade piperonyl sulfoxide alone, and no conclusions can be drawn from the data in this report as to the possible

carcinogenic effects of the combination of the test compound with pyrethrins.

It is concluded that under the conditions of this bioassay, technical-grade piperonyl sulfoxide was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice, but was carcinogenic for male B6C3F1 mice, producing an increased incidence of hepatocellular carcinomas.

VI. BIBLIOGRAPHY

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

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

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

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

Davey, F. R. and Moloney, W. C., Postmortem observations on Fischer rats with leukemia and other disorders. Lab. Invest. 23:327-334, 1970.

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

Hagan, E. C., Jenner, P. M., Jones, W. I., Fitzhugh, O. G., Long, E. L., Brouwer, J. G., and Webb, W. K., Toxic properties of compounds related to safrole. Toxicol. Appl. Pharmacol. 7:18-24, 1965.

Innes, J. R. M., Ulland, B. M., Valerio, M. G., Petrucelli, L., Fishbein, L., Hart, E. R., Pallotta, A. J., Bates, R. R., Falk, H. L., Gart, J. J., Klein, M., Mitchell, I., and Peters, J., Bioassay of pesticides and industrial chemicals for tumorigenicity in mice: a preliminary note. J. Natl Cancer Inst. 42(6): 1101-1106, 1969.

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

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

Long, E. L., Nelson, A. A., Fitzhugh, O. G., and Hansen, W. H., Liver tumors produced in rats by feeding safrole. Arch Path 75:595-604, 1963.

Metcalf, R. L., Insecticides. In: Kirk-Othmer Encyclopedia of Chemical Technology, Vol. 11, Interscience Publishers, New York, 1966, pp. 685-686.

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

Moloney, W. C., Boschetti, A. E., and King, V. P., Spontaneous leukemia in Fischer rats. Cancer Res. 30:41-43, 1970.

National Institute for Occupational Safety and Health, Registry of Toxic Effects of Chemical Substances, Rockville, Maryland, 1976, p. 170.

National Technical Information Service, Evaluation of Carcinogenic, Teratogenic, and Mutagenic Acitvities of Selected Pesticides and Industrial Chemicals, Vol. I. Carcinogenic Study, U.S. Department of Commerce, PB-223 159, August, 1968, p. 64.

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

Sass, B., Rabstein, L. S., Madison, R., Nims, R. M., Peters, R. L., and Kelloff, G. J., Incidence of spontaneous neoplasms in F344 rats throughout the natural life-span. J. Natl Cancer Inst. 54:1449-1456, 1975.

Squire, R. A. and Levitt, M. H., Report of a workshop on classification of specific hepatocelluar lesions in rats. Cancer Res. 35:3214-3223, 1975.

Suspected Carcinogens, 2nd Edition. A Subfile of the NIOSH Registry of Toxic Effects of Chemical Substances, National Institute for Occupational Safety and Health, Cincinnati, Ohio, 1976, p. 36.

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

Todd, G. C., Pierce, E. C., and Clevinger, W. G., Ganglioneuroma of the adrenal medulla in rats. Pathologia Veterinaria 7:139-144, 1970.

United States International Trade Commission, Pesticides and related products. Synthetic Organic Chemicals - United States Production and Sales, 1976, USITC Publication 833, United States International Trade Commission, Washington, D.C., 1977.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED PIPERONYL SULFOXIDE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED PIPERONYL SULFOXIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(48)
TRICHOEPITHELIOMA		1 (2%)	1 (2%)
KERATOACANTHOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(48)
FIBROMA		2 (4%) <-	1 (2%)
FIBROSARCOMA		2 (4%)	
OSTEOSARCOMA, METASTATIC			1 (2%)
NEURILEMOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
SARCOMA, NOS, METASTATIC			1 (2%)
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(48)
MALIG.LYMPHOMA, UNDIFFER-TYPE	2 (10%)	11 (22%)	4 (8%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#BONE MARROW	(20)	(50)	(48)
SARCOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
SARCOMA, NOS	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
<- MULTIPLE OCCURRENCE OF MORPHOLOGY IN THE SAME ORGAN TISSUES IS COUNTED ONCE ONLY

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
SARCOMA, NOS, METASTATIC			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
SARCOMA, NOS, METASTATIC			1 (2%)
#URINARY BLADDER	(18)	(47)	(46)
TRANSITIONAL-CELL CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(45)	(44)
ADENOMA, NOS	6 (33%)	4 (9%)	4 (9%)
#ADRENAL	(20)	(50)	(48)
PHEOCHROMOCYTOMA	1 (5%)	3 (6%)	3 (6%)
SARCOMA, NOS, METASTATIC			1 (2%)
#THYROID	(20)	(49)	(48)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		
C-CELL ADENOMA	2 (10%)	3 (6%)	3 (6%)
#PARATHYROID	(18)	(43)	(46)
CARCINOMA,NOS		1 (2%)	
#PANCREATIC ISLETS	(19)	(50)	(47)
ISLET-CELL ADENOMA	2 (11%)	2 (4%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(48)
FIBROMA		1 (2%)	
FIBROADENOMA	1 (5%)	1 (2%)	1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(48)
CARCINOMA,NOS		4 (8%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#TESTIS	(20)	(50)	(48)
INTERSTITIAL-CELL TUMOR	16 (80%)	46 (92%)	43 (90%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(48)
GLIOMA, NOS		1 (2%)	
ASTROCYTOMA	2 (10%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA	1 (5%)		
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(20)	(50)	(48)
OSTEOSARCOMA			1 (2%)
*SKELETAL MUSCLE	(20)	(50)	(48)
OSTEOSARCOMA, METASTATIC			1 (2%)
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(48)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	8	5
MORIBUND SACRIFICE	3	6	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	36	39
ANIMAL MISSING			2

@ 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*	19	48	46
TOTAL PRIMARY TUMORS	36	85	70
TOTAL ANIMALS WITH BENIGN TUMORS	18	47	44
TOTAL BENIGN TUMORS	29	64	61
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	19	6
TOTAL MALIGNANT TUMORS	7	21	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED PIPERONYL SULFOXIDE 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)
TRICHOEPITHELIOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
*LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
C-CELL CARCINOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		2 (4%)
MALIG.LYMPHOMA, UNDIFFER-TYPE		5 (10%)	10 (20%)
#SPLEEN	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
#CERVICAL LYMPH NODE	(19)	(50)	(50)
C-CELL CARCINOMA, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			

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	(20)	(50)	(48)
ADENOMA, NOS	7 (35%)	7 (14%)	12 (25%)
#ADRENAL	(20)	(50)	(50)
CORTICAL ADENOMA			1 (2%)
GANGLIONEUROMA	1 (5%)		
GANGLIONEUROBLASTOMA		1 (2%)	
#THYROID	(19)	(50)	(50)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA		4 (8%)	
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(20)	(47)	(47)
ISLET-CELL ADENOMA	1 (5%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA	4 (20%)	1 (2%)	
*CLITORAL GLAND	(20)	(50)	(50)
CARCINOMA,NOS	1 (5%)		
*VAGINA	(20)	(50)	(50)
ENDOMETRIAL STROMAL SARCOMA, INV			1 (2%)
#UTERUS	(20)	(50)	(47)
ENDOMETRIAL STROMAL POLYP		5 (10%)	3 (6%)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#CERVIX UTERI	(20)	(50)	(47)
ENDOMETRIAL STROMAL POLYP		1 (2%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(47)
ADENOCARCINOMA, NOS		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
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*MUSCLE OF NECK	(20)	(50)	(50)
C-CELL CARCINOMA, INVASIVE			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	6	8
MORIBUND SACRIFICE		4	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	40	36
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	24	27
TOTAL PRIMARY TUMORS	18	29	34
TOTAL ANIMALS WITH BENIGN TUMORS	11	18	16
TOTAL BENIGN TUMORS	15	20	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	8	15
TOTAL MALIGNANT TUMORS	3	9	16
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
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 B

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

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	18	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(50)	(50)
ISLET-CELL CARCINOMA, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST	1 (6%)	4 (8%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (17%)	6 (12%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (17%)	4 (8%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(18)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	3 (17%)	5 (10%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
*BLOOD	(18)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (6%)		
#MESENTERIC L. NODE	(18)	(49)	(50)
ISLET-CELL CARCINOMA, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
MALIGNANT LYMPHOMA, NOS	1 (6%)	1 (2%)	
#SMALL INTESTINE	(18)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (6%)	1 (2%)	
#THYMUS	(17)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(50)
ISLET-CELL CARCINOMA, METASTATIC			1 (2%)
HEPATOCELLULAR CARCINOMA	6 (33%)	31 (62%)	46 (92%)
HEMANGIOSARCOMA		1 (2%)	3 (6%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(50)	(49)
ADENOMA, NOS	1 (6%)		
#THYROID	(17)	(46)	(50)
FOLLICULAR-CELL ADENOMA		1 (2%)	
#PANCREATIC ISLETS	(17)	(47)	(50)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(18)	(50)	(50)
ADENOMA, NOS	1 (6%)		3 (6%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*ABDOMINAL CAVITY	(18)	(50)	(50)
NEUROFIBROSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
SITE UNKNOWN			
LIPOMA		3	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	6	7	6
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	43	43
ANIMAL MISSING	1		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	39	47
TOTAL PRIMARY TUMORS	20	55	63
TOTAL ANIMALS WITH BENIGN TUMORS	5	9	6
TOTAL BENIGN TUMORS	5	10	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	12	38	47
TOTAL MALIGNANT TUMORS	15	45	57
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	4	2
TOTAL SECONDARY TUMORS	1	5	5
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (6%)	2 (4%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	4 (21%)	10 (20%)	5 (10%
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)
*BLOOD	(19)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (5%)		
#BONE MARROW	(18)	(50)	(48)
OSTEOSARCOMA		1 (2%)	
#SPLEEN	(18)	(50)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#LIVER	(18)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (6%)		1 (2%)
#SMALL INTESTINE	(18)	(49)	(47)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#THYMUS	(17)	(43)	(47)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(49)
HEPATOCELLULAR CARCINOMA	1 (6%)	3 (6%)	6 (12%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(18)	(50)	(49)
TUBULAR-CELL ADENOMA			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(47)	(45)
ADENOMA, NOS		3 (6%)	
#THYROID	(18)	(45)	(47)
ADENOCARCINOMA, NOS			1 (2%)
FOLLICULAR-CELL ADENOMA		1 (2%)	4 (9%)
#PANCREATIC ISLETS	(18)	(46)	(47)
ISLET-CELL CARCINOMA, INVASIVE			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(19)	(50)	(50)
MYOEPITHELIOMA	1 (5%)		1 (2%)
FIBROADENOMA		1 (2%)	
#UTERUS/ENDOMETRIUM	(17)	(47)	(46)
CARCINOMA,NOS			1 (2%)
#OVARY	(17)	(46)	(47)
FOLLICULAR-CELL ADENOMA	1 (6%)		
'RVOUS SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(19)	(50)	(50)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SITE UNKNOWN			
LIPOMA	1	2	3
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	9	9
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	40	41
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

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*	10	23	25
TOTAL PRIMARY TUMORS	12	26	29
TOTAL ANIMALS WITH BENIGN TUMORS	4	8	9
TOTAL BENIGN TUMORS	4	8	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	16	18
TOTAL MALIGNANT TUMORS	8	18	19
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 C

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

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(48)
CYST, NOS		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
GRANULOMA, NOS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
CONGESTION, ACUTE		1 (2%)	
EDEMA, NOS		1 (2%)	
BRONCHOPNEUMONIA, NOS		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
ALVEOLAR MACROPHAGES	1 (5%)		4 (8%)
#LUNG/ALVEOLI	(20)	(50)	(48)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, EPITHELIAL	2 (10%)	4 (8%)	3 (6%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(48)
ECTOPIA	1 (5%)		2 (4%)
ABSCESS, NOS		1 (2%)	
FIBROSIS, FOCAL		2 (4%)	
INFARCT, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID	2 (10%)		
HEMATOPOIESIS			1 (2%)
#LYMPH NODE	(20)	(49)	(48)
CONGESTION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE			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
#MANDIBULAR L. NODE	(20)	(49)	(48)
DILATATION, NOS		3 (6%)	
#MESENTERIC L. NODE	(20)	(49)	(48)
DILATATION, NOS	1 (5%)	1 (2%)	3 (6%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(48)
FIBROSIS, MULTIFOCAL	1 (5%)		
#HEART/ATRIUM	(20)	(50)	(48)
THROMBOSIS, NOS	1 (5%)	1 (2%)	
#MYOCARDIUM	(20)	(50)	(48)
INFLAMMATION, SUPPURATIVE		1 (2%)	
FIBROSIS	2 (10%)	2 (4%)	3 (6%)
NECROSIS, FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NECROSIS, NOS	1 (5%)	1 (2%)	
NECROSIS, FOCAL			1 (2%)
NECROSIS, DIFFUSE			1 (2%)
METAMORPHOSIS FATTY	1 (5%)	5 (10%)	3 (6%)
HEPATOCYTOMEGALY	2 (10%)	3 (6%)	4 (9%)
HYPERPLASIA, FOCAL	4 (20%)	4 (8%)	4 (9%)
*BILE DUCT	(20)	(50)	(48)
HYPERPLASIA, NOS	4 (20%)	8 (16%)	6 (13%)
#PANCREAS	(19)	(50)	(47)
PERIARTERITIS		1 (2%)	
#PANCREATIC ACINUS	(19)	(50)	(47)
ATROPHY, NOS	2 (11%)	15 (30%)	7 (15%)
#STOMACH	(20)	(50)	(48)
ULCER, 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
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
EROSION		1 (2%)	
#GASTRIC MUCOSA	(20)	(50)	(48)
ACANTHOSIS		1 (2%)	
#COLON	(19)	(47)	(45)
PARASITISM			2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
INFLAMMATION, SUPPURATIVE		1 (2%)	
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	12 (60%)	22 (44%)	31 (65%)
#URINARY BLADDER	(18)	(47)	(46)
HYPERPLASIA, EPITHELIAL	1 (6%)	1 (2%)	
*URETHRA	(20)	(50)	(48)
HYPERPLASIA, EPITHELIAL	1 (5%)		
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(20)	(50)	(48)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(20)	(50)	(48)
HYPERPLASIA, FOCAL	2 (10%)	4 (8%)	5 (10%)
#THYROID	(20)	(49)	(48)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL	2 (10%)	13 (27%)	12 (25%)
HYPERPLASIA, FOLLICULAR-CELL		3 (6%)	
#PARATHYROID	(18)	(43)	(46)
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#PANCREATIC ISLETS	(19)	(50)	(47)
HYPERPLASIA, NOS	1 (5%)	2 (4%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(48)
CYST, 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
INFLAMMATICN, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(48)
ABSCESS, NOS		1 (2%)	3 (6%)
METAPLASIA, SQUAMOUS		2 (4%)	
#PROSTATE	(19)	(50)	(45)
INFLAMMATION, SUPPURATIVE		1 (2%)	4 (9%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#TESTIS	(20)	(50)	(48)
CALCIFICATION, FOCAL			1 (2%)
ATROPHY, NOS	1 (5%)		
*EPIDIDYMIS	(20)	(50)	(48)
GRANULOMA, SPERMATIC			1 (2%)
*SCROTUM	(20)	(50)	(48)
NECROSIS, FOCAL		2 (4%)	2 (4%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(50)	(48)
HEMORRHAGE			1 (2%)
#BRAIN	(20)	(50)	(48)
HEMORRHAGE	1 (5%)	2 (4%)	2 (4%)
NECROSIS, FOCAL		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/CRYSTALLINE LENS	(20)	(50)	(48)
CALCIFICATION, FOCAL			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(48)
NECROSIS, FOCAL		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
*PLEURA	(20)	(50)	(48)
FIBROSIS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (5%)		4 (8%)
ADIPOSE TISSUE			
NECROSIS, FOCAL		1	
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY			2

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 SULFOXIDE 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/BRONCHUS	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG	(20)	(50)	(50)
EDEMA, NOS		2 (4%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
PERIVASCULITIS		1 (2%)	
ALVEOLAR MACROPHAGES	1 (5%)	4 (8%)	3 (6%)
#LUNG/ALVEOLI	(20)	(50)	(50)
ALVEOLAR MACROPHAGES		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
HEMATOPOIETIC SYSTEM			
#MESENTERIC L. NODE	(19)	(50)	(50)
DILATATION, NOS			3 (6%)
HEMORRHAGE		1 (2%)	
NECROSIS, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(19)	(50)	(50)
FIBROSIS, FOCAL		1 (2%)	
#HEART/ATRIUM	(19)	(50)	(50)
THROMBOSIS, NOS			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
#MYOCARDIUM	(19)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
FIBROSIS	1 (5%)		
#ENDOCARDIUM	(19)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(50)
INFLAMMATION, CHRONIC DIFFUSE		1 (2%)	
GRANULOMA, NOS	1 (5%)	3 (6%)	
NECROSIS, NOS		2 (4%)	1 (2%)
NECROSIS, FOCAL		2 (4%)	
METAMORPHOSIS FATTY		2 (4%)	3 (6%)
HEPATOCYTOMEGALY		2 (4%)	12 (24%)
HYPERPLASIA, FOCAL	12 (60%)	11 (23%)	5 (10%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS		8 (16%)	4 (8%)
#PANCREAS	(20)	(47)	(47)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#PANCREATIC ACINUS	(20)	(47)	(47)
ATROPHY, NOS	3 (15%)	8 (17%)	6 (13%)
#STOMACH	(20)	(50)	(49)
ULCER, NOS		1 (2%)	
#GASTRIC MUCOSA	(20)	(50)	(49)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#GASTRIC SUBMUCOSA	(20)	(50)	(49)
EDEMA, NOS		1 (2%)	
#COLON	(19)	(47)	(47)
INFLAMMATION, NECROTIZING		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC	7 (35%)	14 (28%)	21 (42%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#RENAL PAPILLA	(20)	(50)	(50)
NECROSIS, NOS		1 (2%)	
#KIDNEY/PELVIS	(20)	(50)	(50)
DILATATION, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		2 (4%)	
HYPERPLASIA, EPITHELIAL	1 (5%)	2 (4%)	
#URINARY BLADDER	(19)	(47)	(44)
CALCULUS, NOS			1 (2%)
HEMORRHAGE		1 (2%)	
INFLAMMATION, HEMORRHAGIC		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL		2 (4%)	1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(20)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
HYPERPLASIA, FOCAL	1 (5%)	1 (2%)	2 (4%)
#ADRENAL MEDULLA	(20)	(50)	(50)
HYPERPLASIA, FOCAL			2 (4%)
#THYROID	(19)	(50)	(50)
HYPERPLASIA, C-CELL	8 (42%)	17 (34%)	14 (28%)
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)
REPRODUCTIVE SYSTEM			
*CLITORAL GLAND	(20)	(50)	(50)
CYSTIC DUCTS			1 (2%)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
METAPLASIA, SQUAMOUS	1 (5%)	2 (4%)	
#UTERUS/ENDOMETRIUM	(20)	(50)	(47)
HYPERPLASIA, CYSTIC		2 (4%)	
METAPLASIA, SQUAMOUS			1 (2%)
#OVARY/PAROVARIAN	(20)	(50)	(47)
NECROSIS, NOS			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
NECROSIS, FOCAL	1 (5%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID	2 (10%)	1 (2%)	4 (8%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	3	3

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

APPENDIX D

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

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	18	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(50)	(50)
CYST, NOS		1 (2%)	
ABSCESS, NOS			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(50)	(49)
INFARCT, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL		2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	5 (28%)	16 (32%)	2 (4%)
#MESENTERIC L. NODE	(18)	(49)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(50)	(49)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL		2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(50)
NECROSIS, FOCAL	2 (11%)	1 (2%)	1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCYTOMEGALY			1 (2%)
HYPERPLASIA, NODULAR	3 (17%)	5 (10%)	3 (6%)
ANGIECTASIS	1 (6%)		
#PANCREAS	(17)	(47)	(50)
CYSTIC DUCTS			1 (2%)
#STOMACH	(18)	(50)	(49)
INFLAMMATION, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(18)	(50)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	
HYPERPLASIA, LYMPHOID		2 (4%)	
ENDOCRINE SYSTEM			
#PITUITARY	(16)	(50)	(49)
CYST, NOS		1 (2%)	
#THYROID	(17)	(46)	(50)
FOLLICULAR CYST, NOS		1 (2%)	
#THYROID FOLLICLE	(17)	(46)	(50)
CYST, NOS		2 (4%)	1 (2%)
#PANCREATIC ISLETS	(17)	(47)	(50)
HYPERPLASIA, NOS		3 (6%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(49)	(50)
ABSCESS, NOS			1 (2%)
*SEMINAL VESICLE	(18)	(50)	(50)
CAST, NOS	3 (17%)	4 (8%)	4 (8%)
#TESTIS	(18)	(49)	(50)
INFARCT, NOS		1 (2%)	
ATROPHY, NOS			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(18)	(50)	(50)
MINERALIZATION	4 (22%)	5 (10%)	10 (20%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERICARDIUM	(18)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MOPPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	1
ANIMAL MISSING/NO NECROPSY	1		
AUTOLYSIS/NO NECROPSY	1		

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

TABLE D2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	19	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(18)	(50)	(48)
AMYLOIDOSIS			1 (2%)
HYPERPLASIA, LYMPHOID	1 (6%)	4 (8%)	1 (2%)
HEMATOPOIESIS	11 (61%)	28 (56%)	9 (19%)
#LYMPH NODE	(18)	(49)	(49)
CYST, NOS		1 (2%)	
#MESENTERIC L. NODE	(18)	(49)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#THYMUS	(17)	(43)	(47)
HYPERPLASIA, LYMPHOID	1 (6%)		2 (4%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(47)	(49)
INFLAMMATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(18)	(50)	(49)
NECROSIS, FOCAL	1 (6%)		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
CYTOPLASMIC VACUOLIZATION	1 (6%)	2 (4%)	1 (2%)
HEPATOCYTOMEGALY			1 (2%)
HYPERPLASIA, NODULAR	2 (11%)	7 (14%)	8 (16%)
*BILE DUCT	(19)	(50)	(50)
INFLAMMATION, NOS			3 (6%)
#PANCREAS	(18)	(46)	(47)
CYSTIC DUCTS	1 (6%)	2 (4%)	
#PANCREATIC DUCT	(18)	(46)	(47)
CYST, NOS		1 (2%)	
#STOMACH	(18)	(50)	(49)
INFLAMMATION, NOS		2 (4%)	
#COLON	(18)	(48)	(48)
GRANULOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(18)	(50)	(49)
GLOMERULOSCLEROSIS, NOS			1 (2%)
INFARCT, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#URINARY BLADDER	(18)	(45)	(45)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(17)	(47)	(45)
CYST, NOS		1 (2%)	1 (2%)
#THYROID	(18)	(45)	(47)
CYSTIC FOLLICLES			1 (2%)
FOLLICULAR CYST, NOS	1 (6%)	1 (2%)	
#THYROID FOLLICLE	(18)	(45)	(47)
CYST, NOS		4 (9%)	2 (4%)
REPRODUCTIVE SYSTEM			
#UTERUS	(17)	(47)	(46)
CYST, NOS			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
INFLAMMATION, NOS		2 (4%)	1 (2%)
POLYPOID HYPERPLASIA		1 (2%)	1 (2%)
#UTERUS/ENDOMETRIUM	(17)	(47)	(46)
CYST, NOS	9 (53%)	19 (40%)	11 (24%)
HYPERPLASIA, CYSTIC			1 (2%)
#OVARY	(17)	(46)	(47)
CYST, NOS	8 (47%)	8 (17%)	9 (19%)
MULTIPLE CYSTS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(18)	(49)	(47)
MINERALIZATION	5 (28%)	12 (24%)	4 (9%)
INFLAMMATION, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	2	6
ANIMAL MISSING/NO NECROPSY	1		

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	2/20 (10)	12/50 (24)	4/48 (8)
P Values (c,d,)	N.S.	N.S.	N.S.
Relative Risk (f)		2.400	0.833
Lower Limit		0.614	0.133
Upper Limit		20.902	8.776
Weeks to First Observed Tumor	96	10	77
Pituitary: Adenoma, NOS (b)	6/18 (33)	4/45 (9)	4/44 (9)
P Values (c,d)	P = 0.026(N)	P = 0.026(N)	P = 0.028(N)
Relative Risk (f)		0.267	0.273
Lower Limit		0.066	0.067
Upper Limit		1.005	1.027
Weeks to First Observed Tumor	96	100	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	1/20 (5)	3/50 (6)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	1.250
Lower Limit		0.106	0.110
Upper Limit		61.724	64.251
Weeks to First Observed Tumor	105	105	105
Thyroid: C-cell Adenoma (b)	2/20 (10)	3/49 (6)	3/48 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.612	0.625
Lower Limit		0.078	0.079
Upper Limit		6.996	7.137
Weeks to First Observed Tumor	105	105	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets:			
Islet-cell Adenoma (b)	2/19 (11)	2/50 (4)	3/47 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.380	0.606
Lower Limit		0.030	0.077
Upper Limit		5.009	6.913
Weeks to First Observed Tumor	105	105	105
Preputial Gland: Carcinoma, NOS (b)	0/20 (0)	4/50 (8)	0/48 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.386	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	77	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	16/20 (80)	46/50 (92)	43/48 (90)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.150	1.120
Lower Limit		0.929	0.900
Upper Limit		1.451	1.456
Weeks to First Observed Tumor	83	77	69
Brain: Astrocytoma (b)	2/20 (10)	0/50 (0)	0/48 (0)
P Values (c,d)	P = 0.026(N)	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.044		
Relative Risk (f)		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.345	1.400
Weeks to First Observed Tumor	83	--	--

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

(continued)

(a) Dosed groups received 1,500 or 3,000 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 Sulfoxide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	2/20 (10)	0/50 (0)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	0.200
Lower Limit		0.000	0.004
Upper Limit		1.345	3.681
Weeks to First Observed Tumor	105	--	105
Hematopoietic System: Lymphoma (b)	2/20 (10)	5/50 (10)	12/50 (24)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.000	2.400
Lower Limit		0.184	0.614
Upper Limit		10.007	20.902
Weeks to First Observed Tumor	82	102	81

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	7/20 (35)	7/50 (14)	12/48 (25)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	0.714
Lower Limit		0.144	0.318
Upper Limit		1.187	1.875
Weeks to First Observed Tumor	82	86	100
Thyroid: C-cell Adenoma or Carcinoma (b)	0/19 (0)	5/50 (10)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.501	0.021
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	82	102	81

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma (b)	4/20 (20)	1/50 (2)	0/50 (0)
P Values (c,d)	P = 0.002(N)	P = 0.021(N)	P = 0.005(N)
Departure from Linear Trend (e)	P = 0.036		
Relative Risk (f)		0.100	0.000
Lower Limit		0.002	0.000
Upper Limit		0.944	0.427
Weeks to First Observed Tumor	105	105	--
Uterus or Cervix Uteri: Endometrial Stromal Polyp (b)	0/20 (0)	6/50 (12)	3/47 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.667	0.266
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	100	93

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

(continued)

(a) Dosed groups received 3,000 or 6,000 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.

APPENDIX F

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	3/18 (17)	4/50 (8)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.480	0.480
Lower Limit		0.093	0.093
Upper Limit		3.058	3.058
Weeks to First Observed Tumor	105	104	98
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	6/18 (33)	10/50 (20)	7/50 (14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.600	0.420
Lower Limit		0.243	0.147
Upper Limit		1.776	1.346
Weeks to First Observed Tumor	103	104	98

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	5/18 (28)	8/50 (16)	3/50 (6)
P Values (c,d)	P = 0.014(N)	N.S.	P = 0.026(N)
Relative Risk (f)		0.576	0.216
Lower Limit		0.200	0.039
Upper Limit		2.012	1.012
Weeks to First Observed Tumor	23	81	104
Liver: Hemangiosarcoma (b)	0/18 (0)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.020	0.227
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	102

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	6/18 (33)	31/50 (62)	46/50 (92)
P Values (c,d)	P less than 0.001	P = 0.034	P less than 0.001
Relative Risk (f)		1.860	2.760
Lower Limit		0.966	1.570
Upper Limit		4.545	4.868
Weeks to First Observed Tumor	71	92	83
Eye/Lacrimal Gland: Adenoma, NOS (b)	1/18 (6)	0/50 (0)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.080
Lower Limit		0.000	0.096
Upper Limit		6.729	55.565
Weeks to First Observed Tumor	105	--	103

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Site Unknown: Lipoma (b)	0/18 (0)	3/50 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.227	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

(a) Dosed groups received 350 or 700 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 Sulfoxide in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	2/18 (11)	2/48 (4)	2/49 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.375	0.367
Lower Limit		0.030	0.029
Upper Limit		4.932	4.834
Weeks to First Observed Tumor	105	98	102
Hematopoietic System: Lymphoma or Lymphocytic Leukemia (b)	5/19 (26)	12/50 (24)	10/50 (20)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.912	0.760
Lower Limit		0.360	0.284
Upper Limit		2.959	2.547
Weeks to First Observed Tumor	88	51	85

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	1/18 (6)	3/50 (6)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.080	2.204
Lower Limit		0.096	0.302
Upper Limit		55.565	99.144
Weeks to First Observed Tumor	105	105	104
Pituitary: Adenoma, NOS (b)	0/17 (0)	3/47 (6)	0/45 (0)
P Values (c,d)	N.S.	N.S.	--
Relative Risk (f)		Infinite	--
Lower Limit		0.229	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma (b)	0/18 (0)	1/45 (2)	4/47 (9)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.372
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	102
Site Unknown: Lipoma (b)	1/19 (5)	2/50 (4)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.760	1.140
Lower Limit		0.043	0.101
Upper Limit		43.961	58.635
Weeks to First Observed Tumor	105	105	60

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

(continued)

(a) Dosed groups received time-weighted average doses of 295 or 754 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.

Review of the Bioassay of Piperonyl Sulfoxide* 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 Sulfoxide for carcinogenicity.

The primary reviewer agreed with the conclusion in the report that Piperonyl Sulfoxide was not carcinogenic in either sex of rats or female mice, but produced an increased incidence of hepatocellular carcinomas in treated male mice under the conditions of test. He briefly described the experimental design and noted the incidence of hepatocellular carcinomas in the control and treated male mice.

A motion was approved unanimously that the report on the bioassay of Trimethylthiourea 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.

☆U.S. GOVERNMENT PRINTING OFFICE: 1978-281-217/3264

DHEW Publication No. (NIH) 79-1379