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CONTRIBUTORS: This report presents the results of the bioassay of chloramben 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.

The experimental design was determined by Drs. J. H. Weisburger[1,2] and R. R. Bates[1,3]; the doses were selected by Drs. T. E. Shellenberger[4,5], J. H. Weisburger, and R. R. Bates. Animal treatment and observation were supervised by Drs. T. E. Shellenberger and H. P. Burchfield[4], with the technical assistance of Ms. D. H. Monceaux[4] and Mr. D. Broussard[4].

Histopathology was performed by Drs. E. Bernal[4] and B. Buratto[4] at Gulf South Research Institute, and the diagnoses included in this report represent the interpretation of these pathologists. Pathologists from NCI and Tracor Jitco have reviewed selected slides and concur with the overall pathologic evaluation of the study.

Animal pathology tables and survival tables were compiled by 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].

This report was prepared at Tracor Jitco under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg[7], Director of the Bioassay Program; Dr. J. F. Robens[7], toxicologist; Ms. L. A. Waitz[7] and Ms. Y. E. Presley[7], technical writers; and Dr. E. W. Gunberg[7], technical editor.

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SUMMARY

A bioassay of technical-grade chloramben for possible carcinogenicity was conducted by administering the test material in feed to Osborne-Mendel rats and B6C3F1 mice. Groups of 50 rats and 50 mice of both sexes were administered chloramben at one of two doses, either 10,000 or 20,000 ppm. The rats were treated for 80 weeks, then observed for 32 or 33 weeks; the mice were treated for 80 weeks, then observed for 11 or 12 weeks. Matched controls consisted of groups of 10 untreated rats and 10 untreated mice of each sex; pooled controls, used for statistical evaluation, consisted of these matched controls combined with 75 untreated male and 75 untreated female rats or 70 untreated male and 70 untreated female mice from similarly performed bioassays of six other test chemicals. Surviving rats were killed at 112 or 113 weeks; surviving mice were killed at 91 or 92 weeks.

Body weights and mortality of the treated animals were not markedly affected by chloramben under the conditions of the bioassay. The various clinical signs observed were common to both treated and control groups.

In male rats, hemangiomas occurred at a significantly higher incidence in the low-dose animals than in the pooled controls (controls 0/73, low-dose 5/48, P = 0.009). This lesion was not considered to be related to the administration of chloramben, since the tumor did not occur at a significantly higher incidence in the high-dose group than in the pooled-control group, and the incidences did not show a significant dose-related trend.

In both male and female mice, the incidences of hepatocellular carcinoma showed significant dose-related trends using pooled controls (for males: controls 9/69, low-dose 16/48, high-dose 14/48, P = 0.029; for females: controls 2/67, low-dose 7/48, high-dose 10/50, P = 0.004). Direct comparisons showed significantly higher incidences of the tumor in the low-dose males (P = 0.008) and in the high-dose females (P = 0.003) than in the pooled controls. Probability levels of P = 0.028 in high-dose males and P = 0.027 in low-dose females were attained. In male mice, however, the incidence of hepatocellular carcinoma was

considered to be only marginally associated with the administration of chloramben because of the variations in the spontaneous incidence of this lesion in male mice encountered at this laboratory.

In conclusion, under the conditions of this bioassay, there were no tumors in Osborne-Mendel rats that were significantly related to administration of the chemical. In B6C3F1 female mice, chloramben was carcinogenic, producing hepatocellular carcinomas in treated animals.

TABLE OF CONTENTS

APPENDIXES

TABLES

FIGURES

I. INTRODUCTION

Chloramben (CAS 133-90-4; NCI C00055) has been used since 1958 as a preemergent herbicide to control shallow, germinating, broadleaf weeds and annual grasses. Applied as a spray at the time of planting, chloramben remains effective in the soil for several weeks until crops have become well established (Amchem Products, 1976). It is currently registered for use in the cultivation of several vegetable crops. The residue tolerance on most of these vegetables is 0.1 ppm (EPA, 1974 and 1975). Chloramben is not known to be persistent in the environment (Edwards, 1976). Soils sampled 3 months after treatment showed no detectable toxic residues (Burgis, 1972), although a metabolite, N-(3-carboxy-2,5dichlorophenyl)glucosylamine, has been found in treated plants (Swanson et al., 1966). Chloramben is of low mammalian toxicity; the oral LD_{50} in rats is 5,260 mg/kg (Spencer, 1973). The chemical was selected for testing because its extensive use as a herbicide results in human exposure.

II. MATERIALS AND METHODS

A. Chemical

Chloramben, which is the generic name for 3-amino-2,5-dichlorobenzoic acid, was obtained in several batches from Amchem Products, Inc., Ambler, Pennsylvania, as technical-grade Amiben$^{T.M.}$. These batches were used sequentially, in the general order in which they were obtained. The purity of these batches, according to the manufacturer, ranged from 90-95%. Analyses at Gulf South Research Institute (melting point; elemental analysis; infrared, ultraviolet, nuclear magnetic resonance, and mass spectrometry; thin-layer and gas-liquid chromatography) confirmed the identity of these batches, and analyses were consistent with the manufacturer's assay. No attempt was made to identify or quantitate impurities. The chemical was stored at approximately 4°C in the original container.

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 chloramben for each dietary concentration. A given amount of the test material 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 material. 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.

The stability of chloramben in feed was tested by determining the concentration of the chemical in formulated diets at intervals over a 7-day period. Diets containing 10,000 or 20,000 ppm chloramben showed no change in concentration on standing at ambient temperature for this period.

As a quality control test on the accuracy of preparation of the diets, the concentration of chloramben 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 0.5% of the theoretical concentration, and the coefficient of variation was 4.9%. 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. Upon 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 test 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 supplied *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 chloramben, along with their respective matched controls, were housed in a room by themselves. Mice receiving chloramben were maintained in a room housing mice administered chlorothalonil (CAS 1897-45-6), picloram (CAS 1918-02-1), or endrin (CAS 72-20-8), together with their respective matched controls.

E. Subchronic Studies

Feeding studies using rats and mice were conducted to estimate the maximum tolerated doses of chloramben, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. In the subchronic studies, chloramben was added to the animal feed in twofold increasing concentrations, ranging from 62.5 to 2,000 ppm for rats and 1,250 to 30,000 ppm for mice. Control and treated groups each consisted of five male and five female animals. The chemical was provided in feed to the treated groups for 6 weeks, followed by 2 weeks of observation. Because there were no deaths and no effects on body weights in the rats at 62.5 to 2,000 ppm, indicating that the maximum tolerated dose had not been reached, a second study was performed on the rats using doses ranging from 2,000 to 32,000 ppm.

At 16,000 ppm none of the rats died, and there was no effect on body weights; at 32,000 ppm, the treated animals lost weight. The low and high doses for the chronic studies using rats were set at 10,000 and 20,000 ppm.

There were no marked adverse effects on mice receiving dietary concentrations as high as 30,000 ppm. The low and high doses for the chronic studies using mice were set at 10,000 and 20,000 ppm, consistent with those set for rats.

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

Table 1. Design of Chloramben Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Chloramben in Diet (ppm)	Time on Study Treated (weeks)	Untreated[b] (weeks)
Male				
Matched-Control	10	0		113
Low-Dose	50	10,000	80	
		0		32
High-Dose	50	20,000	80	
		0		33
Female				
Matched-Control	10	0		113
Low-Dose	50	10,000	80	
		0		33
High-Dose	50	20,000	80	
		0		33

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

[b]When diets containing chloramben were discontinued, treated rats and their matched controls were fed diets without corn oil for 3 weeks, then control diets (2% corn oil added) for an additional 29 or 30 weeks.

Table 2. Design of Chloramben Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Chloramben in Diet (ppm)	Time on Study Treated (weeks)	Time on Study Untreated[b] (weeks)
Male				
Matched-Control	10	0		91
Low-Dose	50	10,000	80	
		0		11-12
High-Dose	50	20,000	80	
		0		12
Female				
Matched-Control	10	0		91
Low-Dose	50	10,000	80	
		0		11
High-Dose	50	20,000	80	
		0		12

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

[b]When diets containing chloramben were discontinued, treated mice and their matched controls received the control diets (2% corn oil added) until termination of the study.

comparisons. Matched controls from the current study of chloramben were combined with matched controls from studies performed on malathion (CAS 121-75-5), tetrachlorvinphos (CAS 961-11-5), toxaphene (CAS 8001-35-2), lindane (CAS 58-89-9), endrin, and chlorothalonil. The pooled controls for statistical tests using rats consisted of 75 males and 75 females; using mice, 70 males and 70 females. The studies of chemicals other than chloramben were also conducted at Gulf South Research Institute and overlapped the chloramben 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, animal 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.

The data of the experiments in this bioassay program are subjected to the statistical analyses described in the subsequent paragraphs of this section. The analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival are estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals are statistically censored as of the time they are missing or are dead due to other than natural causes; animals dying from natural causes are statistically uncensored. Statistical analyses for a possible dose-related effect on survival employ 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 are reported for all tests except the departure from linearity test, which is noted when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions is 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 include only those animals for which such sites are examined histologically. However, when macroscopic examination is required to detect lesions and when this examination is followed by histologic sampling (e.g., skin or mammary tumors), or when lesions could appear at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of the incidences of tumors is to determine whether animals receiving the test chemical develop a significantly higher proportion of tumors than do control animals. Statistical analyses of the incidences of specific types of tumors are made using the one-tailed Fisher exact test (Cox, 1970) to compare a control group with groups of treated animals at each dose. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction which ensures 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. When appropriate the correction is discussed in the narrative section, but it is not used 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), is 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.

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

When appropriate, life-table methods are applied to the incidence of tumors. Curves of the proportions surviving without a tumor being observed are computed according to Saffiotti et al. (1972). The times at which animals die naturally or are killed are entered as the time point of tumor observation. Cox's methods of comparing these curves are used for two groups, and Tarone's extension to testing for linear trend is used for three groups. The tests for the incidence of tumors using life-table methods are one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity (< 0.05, two-tailed test) are also noted.

The approximate 95% confidence interval for the relative risk between each of the treated groups and its control is calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is 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 that in a control group is expressed by 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 are included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of similar 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, the occurrence of a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) will also obtain. When the lower limit is less than unity and the upper limit is greater than unity, the former indicates the absence of a significant result while the latter 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 high-dose male and female rats were slightly lower than those of the corresponding matched controls (figure 1). The weights of the low-dose males were, for unknown reasons, slightly higher than those of the male controls; weights of low-dose females were essentially unaffected by chloramben.

The treated animals were generally comparable to the controls in appearance and behavior throughout the bioassay. During the second 6 months, clinical signs including epistaxis, diarrhea, and hematuria were noted at low incidences. During the second year, clinical signs including rough and discolored hair coats, dermatitis, pale mucous membranes, tachypnea, ataxia of hind legs, hyperactivity, and vaginal bleeding were noted with increasing frequency. Several animals appeared emaciated.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival of male and female rats receiving chloramben at the doses used in this experiment, together with those of the controls, are shown in figure 2. The Tarone test results for positive dose-related trend in mortality over the period are not

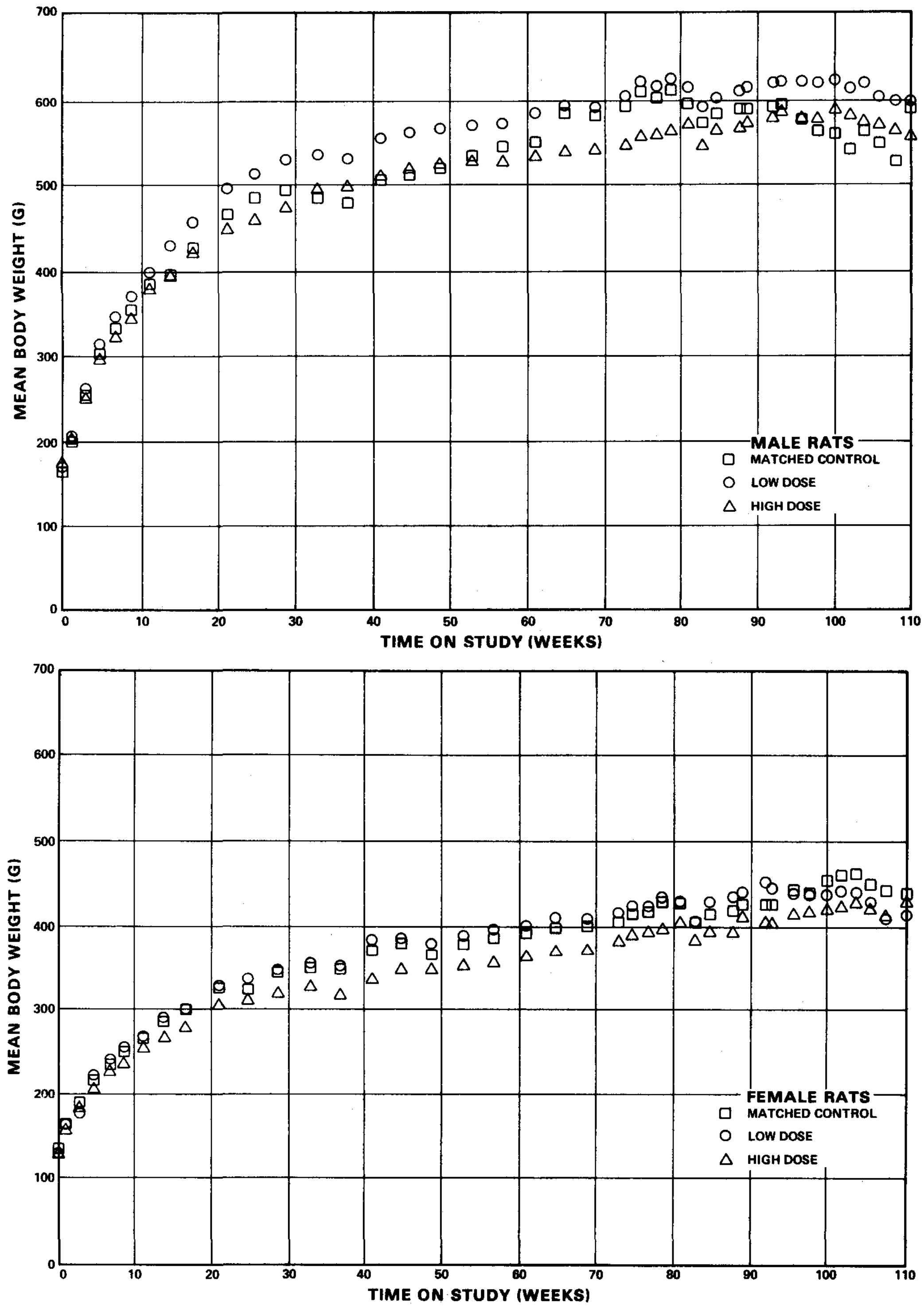


Figure 1. Growth Curves for Rats Fed Chloramben in the Diet

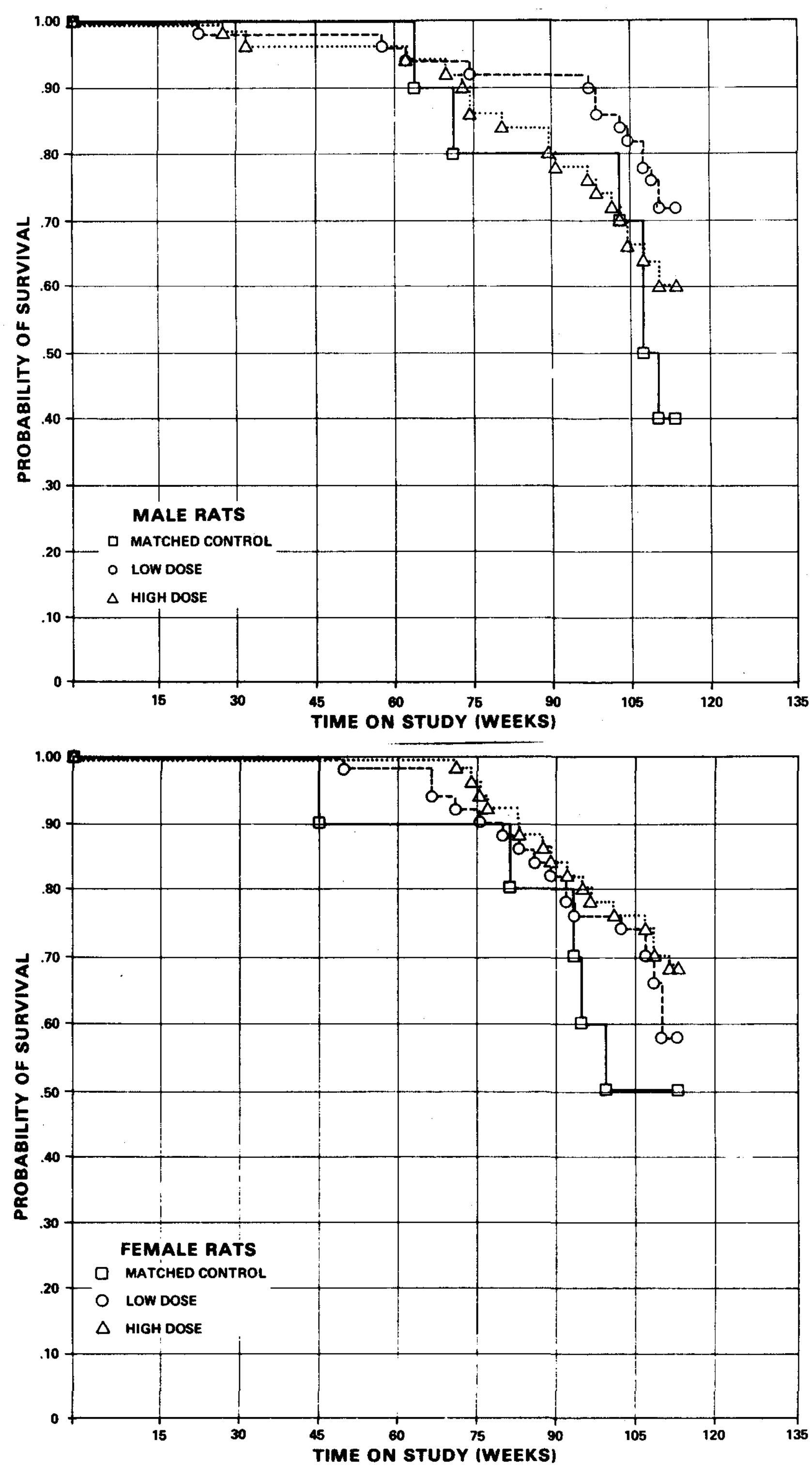


Figure 2. Survival Curves for Rats Fed Chloramben in the Diet

significant in either sex. In male rats, 61% of the high-dose group, 74% of the low-dose group, and 40% of the controls lived to the end of the study. In females, 68% of the high-dose group, 58% of the low-dose group, and 50% of the controls lived to the end of the study. A pooled-control group was used, providing adequate numbers of control animals for meaningful statistical analyses of the incidences of late-developing tumors.

C. Pathology (Rats)

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

A variety of tumors occurred randomly in both the control and treated rats. For the most part, these lesions are not uncommon in this strain of rat independent of any treatment. In addition to the neoplastic lesions, a number of nonneoplastic lesions also were observed in both the treated and control groups. In general, these nonneoplastic lesions are routinely encountered in aged rats of this strain.

C-cell adenomas of the thyroid occurred among both treated male and female rats, whereas adrenal cortical adenomas occurred only among treated females. There was a higher incidence of hyper-plastic changes of both follicular cells and C cells of the thyroid in the treated rats than in the controls, particularly in the treated males. The incidences of C-cell adenomas of the thyroid and cortical adenomas of the adrenal in the treated groups are not unusual in untreated rats of this strain.

Although follicular-cell hyperplasia of the thyroid occurred only in the treated animals and not in the controls, the incidence of the lesion was not dose related. These thyroid lesions suggest that the test chemical may have a goitrogenic effect, but insufficient numbers of controls were available to draw firm conclusions.

In the judgment of the pathologists, chloramben did not induce tumors in rats during 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 male rats, the Cochran-Armitage test for positive dose-related linear trend in proportions for hemangioma, using pooled controls, has a probability level of P = 0.074; but a significant departure from linear trend (P = 0.042) is indicated, due to the higher incidence in the low-dose group than in the high-dose group. The Fisher exact test shows a significantly higher incidence of this tumor in the low-dose group (P = 0.009), but not in the high-dose group, than in the pooled controls. The 95% confidence interval shows a lower limit greater than one for the relative risk comparing the low-dose group with the control group. This indicates that there is a theoretical possibility of the induction of tumors by the test chemical. Because of the lack of statistical significance of the incidence of hemangioma in the high-dose group, the true significance of this tumor in male rats is questionable.

In female rats, the statistical test results on hemangioma are not significant at the 0.05 level. There are no other incidences of tumors at any specific site in either sex which are significant using either the Cochran-Armitage test or the Fisher exact test.

IV. RESULTS - MICE

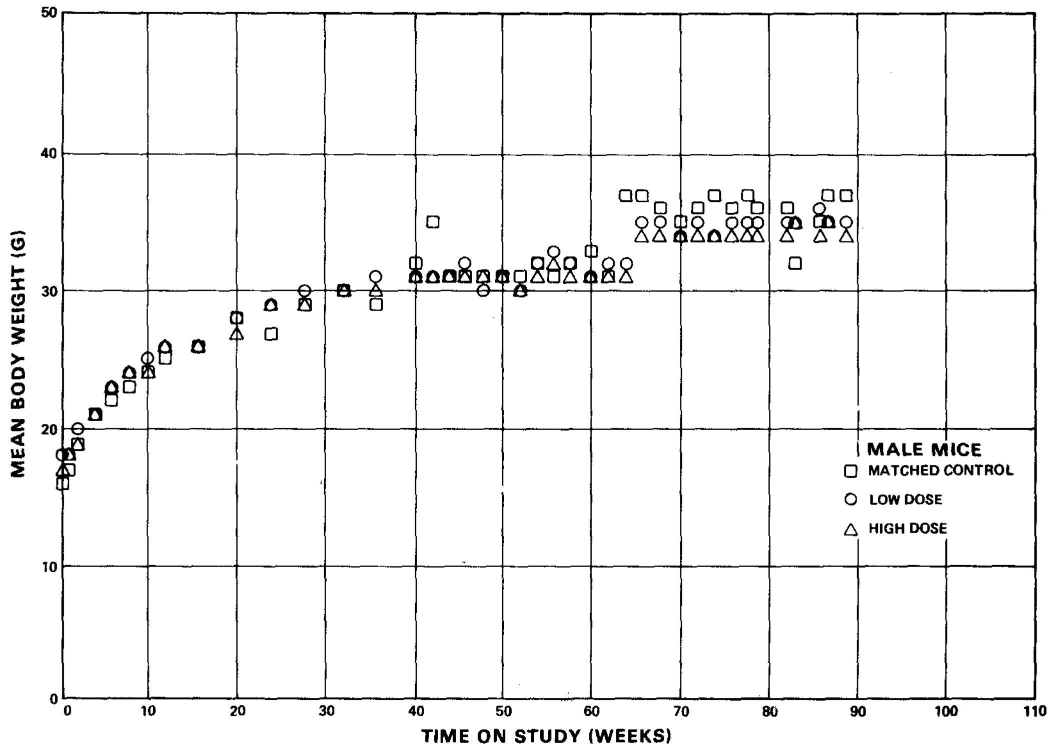
A. Body Weights and Clinical Signs (Mice)

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

The treated animals were generally comparable to the controls in appearance and behavior throughout the bioassay. During the second year, clinical signs including alopecia, rough hair coats, hyperactivity, dyspnea, abdominal distention, and hunched appearance were observed. Many males, treated and control, were observed fighting. The equilibrium of one low-dose male appeared to have been impaired.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival of male and female mice receiving chloramben at the doses used in this experiment, together with those of the controls, are shown in figure 4. The Tarone test results for positive dose-related trend in mortality over the period are not significant in either sex. In male mice, 90% of the high-dose group, 84% of the low-dose group, and 80% of the controls lived to the end of the study. Similarly, in female mice, 90% of the high-dose group, 76% of the low-dose group, and 90% of the



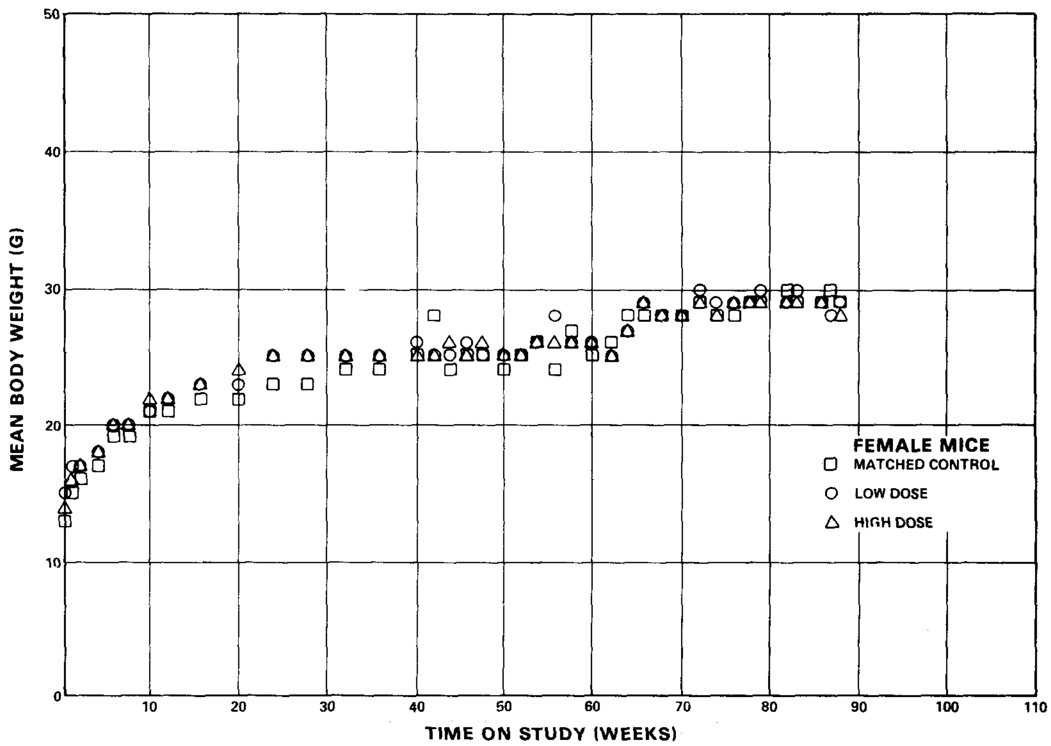
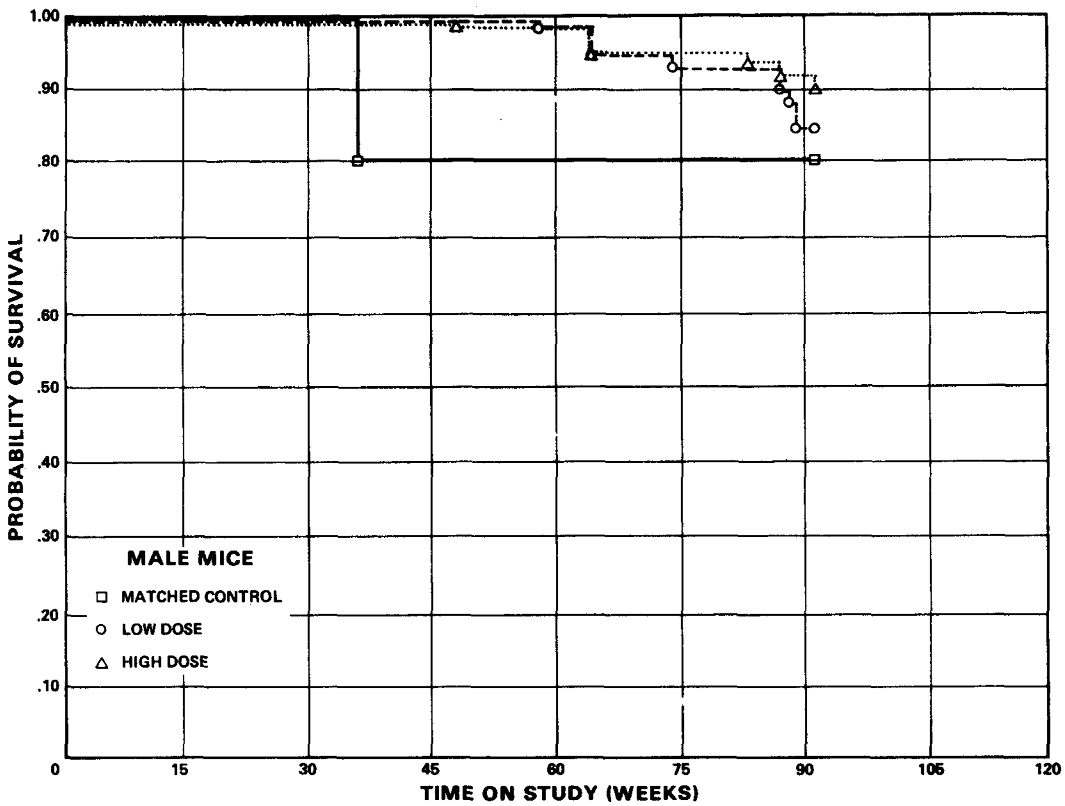


Figure 3. Growth Curves for Mice Fed Chloramben in the Diet



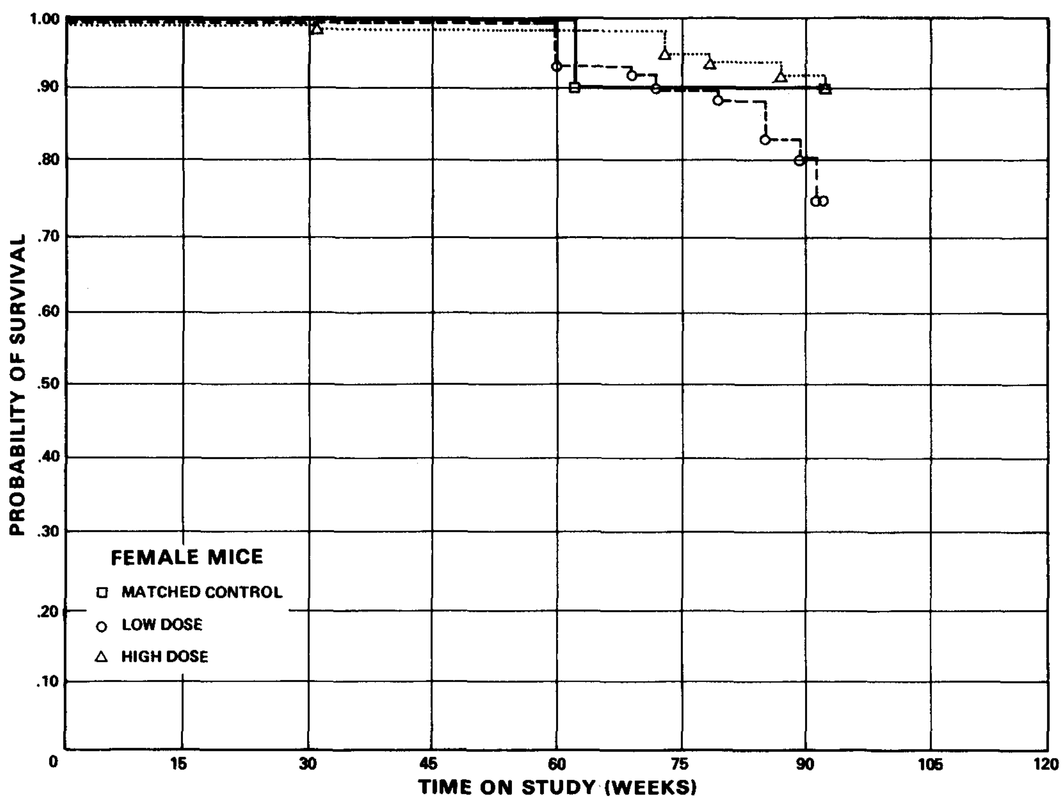


Figure 4. Survival Curves for Mice Fed Chloramben in the Diet

controls lived to the end of the study. Survival of both sexes was adequate for meaningful statistical analyses of the incidence of tumors in these mice.

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.

For the most part, the lesions were of the type commonly encountered in this strain of mice. The incidence of hepatocellular carcinoma in both male and female treated mice was higher than that in the controls (males: controls 2/10 [20%], low-dose 16/48 [33%], high-dose 14/48 [29%]; females: controls 0/9 [0%], low-dose 7/48 [15%], high-dose 10/50 [20%]). Spontaneous hepatocellular carcinoma is not uncommon in this strain of mouse, particularly in the males. In the judgment of the pathologists, the incidence in males was insufficient to indicate a clear relationship to treatment; however, the relationship between treatment with chloramben and the incidence of hepatocellular carcinoma appears to be significant in female mice.

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 male mice, the Cochran-Armitage test for positive dose-related trend in the proportions of hepatocellular carcinoma is significant (P = 0.029) using the pooled controls, and the Fisher exact test shows higher incidences of this tumor in both the low-dose group (P = 0.008) and the high-dose group (P = 0.028) than in the pooled controls. The probability level of 0.028 is above the level of 0.025 required for significance by the multiple comparison procedure for the Fisher exact test. The 95% confidence intervals using the pooled controls have lower limits greater than one.

The significant results in the males are confirmed in the females, since the Cochran-Armitage test result for positive dose-related trend in the proportions of hepatocellular carcinoma is significant (P = 0.004) using the pooled controls, and the Fisher exact test shows significantly higher incidences of this tumor in both the low-dose group (P = 0.027) and the high-dose group (P = 0.003) than in the pooled controls. The probability level of 0.027 is above the level of 0.025 required for significance by the multiple comparison procedure for the Fisher exact test. The 95% confidence intervals for the relative risk comparing the high-dose group with the pooled controls have lower limits greater than one. These tests show that, statistically, there is an association between chloramben treatment and the occurrence of hepatocellular carcinoma in mice at the doses used in this experiment.

V. DISCUSSION

Mean body weights and rates of mortality of the treated animals were not markedly affected by chloramben under the conditions of the bioassay. The various clinical signs observed were common to both treated and control groups. Survival was adequate for meaningful statistical analyses of the incidence of tumors. Thus, the concentrations of chloramben used in both rats and mice, i.e., 10,000 and 20,000 ppm, can be considered to be only slightly toxic. However, these concentrations are high when compared with the possible exposure of humans to residues of the herbicide.

In rats, hemangiomas occurred at a significantly higher incidence in low-dose males (5/48 [10%]) than in pooled controls (0/73); however, the incidence of this tumor was not significant for high-dose males compared with pooled controls, and the dose-related trend was not statistically significant. In addition, the pathologists did not consider the hemangioma to be related to administration of the chemical. Thus, the occurrences of hemangiomas are not considered to be related to the administration of chloramben.

In both male and female mice, the incidences of hepatocellular carcinoma showed significant dose-related trends using pooled controls (for males: controls 9/69, low-dose 16/48, high-dose 14/48, P = 0.029; for females: controls 2/67, low-dose 7/48, high-dose 10/50, P = 0.004). Direct comparisons showed significantly higher incidences of the tumor in the low-dose males (P = 0.008) and in the high-dose females (P = 0.003) than in the pooled controls. Probability levels of P = 0.028 in high-dose males and P = 0.027 in low-dose females were attained. Very few related lesions were observed. Two additional male animals, but only one female, had hepatocellular adenoma or neoplastic nodule. No hyperplastic lesions of the liver were observed in either sex.

The variability in the incidence of hepatocellular carcinoma among historical control mice at Gulf South Research Institute was considered. In a few control groups in the bioassay program at this testing laboratory, as many as 3/10 or 4/10 male mice had hepatocellular carcinoma. The mean of the incidences for the male controls at Gulf South Research Institute was 16.8%. Because of the variation (0-40%) in the historical incidences of spontaneous hepatocellular carcinomas in control male mice at this laboratory, the incidences of these lesions in treated male mice reported in this study are considered as marginal. This is consistent with the pathologists' view that the incidence in males was insufficient to indicate a clear relationship to treatment. Control groups of female mice had no more than 2/9 animals with hepatocellular carcinoma, and the mean of the incidences for all females in the historical group was 2.3%.

In conclusion, under the conditions of this bioassay, chloramben was not carcinogenic in either sex of the Osborne-Mendel rats. Hemangiomas were present at a slightly higher incidence in low-dose male rats than in pooled controls. However, the bioassay does not conclusively demonstrate the relationship of these lesions to treatment. In B6C3F1 male mice, the incidence of hepatocellular carcinoma was considered as only marginally associated with the administration of chloramben. In B6C3F1 female mice, chloramben was carcinogenic, producing hepatocellular carcinomas in treated animals.

VI. BIBLIOGRAPHY

Amchem Products, Amiben T.M.; Preemergence Herbicide, Amchem Products, Inc., Ambler, Pa., accepted 1976.

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-265.

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

Burgis, D. S., Herbicide tests on pepper transplants and seeded peppers. Proc. Fla. State Hort. Soc. 84:183-186, 1972.

Cox, D. R., Regression models and life tables. J. R. Statist. Soc. B 34 (2):187-220, 1972.

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

Edwards, C. A., Persistent Pesticides in the Environment, CRC Press, Cleveland, Ohio, 1976, pp. 107-108.

Environmental Protection Agency, Chloramben. EPA Compendium of Registered Pesticides, U. S. Government Printing Office, Washington, D.C., 1975, I-C-13.1.

Environmental Protection Agency, Chloramben. EPA Compendium of Registered Pesticides, U.S. Government Printing Office, Washington, D. C., 1974, I-C-13.2.

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

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

Leigh, E. S. J., Jr. and Lisk, D. L., Excretory pathway of Amiben in a lactating cow. J. Agr. Food Chem. 18:482-484, 1970.

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

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

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

Spencer, E. Y., Guide to Chemicals Used in Crop Protection, Research Institute, University of Western Ontario, London, Ontario, 1973, p. 90.

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

Swanson, C. R., Kadunce, R. E., Hodgson, R. H., and Frear, D. S., Amiben metabolism in plants, I. Isolation and identification of an N-glucosyl complex. Weeds 14:319-323, 1966.

Tarone, R. E., Tests for trend in life table analysis. Biometrika 62(3):679-682, 1975.

APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	47	47
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(48)	(49)
FIBROUS HISTIOCYTOMA			1 (2%)
FIBROUS HISTIOCYTOMA, MALIGNANT		2 (4%)	
*SUBCUT TISSUE	(9)	(48)	(49)
LEIOMYOMA		1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(9)	(48)	(49)
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(7)	(47)	(47)
HEMANGIOMA		3 (6%)	2 (4%)
CIRCULATORY SYSTEM			
#HEART	(9)	(47)	(48)
ADENOCARCINOMA, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(9)	(46)	(46)
NEOPLASTIC NODULE		4 (9%)	1 (2%)
HEMANGIOMA		1 (2%)	1 (2%)
#PANCREATIC DUCT	(8)	(46)	(48)
ADENOCARCINOMA, NOS	1 (13%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(9)	(47)	(48)
† HAMARTOMA			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(44)	(40)
CARCINOMA,NOS			1 (3%)
CHROMOPHOBE ADENOMA	1 (11%)	5 (11%)	8 (20%)
#ADRENAL	(8)	(46)	(48)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA	1 (13%)		
#THYROID	(8)	(47)	(48)
C-CELL ADENOMA		4 (9%)	3 (6%)
#PANCREATIC ISLETS	(8)	(46)	(48)
ISLET-CELL ADENOMA		2 (4%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(48)	(49)
INFILTRATING DUCT CARCINOMA			1 (2%)
FIBROMA			1 (2%)
LIPOMA		1 (2%)	1 (2%)
NERVOUS SYSTEM			
#BRAIN	(9)	(47)	(48)
MENINGIOMA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKULL	(9)	(48)	(49)
OSTEOMA			1 (2%)

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
† NONNEOPLASTIC PROLIFERATIVE LESION COMPOSED OF LIPOCYTES, TUBULAR STRUCTURES, AND FIBROBLASTS IN VARYING PROPORTIONS.

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(9)	(48)	(49)
HAMARTOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(9)	(48)	(49)
OSTEOSARCOMA			1 (2%)
DIAPHRAGM			
HEMANGIOMA		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@	1	5	5
MORIBUND SACRIFICE	5	9	15
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	4	36	30
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	22	21
TOTAL PRIMARY TUMORS	3	27	28
TOTAL ANIMALS WITH BENIGN TUMORS	2	16	16
TOTAL BENIGN TUMORS	2	20	23
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	3	4
TOTAL MALIGNANT TUMORS	1	3	4
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		4	1
TOTAL UNCERTAIN TUMORS		4	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(10)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (10%)		
FIBROMA			1 (2%)
LEIOMYOMA			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(49)	(50)
HEMANGIOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(48)	(50)
NEOPLASTIC NODULE		2 (4%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	
LIPOSARCOMA		1 (2%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(45)	(46)
CARCINOMA,NOS		3 (7%)	1 (2%)
ADENOMA, NOS	1 (11%)		
CHROMOPHOBE ADENOMA	1 (11%)	11 (24%)	6 (13%)
#ADRENAL	(10)	(49)	(50)
CORTICAL ADENOMA		3 (6%)	4 (8%)
#THYROID	(8)	(48)	(50)
C-CELL ADENOMA		4 (8%)	4 (8%)
#PANCREATIC ISLETS	(9)	(48)	(50)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROMA			1 (2%)
LIPOMA			1 (2%)
FIBROADENOMA	2 (20%)	7 (14%)	7 (14%)
#UTERUS	(10)	(44)	(47)
ADENOCARCINOMA, NOS		1 (2%)	
ENDOMETRIAL STROMAL POLYP		3 (7%)	2 (4%)
ENDOMETRIAL STROMAL SARCOMA			2 (4%)
#UTERUS/ENDOMETRIUM	(10)	(44)	(47)
PAPILLARY ADENOCARCINOMA			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(10)	(48)	(50)
CARCINOMA, NOS, INVASIVE			1 (2%)
GLIOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*EAR CANAL	(10)	(50)	(50)
LEIOMYOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (10%)	1 (2%)	1 (2%)
MESOTHELIOMA, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		3	4
MORIBUND SACRIFICE	5	18	12
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	5	29	34
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	34	30
TOTAL PRIMARY TUMORS	6	41	35
TOTAL ANIMALS WITH BENIGN TUMORS	4	27	26
TOTAL BENIGN TUMORS	4	30	29
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	8	5
TOTAL MALIGNANT TUMORS	2	8	5
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT		3	1
TOTAL UNCERTAIN TUMORS		3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

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

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST		5 (10%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(49)	(49)
PLASMA-CELL MYELOMA		1 (2%)	
#KIDNEY	(9)	(48)	(48)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(10)	(48)	(48)
HEPATOCELLULAR ADENOMA		1 (2%)	
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA	2 (20%)	16 (33%)	14 (29%)
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			
#PITUITARY	(10)	(41)	(39)
ADENOMA, NOS			1 (3%)
#THYROID	(9)	(42)	(47)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#TESTIS	(10)	(43)	(47)
SEMINOMA/DYSGERMINOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(10)	(49)	(49)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	

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@		2	2
MORIBUND SACRIFICE	2	5	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	8	43	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	20	20
TOTAL PRIMARY TUMORS	2	21	22
TOTAL ANIMALS WITH BENIGN TUMORS		1	5
TOTAL BENIGN TUMORS		1	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	18	16
TOTAL MALIGNANT TUMORS	2	19	17
TOTAL ANIMALS WITH SECONDARY TUMORS#		5	
TOTAL SECONDARY TUMORS		5	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

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

TABLE B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(10)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
GRANULOCYTIC LEUKEMIA		1 (2%)	
#SPLEEN	(10)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)
#LIVER	(9)	(48)	(50)
GRANULOCYTIC LEUKEMIA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(48)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	
HEPATOCELLULAR CARCINOMA		7 (15%)	10 (20%)
#STOMACH	(10)	(48)	(49)
PAPILLOMA, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#OVARY	(7)	(46)	(48)
CYSTADENOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
SITE UNKNOWN			
HEMANGIOSARCOMA			1

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	10	50	50
NATURAL DEATH@		1	1
MORIBUND SACRIFICE	1	11	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	38	45
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*		12	13
TOTAL PRIMARY TUMORS		13	14
TOTAL ANIMALS WITH BENIGN TUMORS		4	1
TOTAL BENIGN TUMORS		4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS		9	12
TOTAL MALIGNANT TUMORS		9	13
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 CHLORAMBEN IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	9	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	9	47	47
INTEGUMENTARY SYSTEM			
*SKIN	(9)	(48)	(49)
INFLAMMATION, NOS			1 (2%)
GRANULOMA, NOS	1 (11%)		
RESPIRATORY SYSTEM			
#LUNG	(9)	(47)	(48)
ATELECTASIS	1 (11%)		1 (2%)
#LUNG/ALVEOLI	(9)	(47)	(48)
EMPHYSEMA, NOS		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	
CALCIFICATION, METASTATIC		2 (4%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(7)	(47)	(47)
FIBROSIS, FOCAL			1 (2%)
HEMATOPOIESIS			1 (2%)
#LYMPH NODE	(9)	(40)	(43)
DILATATION, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(9)	(47)	(48)
THROMBOSIS, NOS	1 (11%)		
MEDIAL CALCIFICATION		1 (2%)	
#MYOCARDIUM	(9)	(47)	(48)
INFLAMMATION, CHRONIC			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL			1 (2%)
FIBROSIS		1 (2%)	1 (2%)
FIBROSIS, FOCAL		6 (13%)	4 (8%)
*AORTA	(9)	(48)	(49)
ANEURYSM			1 (2%)
ARTERIOSCLEROSIS, NOS	1 (11%)		
MEDIAL CALCIFICATION		2 (4%)	1 (2%)
*CORONARY ARTERY	(9)	(48)	(49)
MEDIAL CALCIFICATION		1 (2%)	
*SPLENIC ARTERY	(9)	(48)	(49)
MEDIAL CALCIFICATION		1 (2%)	
DIGESTIVE SYSTEM			
#SUBMAXILLARY GLAND	(9)	(47)	(48)
FIBROSIS, FOCAL		1 (2%)	
#LIVER	(9)	(46)	(46)
FIBROSIS	1 (11%)		
DEGENERATION, BALLOONING			2 (4%)
DEGENERATION PARENCHYMATOUS	1 (11%)	2 (4%)	
NECROSIS, FOCAL		2 (4%)	1 (2%)
METAMORPHOSIS FATTY	2 (22%)	6 (13%)	4 (9%)
ANGIECTASIS	1 (11%)	1 (2%)	
#LIVER/CENTRILOBULAR	(9)	(46)	(46)
NECROSIS, NOS	1 (11%)		
#STOMACH	(9)	(46)	(44)
CALCIFICATION, NOS		1 (2%)	
CALCIFICATION, METASTATIC		1 (2%)	
HYPERKERATOSIS		1 (2%)	
#GASTRIC MUCOSA	(9)	(46)	(44)
CALCIFICATION, NOS		1 (2%)	1 (2%)
CALCIFICATION, METASTATIC	1 (11%)		
#GASTRIC SUBMUCOSA	(9)	(46)	(44)
CALCIFICATION, NOS		1 (2%)	
#SMALL INTESTINE	(9)	(45)	(45)
NECROSIS, NOS		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
#CECUM	(7)	(44)	(43)
INFLAMMATION, ACUTE		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(9)	(47)	(48)
GLOMERULONEPHRITIS, NOS	2 (22%)	1 (2%)	
INFLAMMATION, CHRONIC	3 (33%)	6 (13%)	22 (46%)
FIBROSIS, DIFFUSE		1 (2%)	
NEPHROSIS, NOS		15 (32%)	3 (6%)
CALCIFICATION, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(44)	(40)
CYST, NOS		11 (25%)	5 (13%)
HYPERPLASIA, NOS		2 (5%)	1 (3%)
HYPERPLASIA, FOCAL		4 (9%)	
ANGIECTASIS	1 (11%)		
#ADRENAL	(8)	(46)	(48)
HEMORRHAGE		1 (2%)	
#ADRENAL CORTEX	(8)	(46)	(48)
DEGENERATION, CYSTIC		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
ANGIECTASIS			1 (2%)
#THYROID	(8)	(47)	(48)
EPIDERMAL INCLUSION CYST		1 (2%)	
CYSTIC FOLLICLES		3 (6%)	1 (2%)
ATROPHY, NOS		5 (11%)	
HYPERPLASIA, C-CELL		8 (17%)	6 (13%)
HYPERPLASIA, FOLLICULAR-CELL		9 (19%)	4 (8%)
#PARATHYROID	(4)	(28)	(33)
HYPERPLASIA, NOS		1 (4%)	3 (9%)
HYPERPLASIA, SECONDARY		5 (18%)	
HYPERPLASIA, DIFFUSE		1 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(9)	(48)	(49)
HYPERPLASIA, NOS			2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DYSPLASIA, NOS			1 (2%)
#PROSTATE	(9)	(45)	(46)
EDEMA, NOS			1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
INFLAMMATION ACUTE AND CHRONIC		1 (2%)	
#TESTIS	(9)	(46)	(48)
EDEMA, NOS			1 (2%)
PERIARTERITIS		1 (2%)	1 (2%)
NECROSIS, FIBRINOID	1 (11%)		
ATROPHY, NOS	1 (11%)	14 (30%)	7 (15%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*FEMUR	(9)	(48)	(49)
OSTEOPOROSIS		3 (6%)	
BODY CAVITIES			
*MESENTERY	(9)	(48)	(49)
PERIARTERITIS		1 (2%)	3 (6%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
FIBROSIS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	3	5	4
AUTO/NECROPSY/NO HISTO		1	2
AUTOLYSIS/NO NECROPSY	1	2	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 CHLORAMBEN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(10)	(50)	(50)
GRANULOMA, NOS		1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(49)	(50)
HEMATOPOIESIS			2 (4%)
#LYMPH NODE	(9)	(41)	(41)
INFLAMMATION, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(10)	(49)	(50)
HEMORRHAGE			1 (2%)
#MYOCARDIUM	(10)	(49)	(50)
FIBROSIS, FOCAL			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(10)	(48)	(49)
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
#LIVER	(10)	(48)	(50)
INFLAMMATION, ACUTE SUPPURATIVE			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
DEGENERATION PARENCHYMATOUS	3 (30%)		
METAMORPHOSIS FATTY		1 (2%)	
ATYPIA, NOS	1 (10%)		
HYPERPLASIA, DIFFUSE	1 (10%)		
ANGIECTASIS	2 (20%)		
*BILE DUCT	(10)	(50)	(50)
INFLAMMATION, ACUTE SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC FOCAL		4 (8%)	
HYPERPLASIA, FOCAL			1 (2%)
#PANCREAS	(9)	(48)	(50)
CALCULUS, NOS			1 (2%)
DILATATION/DUCTS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#STOMACH	(10)	(48)	(49)
ULCER, NOS		1 (2%)	
EROSION		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(10)	(49)	(50)
HYDRONEPHROSIS		1 (2%)	
GLOMERULONEPHRITIS, NOS	1 (10%)		
INFLAMMATION, CHRONIC	1 (10%)	5 (10%)	2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(9)	(45)	(46)
CYST, NOS		2 (4%)	
CONGESTION, NOS		1 (2%)	2 (4%)
HEMORRHAGE			1 (2%)
HYPERPLASIA, NOS		2 (4%)	2 (4%)
HYPERPLASIA, FOCAL		1 (2%)	4 (9%)
ANGIECTASIS	1 (11%)		1 (2%)
#ADRENAL	(10)	(49)	(50)
CYST, NOS		2 (4%)	3 (6%)
HEMORRHAGE		2 (4%)	3 (6%)
HEMORRHAGIC CYST			5 (10%)
DEGENERATION, CYSTIC			4 (8%)
ANGIECTASIS		4 (8%)	
#ADRENAL CORTEX	(10)	(49)	(50)
DILATATION, NOS			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
HEMORRHAGE		1 (2%)	
DEGENERATION, NOS			1 (2%)
DEGENERATION, CYSTIC			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
ANGIECTASIS		4 (8%)	9 (18%)
#THYROID	(8)	(48)	(50)
HYPERPLASIA, C-CELL	2 (25%)	3 (6%)	6 (12%)
HYPERPLASIA, FOLLICULAR-CELL		6 (13%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(10)	(50)	(50)
HYPERPLASIA, NOS		4 (8%)	5 (10%)
DYSPLASIA, NOS			3 (6%)
FIBROCYSTIC DISEASE	1 (10%)		
#UTERUS	(10)	(44)	(47)
HEMORRHAGE			1 (2%)
#OVARY	(10)	(47)	(50)
FOLLICULAR CYST, NOS	1 (10%)		
NERVOUS SYSTEM			
#BRAIN	(10)	(48)	(50)
HYDROCEPHALUS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(10)	(50)	(50)
PERIARTERITIS		2 (4%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		5	5
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 CHLORAMBEN IN THE DIET

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(10)	(49)	(48)
CONGESTION, NOS	1 (10%)		
EDEMA, NOS	1 (10%)		
INFLAMMATION, CHRONIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(9)	(48)	(48)
ANGIECTASIS			1 (2%)
#MESENTERIC L. NODE	(8)	(46)	(44)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(10)	(41)	(46)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LIVER	(10)	(48)	(48)
INFARCT, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(9)	(48)	(48)
HYDRONEPHROSIS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
ENDOCRINE SYSTEM			
#ADRENAL	(10)	(48)	(48)
CYST, NOS		1 (2%)	
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	7	26	28
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY		1	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 CHLORAMBEN IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	10	50	50
ANIMALS NECROPSIED	10	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	10	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(10)	(48)	(49)
CONGESTION, NOS		1 (2%)	
INFLAMMATION, NOS		2 (4%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#LYMPH NODE	(10)	(46)	(49)
INFLAMMATION, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(9)	(48)	(50)
INFLAMMATION, NOS		3 (6%)	
INFLAMMATION, ACUTE		2 (4%)	
GRANULOMA, NOS	1 (11%)		
METAMORPHOSIS FATTY		1 (2%)	
HEMATOPOIESIS		1 (2%)	
*BILE DUCT	(10)	(50)	(50)
INFLAMMATION, NOS			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ACINUS	(9)	(48)	(49)
ATROPHY, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(10)	(48)	(50)
HYDRONEPHROSIS			1 (2%)
GLOMERULONEPHRITIS, NOS	1 (10%)		
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, INTERSTITIAL		2 (4%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(10)	(47)	(50)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(10)	(48)	(48)
INFLAMMATION, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(10)	(48)	(48)
HYPERPLASIA, CYSTIC	2 (20%)	1 (2%)	1 (2%)
#OVARY	(7)	(46)	(48)
FOLLICULAR CYST, NOS	1 (14%)	1 (2%)	2 (4%)
INFLAMMATION, NOS		13 (28%)	3 (6%)
INFLAMMATION, SUPPURATIVE	1 (14%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN	(10)	(47)	(48)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(10)	(50)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	6	18	30
AUTO/NECROPSY/NO HISTO		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS

IN RATS FED CHLORAMBEN IN THE DIET

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
All Sites: Hemangioma[b]	0/9 (0.00)	0/73 (0.00)	5/48 (0.10)	3/49 (0.06)
P Values[c,d]	N.S.	N.S.	P = 0.009**	N.S.
Departure from Linear Trend[e]		P = 0.042		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.269	0.125
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			Infinite	Infinite
Lower Limit			1.903	0.889
Upper Limit			Infinite	Infinite
Weeks to First Observed Tumor	--	--	103	113
Liver: Neoplastic Nodule[b]	0/9 (0.00)	1/72 (0.01)	4/46 (0.09)	1/46 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Departure from Linear Trend[e]		P = 0.035		
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.205	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			6.261	1.565
Lower Limit			0.641	0.020
Upper Limit			301.199	120.232
Weeks to First Observed Tumor	--	--	112	113

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	1/9 (0.11)	7/63 (0.11)	5/44 (0.12)	8/40 (0.20)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.023	1.800
Lower Limit			0.145	0.310
Upper Limit			46.226	77.552
Relative Risk (Pooled Control)[f]			1.023	1.800
Lower Limit			0.271	0.616
Upper Limit			3.472	5.325
Weeks to First Observed Tumor	110	--	96	101
Pituitary: Carcinoma, NOS[b]	0/9 (0.00)	1/63 (0.02)	0/44 (0.00)	1/40 (0.03)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.013
Upper Limit			--	Infinite
Relative Risk (Pooled Control)[f]			0.000	1.575
Lower Limit			0.000	0.020
Upper Limit			26.739	120.584
Weeks to First Observed Tumor	--	--	--	113

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma, NOS[b]	1/9 (0.11)	8/63 (0.13)	5/44 (0.12)	9/40 (0.23)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			1.023	2.025
Lower Limit			0.145	0.362
Upper Limit			47.226	86.067
Relative Risk (Pooled Control)[f]			0.895	1.772
Lower Limit			0.244	0.658
Upper Limit			2.867	4.785
Weeks to First Observed Tumor	110	--	96	113
Thyroid: C-cell Adenoma[b]	0/8 (0.00)	2/63 (0.03)	4/47 (0.09)	3/48 (0.06)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.182	0.115
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			2.681	1.969
Lower Limit			0.401	0.235
Upper Limit			28.550	22.746
Weeks to First Observed Tumor	--	--	112	101

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Cortical Adenoma[b]	0/8 (0.00)	2/70 (0.03)	1/46 (0.02)	0/48 (0.00)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	--
Lower Limit			0.010	--
Upper Limit			Infinite	--
Relative Risk (Pooled Control)[f]			0.761	0.000
Lower Limit			0.013	0.000
Upper Limit			14.128	4.926
Weeks to First Observed Tumor	--	--	112	--

[a]Treated groups received doses of 10,000 or 20,000 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 Chloramben in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
All Sites: Hemangioma[b]	0/10 (0.00)	0/74 (0.00)	0/50 (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]			--	Infinite
Lower Limit			--	0.078
Upper Limit			--	Infinite
Weeks to First Observed Tumor	--	--	--	113
Liver: Neoplastic Nodule[b]	0/10 (0.00)	1/73 (0.01)	2/48 (0.04)	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			0.068	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			3.042	1.460
Lower Limit			0.163	0.019
Upper Limit			175.641	112.322
Weeks to First Observed Tumor	--	--	113	113

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma[b]	1/9 (0.11)	12/65 (0.18)	11/45 (0.24)	6/46 (0.14)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			2.200	1.174
Lower Limit			0.414	0.181
Upper Limit			92.100	52.803
Relative Risk (Pooled Control)[f]			1.324	0.707
Lower Limit			0.578	0.234
Upper Limit			2.950	1.867
Weeks to First Observed Tumor	91	--	75	94
Pituitary: Carcinoma, NOS[b]	0/9 (0.00)	1/65 (0.02)	3/45 (0.07)	1/46 (0.02)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.135	0.011
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			4.333	1.413
Lower Limit			0.359	0.018
Upper Limit			222.252	108.514
Weeks to First Observed Tumor	--	--	106	113

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma, NOS[b]	1/9 (0.11)	13/65 (0.20)	14/45 (0.31)	7/46 (0.16)
P Values[c,d]	N.S.	N.S. (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]			2.800	1.370
Lower Limit			0.550	0.224
Upper Limit			114.824	60.268
Relative Risk (Pooled Control)[f]			1.556	0.761
Lower Limit			0.748	0.276
Upper Limit			3.206	1.873
Weeks to First Observed Tumor	94	--	75	94
Thyroid: C-cell Adenoma[b]	0/8 (0.00)	1/61 (0.02)	4/48 (0.09)	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			0.179	0.170
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			5.083	4.880
Lower Limit			0.527	0.503
Upper Limit			244.874	235.283
Weeks to First Observed Tumor	--	--	91	111

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Adrenal: Cortical Adenoma[b]	0/10 (0.00)	1/70 (0.01)	3/49 (0.06)	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			0.137	0.206
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			4.286	5.600
Lower Limit			0.358	0.575
Upper Limit			220.214	269.964
Weeks to First Observed Tumor	--	--	70	70
Mammary Gland: Fibroadenoma[b]	2/10 (0.20)	11/74 (0.15)	7/50 (0.14)	7/50 (0.14)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			0.700	0.700
Lower Limit			0.173	0.173
Upper Limit			6.482	6.482
Relative Risk (Pooled Control)[f]			0.942	0.942
Lower Limit			0.330	0.330
Upper Limit			2.455	2.455
Weeks to First Observed Tumor	45	--	49	87

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

(continued)

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	0/10 (0.00)	6/69 (0.09)	3/44 (0.07)	2/47 (0.04)
P Values[c,d]	N.S.	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.152	0.069
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.784	0.489
Lower Limit			0.132	0.049
Upper Limit			3.449	2.589
Weeks to First Observed Tumor	--	--	108	77

[a]Treated groups received doses of 10,000 or 20,000 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 CHLORAMBEN IN THE DIET

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

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/10 (0.00)	4/66 (0.06)	0/49 (0.00)	4/48 (0.08)
P Values[c,d]	P = 0.043	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]			--	Infinite
Lower Limit			--	0.215
Upper Limit			--	Infinite
Relative Risk (Pooled Control)[f]			0.000	1.375
Lower Limit			0.000	0.266
Upper Limit			1.455	7.004
Weeks to First Observed Tumor	--	--	87	92
Liver: Hepatocellular Carcinoma[b]	2/10 (0.20)	9/69 (0.13)	16/48 (0.33)	14/48 (0.29)
P Values[c,d]	N.S.	P = 0.029	P = 0.008**	P = 0.028**
Relative Risk (Matched Control)[f]			1.667	1.458
Lower Limit			0.515	0.439
Upper Limit			13.777	12.223
Relative Risk (Pooled Control)[f]			2.556	2.236
Lower Limit			1.164	0.984
Upper Limit			5.926	5.321
Weeks to First Observed Tumor	91	--	87	64

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

(continued)

[a]Treated groups received doses of 10,000 or 20,000 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 Chloramben in the Diet[a]

Topography: Morphology	Matched Control	Pooled Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/10 (0.00)	3/69 (0.04)	1/48 (0.02)	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			0.012	0.012
Upper Limit			Infinite	Infinite
Relative Risk (Pooled Control)[f]			0.479	0.460
Lower Limit			0.009	0.009
Upper Limit			5.734	5.510
Weeks to First Observed Tumor	--	--	91	92
Liver: Hepatocellular Carcinoma[b]	0/9 (0.00)	2/67 (0.03)	7/48 (0.15)	10/50 (0.20)
P Values[c,d]	N.S.	P = 0.004	P = 0.027**	P = 0.003**
Relative Risk (Matched Control)[f]			Infinite	Infinite
Lower Limit			0.413	0.607
Upper Limit			Infinite	Infinite
Relative Risk Pooled Control)[f]			4.885	6.700
Lower Limit			0.982	1.515
Upper Limit			46.360	60.419
Weeks to First Observed Tumor	--	--	91	78

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

(continued)

[a]Treated groups received doses of 10,000 or 20,000 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 CHLORAMBEN

APPENDIX G

Analysis of Formulated Diets for

Concentrations of Chloramben

A 10-g sample of the formulated diet was shaken with 125 ml of methanol at room temperature for 16 hours, then filtered through Celite with methanol washes, and reduced in volume to a theoretical chloramben concentration of about 400 ng/ml.

The chloramben then was converted to its methyl ester for gas-liquid chromatographic (glc) analysis by a modification of the procedure of Leigh and Lisk (1970). To a 1-ml aliquot of the above extract in a 10-ml volumetric flask was added 3 ml of 14% $BF_3 : CH_3OH$. After 2 hours at 75°C, the flask was cooled and 2 ml of hexane was added. An aqueous solution of Na_2SO_4 (2%, w/v) was added to bring the total volume to 10 ml, and the sample was shaken vigorously for 1 minute and then allowed to separate. The (upper) hexane layer was quantitatively analyzed for chloramben by glc (electron capture detector, 10% DC-200 on Gas Chrom Q column), Recoveries were checked with chloramben-spiked samples carried through the workup and analysis, and external standards were used for calibration.

Theoretical Concentration in Diet (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
10,000	41	10,000	4.9%	9,100-11,330
20,000	38	20,105	4.9%	18,200-22,000

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