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Carcinogen Bioassay and Program Resources Branch
Carcinogenesis Program
Division of Cancer Cause and Prevention
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CONTRIBUTORS: This report presents the results of the bioassay of picloram for possible carcinogenicity, conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Gulf South Research Institute, New Iberia, Louisiana, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogenesis bioassay program.

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Animal pathology tables and survival tables were complied at EG&G Mason Research Institute[6]. Statistical analyses were performed by Dr. J. R. Joiner[7], using methods selected for the bioassay program by Dr. J. J. Gart[8]. Chemicals used in this bioassay were analyzed under the direction of Dr. H. P. Burchfield, and the analytical results were reviewed by Dr. S. S. Olin[7].

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SUMMARY

A bioassay of technical-grade picloram for possible carcinogenicity was conducted by administering the test chemical in feed to Osborne-Mendel rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered picloram in the diet at one of the following doses for 80 weeks. Time-weighted average doses for the rats were 7,437 or 14,875 ppm; those for the mice were 2,531 or 5,062 ppm. The rats were then observed for 33 weeks, the mice for 10 weeks. Matched controls consisted of groups of 10 untreated rats or 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of the matched-control groups combined with 30 untreated male and 30 untreated female rats or mice from similar bioassays of three other test chemicals. All surviving rats were killed at 113 weeks; all surviving mice were killed at 90 weeks. Survival was adequate for meaningful statistical analyses of the incidences of tumors in rats and mice of both sexes.

Mean body weights of the high-dose rats were lower than those of the matched controls during the first part of the study; however, beginning at approximately 80 weeks, mean weights of controls were lower than those of treated animals. Body weights of the mice were unaffected by the picloram.

In rats, a relatively high incidence of follicular hyperplasia, C-cell hyperplasia, and C-cell adenoma of the thyroid occurred in both sexes. However, the statistical tests for adenoma did not show sufficent evidence for association of the tumor with picloram administration.

An increased incidence of hepatic neoplastic nodules was observed in treated male and female rats as compared with untreated animals. This lesion is considered to be a benign tumor. In male rats the lesion appeared only in three animals of the

low-dose treatment group and was not significant when compared with the controls; however, the test for positive dose-related trend in females was significant (pooled controls 0/39, low-dose 5/50, high-dose 7/49, P = 0.016) and the incidence in the high-dose group was significant (P = 0.014) when compared with that in the pooled-control group.

There was also one hepatocellular carcinoma in a low-dose male rat and one in a high-dose female rat. In both males and females, there was a possibly treatment-related lesion of the liver diagnosed as foci of cellular alteration. The incidences of this latter lesion were, female rats: matched controls 1/10, low-dose 8/50, high-dose 18/49; male rats: matched controls 0/10, low-dose 12/49, high-dose 5/49. Thus, there is evidence that picloram affected the livers of rats of both sexes, but more particularly those of the females.

No tumors were found in male or female mice or male rats at incidences that could be significantly associated with treatment, and it is concluded that picloram was not carcinogenic for B6C3F1 mice or male Osborne-Mendel rats.

In female rats, however, the incidence of neoplastic nodules of the liver, benign tumors, was associated with treatment with picloram. It is concluded that under the conditions of the bioassay, the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne-Mendel rats.

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I. INTRODUCTION

Picloram (CAS 1918-02-1; C00237), which is the generic name for 4-amino-3,5,6-trichloropicolinic acid, is a systemic herbicide (Neumeyer, 1973; Spencer, 1973) registered by EPA for only nonfood use to control broadleaf weeds and woody plants (EPA Compendium, 1974). The chemical can replace the plant growth hormone indoleacetic acid, and inhibit the synthesis of protein in plants (Bradley, 1974; EPA Compendium, 1974; Neumeyer, 1973). The persistence of picloram in the soil poses environmental problems associated with contamination of the soil and of surface and subsurface water (Edwards, 1976; EPA Compendium, 1974; Hamaker et al., 1963).

Picloram was selected for testing because of its herbicidal use, and because its persistence in soil and water suggested a potential for long-term, low-level human exposure.

II. MATERIALS AND METHODS

A. Chemical

Technical-grade picloram was obtained in three batches of Lot No. 623816 from Dow Chemical Co., Midland, Michigan, for use in the chronic study. The product was at least 90% pure 4-amino-3,5,6-trichloropicolinic acid by the manufacturer's assay, and its identity was confirmed by melting point, elemental analyses, and spectral analyses (infrared, ultraviolet, mass, and nuclear magnetic resonance). Thin-layer chromatography revealed several impurities; no attempt was made to identify or quantitate these impurities.

The picloram was stored at 5°C in the original amber glass containers until used.

B. Dietary Preparation

All diets were formulated using finely ground Wayne® Lab Blox (Allied Mills, Inc., Chicago, Ill.) to which was added the required amount of picloram for each dietary concentration. A given amount of the test chemical was first hand-mixed with an approximately equal amount of feed. This mixture was then added slowly with mechanical mixing to a larger quantity of feed to give the desired concentration of the chemical. Acetone

(Mallinckrodt Inc., St. Louis, Mo.) and corn oil (Louana®, Opelousas Refinery Co., Opelousas, La.) were then added to the feed, each in an amount corresponding to 2% of the final weight of feed. The diets were mixed mechanically for not less than 25 minutes to assure homogeneity of the mixture and evaporation of the acetone. Formulated diets were stored at approximately 17°C until used, but no longer than 1 week.

As a quality control test on the accuracy of preparation of the diets, the concentration of picloram was determined in different batches of formulated diets during the chronic study. The results are summarized in Appendix G. At each dietary concentration, the mean of the analytical concentrations for the samples tested was within 2.5% of the theoretical concentration, and the coefficient of variation was never more than 6.7%. Thus, the evidence indicates that the formulated diets were prepared accurately.

C. Animals

Rats and mice of both sexes, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Osborne-Mendel strain obtained from Battelle Memorial Institute, Columbus, Ohio, and the mice were B6C3F1 hybrids obtained from Charles River

Breeding Laboratories, Inc., Wilmington, Massachusetts. On arrival at the laboratory, all animals were quarantined for an acclimation period (rats for 10 days, mice for 12 days) and were then assigned to control and treated groups.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature range was 22-24°C, and the relative humidity was maintained at 40-70%. The air in each room was changed 10-12 times per hour. Fluorescent lighting provided illumination 10 hours per day. Food and water were presented ad libitum.

The rats were housed individually in hanging galvanized steel mesh cages, and the mice were housed in plastic cages with filter bonnets, five per cage for females, and two or three per cage for males. Initially, rats were transferred once per week to clean cages; later in the study, cages were changed every 2 weeks. Mice were transferred once per week to clean cages with filter bonnets; bedding used for the mice was Absorb-Dri® (Lab Products, Inc., Garfield, N.J.). For rats, absorbent sheets under the cages were changed three times per week. Feeder jars and water bottles were changed and sterilized three times per week.

Cages for control and treated mice were placed on separate racks

in the same room. Animal racks for both species were rotated laterally once per week; at the same time, each cage was changed to a different position in the row within the same column. Rats receiving picloram, along with their matched controls, were housed in a room by themselves. Mice receiving picloram were maintained in a room housing mice administered chlorothalonil (CAS 1897-45-6), chloramben (CAS 133-90-4), or endrin (CAS 72-20-8), together with their respective matched controls.

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses of picloram, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the bioassay. In these subchronic studies, picloram was added to the animal feed in twofold increasing concentrations, ranging from 1,250 to 20,000 ppm for rats and 1,250 to 30,000 ppm for mice. The treated and matched-control groups each consisted of five male and five female animals. Picloram was provided in feed to the treated groups for 6 weeks, followed by a 2-week period of observation.

For the first 3 weeks of the study, the weights of the male rats receiving 20,000 ppm picloram were slightly lower than those of

the matched controls. However, the mean body weights of the female rats receiving 10,000 or 20,000 ppm were similar to those of the matched controls, except during week 1. No deaths occurred in either sex at doses of 10,000 or 20,000 ppm. The low and high doses for both male and female rats were set at 10,000 and 20,000 ppm for the chronic studies.

Weight gains of male and female mice receiving 5,000 and 10,000 ppm picloram were comparable to those of the matched controls. No deaths occurred in either sex at doses of 5,000 or 10,000 ppm. Of the animals receiving 20,000 ppm, 4/5 males and 3/5 females died. The low and high doses for both male and female mice for the chronic studies were set at 5,000 and 10,000 ppm.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2.

Since the numbers of animals in the matched-control groups were small, pooled-control groups also were used for statistical comparisons. Matched controls from the current studies on picloram were combined with matched controls from studies on phosphamidon (CAS 13171-21-6), tetrachlorvinphos (CAS 961-11-5), and chloramben (CAS 133-90-4). The pooled controls for statistical tests using rats consisted of 40 males and 40 females; using mice, 40 males and 40 females. The studies on chemicals other

Table 1. Design of Picloram Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Picloram in Diet (ppm)	Time on Study: Treated (weeks)[b]	Time on Study: Untreated (weeks)[c]	Time-Weighted Average Dose[d] (ppm)
Male					
Matched-Control	10	0		113	
Low-Dose	50	10,000	39		7,437
		5,000	41		
		0		33	
High-Dose	50	20,000	39		14,875
		10,000	41		
		0		33	
Female					
Matched-Control	10	0		113	
Low-Dose	50	10,000	39		7,437
		5,000	41		
		0		33	
High-Dose	50	20,000	39		14,875
		10,000	41		
		0		33	

[a]All animals were 35 days of age when placed on study.

[b]Doses of picloram were lowered at 39 weeks on study, since it was believed that excessive mortality would occur before termination of the study, based on the pattern of mortality, weight changes, and the general condition of rats used in similar studies on other chemicals at Gulf South Research Institute.

[c]When test diets were discontinued, treated and control rats were fed control diets without corn oil for 4 weeks, then control diets (2% corn oil added) for an additional 29 weeks.

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

Table 2. Design of Picloram Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Picloram in Diet (ppm)	Time on Study: Treated (weeks)[b]	Time on Study: Untreated (weeks)[c]	Time-Weighted Average Dose[c] (ppm)
Male					
Matched-Control	10	0		90	
Low-Dose	50	5,000	1		2,531
		2,500	79		
		0		10	
High-Dose	50	10,000	1		5,062
		5,000	79		
		0		10	
Female					
Matched-Control	10	0		90	
Low-Dose	50	5,000	1		2,531
		2,500	79		
		0		10	
High-Dose	50	10,000	1		5,062
		5,000	79		
		0		10	

[a]All animals were 35 days of age when placed on study.

[b]Doses of picloram were lowered at 1 week on study, since it was believed that excessive mortality would occur before termination of the study, based on the pattern of mortality, weight changes, and the general condition of mice used in similar studies on other chemicals at Gulf South Research Institute.

[c]When test diets were discontinued, treated and control mice were fed control diets (2% corn oil added).

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

than picloram were also conducted at Gulf South Research Institute and overlapped the picloram study by at least 1 year. The matched-control groups for the different test chemicals were of the same strain and from the same supplier, and they were examined by the same pathologists.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, weighed at regular intervals, and palpated for masses at each weighing. Animals that were moribund at the time of clinical examination were killed and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, gallbladder (mice), pancreas, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, and brain. Occasionally, additional tissues 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 tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or 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). 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 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 relation-

ship. 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 animals died naturally or were 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

significant result (a $P < 0.025$ 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.

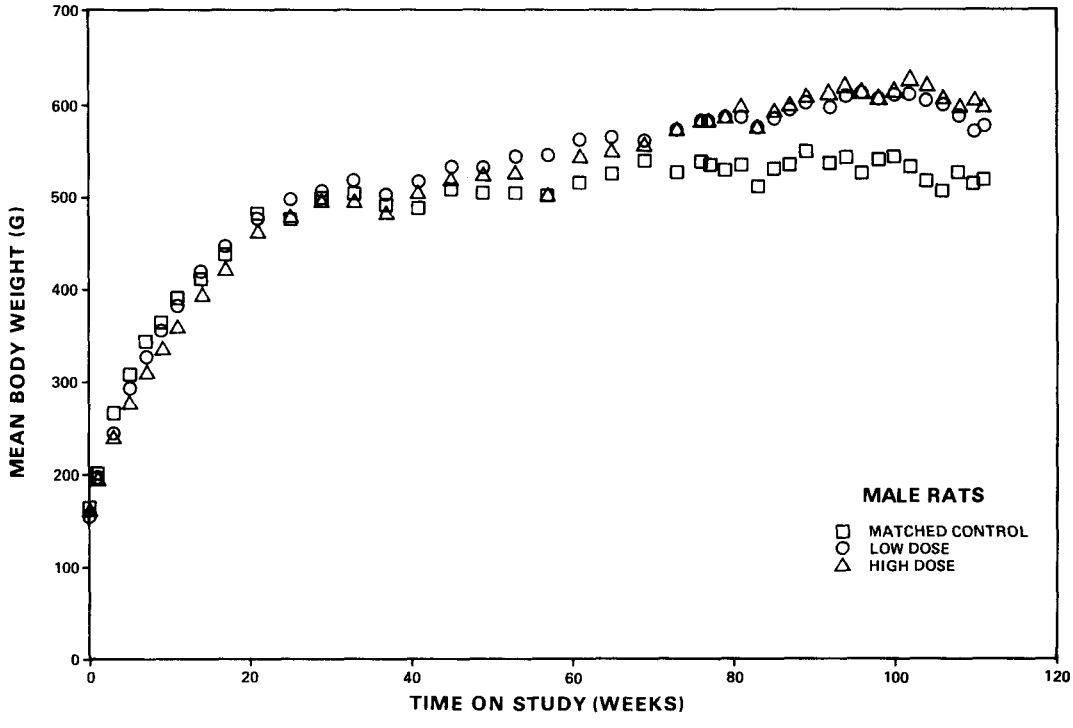
III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

The mean body weights of the rats fed picloram were slightly lower than those of the matched controls until week 16 (figure 1). For the remainder of the study, however, the weights of the matched controls, particularly the males, were increasingly lower than those of the treated groups.

During the first 6 months of the study, the treated rats were generally comparable to the controls in appearance and behavior. During the second 6 months, clinical signs including diarrhea, hematuria, and rough hair coats were observed at a moderate incidence. At week 35 a few animals in each group, treated and matched-control, had an enlarged and protruding eyeball with a developing opacity of the corneal surface, and in some cases a definite thickening of the palpebral conjunctival membranes; this condition was diagnosed as viral conjunctivitis and left affected animals blind. This disease was considered to be incidental in the colony and was not related to treatment with picloram.

During the second year of the study, clinical signs were observed with an increased frequency in the treated rats. These signs



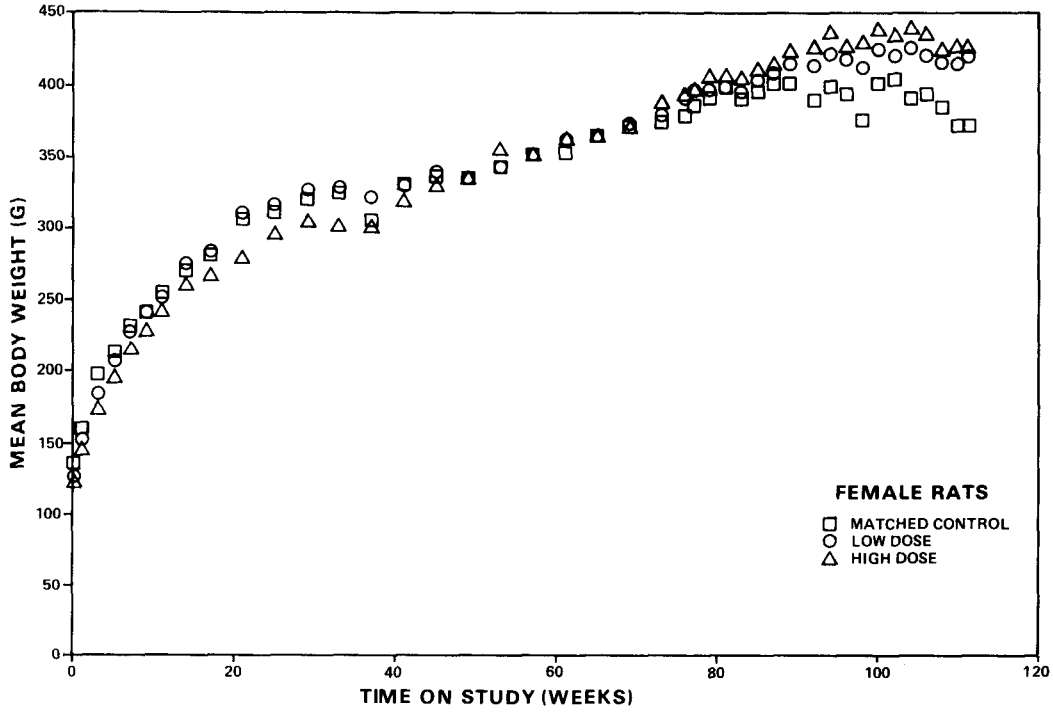


Figure 1. Growth Curves For Rats Fed Picloram In The Diet

included pale mucous membranes, dermatitis, alopecia, tachypnea, discolored (dark) urine, diarrhea, and vaginal bleeding.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats receiving picloram at the doses of this experiment, together with those of the matched controls, are shown in figure 2. In both sexes, the Tarone test results for positive dose-related trend in mortality over the period are not significant at the 0.05 level. In male rats, 49% of the high-dose group, 68% of the low-dose group, and 50% of the matched-control group lived to the end of the study. Deaths in the high-dose group which occurred prior to termination of the study were not tumor associated. In females, survivals among the three groups are comparable; 69% of the high-dose group, 66% of the low-dose group, and 70% of the controls lived to the end of the study. Survival was adequate for meaningful statistical analyses of the incidences of tumors in these groups.

C. Pathology (Rats)

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

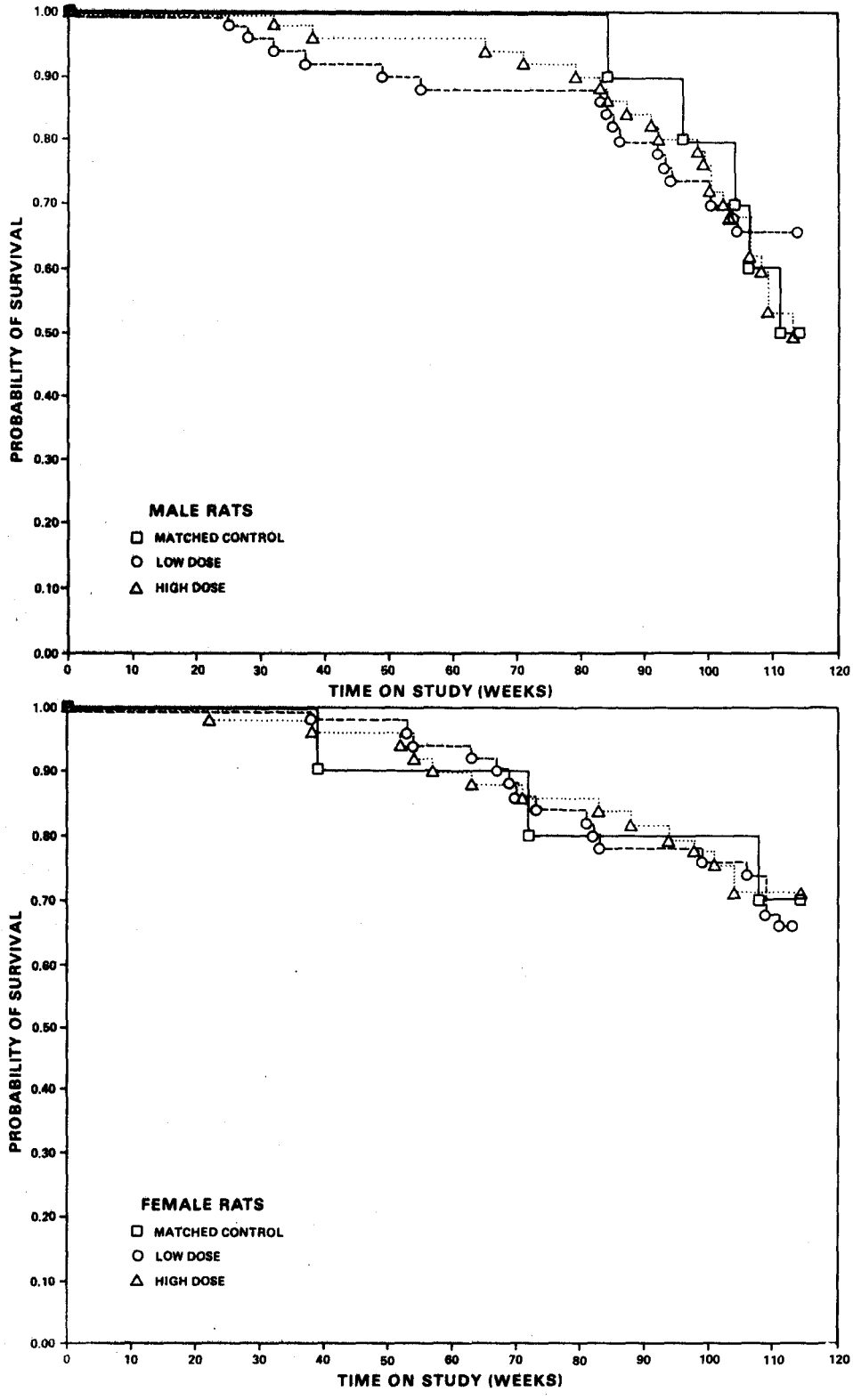


Figure 2. Survival Curves For Rats Fed Picloram In The Diet

Similar lesions occurred among animals of the matched-control and treated groups. For the most part, these lesions are commonly encountered in aging rats of the Osborne-Mendel strain. Furthermore, the incidence in which they occurred in treated rats was similar to that in which they were observed to occur spontaneously in similar studies. However, there was a relatively high incidence of follicular and C-cell hyperplasia and/or neoplasia of the thyroid gland, neoplastic nodules of the liver, and cellular alteration of the liver exclusively or almost exclusively in picloram-treated rats. The neoplastic nodules were composed predominantly of eosinophilic hepatocytes and lesser numbers of clear hepatocytes. Foci of cellular alteration composed of eosinophilic and/or clear hepatocytes were seen in the livers of several treated rats. The significance of these changes in both the thyroid and the liver is difficult to assess because of the disproportionately small number of matched-control animals.

Although neoplastic nodules and foci of cellular alteration were observed in the liver of only treated male and female rats and may have been treatment related, in the judgment of the pathologist, there was no conclusive evidence of carcinogenicity induced by picloram in Osborne-Mendel rats at the doses administered and for the period of time rats were fed in this study.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

In the analyses of the incidence of neoplastic nodule of the liver in female rats, the Cochran-Armitage test result for positive dose-related trend in proportions is significant (P = 0.016) using the pooled controls, and the Fisher exact test shows a significantly higher incidence of this tumor in the high-dose group (P = 0.014) when compared with the pooled controls. The statistical conclusion is that the occurrence of neoplastic nodule of the liver in female rats is associated with picloram at the doses administered in this experiment. The statistical test results of the incidence of this tumor in male rats are not significant at the 0.05 level.

In female rats, although the Cochran-Armitage test result for positive dose-related trend in proportions for C-cell adenoma of the thyroid is significant (P = 0.029) using the pooled controls, the Fisher exact test for comparisons of the incidences in the treated groups with those in the control groups do not confirm

this result. Statistical tests of the incidence of this tumor in male rats are not significant at the 0.05 level.

There are no other tumors for which the statistical test results of the incidences are significant in the positive direction. In all of the confidence intervals shown in the tables for the incidences of tumors, other than those in the liver, the value of one or less than one is included, indicating the absence of significant positive results. It should also be noted that these intervals, except the incidence of chromophobe adenoma of the pituitary in low-dose male rats, have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by picloram, which could not be detected under the conditions of this test. Elimination of those animals dying before week 52 on study does not affect the conclusions of this analysis.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the treated male and female mice were essentially unaffected by the picloram over the period of the bioassay (figure 3).

During the first 16 weeks of the study, the treated mice were generally comparable to the controls in appearance and behavior. At week 17, one low-dose female and five high-dose females had generalized body tremors. During the remainder of the first year, the general condition of both treated and control mice was good. A few individual animals lost weight, and had alopecia and fight wounds.

During the second year of the study, clinical signs were noted with increasing frequency among the treated mice. At week 52, a majority of treated males appeared to be slightly hyperactive. Rough hair coats were observed in low-dose males beginning at week 57 and in high-dose males beginning at week 68 and continuing to termination of the study. Other clinical signs included abdominal distention, predominantly among the low-dose males and females.

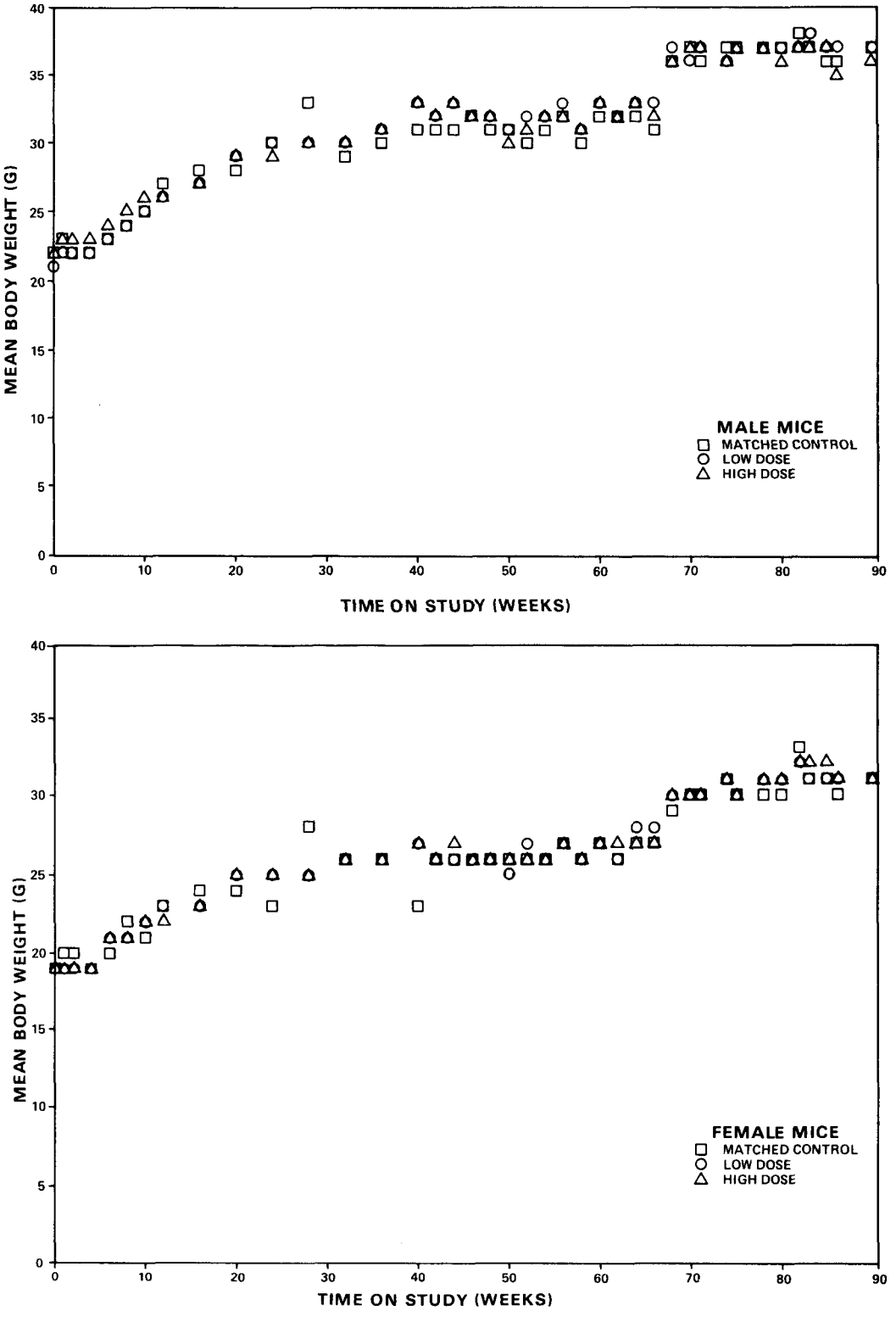


Figure 3. Growth Curves For Mice Fed Picloram In The Diet

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice receiving picloram at the doses of this experiment, together with those of the matched controls, are shown in figure 4. In neither sex was the Tarone test result significant at the 0.05 level for positive dose-related trend in mortality over the period. With over 80% of both male and female groups living to termination of the study, survival was adequate for meaningful statistical analyses of the incidences of late-developing 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.

Neoplastic and nonneoplastic lesions were found in mice of the treated and matched-control groups. The lesions occurred with a wide variation, random distribution, and either in insignificant numbers or with approximately equal frequency among mice of the treated and control groups.

In the judgment of the pathologist, there was no conclusive evidence of carcinogenicity induced by picloram in the B6C3F1

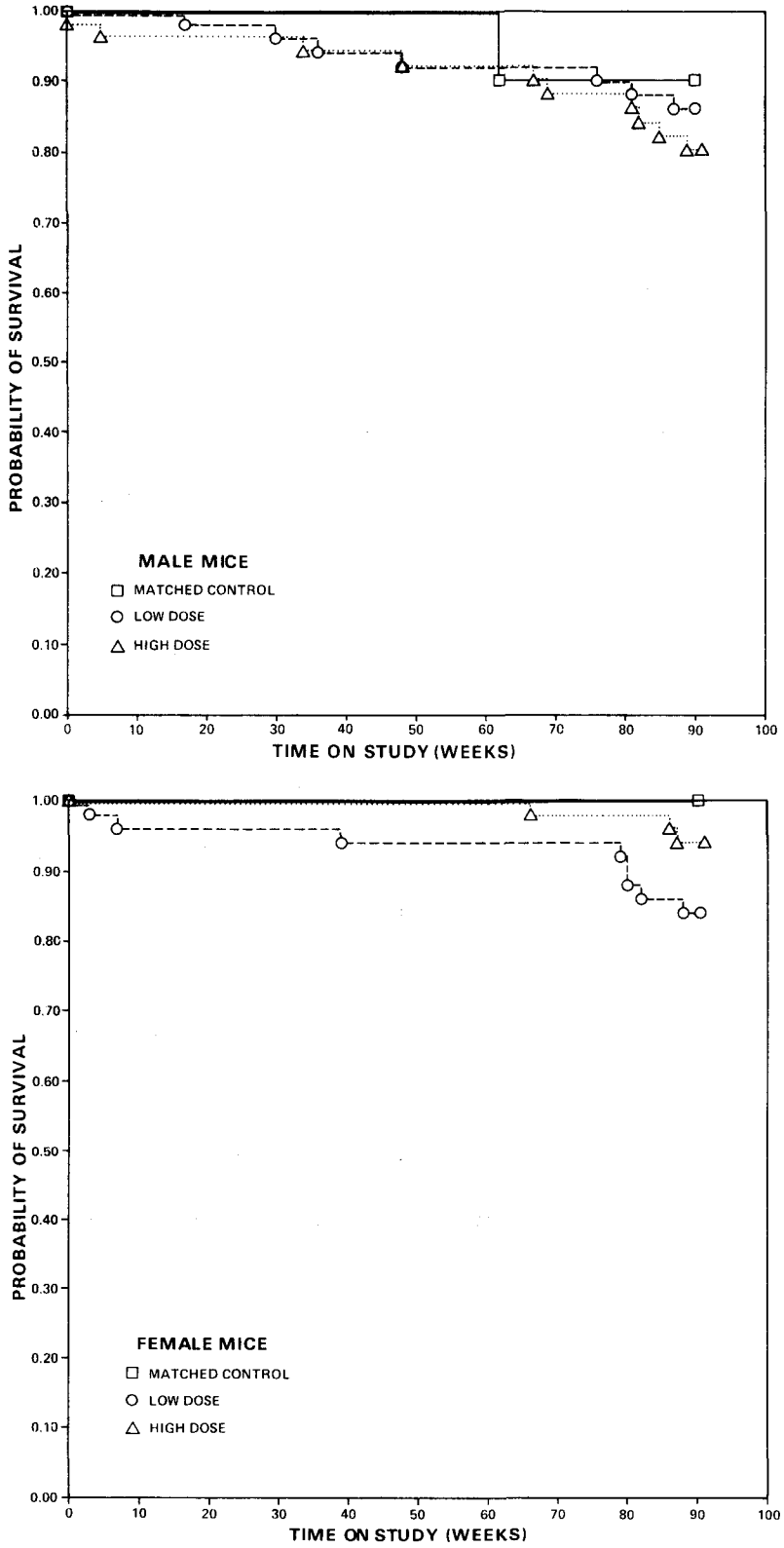


Figure 4. Survival Curves For Mice Fed Picloram In The Diet

hybrid mouse at the doses administered and for the period of time mice were fed in this study.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those specific primary tumors that were observed in at least 5% of one or more treated groups of either sex.

In neither sex were the results of the Cochran-Armitage test for positive dose-related trend in proportions or the Fisher exact test for comparisons of incidences between the treated groups and the control groups significant at the 0.05 level. In all of the 95% confidence intervals shown in the tables for the incidence of tumors, the lower limit has a value of less than one, indicating the absence of positive significant results. It should also be noted that these intervals have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by picloram, which could not be detected under the conditions of this test.

V. DISCUSSION

In this bioassay, picloram did not consistently affect the body weights of the treated animals. Mean body weights of high-dose rats were lower than those of the matched controls early in the study, but beginning at approximately 80 weeks, mean weights of controls were lower than those of the treated animals.

Body weights of mice were unaffected by picloram. No consistent clinical signs attributable to treatment were reported during the first 6 months of the study, except for isolated incidences of tremors and hyperactivity in mice. Later, particularly during the second year, rough hair coats, diarrhea, pale mucous membranes, alopecia, and abdominal distention occurred in both treated rats and mice to a greater degree than in the controls.

Survival was adequate for meaningful statistical analyses of the incidences of tumors in groups of rats and mice of both sexes.

In rats, a relatively high incidence of follicular hyperplasia, C-cell hyperplasia, and C-cell adenoma of the thyroid occurred in both sexes. However, the statistical tests for adenoma did not show sufficient evidence for association of the tumor with picloram administration.

An increased incidence of hepatic neoplastic nodules was observed

in treated male and female rats as compared with untreated animals. This lesion is considered to be a benign tumor. In male rats the lesion appeared only in three animals of the low-dose treatment group and was not significant when compared with the controls; however, the test for positive dose-related trend in females was significant (pooled controls 0/39, low-dose 5/50, high-dose 7/49, $P = 0.016$), and the incidence in the high-dose group was significant ($P = 0.014$) when compared with that in the pooled-control group.

There was also one hepatocellular carcinoma in a low-dose male rat and one in a high-dose female rat. In both males and females, there was a possibly treatment-related lesion of the liver diagnosed as foci of cellular alteration. These latter lesions are frequently associated with the induction of neoplastic nodules and hepatocellular carcinomas in rats (Squire and Levitt, 1975). The incidences of the foci were, female rats: matched controls 1/10, low-dose 8/50, high-dose 18/49; male rats: matched controls 0/10, low-dose 12/49, high-dose 5/49. Thus, there is evidence that picloram affected the livers of rats of both sexes, but more particularly those of the females.

In mice, no tumors occurred in proportions that could be shown to be related to the administration of picloram, either by tests for

dose-related trend or by direct comparisons of the incidences in treated and control groups.

In previous studies of the toxicity of picloram, Lynn (1965) reported LD_{50}s of 2,000 to 4,000 mg/kg in the female mouse and over 8,000 mg/kg in the female rat. McCollister and Leng (1969) reported no adverse effects or tumors in albino rats and Beagle dogs at intakes up to 150 mg/kg/day for 2 years. Intakes of 150 mg/kg/day are lower than the doses used for rats in the present bioassay.

No tumors were found in male or female mice or male rats at incidences that could be significantly associated with treatment, and it is concluded that picloram was not carcinogenic for B6C3F1 mice or male Osborne-Mendel rats.

In female rats, however, the incidence of neoplastic nodules of the liver, benign tumors, was associated with treatment with picloram. It is concluded that under the conditions of the bioassay, the findings are suggestive of the ability of the compound to induce benign tumors in the livers of female Osborne-Mendel rats.

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APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(50)
BASAL-CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(10)	(50)	(50)
FIBROMA			1 (2%)
FIBROUS HISTIOCYTOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(50)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
PHEOCHROMOCYTOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(50)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(10)	(50)	(50)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
HEMANGIOMA		3 (6%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(10)	(49)	(50)
FIBROMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(49)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
NEOPLASTIC NODULE		3 (6%)	
HEPATOCELLULAR CARCINOMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
*BILE DUCT	(10)	(50)	(50)
BILE DUCT ADENOMA			1 (2%)
#PANCREAS	(10)	(49)	(49)
UNDIFFERENTIATED CARCINOMA			1 (2%)
#STOMACH	(10)	(50)	(46)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
PAPILLOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(50)	(50)
TUBULAR-CELL ADENOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(46)	(45)
CARCINOMA,NOS		2 (4%)	
CHROMOPHOBE ADENOMA	2 (22%)	2 (4%)	9 (20%)
#ADRENAL	(10)	(49)	(49)
CORTICAL ADENOMA		2 (4%)	
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(9)	(47)	(49)
C-CELL ADENOMA		6 (13%)	1 (2%)
C-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
FIBROMA	1 (10%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(10)	(49)	(50)
MENINGIOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(10)	(50)	(50)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
*MESENTERY	(10)	(50)	(50)
UNDIFFERENTIATED CARCINOMA METAS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	1 (2%)
DIAPHRAGM			
UNDIFFERENTIATED CARCINOMA METAS			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		6	10
MORIBUND SACRIFICE	5	11	16
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	5	33	24
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	3	23	18
TOTAL PRIMARY TUMORS	3	26	22
TOTAL ANIMALS WITH BENIGN TUMORS	3	14	13
TOTAL BENIGN TUMORS	3	15	17
TOTAL ANIMALS WITH MALIGNANT TUMORS		8	5
TOTAL MALIGNANT TUMORS		8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
TOTAL SECONDARY TUMORS			8
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		3	
TOTAL UNCERTAIN TUMORS		3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(50)
SARCOMA, NOS	1 (10%)		
FIBROUS HISTIOCYTOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(50)	(49)
LIPOSARCOMA, METASTATIC			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (10%)		1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(50)	(49)
NEOPLASTIC NODULE		5 (10%)	7 (14%)
HEPATOCELLULAR CARCINOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(48)
LIPOSARCOMA			1 (2%)
†HAMARTOMA		1 (2%)	1 (2%)

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

† This is considered to be a benign form of the malignant mixed tumor of the kidney and consists of lipocytes, tubular structures, and fibroblasts in varying proportions.

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(48)	(46)
CARCINOMA, NOS	1 (11%)		
ADENOMA, NOS		1 (2%)	
CHROMOPHOBE ADENOMA		7 (15%)	7 (15%)
#ADRENAL	(8)	(50)	(48)
CORTICAL ADENOMA		2 (4%)	4 (8%)
PHEOCHROMOCYTOMA			1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
#THYROID	(9)	(46)	(46)
C-CELL ADENOMA		3 (7%)	7 (15%)
#PANCREATIC ISLETS	(10)	(47)	(49)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
INFILTRATING DUCT CARCINOMA		1 (2%)	1 (2%)
FIBROMA	1 (10%)	1 (2%)	2 (4%)
FIBROADENOMA		6 (12%)	4 (8%)
#UTERUS	(10)	(48)	(45)
SQUAMOUS CELL CARCINOMA		1 (2%)	
GRANULOSA-CELL CARCINOMA, METAST		1 (2%)	
ENDOMETRIAL STROMAL POLYP	1 (10%)	8 (17%)	4 (9%)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#UTERUS/ENDOMETRIUM	(10)	(48)	(45)
ADENOCARCINOMA, NOS		1 (2%)	
#OVARY	(10)	(47)	(48)
GRANULOSA-CELL CARCINOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(10)	(50)	(49)
GRANULAR-CELL TUMOR, BENIGN			1 (2%)
GRANULAR-CELL TUMOR, MALIGNANT		1 (2%)	
OLIGODENDROGLIOMA		1 (2%)	1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SHOULDER JOINT	(10)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*MESENTERY	(10)	(50)	(50)
GRANULOSA-CELL CARCINOMA, METAST		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	2	4
MORIBUND SACRIFICE	2	15	11
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			1
TERMINAL SACRIFICE	7	33	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	32	30
TOTAL PRIMARY TUMORS	5	43	46
TOTAL ANIMALS WITH BENIGN TUMORS	2	25	23
TOTAL BENIGN TUMORS	2	30	33
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	6	6
TOTAL MALIGNANT TUMORS	3	8	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	1
TOTAL SECONDARY TUMORS		4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		5	7
TOTAL UNCERTAIN TUMORS		5	7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE FED PICLORAM IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED PICLORAM IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(49)
SARCOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)	4 (8%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(49)
HEPATOCELLULAR ADENOMA			2 (4%)
NEOPLASTIC NODULE	1 (10%)	2 (4%)	2 (4%)
HEPATOCELLULAR CARCINOMA	4 (40%)	11 (22%)	4 (8%)
URINARY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#THYROID	(10)	(48)	(44)
FOLLICULAR-CELL ADENOMA			2 (5%)
REPRODUCTIVE SYSTEM			
#TESTIS	(10)	(48)	(48)
INTERSTITIAL-CELL TUMOR	1 (10%)		
SEMINOMA/DYSGERMINOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(10)	(50)	(49)
PAPILLARY ADENOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@			3
MORIBUND SACRIFICE	1	7	7
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	43	40
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	5	18	14
TOTAL PRIMARY TUMORS	7	19	17
TOTAL ANIMALS WITH BENIGN TUMORS	2	4	4
TOTAL BENIGN TUMORS	2	4	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	13	8
TOTAL MALIGNANT TUMORS	4	13	8
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	2
TOTAL UNCERTAIN TUMORS	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED PICLORAM IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (10%)	1 (2%)	
OSTEOSARCOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(49)	(50)
LYMPHOCYTIC LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(50)
HEPATOCELLULAR CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(10)	(47)	(45)
FOLLICULAR-CELL ADENOMA		1 (2%)	1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(49)	(50)
ADENOCARCINOMA, NOS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		2	
MORIBUND SACRIFICE		7	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	41	47
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	6	3
TOTAL PRIMARY TUMORS	1	6	3
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	1
TOTAL BENIGN TUMORS	1	2	1
TOTAL ANIMALS WITH MALIGNANT TUMORS		4	2
TOTAL MALIGNANT TUMORS		4	2
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
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 FED PICLORAM IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
GRANULATION, TISSUE	1 (10%)	1 (2%)	
*SUBCUT TISSUE	(10)	(50)	(50)
EDEMA, NOS			1 (2%)
GRANULOMA, NOS		1 (2%)	
GRANULATION, TISSUE		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(50)
EDEMA, NOS	1 (10%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
HEMATOPOIESIS		2 (4%)	1 (2%)
#CERVICAL LYMPH NODE	(10)	(44)	(45)
INFLAMMATION, NOS	1 (10%)		
INFLAMMATION, CHRONIC	1 (10%)		
HYPERPLASIA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(10)	(49)	(50)
ARTERIOSCLEROSIS, NOS			1 (2%)
CALCIFICATION, METASTATIC		1 (2%)	
#HEART/ATRIUM	(10)	(49)	(50)
THROMBUS, ORGANIZED			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(10)	(49)	(50)
FIBROSIS, FOCAL	1 (10%)	3 (6%)	4 (8%)
FIBROSIS, DIFFUSE		1 (2%)	2 (4%)
CALCIFICATION, NOS			1 (2%)
CALCIFICATION, FOCAL			1 (2%)
*AORTA	(10)	(50)	(50)
MEDIAL CALCIFICATION			3 (6%)
*CORONARY ARTERY	(10)	(50)	(50)
ARTERIOSCLEROSIS, NOS	1 (10%)		
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(49)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
METAMORPHOSIS FATTY	2 (20%)		9 (18%)
FOCAL CELLULAR CHANGE		12 (24%)	5 (10%)
*BILE DUCT	(10)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, DIFFUSE			2 (4%)
#PANCREAS	(10)	(49)	(49)
ARTERIOSCLEROSIS, NOS			1 (2%)
#PANCREATIC ACINUS	(10)	(49)	(49)
ATROPHY, NOS			1 (2%)
#STOMACH	(10)	(50)	(46)
ULCER, NOS		1 (2%)	1 (2%)
EROSION			1 (2%)
CALCIFICATION, NOS			4 (9%)
#GASTRIC MUCOSA	(10)	(50)	(46)
CALCIFICATION, NOS			1 (2%)
#GASTRIC MUSCULARIS	(10)	(50)	(46)
CALCIFICATION, NOS		1 (2%)	
#CECUM	(6)	(41)	(40)
INFLAMMATION, ACUTE			1 (3%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(10)	(50)	(50)
CALCULUS, NOS		1 (2%)	
GLOMERULONEPHRITIS, NOS	3 (30%)		
PYELONEPHRITIS, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC	5 (50%)	12 (24%)	26 (52%)
NEPHROSIS, NOS		1 (2%)	
#URINARY BLADDER	(9)	(47)	(46)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(46)	(45)
CYST, NOS		4 (9%)	10 (22%)
HEMORRHAGE			1 (2%)
DEGENERATION, CYSTIC		1 (2%)	2 (4%)
HYPERPLASIA, FOCAL	1 (11%)	1 (2%)	
#ADRENAL	(10)	(49)	(49)
CYST, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	1 (2%)
DEGENERATION, CYSTIC	1 (10%)	2 (4%)	
METAMORPHOSIS FATTY		1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(10)	(49)	(49)
DEGENERATION, NOS		2 (4%)	
DEGENERATION, CYSTIC			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
HYPERPLASIA, FOCAL		3 (6%)	2 (4%)
#THYROID	(9)	(47)	(49)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL		6 (13%)	7 (14%)
HYPERPLASIA, FOLLICULAR-CELL		5 (11%)	6 (12%)
#PARATHYROID	(6)	(32)	(35)
HYPERPLASIA, NOS		1 (3%)	7 (20%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
ADENOSIS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PROSTATE	(9)	(47)	(48)
INFLAMMATION, SUPPURATIVE		2 (4%)	1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
CALCIFICATION, NOS			1 (2%)
ATROPHY, NOS		1 (2%)	
#TESTIS	(10)	(50)	(50)
PERIARTERITIS		1 (2%)	3 (6%)
ATROPHY, NOS	5 (50%)	7 (14%)	14 (28%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(10)	(50)	(50)
CATARACT	1 (10%)		
MUSCULOSKELETAL SYSTEM			
*BONE	(10)	(50)	(50)
OSTEOPOROSIS			1 (2%)
BODY CAVITIES			
*PLEURA	(10)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*MESENTERY	(10)	(50)	(50)
PERIARTERITIS	2 (20%)	2 (4%)	7 (14%)
ARTERIOSCLEROSIS, NOS	1 (10%)		1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(50)
PERIARTERITIS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NC LESION REPORTED	1	11	2
AUTO/NECROPSY/NO HISTO			1

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

TABLE C2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(49)	(49)
HEMATOPOIESIS			1 (2%)
MYELOID METAPLASIA			1 (2%)
#SPLENIC RED PULP	(10)	(49)	(49)
ATROPHY, NOS		1 (2%)	
#CERVICAL LYMPH NODE	(8)	(46)	(46)
INFLAMMATION, NOS	1 (13%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(10)	(50)	(49)
FIBROSIS, FOCAL		1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(10)	(50)	(49)
NECROSIS, FOCAL		1 (2%)	
METAMORPHOSIS FATTY			2 (4%)
FOCAL CELLULAR CHANGE	1 (10%)	8 (16%)	18 (37%)
MYELOID METAPLASIA			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*BILE DUCT	(10)	(50)	(50)
FIBROSIS		2 (4%)	6 (12%)
HYPERPLASIA, NOS		3 (6%)	1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(10)	(47)	(49)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(10)	(49)	(48)
ULCER, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(48)
CYST, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (10%)	2 (4%)	4 (8%)
FIBROSIS, FOCAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(48)	(46)
CYST, NOS	1 (11%)		
CONGESTION, NOS	1 (11%)		1 (2%)
HEMORRHAGE	1 (11%)		
HYPERPLASIA, NOS	1 (11%)	3 (6%)	
HYPERPLASIA, FOCAL		1 (2%)	1 (2%)
#ADRENAL	(8)	(50)	(48)
THROMBOSIS, NOS	1 (13%)		
HEMORRHAGE		5 (10%)	5 (10%)
DEGENERATION, CYSTIC		3 (6%)	2 (4%)
#ADRENAL CORTEX	(8)	(50)	(48)
HEMORRHAGE	1 (13%)		
DEGENERATION, NOS		2 (4%)	
DEGENERATION, CYSTIC		1 (2%)	6 (13%)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#THYROID	(9)	(46)	(46)
HYPERPLASIA, C-CELL		8 (17%)	6 (13%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	6 (13%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
HYPERPLASIA, NOS		6 (12%)	6 (12%)
DYSPLASIA, NOS		1 (2%)	
ADENOSIS	1 (10%)		
#UTERUS	(10)	(48)	(45)
METAPLASIA, SQUAMOUS		1 (2%)	
#OVARY	(10)	(47)	(48)
CYST, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(10)	(50)	(50)
PERIARTERITIS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	8	7
AUTO/NECROPSY/NO HISTO			1

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE FED PICLORAM IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED PICLORAM IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
ULCER, NOS		2 (4%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(48)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#STOMACH	(10)	(49)	(48)
DIVERTICULUM			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(10)	(48)	(44)
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, ADENOMATOUS	1 (10%)		
HYPERPLASIA, FOLLICULAR-CELL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	29	30
AUTOLYSIS/NO NECROPSY			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED PICLORAM IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(50)
INFLAMMATION, CHRONIC	1 (10%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(48)	(50)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(49)	(50)
INFLAMMATION, FOCAL			3 (6%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
BASOPHILIC CYTO CHANGE		1 (2%)	
#PANCREAS	(9)	(49)	(50)
DILATATION/DUCTS			1 (2%)
INFLAMMATION, NOS			1 (2%)
#COLON	(3)		(1)
ULCER, NOS			1 (100%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(50)
INFLAMMATION, CHRONIC			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(10)	(47)	(45)
FOLLICULAR CYST, NOS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(10)	(47)	(47)
HYPERPLASIA, CYSTIC	2 (20%)		
#OVARY	(10)	(47)	(49)
CYST, NOS			1 (2%)
FOLLICULAR CYST, NOS	2 (20%)		
INFLAMMATION, NOS			9 (18%)
INFLAMMATION, SUPPURATIVE		16 (34%)	5 (10%)
NERVOUS SYSTEM			
#BRAIN	(10)	(49)	(49)
HYDROCEPHALUS, NOS			1 (2%)
CORPORA AMYLACEA	1 (10%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	27	26
AUTOLYSIS/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 FED PICLORAM IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Picloram in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Spleen: Hemangioma[b]	0/10 (0.00)	1/36 (0.03)	3/50 (0.06)	1/50 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.134	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.160	0.720
Lower Limit			0.183	0.009
Upper Limit			111.051	55.415
Weeks to First Observed Tumor	--		113	106
Liver: Neoplastic Nodule[b]	0/10 (0.00)	0/38 (0.00)	3/49 (0.06)	0/49 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.020		
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.137	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.470	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--		113	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	2/9 (0.22)	9/36 (0.25)	2/46 (0.04)	9/45 (0.20)
P Values[c,d]	N.S.	N.S.	P = 0.008**(N)	N.S.
Departure from Linear Trend[e]	P = 0.033	P = 0.007		
Relative Risk (Matched Control)[f]			0.196	0.900
Lower Limit			0.018	0.250
Upper Limit			2.519	7.901
Relative Risk (Pooled Control)[f]			0.174	0.800
Lower Limit			0.019	0.318
Upper Limit			0.776	2.042
Weeks to First Observed Tumor	113		113	83

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/9 (0.00)	1/36 (0.03)	6/47 (0.13)	1/49 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.042	P = 0.018		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.347	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			4.596	0.735
Lower Limit			0.595	0.010
Upper Limit			206.379	56.525
Weeks to First Observed Tumor	--		113	113

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Cortical Adenoma[b]	0/10 (0.00)	0/36 (0.00)	2/49 (0.04)	0/49 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.067	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			Infinite	--
Lower Limit			0.220	--
Upper Limit			Infinite	--
Weeks to First Observed Tumor	--		83	--

[a]Treated groups received time-weighted average doses of 7,437 or 14,875 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of tumors in a 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Picloram in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Neoplastic Nodule[b]	0/10 (0.00)	0/39 (0.00)	5/50 (0.10)	7/49 (0.14)
P Values[c,d]	N.S.	P = 0.016	N.S.	P = 0.014**
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.281	0.441
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.989	1.557
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		99	88
Pituitary: Chromophobe Adenoma[b]	0/9 (0.00)	4/37 (0.11)	7/48 (0.15)	7/46 (0.15)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.413	0.430
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			1.349	1.408
Lower Limit			0.375	0.392
Upper Limit			5.873	6.116
Weeks to First Observed Tumor	--		109	94

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Thyroid: C-cell Adenoma[b]	0/9 (0.00)	1/38 (0.03)	3/46 (0.07)	7/46 (0.15)
P Values[c,d]	N.S.	P = 0.029	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.132	0.430
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.478	5.783
Lower Limit			0.209	0.796
Upper Limit			127.174	254.145
Weeks to First Observed Tumor	--		113	114
Adrenal: Cortical Adenoma[b]	0/8 (0.00)	0/37 (0.00)	2/50 (0.04)	4/48 (0.08)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.054	0.179
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			0.222	0.719
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--		81	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Mammary Gland:				
Fibroadenoma[b]	0/10 (0.00)	7/39 (0.18)	6/50 (0.12)	4/50 (0.08)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.356	0.206
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.669	0.446
Lower Limit			0.203	0.103
Upper Limit			2.142	1.626
Weeks to First Observed Tumor	--		63	63
Uterus: Endometrial				
Stromal Polyp[b]	1/10 (0.10)	3/39 (0.08)	8/48 (0.17)	4/45 (0.09)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.667	0.889
Lower Limit			0.278	0.108
Upper Limit			72.240	42.792
Relative Risk (Pooled Control)[f]			2.167	1.156
Lower Limit			0.565	0.208
Upper Limit			11.972	7.468
Weeks to First Observed Tumor	113		81	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Fed Picloram in the Diet[a]

(continued)

[a]Treated groups received time-weighted average doses of 7,437 or 14,875 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of tumors in a 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE FED PICLORAM IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Fed Picloram in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/10 (0.10)	2/38 (0.05)	5/49 (0.10)	3/48 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.020	0.625
Lower Limit			0.141	0.061
Upper Limit			47.261	32.146
Relative Risk (Pooled Control)[f]			1.939	1.188
Lower Limit			0.339	0.143
Upper Limit			19.554	13.675
Weeks to First Observed Tumor	90		76	91
Liver: Hepatocellular Carcinoma[b]	4/10 (0.40)	8/38 (0.21)	11/49 (0.22)	4/49 (0.08)
P Values[c,d]	P = 0.007 (N)	N.S.	N.S.	P = 0.022* (N)
Relative Risk (Matched Control)[f]			0.561	0.204
Lower Limit			0.235	0.053
Upper Limit			2.094	0.963
Relative Risk (Pooled Control)[f]			1.066	0.388
Lower Limit			0.437	0.093
Upper Limit			2.765	1.337
Weeks to First Observed Tumor	62		87	81

Table F1. Analyses of the Indicence of Primary Tumors in Male Mice Fed Picloram in the Diet[a]

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma or Neoplastic Nodule[b]	5/10 (0.50)	9/38 (0.24)	13/49 (0.27)	8/49 (0.16)
P Values[c,d]	P = 0.020 (N)	N.S.	N.S.	P = 0.033* (N)
Relative Risk (Matched Control)[f]			0.531	0.327
Lower Limit			0.264	0.140
Upper Limit			1.595	1.077
Relative Risk (Pooled Control)[f]			1.120	0.689
Lower Limit			0.501	0.257
Upper Limit			2.663	1.827
Weeks to First Observed Tumor	62		87	81

[a]Treated groups received time-weighted average doses of 2,531 or 5,062 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of tumors in a 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 (*) or with the pooled-control group (**) when P < 0.05 for either control group; otherwise, not significant (N.S.) is indicated.

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Picloram in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	1/10 (0.10)	2/39 (0.05)	1/49 (0.02)	0/50 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.204	0.000
Lower Limit			0.003	0.000
Upper Limit			15.723	3.747
Relative Risk (Pooled Control)[f]			0.398	0.000
Lower Limit			0.007	0.000
Upper Limit			7.377	2.634
Weeks to First Observed Tumor	90		90	--
Liver: Hepatocellular Carcinoma[b]	0/10 (0.00)	3/39 (0.00)	0/49 (0.00)	1/50 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.012
Upper Limit			--	Infinite
Relative Risk (Pooled Control)[f]			0.000	0.260
Lower Limit			0.000	0.005
Upper Limit			1.321	3.097
Weeks to First Observed Tumor	--		--	91

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Fed Picloram in the Diet[a]

(continued)

[a]Treated groups received time-weighted average dose of 2,531 or 5,062 ppm.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of tumors in a 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 (*) or with the pooled-control group (**) when $P < 0.05$ for either control group; otherwise, not significant (N.S.) is indicated.

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

APPENDIX G

ANALYSIS OF FORMULATED DIETS FOR CONCENTRATIONS OF PICLORAM

APPENDIX G

Analysis of Formulated Diets for Concentrations of Picloram

A 10-g sample of the dosage mixture to be analyzed was shaken with 125 ml methanol for 16 hours. The mixture was then filtered through Celite with methanol washes, and the combined methanol solution was reduced to 10 ml. After appropriate dilutions, the solution was quantitatively analyzed for picloram by gas-liquid chromatography (electron capture detector, 10% DC-200 on Gas Chrom Q column). External standards were used for calibrations, and recoveries were determined with spiked samples.

Theoretical Concentrations in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
2,500	19	2,494	3.1%	2,340-2,670
5,000	19	4,984	4.0%	4,660-5,400
10,000	23	9,922	6.7%	9,200-11,200
20,000	10	19,560	3.5%	18,700-20,600

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