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FOR POSSIBLE CARCINOGENICITY

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

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CONTRIBUTORS: This report presents the results of the bioassay of lasiocarpine for possible carcinogenicity, conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Stanford Research Institute, Menlo Park, California, initially under direct contract to NCI and currently under a subcontract to Tracer Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. R. R. Bates[1,2], D. C. L. Jones[3], D. P. Sasmore[3], G. W. Newell[3], and R. M. Elashoff[4], and Mr. W. E. Davis[3]. The principal investigator was Dr. D. C. L. Jones; the technical supervisor of animal treatment, observation, and data handling was Mr. W. E. Davis; necropsy and tissue fixation were supervised by Dr. D. P. Sasmore.

Histologic examinations were performed by Dr. V. J. Rosen[5], and the diagnoses included in this report represent his interpretation. Neoplasms and compound-related hyperplastic lesions were reviewed by Dr. W. M. Busey[6], who also prepared the interpretive pathology summary included in this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[7]. The statistical analyses were performed by Dr. J. R. Joiner[8], using methods selected for the bioassay program by Dr. J. J. Gart[9]. Chemicals used in this bioassay were analyzed at Stanford Research Institute, and the analytical results were reviewed by Dr. C. W. Jameson[8].

This report was prepared at Tracor Jitco[8] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg, Director of the Bioassay Program; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Ms. L. A. Waitz and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

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SUMMARY

A bioassay of lasiocarpine for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats.

Groups of 24 rats of each sex were administered lasiocarpine at one of three doses, either 7, 15, or 30 ppm, for 104 weeks. Matched controls consisted of groups of 24 untreated rats of each sex. All surviving rats were killed at 104 weeks.

Mean body weights of the high-dose male and female rats were lower than those of the matched-control groups throughout most of the study, while weights of the mid-dose rats were lower only during the second year, and weights of the low-dose groups were unaffected. There was a positive dose-related trend in mortality for both sexes, with none of the high-dose animals, only five of the mid-dose animals, 23 of the low-dose animals, and 43 of the matched controls surviving to termination of the study. In spite of these early deaths, all male rats except one low-dose animal and one high-dose animal developed tumors, and among the females, 23 low-dose and 22 mid-dose animals developed tumors. Time-adjusted analysis of the incidence of tumors was performed in the female rats.

In male rats, there was a positive dose-related trend ($P < 0.001$) in the incidence of angiosarcoma of the liver; furthermore, the incidences in the mid- and high-dose groups, but not that in the low-dose, were significantly higher ($P < 0.001$, both groups) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 11/24, high-dose 13/24). In females, the incidences in both the low- and mid-dose groups, but not that in the high-dose, were significantly higher ($P = 0.002$ and $P = 0.005$, respectively) than that in the controls (controls 0/24, low-dose 8/24, mid-dose 7/24, high-dose 2/9). Metastatic angiosarcomas were present in the lungs from a few of the rats in all three treated groups of both sexes.

In both male and female rats, there was a positive dose-related

trend in the combined incidence of hepatocellular carcinoma and adenoma of the liver (males, P = 0.003; females, P < 0.001); furthermore, the combined incidence of these tumors in the high-dose females, but not those in the low- and mid-dose, was significantly higher (P < 0.001) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 1/24, high-dose 7/9). The P-value of the combined incidence in the high-dose males (P = 0.025) is above the 0.016 level required by the Bonferroni inequality criterion, when multiple comparison is considered (controls 0/24, low-dose 0/24, mid-dose 3/24, high-dose 5/24). Nodular hyperplasia was observed in additional animals of each treated group of each sex. Thus, lasiocarpine was associated with proliferative lesions of hepatocytes as well as with angiosarcomas arising from endothelial cells of the liver.

The combined incidence of lymphoma or leukemia was significant in both the low- and mid-dose female groups ($P \leq 0.018$), but not in the high-dose group, perhaps because of the early deaths in this group (controls 2/24, low-dose 9/24, mid-dose 11/24, high-dose 1/23). The incidences of these tumors in the males were not significant.

It is concluded that under the conditions of this bioassay, lasiocarpine was carcinogenic in Fischer 344 rats producing hepatocellular tumors and angiosarcomas of the liver in both sexes and hematopoietic tumors in female animals.

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I. INTRODUCTION

Lasiocarpine (CAS 303-34-4; NCI C01478) is a pyrrolizidine alkaloid that is found in the seeds of Heliotropium lasiocarpum, Heliotropium europaeum, and several other plant species, all members of the family Boraginaceae (IARC, 1976). Its hepatotoxic effects in sheep and cattle grazing on Heliotropium plants have been studied in Australia. Acute liver dystrophy in man, caused by H. lasiocarpum poisoning, was reported in the U.S.S.R. between 1931 and 1945 (Bull et al., 1968). Lasiocarpine has been reported to be carcinogenic in rats following its intraperitoneal injection (Svoboda and Reddy, 1972).

This bioassay of lasiocarpine was conducted as a part of a larger study that was designed to assess the combined effects of a group of known or suspected carcinogens. Only the results of the study of the administration of lasiocarpine are reported herein.

II. MATERIALS AND METHODS

A. Chemical

Lasiocarpine is an alkaloid which is the 7((2,3-dihydroxy-2-(l-methoxyethyl)-3-methyl-l-oxobutoxy)methyl)-2,3,5,7a-tetrahydro-l H-pyrrolizin-1-yl ester (1S,(1α(Z),-7(2S,3R)7aα) of 2-methyl-2-butenoic acid. The chemical was obtained in a single batch from Chemasea, Inc., Sidney, Australia. The purity of the batch was determined to be 97% by analyses at Stanford Research Institute. The melting point (capillary) was 92.5-93.5°C uncorrected (literature: 96.5-97°C), and the elemental analysis (C, H, N) was correct for $C_{21}H_{33}NO_7$ the molecular formula of lasiocarpine. The identity of the chemical was confirmed by nuclear magnetic resonance, infrared, and ultraviolet spectra, which were in agreement with the structure and matched the spectra given in the literature.

The lasiocarpine was stored at -4°C in capped bottles.

B. Dietary Preparation

All diets were formulated every 2 weeks using Low Fat Lab Chow® (Ralston Purina Co., St. Louis, Mo.). A stock diet containing 500 ppm lasiocarpine was first prepared by grinding the alkaloid to a fine powder and then mixing by hand a weighed amount of the

powdered material with a small amount of feed. Corn oil and more feed were then added to give a final concentration of 500 ppm lasiocarpine and 3% corn oil (Staley Manufacturing Company, Orange, Calif.). Final mixing was accomplished with a Hobart blender. Each stock diet was analyzed for content of lasiocarpine by a method involving extraction and quantitation by gas-liquid chromatography. Concentrations of 500 ppm ± 10% were considered acceptable for use in preparing the test diets. Lasiocarpine at 500 ppm in the stock diet was found to be stable when held in rat feeders at room temperature for a 2-week period.

To obtain test diets having appropriate concentrations of lasiocarpine, the stock diet was diluted, as required, with control diet containing 3% corn oil and mixed in a Hobart blender for 7 minutes. The stock mixtures were stored in plastic bags at -4°C. Test diets were stored in covered plastic containers at room temperature.

C. Animals

Male and female Fischer 344 rats, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were obtained from Simonsen Laboratory, Gilroy, California. On arrival at the laboratory, all animals were quarantined for 2 weeks as an acclimation period.

Following this period, all males gaining less than 25 grams, all females gaining less than 15 grams, and all unhealthy animals were culled. The remaining animals were assigned to cages, one per cage, until each cage contained three animals. Cages were then numbered and assigned to control and treated groups using a computer-generated randomization table. Rats were ear-clipped for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 22°C with a range from 21-24°C, and the relative humidity was maintained at approximately 45%. The room air was changed 10 times per hour and was maintained under positive pressure relative to the access halls. Fluorescent lighting provided illumination 12 hours per day. Food and water were available ad libitum. Drinking water was softened, filtered, sterilized with ultraviolet light, and supplied by means of an automatic watering system.

The rats were housed three per cage in polycarbonate cages equipped with disposable polyester woven filter tops. Autoclaved hardwood chips (Iso-Dri®, Becton, Dickinson, and Carworth, Warrensburg, N. Y.) were used as bedding. The cages were changed,

washed, and provided with fresh bedding twice per week. Filter tops were replaced once per month.

Rats fed lasiocarpine were housed in the same room as rats treated with N-methyl-N'-nitro-N-nitrosoguanidine (CAS 70-25-7); N-butyl-N-(4-hydroxybutyl)nitrosamine (CAS 3817-11-6); nitrilotriacetic acid, trisodium salt, monohydrate (CAS 18662-53-8); aflatoxin B_1 (CAS 1162-65-8); cycasin (CAS 14901-08-7); or N-nitrosodi-pentylamine (CAS 13256-06-9).

E. Subchronic Studies

Subchronic feeding studies were conducted with male and female Fischer 344 rats to estimate the maximum tolerated dose of lasiocarpine, on the basis of which low, mid, and high concentrations (hereinafter referred to as "low doses", "mid doses", and "high doses") were determined for administration in the chronic studies. In the subchronic studies, lasiocarpine was added to feed in concentrations of 5, 10, 20, 40, 80, or 160 ppm. Treated and control groups each consisted of 15 male and of 15 female rats. The chemical was provided in feed to the treated groups for 8 weeks.

At the end of 2 weeks, the animals receiving 160 and 5 ppm were killed, because of appreciable body weight loss in the 160-ppm group and lack of effect in the 5-ppm group. In the remaining

groups there were few deaths (1/15 males at 20 ppm and 1/15 females at 80 ppm). Weight gain of males at the end of 8 weeks was unaffected at 10 and 20 ppm, 80% of that of controls at 40 ppm, and 23% of that of controls at 80 ppm. In females, weight gain was unaffected at 10 and 20 ppm, 72% of that of controls at 40 ppm, and 14% of that of controls at 80 ppm. There were no gross lesions at necropsy; however, slight hepatocellular pleomorphism was noted at 40 ppm. The low, mid, and high doses for the chronic studies were set at 7, 15, and 30 ppm.

F. Design of Chronic Studies

The design of the chronic studies is shown in table 1.

G. Clinical and Pathologic Examinations

All animals were observed daily for signs of toxicity, and animals that were moribund were killed and necropsied. Animals were weighed individually every other week for 12 weeks, and once every fourth week for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross examination of major organs and tissues from killed animals and from animals found dead. The following tissues were routinely examined microscopically from both control and treated animals: lungs and bronchi,

Table 1. Design of Lasiocarpine Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Lasiocarpine[b]	Time on Study	
			Treated[c] (weeks)	Untreated (weeks)
Matched-Control	24	0		104
Matched-Control	24	0		104
High-Dose	24	30	69[e]	

[a]All animals were approximately 54 ± 2 days of age when placed on study.

[b]All diets contained 3% corn oil.

[c]A11 animals were started on study within 2 days of each other.

[d]All high-dose males died by week 88.

[e]All high-dose females died by week 69.

spleen, liver, kidney, pituitary, and testis. In addition, tissues of the stomach, urinary bladder, thyroid, uterus, ovary, and brain were examined from a majority of the controls; these tissues were taken from treated animals only if a lesion was found at necropsy. Occasionally, additional tissues were examined microscopically. Gross lesions from all animals were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few of the tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. 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 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's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given 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).

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 treated animals at each dose level. When results for a number of treated 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 ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to 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 treated 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 treated 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 treated 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 treated 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 signifi-

cant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs

Mean body weights of high-dose male and female rats were lower than those of all other groups throughout most of the study (figure 1). Weights of mid-dose animals were lower than those of controls only during the second year, and weights of low-dose groups were unaffected by lasiocarpine. Between weeks 80 and 88, surviving treated and control animals were anorexic and lost weight from a suspected viral infection. No treatment was given, and the animals apparently recovered by the next weighing period (week 92).

Mortality was not usually preceded by any clinical signs; occasionally, however, pale mucous membranes, general weakness, abdominal distention, or emaciation were observed before deaths occurred.

B. Survival

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats fed lasiocarpine in the diet at the doses used in this study, together with those of the controls, are shown in figure 2.

In both sexes, the Tarone test result for positive dose-related

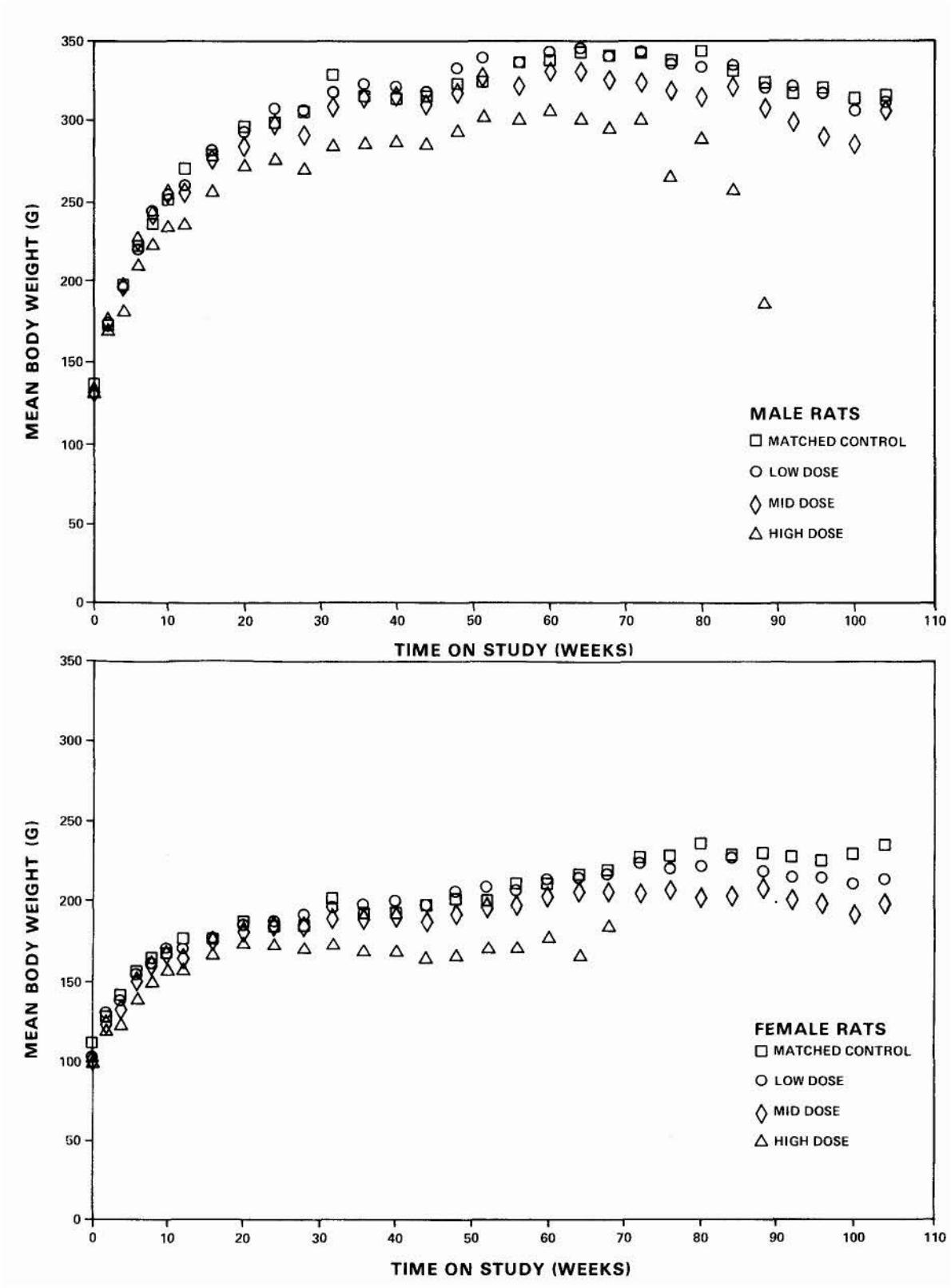

Figure 1. Growth Curves For Rats Fed Lasiocarpine In The Diet

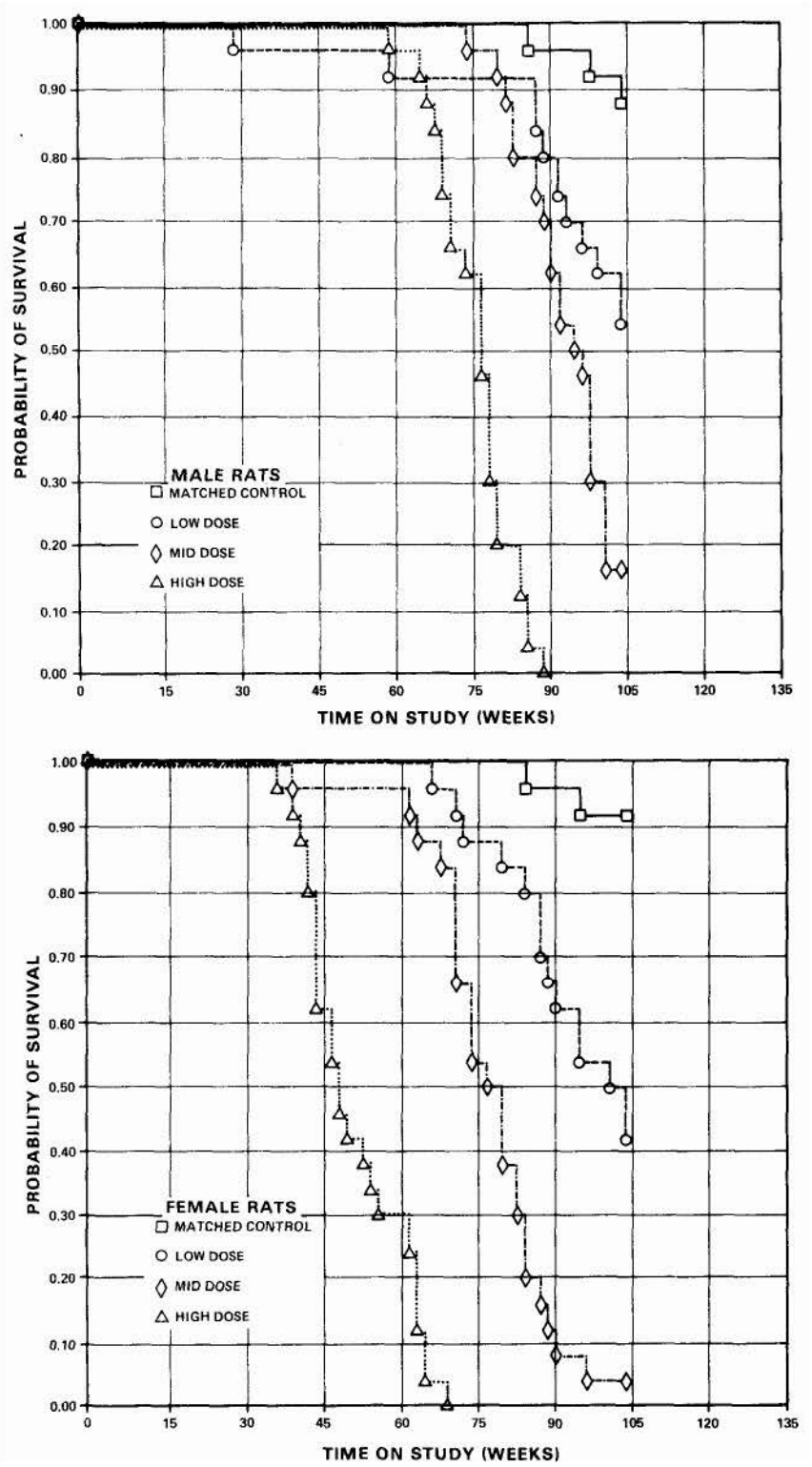

Figure 2. Survival Curves For Rats Fed Lasiocarpine In The Diet

trend in mortality is significant ($P < 0.001$), and departure from linear trend is observed ($P < 0.001$), due to the steep increase in mortality occurring in the treated groups, especially the high-dose groups. In male rats, 88% of the controls, 54% of the low-dose group, 17% of the mid-dose group, and none of the high-dose group survived to termination of the study. In females, 92% of the controls, 42% of the low-dose group, 4% of the mid-dose group, and none of the high-dose group survived to termination of the study. Since over 50% of the female high-dose group died before week 52 on study, the statistical analysis of female rats was done using only those animals surviving more than 52 weeks. The numbers of such female rats were 24 in the control, 24 in the low-dose, 23 in the mid-dose, and 9 in the high-dose groups. Since a tumor occurred in the hematopoietic system at week 39 in female rats, the time-adjusted analysis at this site is based on animals surviving 39 weeks or more. All male rats, except one low-dose and one high-dose animal, developed tumors. In female rats, 23/24 low-dose and 22/24 mid-dose rats developed tumors, but the early deaths of the high-dose females may have reduced the incidences of late—appearing tumors in that group.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in

Appendix A, tables Al and A2; nonneoplastic lesions are summarized in Appendix B, tables B1 and B2.

A variety of neoplastic processes not related to treatment were observed in both the control and treated rats. The incidences of these neoplasms were comparable among the control and treated groups. The following neoplasms were randomly distributed throughout the control and treated groups: squamous-cell carcinoma of the skin, alveolar/bronchiolar adenomas of the lung, and endometrial stromal polyps of the uterus. Lymphomas and leukemias were found more frequently in treated than in control rats.

Angiosarcomas were observed in the livers from several of the male and female lasiocarpine-treated rats. In the males, the incidence of this neoplasm of the liver occurred in a dose-related fashion, whereas in the females, the incidence was greater in the low- and mid-dose groups than in the high-dose group. This inverse dose relationship can probably be explained by the increased deaths in the high-dose females compared with either the controls or low-dose females. Metastatic angiosarcomas were occasionally seen in the lungs from the rats in all three treated groups. The angiosarcomas were either single or multiple in the liver and were characterized by a proliferation of neoplastic endothelial cells, which in some

cases exhibited a high degree of anaplasia. Large blood-filled cysts lined by neoplastic endothelial cells frequently were present. The neoplastic endothelial cells sometimes assumed a cuboidal shape and contained large basophilic nuclei. In some instances, sheets of proliferating endothelial cells were present in the parenchyma of the liver, destroying the hepatic architecture. Adenomas and/or nodular hyperplasia of the hepatocytes were also frequently seen in the liver. The areas of nodular hyperplasia contained swollen hepatocytes with large, bizarre nuclei and possessed tinctorial properties distinctly different from the surrounding liver tissue. In some instances, the foci were compressing the surrounding liver parenchyma. The hepatocellular adenomas morphologically resembled neoplastic nodules (Squire and Levitt, 1975) and were usually composed of large, eosinophilic hepatocytes containing bizarre nuclei. These adenomas were large, occupied several liver lobules, and frequently compressed the surrounding liver parenchyma. In both sexes, hepatocellular carcinomas were found only in the high-dose animals: two in the males and one in the females.

In conclusion, the results of this microscopic examination indicate that the administration of lasiocarpine at the three doses used in this study had a carcinogenic effect in the liver of Fischer 344 rats. This effect was manifested by the induction

of angiosarcomas, several of which metastasized to the lung. No angiosarcomas were present in any of the tissues examined from the control rats, and this neoplasm is rare in the Fischer 344 rat. Lesions diagnosed as adenomas or nodular hyperplasia were seen only in the treated rats, indicating a proliferative hepatocellular effect. This finding lends support to the conclusion that lasiocarpine had a carcinogenic effect in the liver.

D. Statistical Analyses of Results

Tables C1-C3 in Appendix C contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals in one group and with an incidence of at least 5% in one or more of the treated groups. The analysis of female rats is based on those animals surviving 52 weeks or more; however, the analysis of the incidence of lymphoma and the combined incidence of lymphoma and leukemia is based on animals that lived 39 weeks or more, because such a tumor appeared in this system at week 39.

In male rats, the Cochran-Armitage test results for positive dose-related trend in the proportions of angiosarcoma of the liver is significant ($P < 0.001$). The Fisher exact test shows that the incidences in the mid-, and high-dose groups are

significantly higher than the incidence in the matched-control group ($P < 0.001$, and $P < 0.001$, respectively), and the lower limits of the 95% confidence intervals for these relative risks are greater than one. The Fisher exact comparison of incidences in the low-dose groups with incidences in the matched-control groups show a probability level of 0.025, which is above the 0.016 level required by the Bonferroni inequality criterion when multiple comparison is considered.

In female rats, the Fisher exact test shows that the incidences of angiosarcoma of the liver in both the low- and mid-dose groups are significantly higher than that in the controls ($P = 0.002$ and $P = 0.005$, respectively). The incidence of this tumor in the high-dose group is not significant, due to the low number of animals in the denominator of that group; nevertheless, the incidence is 2/9 (22%). The statistical evidence suggests that the incidence of this tumor in rats is associated with lasiocarpine at the doses used in this experiment.

When the analyses of the incidence of leukemia and of the combined incidence of leukemia and lymphoma in female rats are performed, the Fisher exact test shows that the incidences in the low- and mid-dose groups are significantly higher ($P < 0.025$) than those in the corresponding matched controls. The time-adjusted incidence of these tumors in the high-dose group is 1/9

(11%) for leukemia alone and 1/23 (4%) for the combination of lymphoma and leukemia. Statistical tests on the incidence of these tumors in the male rats are not significant. Although the combined incidence in the mid-dose group indicates a P value of 0.030 when compared to the matched-control incidence, this probability level is higher than the 0.016 level required by the application of the Bonferroni inequality criterion to the results of multiple comparison under this situation.

In both sexes, hepatocellular carcinomas are found only in the high-dose groups. None of the Fisher exact test results in either sex are significant. However, the combination of adenoma, NOS (not otherwise specified), and hepatocellular carcinoma indicates a significant difference in incidence ($P < 0.001$) between the high-dose group and the controls in females. The Fisher exact comparison of incidences between the high-dose and matched-control groups in male rats indicate a probability level of 0.025, which is above the 0.016 level required by the Bonferroni inequality criterion, when multiple comparison is considered. The result of the Cochran-Armitage test for positive dose-related trend is also significant for both sexes ($P = 0.003$, males; $P < 0.001$, females). The principle contribution to these significant results is made by the adenomas, NOS. The overall statistical conclusion is that there is a dose relationship

between this chemical and the occurrence of these liver tumors in female rats.

Significant results in the negative direction are observed in the incidence of interstitial-cell tumor of the testis in male rats and in the incidence of chromophobe adenoma of the pituitary in female rats, where the incidences in the controls exceed those in the treated groups. These significant negative results may be explained by the severe mortality of the treated animals.

IV. DISCUSSION

The high and mid doses of lasiocarpine used in this bioassay were toxic, as shown by the decrease in body weights and decrease in survival. Mean body weights of the high-dose groups were lower than those of matched-control groups throughout most of the study, while mean body weights of the mid-dose groups were lower only during the second year, and weights of the low-dose groups were unaffected. There was a positive dose-related trend in mortality for both sexes, and none of the high-dose animals survived to termination of the study. Time-adjusted analysis of the incidence of tumors was performed in the female rats.

Angiosarcomas occurred at several sites in treated rats of both sexes (males: 5 low-dose, 11 mid-dose, 14 high-dose; females: 8 low-dose, 9 mid-dose, 2 high-dose). However, most of these occurred in the liver. In male rats, there was a positive dose-related trend ($P < 0.001$) in the incidence of angiosarcoma of the liver; furthermore, the incidences in the mid- and high-dose groups, but not that in the low-dose, were significantly higher ($P < 0.001$, both groups) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 11/24, high-dose 13/24). In females, the incidences in both the low- and mid-dose groups, but not that in the high-dose, were significantly higher ($P = 0.002$ and $P = 0.005$, respectively) than that in the controls

(controls 0/24, low-dose 8/24, mid-dose 7/24, high-dose 2/9). Metastatic angiosarcomas were observed in the lungs from a few of the rats of both sexes in all three treated groups (males: three low-dose, five mid-dose, and seven high-dose; females: three low-dose, four mid-dose, and one high-dose). No angiosarcomas were present in control rats and this neoplasm is rare among Fischer 344 rats.

In both male and female rats, there was a positive dose-related trend in the combined incidence of hepatocellular carcinoma and adenoma of the liver (males, $P = 0.003$; females, $P < 0.001$); furthermore, the combined incidence of these tumors in the high-dose females, but not those in the low- and mid-dose, was significantly higher ($P < 0.001$) than that in the controls (controls 0/24, low-dose 5/24, mid-dose 1/24, high-dose 7/9). The P-value of the combined incidence in the high-dose males ($P = 0.025$) is above the 0.016 level required by the Bonferroni inequality criterion, when multiple comparison is considered (controls 0/24, low-dose 0/24, mid-dose 3/24, high-dose 5/24). Nodular hyperplasia was observed in additional animals of each treated group of each sex. Thus, lasiocarpine was associated with proliferative hepatocellular lesions as well as with the angiosarcomas arising from endothelial cells of the liver.

The combined incidence of lymphoma or leukemia (controls 2/24,

low-dose 9/24, mid-dose 11/24, high-dose 1/23) was significant in both the low- and mid-dose female groups ($P \leq 0.018$), but not in the high-dose group, perhaps because of the early deaths in this group. The incidences of these tumors in the males were not significant.

Previous studies have also shown that the liver is the target organ for lasiocarpine. Rats injected with lasiocarpine developed centrilobular necrosis that sometimes appeared several weeks or months after the treatment (Schoental and Magee, 1957). Midzonal hemorrhagic necrosis was reported to occur in injected rats in acute toxicity studies (Bull et al., 1958). In chronic toxicity studies, injected rats developed enlarged livers which consisted almost entirely of regenerated parenchyma (Bull et al., 1968; Svoboda et al., 1971); this alteration was believed to be related to the prolonged antimitotic effect of the chemical. Svoboda and Reddy (1972) found carcinomas of the liver and squamous-cell carcinomas of the skin in Fischer 344 rats that were injected with lasiocarpine twice per week for 4 weeks, and then injected once per week for 52 weeks.

It is concluded that under the conditions of this bioassay, lasiocarpine was carcinogenic in Fischer 344 rats producing hepatocellular tumors and angiosarcomas of the liver in both sexes and hematopoietic tumors in female animals.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS FED LASIOCARPINE IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED LASIOCARPINE IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24	24	24	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	24	24	24
INTEGUMENTARY SYSTEM				
*SKIN	(24)	(24)	(24)	(24)
SQUAMOUS CELL CARCINOMA		1 (4%)		
*SUBCUT TISSUE	(24)	(24)	(24)	(24)
SARCOMA, NOS			1 (4%)	
RESPIRATORY SYSTEM				
#LUNG	(23)	(24)	(24)	(24)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (9%)			1 (4%)
PHEOCHROMOCYTOMA, METASTATIC		1 (4%)		
SARCOMA, NOS	1 (4%)			
ANGIOSARCOMA				1 (4%)
ANGIOSARCOMA, METASTATIC		3 (13%)	5 (21%)	7 (29%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(24)	(24)	(24)	(24)
MALIGNANT LYMPHOMA, NOS	1 (4%)	2 (8%)	3 (13%)	1 (4%)
UNDIFFERENTIATED LEUKEMIA	1 (4%)		2 (8%)	2 (8%)
GRANULOCYTIC LEUKEMIA	1 (4%)	1 (4%)	6 (25%)	4 (17%)
#LYMPH NODE	(2)	(4)	(10)	(8)
LEIOMYOSARCOMA, METASTATIC			1 (10%)	
ANGIOSARCOMA, METASTATIC		1 (25%)	1 (10%)	
#LIVER	(23)	(24)	(23)	(23)
GRANULOCYTIC LEUKEMIA	1 (4%)			
#THYMUS		(1)	(1)	(2)
ANGIOSARCOMA, METASTATIC			1 (100%)	
CIRCULATORY SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(23)	(24)	(23)	(23)
ADENOMA, NOS			3 (13%)	3 (13%)
HEPATOCELLULAR CARCINOMA				2 (9%)
LEIOMYOSARCOMA, METASTATIC		1 (4%)	1 (4%)	
ANGIOMA			1 (4%)	
ANGIOSARCOMA		5 (21%)	11 (48%)	13 (57%)
#DUODENUM		(3)	(2)	(2)
ADENOCARCINOMA, NOS			1 (50%)	
SARCOMA, NOS		1 (33%)		1 (50%)
LEIOMYOSARCOMA		1 (33%)	1 (50%)	1 (50%)
FIBROADENOMA		1 (33%)		
#COLON			(2)	
ADENOMATOUS POLYP, NOS			1 (50%)	
URINARY SYSTEM				
#KIDNEY	(23)	(24)	(24)	(24)
ANGIOSARCOMA, METASTATIC		1 (4%)		
#KIDNEY/CORTEX	(23)	(24)	(24)	(24)
ADENOMA, NOS		1 (4%)		
ENDOCRINE SYSTEM				
#PITUITARY	(22)	(23)	(23)	(24)
CHROMOPHOBE ADENOMA			1 (4%)	
#ADRENAL	(1)	(1)		
PHEOCHROMOCYTOMA, MALIGNANT		1 (100%)		
#THYROID	(16)	(1)	(1)	
C-CELL CARCINOMA		1 (100%)		
REPRODUCTIVE SYSTEM				
#TESTIS	(24)	(24)	(24)	(24)
INTERSTITIAL-CELL TUMOR	24 (100%)	21 (88%)	22 (92%)	8 (33%)
NERVOUS SYSTEM				
NONE				

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
*EYE/LACRIMAL GLAND	(24)	(24)	(24)	(24)
UNDIFFERENTIATED CARCINOMA			1 (4%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
*MULTIPLE ORGANS	(24)	(24)	(24)	(24)
MESOTHELIOMA, MALIGNANT	1 (4%)			
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	24	24	24	24
NATURAL DEATH@		4	6	10
MORIBUND SACRIFICE	3	7	14	14
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	21	13	4	
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	24	23	24	23
TOTAL PRIMARY TUMORS	32	36	54	37
TOTAL ANIMALS WITH BENIGN TUMORS	24	21	23	10
TOTAL BENIGN TUMORS	26	23	28	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	12	20	23
TOTAL MALIGNANT TUMORS	6	13	26	25
TOTAL ANIMALS WITH SECONDARY TUMORS#		5	6	7
TOTAL SECONDARY TUMORS		7	9	7
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 A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED LASIOCARPINE IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24	24	24	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	24	24	23
INTEGUMENTARY SYSTEM				
*SKIN	(24)	(24)	(24)	(24)
SQUAMOUS CELL CARCINOMA	1 (4%)			
*SUBCUT TISSUE	(24)	(24)	(24)	(24)
ANGIOSARCOMA			1 (4%)	
RESPIRATORY SYSTEM				
#LUNG	(24)	(23)	(24)	(23)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (4%)		
ANGIOSARCOMA			2 (8%)	
ANGIOSARCOMA, METASTATIC		3 (13%)	4 (17%)	1 (4%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(24)	(24)	(24)	(24)
MALIGNANT LYMPHOMA, NOS	1 (4%)	2 (8%)	3 (13%)	
UNDIFFERENTIATED LEUKEMIA			1 (4%)	
LYMPHOCYTIC LEUKEMIA		1 (4%)		
GRANULOCYTIC LEUKEMIA	1 (4%)	6 (25%)	7 (29%)	1 (4%)
#THYMUS		(1)	(5)	
ANGIOSARCOMA, METASTATIC		1 (100%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(24)	(22)	(24)	(23)
ADENOMA, NOS		5 (23%)	1 (4%)	6 (26%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA				1 (4%)
ANGIOSARCOMA		8 (36%)	7 (29%)	2 (9%)
#STOMACH	(23)	(4)	(1)	(8)
SQUAMOUS CELL PAPILLOMA	1 (4%)			
ADENOCARCINOMA, NOS		1 (25%)		
ADENOMATOUS POLYP, NOS		2 (50%)		
URINARY SYSTEM				
#URINARY BLADDER	(16)		(1)	(1)
PAPILLARY CARCINOMA	1 (6%)			
ENDOCRINE SYSTEM				
#PITUITARY	(24)	(24)	(23)	(23)
CHROMOPHOBE ADENOMA	10 (42%)	5 (21%)		
BASOPHIL ADENOMA		1 (4%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(24)	(24)	(24)	(24)
ADENOMA, NOS	4 (17%)			
ADENOCARCINOMA, NOS		1 (4%)		
#UTERUS	(22)	(15)	(12)	(2)
CARCINOMA-IN-SITU, NOS		1 (7%)		
ADENOCARCINOMA, NOS	1 (5%)	1 (7%)		
ENDOMETRIAL STROMAL POLYP	6 (27%)	9 (60%)	4 (33%)	
ENDOMETRIAL STROMAL SARCOMA	1 (5%)			
CARCINOSARCOMA		1 (7%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
ADIPOSE TISSUE				
ANGIOSARCOMA, METASTATIC				1
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	24	24	24	24
NATURAL DEATH@		5	7	14
MORIBUND SACRIFICE	2	9	16	10
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	22	10	1	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	22	22	8
TOTAL PRIMARY TUMORS	27	45	26	10
TOTAL ANIMALS WITH BENIGN TUMORS	17	16	4	6
TOTAL BENIGN TUMORS	21	23	5	6
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	19	20	4
TOTAL MALIGNANT TUMORS	6	22	21	4
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	4	1
TOTAL SECONDARY TUMORS		4	4	2
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 NONNEOPLASTIC LESIONS IN RATS FED LASIOCARPINE IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED LASIOCARPINE IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24	24	24	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	24	24	24
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(23)	(24)	(24)	(24)
CONGESTION, NOS	2 (9%)	4 (17%)	5 (21%)	3 (13%)
INFLAMMATION, INTERSTITIAL				1 (4%)
ABSCESS, NOS		1 (4%)		
HEMATOPOIETIC SYSTEM				
#SPLEEN	(23)	(24)	(23)	(23)
CONGESTION, NOS		2 (8%)	1 (4%)	
INFARCT, NOS	1 (4%)			
HEMATOPOIESIS			1 (4%)	
#LYMPH NODE	(2)	(4)	(10)	(8)
CONGESTION, NOS				2 (25%)
HYPERPLASIA, NOS		1 (25%)	1 (10%)	
#THYMUS		(1)	(1)	(2)
CONGESTION, NOS				2 (100%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(23)	(24)	(23)	(23)
INFLAMMATION, GRANULOMATOUS		2 (8%)		

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

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
GRANULOMA, NOS	2 (9%)	2 (8%)		
INFLAMMATION, FOCAL GRANULOMATOU		1 (4%)		
PELICSIS HEPATIS			1 (4%)	
INFARCT, NOS				1 (4%)
HYPERPLASIA, NODULAR		4 (17%)	1 (4%)	6 (26%)
*BILE DUCT	(24)	(24)	(24)	(24)
INFLAMMATION, CHRONIC		1 (4%)		
#STOMACH	(23)	(3)	(2)	(4)
DIVERTICULUM	1 (4%)			
EROSION		2 (67%)	1 (50%)	1 (25%)
URINARY SYSTEM				
#KIDNEY	(23)	(24)	(24)	(24)
INFARCT, HEALED		1 (4%)		
ENDOCRINE SYSTEM				
#THYROID	(16)	(1)	(1)	
CYST, NOS	1 (6%)			
REPRODUCTIVE SYSTEM				
#PROSTATE		(1)		
HEMORRHAGE		1 (100%)		
#TESTIS	(24)	(24)	(24)	(24)
ATROPHY, NOS		1 (4%)	1 (4%)	1 (4%)
NERVOUS SYSTEM				
#BRAIN	(23)		(2)	(2)
CONGESTION, NOS			1 (50%)	
HEMORRHAGE			1 (50%)	
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

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

TABLE B1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
BODY CAVITIES				
*INGUINAL REGION	(24)	(24)	(24)	(24)
NECROSIS, FAT	1 (4%)	4 (17%)	4 (17%)	1 (4%)
ALL OTHER SYSTEMS				
THORAX				
HEMORRHAGE		1		
SPECIAL MORPHOLOGY SUMMARY				
NONE				

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED LASIOCARPINE IN THE DIET

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	24	24	24	24
ANIMALS NECROPSIED	24	24	24	24
ANIMALS EXAMINED HISTOPATHOLOGICALLY	24	24	24	23
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG/BRONCHUS	(24)	(23)	(24)	(23)
BRONCHIECTASIS	1 (4%)	1 (4%)		
#LUNG	(24)	(23)	(24)	(23)
CONGESTION, NOS	4 (17%)	2 (9%)	3 (13%)	6 (26%)
INFLAMMATION, INTERSTITIAL		1 (4%)	1 (4%)	
GRANULOMA, NOS	1 (4%)			
HEMOSIDEROSIS			1 (4%)	
HEMATOPOIETIC SYSTEM				
#SPLEEN	(24)	(24)	(24)	(23)
CONGESTION, NOS			1 (4%)	
FIBROSIS				1 (4%)
HEMATOPOIESIS		3 (13%)	2 (8%)	
#LYMPH NODE	(5)	(2)	(6)	
LYMPHANGIECTASIS	1 (20%)			
GRANULOMA, NOS		1 (50%)		
#MESENTERIC L. NODE	(5)	(2)	(6)	
EDEMA, NOS			1 (17%)	
HYPERPLASIA, NOS			1 (17%)	
#THYMUS		(1)	(5)	
HYPERPLASIA, NOS			1 (20%)	
CIRCULATORY SYSTEM				
NONE				

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

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(24)	(22)	(24)	(23)
GRANULOMA, NOS	8 (33%)			
PELIOSIS HEPATIS		1 (5%)		
NECROSIS, FOCAL	2 (8%)			
INFARCT, NOS				1 (4%)
HYPERPLASIA, NODULAR		6 (27%)	10 (42%)	13 (57%)
#STOMACH	(23)	(4)	(1)	(6)
EROSION		1 (25%)		
#GASTRIC MUCOSA	(23)	(4)	(1)	(6)
EROSION				1 (13%)
#SMALL INTESTINE				(1)
HEMORRHAGE				1 (100%)
#CECUM		(1)	(1)	(5)
CONGESTION, NOS				1 (20%)
EDEMA, NOS			1 (100%)	3 (60%)
INFLAMMATION, CHRONIC				1 (20%)
URINARY SYSTEM				
#KIDNEY	(24)	(24)	(24)	(23)
HEMOSIDEROSIS		1 (4%)		
#URINARY BLADDER	(16)		(1)	(1)
HEMORRHAGE				1 (100%)
ENDOCRINE SYSTEM				
#PITUITARY	(24)	(24)	(23)	(23)
CONGESTION, NOS	1 (4%)		1 (4%)	
HEMORRHAGE			1 (4%)	1 (4%)
REPRODUCTIVE SYSTEM				
#UTERUS	(22)	(15)	(12)	(2)
HYDROMETRA	1 (5%)			

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

TABLE B2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	MID DOSE	HIGH DOSE
PYOMETRA	4 (18%)	1 (7%)		
ATROPHY, NOS				1 (50%)
#UTERUS/ENDOMETRIUM	(22)	(15)	(12)	(2)
INFLAMMATION, NOS		1 (7%)		
HYPERPLASIA, CYSTIC	1 (5%)		3 (25%)	
#OVARY	(20)	(2)	(1)	
CYST, NOS	4 (20%)			
ABSCESS, NOS			1 (100%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1			1
AUTO/NECROPSY/NO HISTO				1

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

APPENDIX C

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS FED LASIOCARPINE IN THE DIET

Table C1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Lasiocarpine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Hematopoietic System: Lymphoma[b]	1/24 (4)	2/24 (8)	3/24 (13)	1/24 (4)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.000	3.000	1.000
Lower Limit		0.111	0.265	0.013
Upper Limit		112.815	150.246	75.218
Weeks to First Observed Tumor	103	88	80	59
Hematopoietic System:				
Leukemia[b]	3/24 (13)	1/24 (4)	8/24 (33)	6/24 (25)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.333	2.667	2.000
Lower Limit		0.007	0.739	0.490
Upper Limit		3.801	13.700	10.992
Weeks to First Observed Tumor	86	103	82	68

Table C1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Lymphoma or Leukemia[b]	4/24 (17)	3/24 (13)	11/24 (46)	7/24 (29)
P Values[c,d]	N.S.	N.S.	P = 0.030	N.S.
Relative Risk (Matched Control)[f]		0.750	2.750	1.750
Lower Limit		0.121	0.968	0.515
Upper Limit		3.951	9.911	7.075
Weeks to First Observed Tumor	86	88	80	59
Liver: Hepatocellular Carcinoma[b]	0/24 (0)	0/24 (0)	0/24 (0)	2/24 (8)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	--	Infinite
Lower Limit		--	--	0.305
Upper Limit		--	--	Infinite
Weeks to First Observed Tumor	--	--	--	84

Table C1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma, NOS[b]	0/24 (0)	0/24 (0)	3/24 (13)	5/24 (21)
P Values[c,d]	P = 0.003	N.S.	N.S.	P = 0.025
Relative Risk (Matched Control)[f]		--	Infinite	Infinite
Lower Limit		--	0.622	1.309
Upper Limit		--	Infinite	Infinite
Weeks to First Observed Tumor	--	--	104	66
Liver: Angiosarcoma[b]	0/24 (0)	5/24 (21)	11/24 (46)	13/24 (54)
P Values[c,d]	P < 0.001	P = 0.025	P < 0.001	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite	Infinite
Lower Limit		1.309	3.478	4.223
Upper Limit		Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	87	74	64

Table C1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
	24/24 (100)	21/24 (88)	22/24 (92)	8/24 (33)
P Values[c,d]	P < 0.001 (N)	N.S.	N.S.	P < 0.001 (N)
Relative Risk (Matched Control)[f]		0.875	0.917	0.333
Lower Limit		0.000	0.000	0.000
Upper Limit		1.143	1.091	3.000
Weeks to First Observed Tumor	86	87	74	70

[a]Treated groups received doses of 7, 15, or 30 ppm.

[b]Number of tumor-bearing animals/number of animals necropsied (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P < 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when P < 0.05 for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched control group.

Table C2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Lung: Angiosarcoma[b]	0/24 (0)	0/24 (0)	2/24 (8)	0/24 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite	--
Lower Limit		--	0.305	--
Upper Limit		--	Infinite	--
Weeks to First Observed Tumor	--	--	68	--
Hematopoietic System: Malignant Lymphoma, NOS[b]	1/24 (4)	2/24 (8)	3/24 (13)	0/24 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.000	3.000	0.000
Lower Limit		0.111	0.265	0.000
Upper Limit		112.815	150.246	18.289
Weeks to First Observed Tumor	104	70	39	--

Table C2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Hematopoietic System: Leukemia[b]	1/24 (4)	7/24 (29)	8/24 (33)	1/24 (4)
P Values[c,d]	N.S.	P = 0.024	P = 0.013	N.S.
Relative Risk (Matched Control)[f]		7.000	8.000	1.000
Lower Limit		1.010	1.212	0.013
Upper Limit		297.414	333.388	75.218
Weeks to First Observed Tumor	95	72	63	62
Hematopoietic System: Lymphoma or Leukemia[b]	2/24 (8)	9/24 (38)	11/24 (46)	1/24 (4)
P Values[c,d]	N.S.	P = 0.018	P = 0.004	N.S.
Relative Risk (Matched Control)[f]		4.500	5.500	0.500
Lower Limit		1.072	1.395	0.009
Upper Limit		38.367	45.074	8.943
Weeks to First Observed Tumor	95	70	39	62

Table C2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	0/24 (0)	0/24 (0)	0/24 (0)	1/24 (5)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	--	Infinite
Lower Limit		--	--	0.055
Upper Limit		--	--	Infinite
Weeks to First Observed Tumor	--	--	--	63
Liver: Hepatocellular Carcinoma or Adenoma, NOS[b]	0/24 (0)	5/24 (21)	1/24 (4)	7/24 (29)
P Values[c,d]	P = 0.013	P = 0.025	N.S.	P = 0.005
Relative Risk (Matched Control)[f]		Infinite	Infinite	Infinite
Lower Limit		1.309	0.055	2.021
Upper Limit		Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	104	82	52

Table C2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Liver: Angiosarcoma[b]	0/24 (0)	8/24 (33)	7/24 (29)	2/24 (8)
P Values[c,d]	N.S.	P = 0.002	P = 0.005	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite	Infinite
Lower Limit		2.382	2.021	0.305
Upper Limit		Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	84	68	56
Pituitary: Chromophobe Adenoma[b]	10/24 (42)	5/24 (21)	0/24 (0)	0/24 (0)
P Values[c,d]	P < 0.001 (N)	N.S.	P < 0.001 (N)	P < 0.001 (N)
Relative Risk (Matched Control)[f]		0.500	0.000	0.000
Lower Limit		0.160	0.000	0.000
Upper Limit		1.348	0.322	0.322
Weeks to First Observed Tumor	84	94	--	--

Table C2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	6/24 (25)	9/24 (38)	4/24 (17)	0/24 (0)
P Values[c,d]	P = 0.004 (N)	N.S.	N.S.	P = 0.011 (N)
Relative Risk (Matched Control)[f]		1.500	0.667	0.000
Lower Limit		0.568	0.158	0.000
Upper Limit		4.264	2.440	0.601
Weeks to First Observed Tumor	104	70	71	—

[a]Treated groups received doses of 7, 15, or 30 ppm.

[b]Number of tumor-bearing animals/number of animals necropsied (percent).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P < 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when P < 0.05 for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Table C3. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Lung: Angiosarcoma[b] (52)	0/24 (0)	0/24 (0)	2/24 (8)	0/9 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite	--
Lower Limit		--	0.305	--
Upper Limit		--	Infinite	--
Weeks to First Observed Tumor	--	--	68	--
Hematopoietic System: Malignant Lymphoma, NOS[b] (39)	1/24 (4)	2/24 (8)	3/24 (13)	0/23 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.000	3.000	0.000
Lower Limit		0.111	0.265	0.000
Upper Limit		112.815	150.246	19.050
Weeks to First Observed Tumor	104	70	39	--

Table C3.Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Hematopoietic System: Leukemia[b] (52)	1/24 (4)	7/24 (29)	8/23 (35)	1/9 (11)
P Values[c,d]	N.S.	P = 0.024	P = 0.009	N.S.
Relative Risk (Matched Control)[f]		7.000	8.348	2.667
Lower Limit		1.010	1.267	0.036
Upper Limit		297.414	346.345	183.950
Weeks to First Observed Tumor	95	72	63	62
Hematopoietic System: Lymphoma or Leukemia[b] (39)	2/24 (8)	9/24 (38)	11/24 (46)	1/23 (4)
P Values[c,d]	N.S.	P = 0.018	P = 0.004	N.S.
Relative Risk (Matched Control)[f]		4.500	5.500	0.522
Lower Limit		1.072	1.395	0.009
Upper Limit		38.367	45.074	9.307
Weeks to First Observed Tumor	95	70	39	62

Table C3. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Liver: Hepatocellular Carcinoma[b] (52)	0/24 (0)	0/24 (0)	0/24 (0)	1/9 (11)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	--	Infinite
Lower Limit		--	--	0.145
Upper Limit		--	--	Infinite
Weeks to First Observed Tumor	--	--	--	63
Liver: Hepatocellular Carcinoma or Adenoma, NOS[b](52)	0/24 (0)	5/24 (21)	1/24 (4)	7/9 (78)
P Values[c,d]	P < 0.001	P = 0.025	N.S.	P < 0.001
Relative Risk (Matched Control)[f]		Infinite	Infinite	Infinite
Lower Limit		1.309	0.055	5.829
Upper Limit		Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	104	82	52

Table C3. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Liver: Angiosarcoma[b] (52)	0/24 (0)	8/24 (33)	7/24 (30)	2/9 (22)
P Values[c,d]	N.S.	P = 0.002	P = 0.005	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite	Infinite
Lower Limit		2.382	2.021	0.822
Upper Limit		Infinite	Infinite	Infinite
Weeks to First Observed Tumor	--	84	68	56
Pituitary: Chromophobe Adenoma[b] (52)	10/24 (42)	5/24 (21)	0/24 (0)	0/9 (0)
P Values[c,d]	P = 0.001 (N)	N.S.	P < 0.001 (N)	P = 0.021 (N
Relative Risk (Matched Control)[f]		0.500	0.000	0.000
Lower Limit		0.160	0.000	0.000
Upper Limit		1.348	0.322	0.782
Weeks to First Observed Tumor	84	94	—	—

Table C3. Time-adjusted Analyses of the Incidence of Primary Tumors in Female Rats Fed Lasiocarpine in the Diet[3]

(continued)

Topography: Morphology	Matched Control	Low Dose	Mid Dose	High Dose
Uterus: Endometrial Stromal Polyp[b] (52)	6/24 (25)	9/24 (38)	4/24 (17)	0/9 (0)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.500	0.667	0.000
Lower Limit		0.568	0.158	0.000
Upper Limit		4.264	2.440	1.460
Weeks to First Observed Tumor	104	70	71	_ _

[a]Treated groups received doses of 7, 15, or 30 ppm.

[b]Number of tumor-bearing animals/number of animals necropsied (percent). (Based on animals that lived at least as long as shown in parentheses after the description of the morphology).

[c]Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P < 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the matched-control group when P < 0.05; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]The probability level for departure from linear trend is given when P < 0.05 for any comparison.

[f]The 95% confidence interval of the relative risk between each treated group and the matched-control group.

Review of the Bioassay of Lasiocarpine*for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens
November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and 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 organic 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 NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Lasiocarpine was reviewed.

Lasiocarpine, a known experimental carcinogen, was tested in rats as part of another study designed to investigate the combined effects of chemicals. Under the conditions of test, Lasiocarpine induced angiosarcomas in both sexes of treated rats. Although the report indicated that it also caused granulocytic leukemia in the treated females, it was pointed out that the incidence was approximately the same as observed in female control animals from the Hexa-chlorophene study. One member emphasized the limited number of animals per group and the high early mortality.

A motion was made that Lasiocarpine was carcinogenic under the conditions of test. It was added that the study was less than ideal in that the animal groups were smaller than the current standard and there was high mortality in the mid- and high-dose groups. The motion was seconded and approved unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Lawrence Garfinkel, American Cancer Society
Henry C. Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
John H. Weisburger, American Health Foundation

* 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.

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