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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF CHLOROPICRIN
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

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

CONTRIBUTORS: This bioassay of chloropicrin was conducted by Hazleton Laboratories America, Inc., Vienna, Virginia, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (1,2) and Dr. E. K. Weisburger (1). The principal investigators for the contract were Dr. M. B. Powers (3), Dr. R. W. Voelker (3), Dr. W. A. Olson (3,4) and Dr. W. M. Weatherholtz (3). Chemical analysis was performed by Dr. C. L. Guyton (3,5) and the analytical results were reviewed by Dr. N. Zimmerman (6); the technical supervisor of animal treatment and observation was Ms. K. J. Petrovics (3).

Histopathologic examinations were performed by Dr. R. H. Habermann (3) and reviewed by Dr. R. W. Voelker (3) at the Hazleton Laboratories America, Inc., and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology, and summary tables was performed by FG&G Mason Research Institute (8); the statistical analysis was performed by Mr. W. W. Belew (6) and Dr. J. R. Joiner (7), using methods selected for the Bioassay Program by Dr. J. J. Gart (9).

This report was prepared at METREK, a Division of The MITRE Corporation (6) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (6), the task leader, Dr. M. R. Kornreich (6), the senior biologist, Ms. P. Walker (6), and the technical editor, Ms. P. A. Miller (6). The final report was reviewed by members of the participating organizations.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (9), Mr. J. Nam (9), Dr. H. M. Pettigrew (9), and Dr. R. E. Tarone (9).

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. D. G. Goodman (1), Dr. R. A. Griesemer (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. R. A. Squire (1,10), and Dr. J. M. Ward (1).

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SUMMARY

A bioassay of technical-grade chloropicrin for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. Chloropicrin in corn oil was administered 5 days a week by gavage, at either of two dosages, to groups of 50 male and 50 female animals of each species. Time-weighted average dosages of 25 mg/kg/day for low dose male rats and 20 mg/kg/day for low dose female rats were administered during weeks 1 through 33, then administered cyclically (1 dose-free week followed by 4 weeks of administration) from weeks 34 through 78. Time-weighted average dosages of 26 mg/kg/day for high dose male rats and 22 mg/kg/day for high dose female rats were administered from weeks 1 through 17, weeks 31 through 33, and cyclically (1 dose-free week followed by 4 weeks of administration) during weeks 34 through 78. Time-weighted average dosages of 66 and 33 mg/kg/day, respectively, for male and female mice were administered for 78 weeks. These dosing regimens were followed by observation periods of 32 weeks for rats and 13 weeks for mice.

For each species, 20 animals of each sex were placed on test as vehicle controls. These animals were gavaged with corn oil. Twenty animals of each sex were placed on test as untreated controls for each species. These animals were not gavaged.

A high incidence of early death was observed among chloropicrin-dosed rats. Deaths among dosed rats occurred as early as week 1 for females and week 6 for males. Median survival was week 48 for high dose males, week 54 for low dose males, week 70 for high dose females and week 59 for low dose females. Statistical tests indicate a positive association between chloropicrin dosage and mortality of rats.

No neoplasms were observed at higher incidences in dosed than control rats. In rats of both sexes, incidences of adenoma of the pituitary and of adenocarcinoma or fibroadenoma of the mammary gland were higher in control groups than dosed groups. It is likely that most dosed rats did not survive long enough to be at risk from late-appearing tumors.

A rapid decrease in survival after the first year of the study was observed among high dose mice of both sexes. Survival of high dose male mice decreased from 80 percent in week 54 to 26 percent in week 90. Survival of high dose female mice decreased from 82 percent in week 54 to 36 percent in week 90. Statistical tests indicated a positive association between chloropicrin dosage and mortality of mice.

In chloropicrin-dosed mice, proliferative lesions of the squamous epithelium of the forestomach included two carcinomas and a papilloma. Although these tumors were uncommon in control animals, statistical analysis did not demonstrate that they were related to administration of chloropicrin. Other proliferative lesions of the forestomach occurring at an increased incidence in dosed mice were acanthosis and hyperkeratosis. No statistically significant increase of tumor incidence was observed in mice.

The bioassay of chloropicrin using Osborne-Mendel rats did not permit an evaluation of carcinogenicity because of the short survival time of dosed animals. The bioassay of chloropicrin using B6C3F1 mice did not provide conclusive statistical evidence for the carcinogenicity of this compound.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

Chloropicrin (NCI No. C00533) is an agricultural fumigant, once widely used but now being phased out (Feldmesser, 1977). In the late 1960s, scientists at the National Cancer Institute noted that a group of pesticides used extensively in agriculture had not been adequately tested for carcinogenicity. In 1969 the Report of the Secretary's Commission on Pesticides and Their Relationship to Environmental Health (U.S. Department of Health, Education and Welfare, 1969) further emphasized the need for chronic toxicity studies of certain specific pesticides.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for chloropicrin is trichloronitromethane.* It is also called nitrochloroform.

Domestic production of chloropicrin in 1974 was approximately 4.8 million pounds (U.S. International Trade Commission, 1976). It was developed as a tear gas, but was found to be useful as a fumigant in 1918 (Matsumura, 1975). The primary use of chloropicrin as a fumigant was in the treatment of stored grain (Rogers, 1966). It also functions as a nematicide, fungicide, and insecticide when used as a soil fumigant prior to planting. Chloropicrin is lethal to most plants, necessitating the aeration of soil after treatment for one to two weeks before planting (Rogers, 1966). Soil persistence of the

*The CAS registry number is 76-06-2.

chemical ranges from one to three weeks (Pesticide Information Manual, 1966). Because of its odor and lachrymatory properties, chloropicrin has been used increasingly since the 1950s as a warning agent in fumigant formulations, such as 2 percent chloropicrin and 98 percent methyl bromide (Pesticide Chemicals Official Compendium, 1966; Feldmesser, 1977).

II. MATERIALS AND METHODS

A. Chemicals

A single batch of technical-grade chloropicrin was purchased by Hazleton Laboratories America, Inc., Vienna, Virginia, from Morton International, Inc. The purity of the compound was initially determined by Hazleton Laboratories using gas-liquid chromatography (GLC) and infrared spectrophotometry. The GLC total area analysis revealed six peaks; the fourth peak eluted from the column, presumed to be chloropicrin, accounted for 98 percent of the total area, while none of the other individual peaks accounted for more than 1 percent of the total area. This indication of purity in the range of 98 percent was consistent with the supplier's Volhard assay determination of 98 percent chloropicrin. The infrared spectrum of the tested chloropicrin was consistent with that expected from the structure of the compound.

A second purity determination using GLC and infrared spectrophotometry was performed by Hazleton Laboratories 20 months after the initial analysis to establish the stability of the chloropicrin under storage conditions. The GLC total area analysis again showed six peaks. The major peak accounted for approximately 99 percent of the total area, with the other five observed peaks totaling less than 1 percent of the total area. The infrared spectrum was consistent with that of the previous analysis.

Throughout this report the term chloropicrin is used to represent this technical-grade material.

B. Dosage Preparation

Fresh solutions of chloropicrin in Duke's® corn oil (S. F. Sauer Company, Richmond, Virginia) were prepared weekly, sealed, and stored in dark bottles at 1°C. Concentrations of chloropicrin in corn oil of 2 and 5 percent (on a weight/volume basis) were utilized for rats and mice, respectively. When the dose administered to mice was increased during the chronic test, the chloropicrin concentration in corn oil was increased to 7 percent.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Osborne-Mendel rat was selected on the basis of a comparative study of the tumorigenic responsiveness to carbon tetrachloride of five different strains of rats (Reuber and Glover, 1970). The B6C3F1 mouse was selected because it has been used by the NCI for carcinogenesis bioassays and has proved satisfactory in this capacity.

Rats and mice of both sexes were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. The Osborne-Mendel rats were procured from the Battelle Memorial Institute, Columbus, Ohio, and the B6C3F1 mice were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Upon receipt, animals were quarantined for at least 10 days, observed for visible signs of disease or parasites, and assigned to the various dosed and control groups.

D. Animal Maintenance

All animals were housed by species in temperature- and humidity-controlled rooms. The temperature range was 20° to 24°C and the relative humidity was maintained between 45 and 55 percent. The air conditioning system in the laboratory provided filtered air at a rate of 12 complete changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle. The rats were individually housed in suspended galvanized-steel wire-mesh cages with perforated floors. Mice were housed by sex in groups of ten in solid-bottom polypropylene cages equipped with filter tops. Sanitized cages with fresh bedding (Sanichips®, Pinewood Sawdust Company, Moonachie, New Jersey) were provided once each week for mice. Rats received sanitized cages with no bedding with the same frequency. Food hoppers were changed and heat-sterilized once a week for the first 10 weeks and once a month thereafter. Fresh heat-sterilized glass water bottles were provided three times a week. Food (Wayne Lab-Blox® meal, Allied Mills, Inc., Chicago, Illinois) and water were available ad libitum.

The rats dosed with chloropicrin and the vehicle controls were housed in the same room with other rats intubated with* methylchloroform (71-55-6) and trichlorofluoromethane (75-69-4). The untreated control rats were housed with other rats intubated with 1,1,2,2-tetrachloroethane (79-34-5), allyl chloride (107-05-1), 1,2-dibromoethane

*CAS registry numbers are given in parentheses.

(106-93-4), carbon tetrachloride (56-23-5), and chloroform (67-66-3). All mice used in the chloropicrin study, including controls, were housed in the same room as other mice intubated with 1,1,2,2-tetrachloroethane (79-34-5), chloroform (67-66-3), dibromochloropropane (96-12-8), allyl chloride (107-05-1), 1,2-dibromoethane (106-93-4), 1,2-dichloroethane (107-06-2), 1,1-dichloroethane (75-34-3), trichloroethylene (79-01-6), 3-sulfolene (77-79-2), iodoform (75-47-8), methylchloroform (71-55-6), 1,1,2-trichloroethane (79-00-5), tetrachloroethylene (127-18-4), hexachloroethane (67-72-1), carbon disulfide (75-15-0), trichlorofluoromethane (75-69-4), and carbon tetrachloride (56-23-5).

E. Gastric Intubation

Intubation was performed for five consecutive days per week on a mg/kg body weight basis utilizing the most recently observed group mean body weight as a guide for determining the dose. Mean body weights for each group were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. All animals of one sex within a dosed group received the same dosage. Gavage of dosed animals was performed under a hood to minimize extraneous exposure of other animals and laboratory personnel to the chemical.

F. Selection of Initial Dose Levels

In order to establish the maximum tolerated doses of chloropicrin for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice.

Animals of each species were distributed among six groups, each consisting of five males and five females. Chloropicrin in corn oil was introduced by gavage to five of the six rat groups at dosages of 16, 25, 40, 63, and 100 mg/kg/day and five of the six mouse groups at dosages of 10, 16, 25, 40, and 63 mg/kg/day. The sixth group of each species served as a control group, receiving only corn oil. Intubation occurred 5 days per week for 6 weeks, followed by a 2-week observation period to detect any delayed toxicity.

None of the male rats died at chloropicrin dosages of 40 mg/kg/day or less. Mean body weight depression in the males at 40 and 63 mg/kg/day was 11 and 38 percent, respectively. Except for one female rat treated with 25 mg/kg/day, all females receiving 40 mg/kg/day or less survived the 8-week study. Mean body weight depression at 40 and 63 mg/kg/day for female rats was 17 and 30 percent, respectively. The initial high dosage for male and female rats in the chronic study was set at 46 mg/kg/day.

No male or female mice died during the 8-week study. At levels of 40 and 63 mg/kg/day, mean body weight depression in males was 12 and 20 percent, respectively, and in females was 3 and 6 percent, respectively. The initial high dosage for mice of both sexes in the chronic study was set at 50 mg/kg/day.

G. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, dosages administered, duration of treated and

untreated observation periods, and the time-weighted average dosages) are summarized in Tables 1 and 2.

The treated and vehicle control rats were all approximately 6 weeks old at the time the experiment began. The untreated rats were placed on the study when they were approximately 6 weeks old, but 11 weeks after the initial gavage regimen was begun for dosed and vehicle control rats. Gavage was performed five consecutive days per week. The doses initially utilized for both males and females were 46 and 23 mg/kg/day, respectively. Throughout this report the groups receiving the former dosage are referred to as the high dose groups, while those receiving the latter dosage are referred to as the low dose groups. When the rats were 11 weeks old (week 5 of the experiment), the high and low doses for males were increased to 56 and 28 mg/kg/day, respectively, as the males appeared to be tolerating the chemical, while the dosages for the female treated groups remained unchanged. After week 17, intubation ceased for 13 weeks for the high dose males and females because of apparent compound toxicity. Gavage continued for the low dose groups for both sexes. Effective week 31, the high dose males and females began receiving the same dose as the respective low dose groups. In week 34, intubation ceased for 1 week for all treated groups, followed by 4 weeks of dose administration. This cyclic pattern of dose administration continued for the remainder of the 78-week study period. The vehicle control rats were gavaged with corn oil for 78 weeks. Observation of all rats continued for an additional 32 weeks.

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
CHLOROPICRIN GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	CHLORO-PICRIN DOSAGE[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE DOSAGE OVER A 78-WEEK PERIOD[b]
MALE					
UNTREATED CONTROL	20	0		109	0
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	23	4		25
		28	29		
		28[c]	36	9	
		0		32	
HIGH DOSE	50	46	4		26
		56	13		
		0		13	
		28	3		
		28[c]	36	9	
		0		32	
FEMALE					
UNTREATED CONTROL	20	0		109	0
VEHICLE CONTROL	20	0	78	32	0
LOW DOSE	50	23	33		20
		23[c]	36	9	
		0		32	
HIGH DOSE	50	46	17		22
		0		13	
		23	3		
		23[c]	36	9	
		0		32	

[a] Dosage, given in mg/kg body weight, was administered by gavage five consecutive days per week.

[b] Time-weighted average dosage = $\frac{\Sigma(\text{dosage X weeks received})}{78 \text{ weeks}}$

[c] These dosages were cyclically administered with a pattern of 1 dose-free week followed by 4 weeks (5 days per week) of dosage at the level indicated.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
CHLOROPICRIN GAVAGE EXPERIMENT

	INITIAL GROUP SIZE	CHLOROPICRIN DOSAGE[a]	OBSERVATION PERIOD		TIME-WEIGHTED AVERAGE DOSAGE OVER A 78-WEEK PERIOD[b]
			TREATED (WEEKS)	UNTREATED (WEEKS)	
MALE					
UNTREATED CONTROL	20	0		90	0
VEHICLE CONTROL	20	0	78	13	0
LOW DOSE	50	25	13		33
		35	65		
		0		13	
HIGH DOSE	50	50	13		66
		70	65		
		0		30	
FEMALE					
UNTREATED CONTROL	20	0		90	0
VEHICLE CONTROL	20	0	78	13	0
LOW DOSE	50	25	13		33
		35	65		
		0		13	
HIGH DOSE	50	50	13		66
		70	65		
		0		13	

[a] Dosages, given in mg/kg body weight, were administered by gavage five consecutive days per week.

[b] Time-weighted average dosage = $\frac{\Sigma(\text{dosage X weeks received})}{78 \text{ weeks}}$

The vehicle control and treated mice were all approximately 5 weeks old on the day their first dose was administered. The vehicle control mice were born approximately 2 weeks earlier than the low and high dose groups. Therefore, administration of corn oil to the vehicle controls began correspondingly earlier than did chloropicrin administration to the dosed mice. The dosed mice received initial dosages of 50 and 25 mg/kg/day, respectively. Throughout this report the groups receiving the former dosage are referred to as the high dose groups, while those receiving the latter are referred to as the low dose groups. In week 14 the high and low dosages were increased to 70 and 35 mg/kg/day, respectively. The entire period of chemical administration for mice was 78 weeks and this was followed by a 13-week observation period. The vehicle control mice received corn oil by gavage for 78 weeks. The untreated controls, having the same median birth date as the low and high dose chloropicrin groups, were included in the test for the same period of time as the dosed groups.

H. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected daily for mortality. Body weights, food consumption, and data concerning appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses were recorded at weekly intervals for the first 10 weeks and at monthly intervals thereafter. The presence of

tissue masses was determined by observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by exsanguination under sodium pentobarbital anesthesia, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Slides were prepared from the following tissues: subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

Tissues for which slides were prepared were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to

preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

I. 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 used Tarone's (1975) extensions of Cox's methods for testing 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 was 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, pp. 48-52) was used to compare the tumor incidence of a control group to 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, pp. 6-10) 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, pp. 362-365), was also used. Under the assumption of a linear trend, this test determined 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 was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an

observed tumor were computed as in Saffiotti et al. (1972). The week during which 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 dosed 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 percent of a large number of identical experiments, the true ratio of the

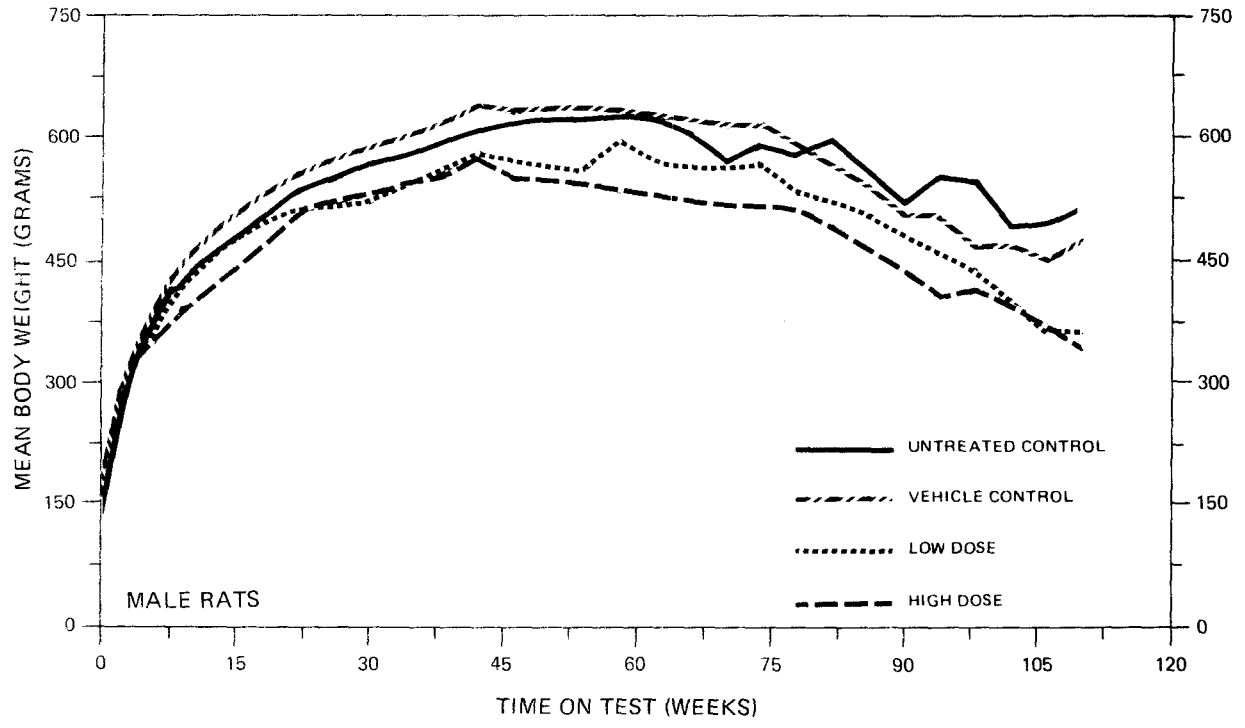
risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence 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. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Mean body weight depression became apparent earlier for male rats than for female rats. Dosed rats continued to gain less weight than control rats for the duration of the bioassay (Figure 1).

Clinical signs were observed in the chloropicrin-dosed groups early in the study. Beginning in week 3, a few rats in all dosed groups started to show the following adverse clinical signs: a hunched or thin appearance, squinted or reddened eyes (often with a brown crust or discharge), reddened ears (through week 5 of the study), and occasional abdominal urine stains. These signs were observed with greater frequency in the dosed groups than in the vehicle controls from week 3 through week 50, but occurred at essentially comparable rates in dosed and vehicle control rats during the second year. Respiratory signs, characterized by wheezing, nasal discharge, and/or labored respiration, were observed at a low to moderate incidence in all dosed and control groups during the last 10 months of the study. Clinical signs associated with aging in the laboratory rat were observed at a similar rate in vehicle control and dosed rats during the second year of the study. These signs included localized alopecia, sores on the tail and other parts of the body, fur discoloration, and palpable nodules or tissue masses.



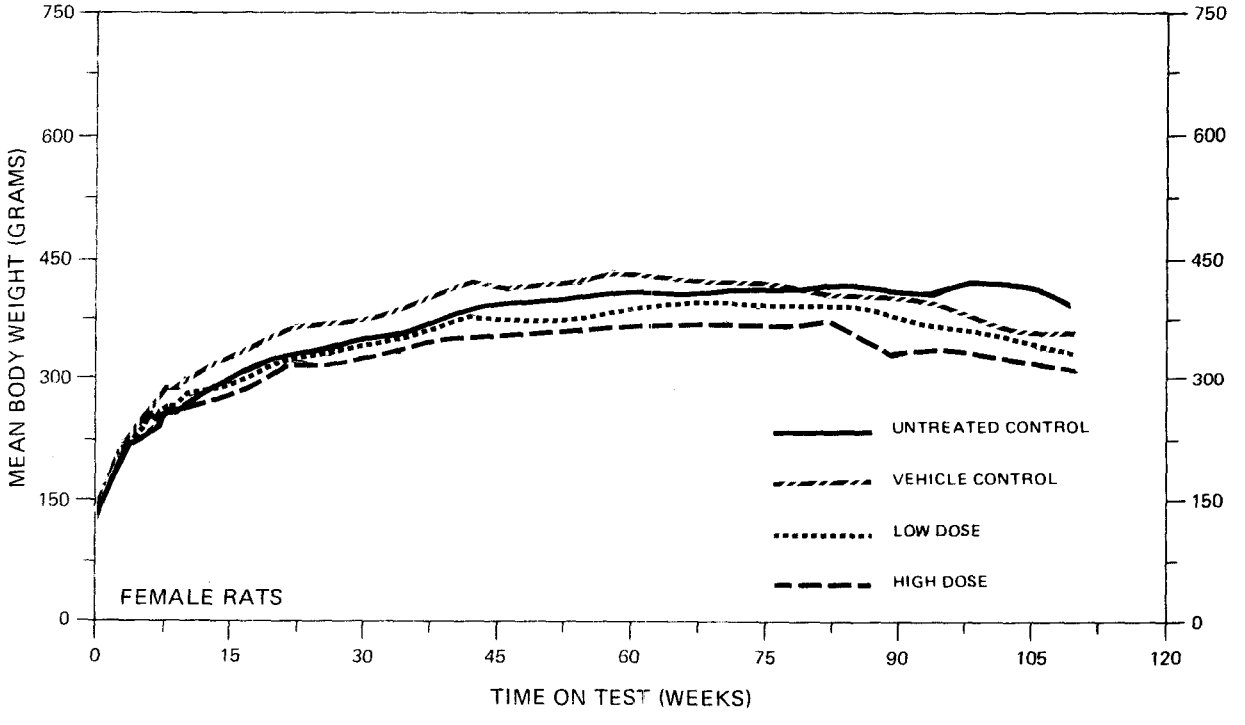


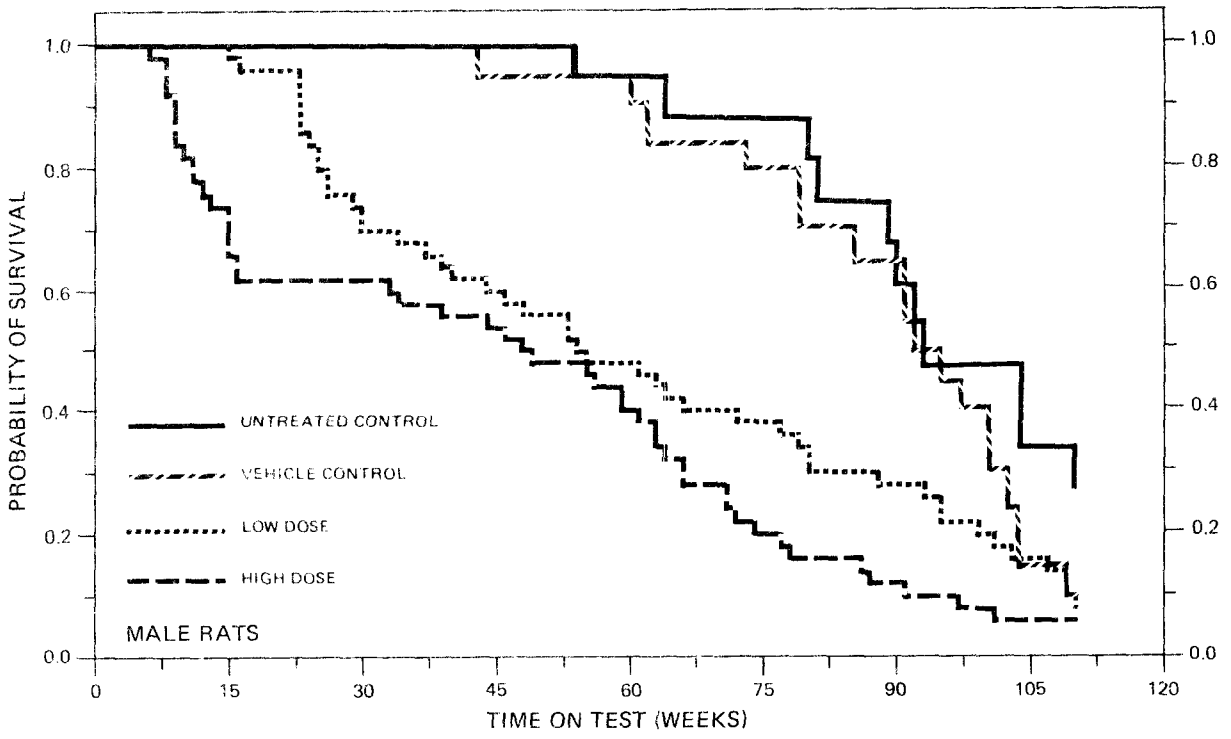
FIGURE 1
GROWTH CURVES FOR CHLOROPICRIN CHRONIC STUDY RATS

B. Survival

The estimated probabilities of survival for male and female rats in the control and chloropicrin-treated groups are shown in Figure 2. High mortality was observed in dosed rats of both sexes.

For male rats the Tarone tests indicated a significant ($P < 0.001$) association between increased dosage and accelerated mortality when comparing to the vehicle control. By week 16, 38 percent (19/50) of the high dose male rats were dead. Fifty percent of the high dose male rats had died by week 48 and 50 percent of the low dose male rats by week 54. Survival to the end of the study was 6 percent (3/50) for high dose male rats and 8 percent (4/50) for low dose male rats. For both vehicle and untreated control groups at least 50 percent of the animals survived past week 89, despite the sacrifice of five males from each in week 61.

Female rats also experienced accelerated, dose-related mortality, as indicated by the significance ($P < 0.001$) of the Tarone test when comparing to the vehicle control. Fatalities among treated female rats were observed as early as week 2. Fifty percent of the female rats were dead by week 70 in the high dose group and by week 59 in the low dose group. Survival at the end of the study was 20 percent (10/50) for high dose females and 22 percent (11/50) for low dose females. For both vehicle and untreated control groups, at least 50 percent of the animals survived over 108 weeks, despite the the sacrifice of five rats from the untreated control in week 61.



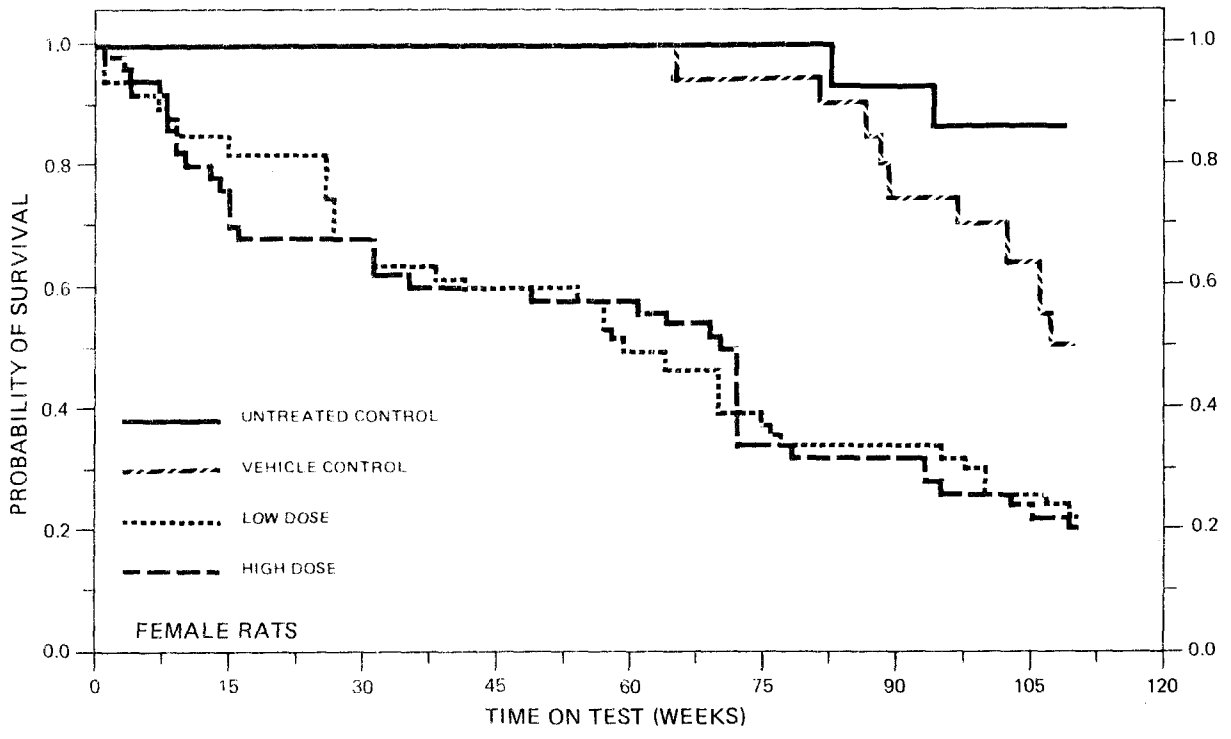


FIGURE 2
SURVIVAL COMPARISONS OF CHLOROPICRIN CHRONIC STUDY RATS

C. Pathology

Histopathologic findings on neoplasms in rats are tabulated in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are tabulated in Appendix C (Tables C1 and C2).

A variety of neoplasms were represented among both dosed and control rats. Each of the types of tumors represented has been encountered previously as a spontaneous lesion in the Osborne-Mendel rat. No appreciable difference in the incidence of neoplasms was noted in the control and treated rats in this study.

Inflammatory, degenerative, and proliferative lesions seen in control and treated animals were similar in number and kind to those naturally occurring lesions found in aged rats.

This study provided no histopathologic evidence for the carcinogenicity of chloropicrin in rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis for every type of tumor that was observed in more than 5 percent of any of the chloropicrin-dosed groups of either sex is included. The untreated controls were not used in these analyses since the comparison to the corn oil-gavaged vehicle control was the comparison of choice. Because of the differences between dosing regimens for the low and high dose groups, the Cochran-Armitage test for dose-response trends was not used in these analyses.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE RATS TREATED WITH CHLOROPICRIN[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	2/20(0.10)	1/20(0.05)	1/50(0.02)	0/49(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.400	0.000
Lower Limit	---	---	0.005	0.000
Upper Limit	---	---	30.802	7.624
Weeks to First Observed Tumor	90	97	110	---
Mammary Gland: Adenocarcinoma or Fibroadenoma[b]	2/20(0.10)	2/20(0.10)	0/50(0.00)	0/50(0.00)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.000	0.000
Lower Limit	---	---	0.000	0.000
Upper Limit	---	---	1.345	1.345
Weeks to First Observed Tumor	90	61	---	---

[a]Treated groups received time-weighted average doses of 25 or 26 mg/kg by gavage.

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

[c]The probability level for the Fisher exact test for the comparison of a treated group with the vehicle control group is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN FEMALE RATS TREATED WITH CHLOROPICRIN[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Adenoma[b]	6/19(0.32)	3/20(0.15)	4/48(0.08)	3/50(0.06)
P Values[c]	---	---	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.556	0.400
Lower Limit	---	---	0.106	0.060
Upper Limit	---	---	3.546	2.801
Weeks to First Observed Tumor	109	89	76	72
Mammary Gland: Adenocarcinoma or Fibroadenoma[b]	4/20(0.20)	7/20(0.35)	7/50(0.14)	6/50(0.12)
P Values	---	---	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.400	0.343
Lower Limit	---	---	0.144	0.114
Upper Limit	---	---	1.187	1.061
Weeks to First Observed Tumor	60	66	95	78

[a]Treated groups received time-weighted average doses of 20 or 22 mg/kg by gavage.

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

[c]The probability level for the Fisher exact test for the comparison of a treated group with the vehicle control group is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. A negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

For both male and female rats none of the Fisher exact tests indicated an association between chemical administration and incidence for any type of tumor. It must be noted, however, that the extreme early mortality observed in dosed animals of both sexes severely restricted the usefulness of these tests for the analysis of tumors.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by chloropicrin that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Progressive mean body weight depression was apparent for female mice treated with chloropicrin. The weight gain patterns for the male mice did not show a consistent weight differential related to chemical administration until week 50 (Figure 3).

During the first 6 months of the study the mice in all dosed groups displayed patterns of appearance and behavior that were comparable with those of the controls. Clinical signs often observed in laboratory mice were observed with similar frequency among the dosed and control groups. These signs included sores on parts of the body; localized alopecia; penile, anal, or vulvar irritation with occasional prolapse of these organs; a hunched posture; a bloated appearance; abdominal urine stains; eyes showing cloudiness, redness, and/or discharge; and palpable nodules and/or swollen areas on the body or legs. From week 26 to cessation of chemical administration in week 78, observations of a hunched or bloated appearance were noted at a slightly greater frequency in the dosed groups than in the controls. During the last 12 weeks of the study the incidence of these signs increased at a comparable rate in the surviving treated and control animals.

B. Survival

The estimated probabilities of survival for male and female mice in the control and chloropicrin-treated groups are shown in Figure 4.

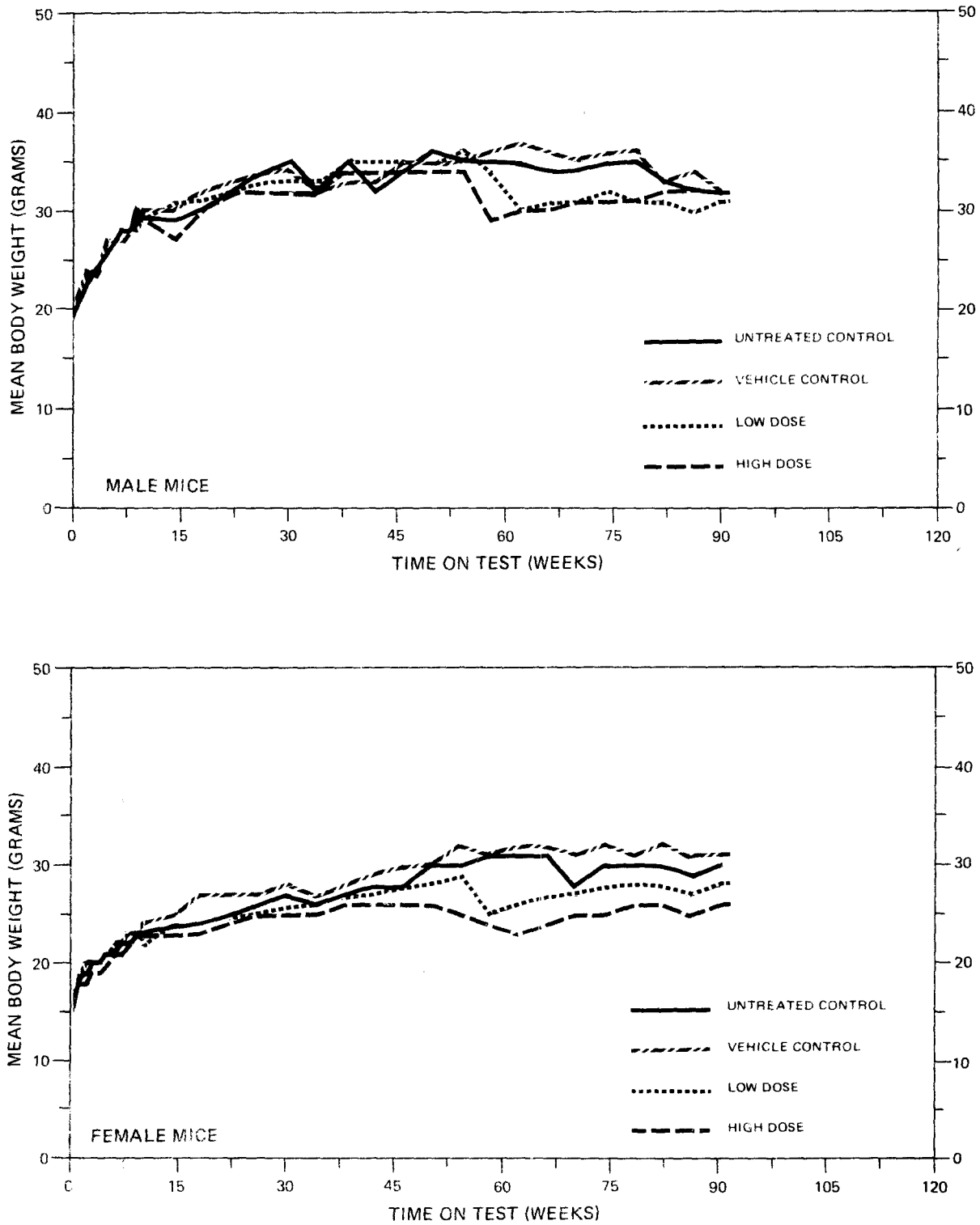
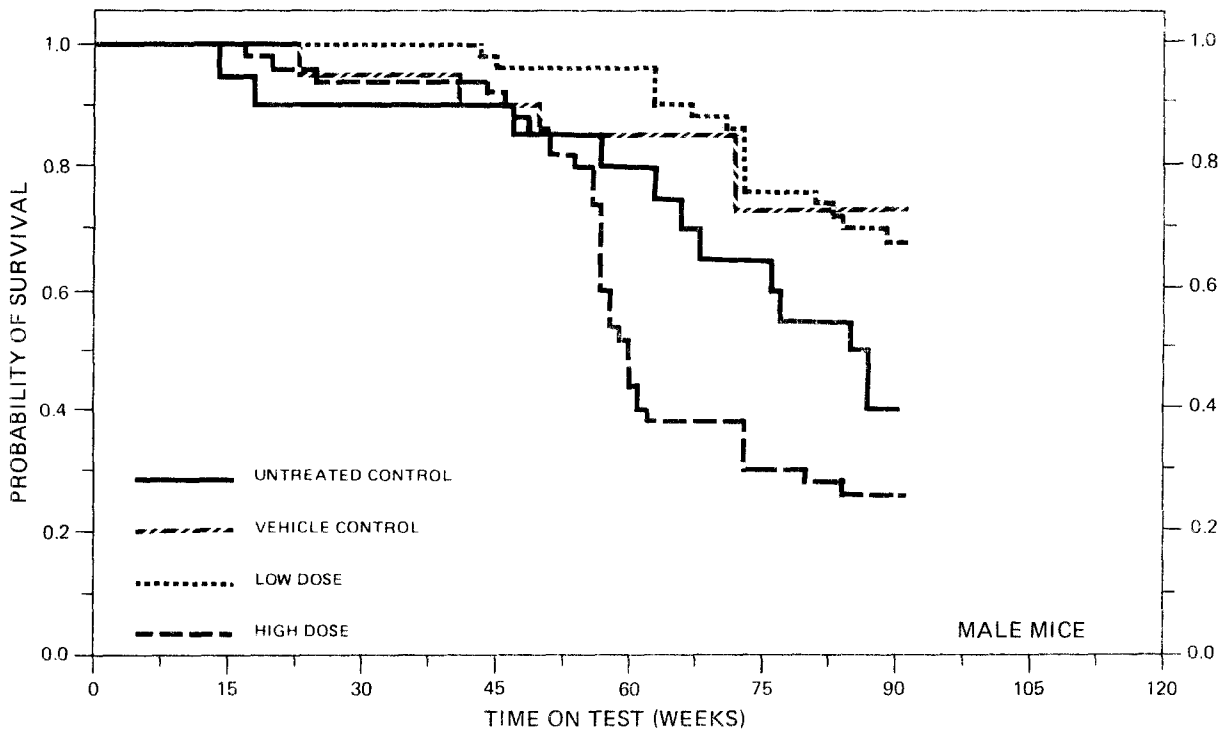


FIGURE 3
GROWTH CURVES FOR CHLOROPICRIN CHRONIC STUDY MICE



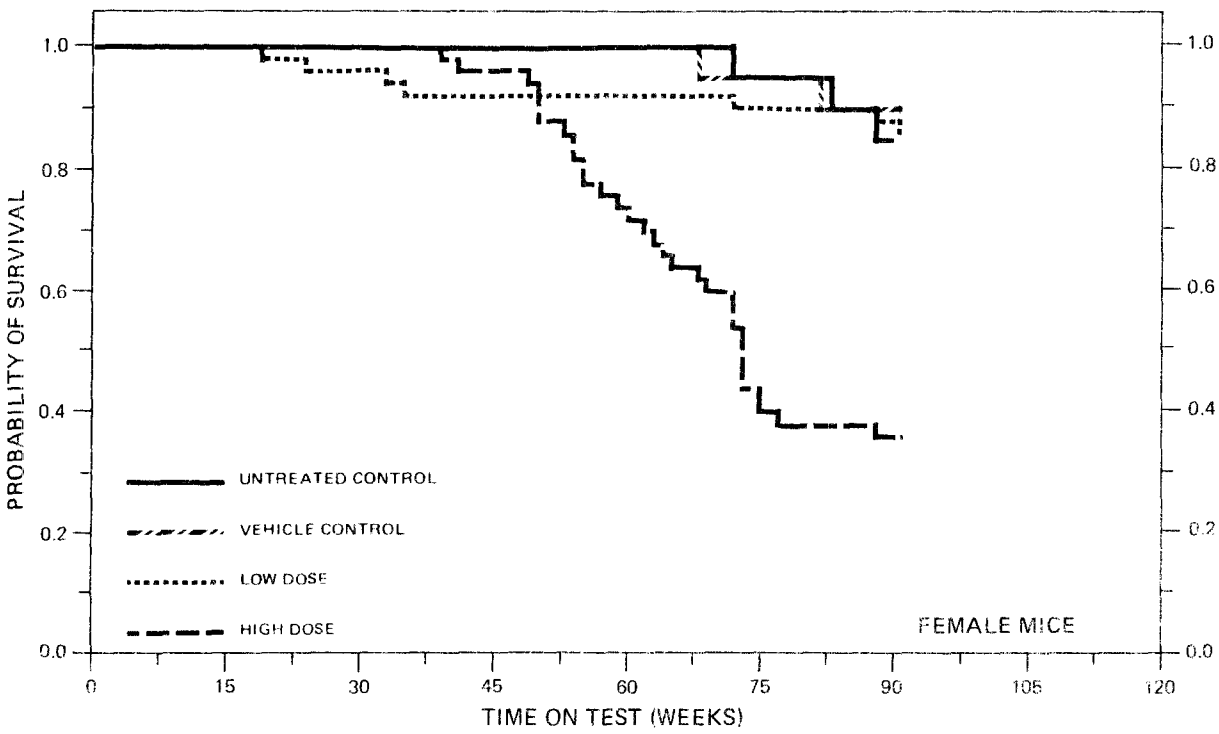


FIGURE 4
SURVIVAL COMPARISONS OF CHLOROPICRIN CHRONIC STUDY MICE

In male mice the Tarone test for association between increased dosage and accelerated mortality was significant when comparing to the vehicle control. The departure from linear trend was significant since the mortality in the high dose and untreated control groups was greater than in the low dose and vehicle control groups. In the high dose group, where 13 mice died between weeks 56 and 58, there was no indication that these deaths were from a common cause. Fifty percent of the male mice survived over 60, 91, and 85 weeks for the high dose, low dose, and untreated control groups, respectively. The usefulness of the vehicle control group was greatly restricted due to the sacrifice of 10 male mice in week 56.

In female mice the Tarone test for association between increased dosage and accelerated mortality was significant when comparing to the vehicle control. The departure from linear trend was also significant, since the accelerated mortality was primarily in the high dose group starting in approximately week 55. Fifty percent of the female mice survived over 73, 91, 91, and 90 weeks in the high dose, low dose, vehicle control, and untreated control groups, respectively.

There was no evidence that early death was associated with tumor incidence.

C. Pathology

Histopathologic findings on neoplasms in mice are tabulated in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are tabulated in Appendix D (Tables D1 and D2).

Squamous-cell carcinomas of the stomach occurred in two high dose male mice. Within these tumors were both well-differentiated and anaplastic areas. Microscopic invasion through the stomach wall was evident. Both of these tumors metastasized to the pancreas and liver, and one also metastasized to the spleen and a mesenteric lymph node. A squamous-cell papilloma of the stomach occurred in one low dose female mouse.

In addition to the tumor-bearing animals, acanthosis and hyperkeratosis of the forestomach occurred in 3/46 low dose males, 1/48 high dose males, 10/48 low dose females, and 9/48 high dose females. These lesions were not observed in any of the control mice.

Other proliferative, inflammatory, and degenerative lesions appeared consistent with lesions that occur spontaneously in this mouse strain, and were observed with similar frequency in the control and treated mice.

Proliferative lesions of the stomach were observed, including two carcinomas and a papilloma. These lesions are uncommon in control animals and are considered to be related to the administration of chloropicrin.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis for every type of tumor that was observed in more than 5 percent of any of the chloropicrin-dosed groups of either sex is included. The untreated

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT SPECIFIC SITES IN MALE MICE TREATED WITH CHLOROPICRIN[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	0/15(0.00)	3/20(0.15)	1/46(0.02)	4/48(0.10)
P Values[c]	---	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	---	P = 0.049	---	---
Relative Risk (Vehicle Control)[d]	---	---	0.145	0.556
Lower Limit	---	---	0.003	0.106
Upper Limit	---	---	1.700	3.546
Weeks to First Observed Tumor	---	56	91	57
Liver: Hepatocellular Carcinoma[b]	1/15(0.07)	2/20(0.10)	4/46(0.09)	2/48(0.04)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	0.870	0.417
Lower Limit	---	---	0.139	0.033
Upper Limit	---	---	9.144	5.490
Weeks to First Observed Tumor	90	56	73	91

[a]Treated groups received time-weighted average doses of 33 or 66 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the vehicle control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the vehicle control group is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH CHLOROPICRIN[a]

TOPOGRAPHY:MORPHOLOGY	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	1/19(0.05)	3/49(0.06)	6/48(0.13)
P Values[c]	---	N.S.	N.S.	N.S.
Relative Risk (Vehicle Control)[d]	---	---	1.163	2.375
Lower Limit	---	---	0.104	0.325
Upper Limit	---	---	59.809	106.788
Weeks to First Observed Tumor	90	91	91	73

[a]Treated groups received time-weighted average doses of 33 or 66 mg/kg by gavage.

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

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the vehicle control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the vehicle control group is given beneath the incidence of tumors in that treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

controls were not used in these analyses since the comparison to the corn oil-gavaged vehicle controls was the comparison of choice.

None of these statistical tests indicated an association between chemical administration and tumor incidence for either male or female mice. Additional analyses were performed based exclusively on mice which survived at least 52 weeks, but none of the additional time-adjusted statistical tests were significant.

In males an unusual tumor type--squamous-cell carcinoma of the forestomach--was observed in 2/48 high dose mice but in none of the other male mice. In historical data collected by Hazleton Laboratories for the NCI Bioassay Program 1/180 vehicle control male B6C3F1 mice had either a squamous-cell papilloma or a squamous-cell carcinoma of the stomach. Under the assumption of a binomial distribution, with a probability of spontaneous incidence of 1/180, the probability that 2 or more of 48 mice would have that tumor was $P = 0.029$. This marginal result was not significant when the Bonferroni criterion was applied.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In all of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that all of the confidence intervals have an upper limit greater than one,

indicating the theoretical possibility of tumor induction in mice by chloropicrin that could not be established under the conditions of this test.

V. DISCUSSION

Because of elevated mortality, it is unlikely that an adequate number of rats lived long enough to be at risk from late-appearing tumors. No statistically significant increase in tumor incidence was observed among mice dosed with chloropicrin, but a few unusual neoplasms were noted.

In mice, proliferative lesions of the squamous epithelium of the forestomach were observed in dosed animals but not in controls. In addition to hyperplasias (acanthosis and hyperkeratosis), two carcinomas were observed in male mice and a papilloma was observed in a female mouse. Both carcinomas metastasized to the pancreas and liver and one carcinoma also metastasized to a mesenteric lymph node. For a binomial distribution with a probability of spontaneous incidence of these neoplasms of 1/180, the probability that 2 or more of 48 mice would have one of these neoplasms was $P = 0.029$; this marginal result was not significant under the Bonferroni criterion.

The method of chloropicrin administration might have played a role in inducing these neoplasms. Chloropicrin is a mucous membrane irritant (Pesticide Information Manual, 1968) and proliferative lesions of the forestomach have been noted in other bioassays where chemical irritants and carcinogens have been administered by gavage.

A variety of neoplasms were observed in rats, but no significant differences in the incidence of these tumors were detected between the dosed and the control groups. However, because of high early

mortality, the number of animals that survived long enough to be at risk from late-appearing tumors does not provide enough data for the results to be considered as conclusive.

The bioassay of chloropicrin using Osborne-Mendel rats did not permit an evaluation of carcinogenicity because of the short survival time of dosed animals. The bioassay of chloropicrin using B6C3F1 mice did not provide conclusive statistical evidence for the carcinogenicity of this compound.

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

January 18, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biostatistics, biochemistry, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Chloropicrin was reviewed.

The primary reviewer said that the bioassay was inadequate to allow a conclusion to be reached with respect to the carcinogenicity of Chloropicrin in treated rats. In the mice, there was no statistical evidence to conclude a carcinogenic effect produced by the chemical. However, the primary reviewer expressed concern that the study may represent a "false negative" based on the known biological activity of Chloropicrin and the fact that it is fairly persistent. He recommended that Chloropicrin be retested.

Due to its extreme toxicity, a Subgroup member said that it is not used widely by itself. It has been employed as a warning agent because of its lacrimatory properties.

It was moved that the report be accepted as written but that Chloropicrin be referred to the Chemical Selection Working Group for possible retest. The motion was seconded and approved by all the Subgroup members except Dr. Rowe, who abstained.

Members Present Were:

Arnold Brown (Acting Chairman), Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph Highland, Environmental Defense Fund
Charles Kensler, Arthur D. Little Company
Verald Rowe, Dow Chemical, U.S.A.
Sheldon Samuels, Industrial Union Department, AFL-CIO
Louise Strong, University of Texas Health Sciences Center
Sidney Wolfe, Health Research Group

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

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH CHLOROPICRIN

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROSARCOMA	1 (5%)			
LIPOSARCOMA		1 (5%)		
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(20)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (5%)			
#SPLEEN	(20)	(20)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#SALIVARY GLAND	(14)	(16)	(20)	(11)
CARCINOMA,NOS	1 (7%)			
#SMALL INTESTINE	(20)	(20)	(50)	(49)
FIBROSARCOMA	1 (5%)			
URINARY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(49)
CHROMOPHOBE ADENOMA	2 (10%)	1 (5%)	1 (2%)	
#ADRENAL	(20)	(20)	(49)	(49)
CORTICAL CARCINOMA	2 (10%)			
#THYROID	(19)	(20)	(49)	(47)
FOLLICULAR-CELL ADENOMA			1 (2%)	
FOLLICULAR-CELL CARCINOMA	1 (5%)	1 (5%)		
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)	2 (10%)		
FIBROADENOMA	1 (5%)			
NERVOUS SYSTEM				
NONE				
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 A1 (CONCLUDED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	11	18	46	47
MORIBUND SACRIFICE				
SCHEDULED SACRIFICE	5			
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	4	2	4	3
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	5	3	
TOTAL PRIMARY TUMORS	11	5	3	
TOTAL ANIMALS WITH BENIGN TUMORS	2	1	2	
TOTAL BENIGN TUMORS	3	1	2	
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	4	1	
TOTAL MALIGNANT TUMORS	8	4	1	
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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
FIBROMA				2 (4%)
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
NONE				
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(49)	(50)
MIXED TUMOR, METASTATIC	1 (5%)			
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(49)	(50)
HEPATOCELLULAR CARCINOMA	1 (5%)			
CORTICAL CARCINOMA, METASTATIC			1 (2%)	
#PANCREAS	(20)	(20)	(50)	(50)
LIPOMA			1 (2%)	
MESOTHELIOMA, METASTATIC			1 (2%)	
#SMALL INTESTINE	(20)	(20)	(50)	(49)
LEIOMYOSARCOMA			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(49)	(50)
CORTICAL CARCINOMA, METASTATIC			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
MIXED TUMOR, MALIGNANT	1 (5%)			
HAMARTOMA +	2 (10%)	1 (5%)		
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(20)	(48)	(50)
CHROMOPHOBE ADENOMA	6 (32%)	3 (15%)	4 (8%)	3 (6%)
#ADRENAL	(20)	(20)	(49)	(50)
CORTICAL CARCINOMA			1 (2%)	
#THYROID	(20)	(20)	(49)	(46)
FOLLICULAR-CELL CARCINOMA			1 (2%)	
C-CELL ADENOMA	2 (10%)			1 (2%)
C-CELL CARCINOMA	2 (10%)			
#PANCREATIC ISLETS	(20)	(20)	(50)	(50)
ISLET-CELL ADENOMA	1 (5%)			
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(20)	(20)	(50)	(50)
ADENOCARCINOMA, NOS	2 (10%)	1 (5%)		1 (2%)
FIBROADENOMA	2 (10%)	7 (35%)	7 (14%)	5 (10%)
#UTERUS	(20)	(20)	(49)	(50)
ADENOCARCINOMA, NOS			1 (2%)	
ENDOMETRIAL STROMAL POLYP		1 (5%)		
HEMANGIOMA	1 (5%)			
#UTERUS/ENDOMETRIUM	(20)	(20)	(49)	(50)
CARCINOMA, NOS				1 (2%)
#OVARY/OVIDUCT	(20)	(20)	(49)	(50)
MESOTHELIOMA, METASTATIC			1 (2%)	
#OVARY	(20)	(19)	(49)	(50)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)	
CYSTADENOCARCINOMA, NOS	1 (5%)			
GRANULOSA-CELL TUMOR		1 (5%)		
MESOTHELIOMA, METASTATIC			1 (2%)	
NERVOUS SYSTEM				
NONE				

\# 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 PROLIFERATIVE LIPOCYTES, TUBULAR STRUCTURES, FIBROBLASTS, AND VASCULAR SPACES IN VARYING PROPORTIONS.

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(20)	(20)	(50)	(50)
MESOTHELIOMA, MALIGNANT			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	2	9	39	40
MORIBUND SACRIFICE		1		
SCHEDULED SACRIFICE	5			
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	13	10	11	10
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	11	14	9
TOTAL PRIMARY TUMORS	21	14	17	13
TOTAL ANIMALS WITH BENIGN TUMORS	12	11	10	9
TOTAL BENIGN TUMORS	14	12	12	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	1	5	2
TOTAL MALIGNANT TUMORS	7	1	5	2
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		3	
TOTAL SECONDARY TUMORS	1		6	
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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH CHLOROPICRIN

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS MISSING			1	
ANIMALS NECROPSIED	18	20	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	15	20	46	47
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(20)	(46)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (15%)	1 (2%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA				1 (2%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(18)	(20)	(46)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (5%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)	
#SPLEEN	(15)	(20)	(46)	(46)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
#MESENTERIC L. NODE	(15)	(19)	(46)	(47)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(20)	(46)	(48)
SQUAMOUS CELL CARCINOMA, METASTA				2 (4%)
HEPATOCELLULAR CARCINOMA	1 (7%)	2 (10%)	4 (9%)	2 (4%)
#PANCREAS	(14)	(20)	(46)	(46)
SQUAMOUS CELL CARCINOMA, METASTA				2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
#STOMACH	(15)	(20)	(46)	(48)
SQUAMOUS CELL CARCINOMA				2 (4%)
URINARY SYSTEM				
#KIDNEY	(15)	(20)	(46)	(48)
SQUAMOUS CELL CARCINOMA, METASTA				1 (2%)
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
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 B1 (CONCLUDED)

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	12	4	16	37
MORIBUND SACRIFICE				
SCHEDULED SACRIFICE		10		
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	6	33	13
ANIMAL MISSING			1	
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	5	7	9
TOTAL PRIMARY TUMORS	1	6	7	10
TOTAL ANIMALS WITH BENIGN TUMORS		3	1	4
TOTAL BENIGN TUMORS		3	1	4
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	3	6	5
TOTAL MALIGNANT TUMORS	1	3	6	6
TOTAL ANIMALS WITH SECONDARY TUMORS#				2
TOTAL SECONDARY TUMORS				7
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	19	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	19	49	48
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(20)	(19)	(50)	(48)
SQUAMOUS CELL CARCINOMA				1 (2%)
LEIOMYOSARCOMA	1 (5%)			
RESPIRATORY SYSTEM				
#LUNG	(20)	(19)	(49)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	1 (5%)	3 (6%)	6 (13%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(20)	(19)	(50)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		1 (2%)	2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	1 (5%)	3 (6%)	
#SPLEEN	(20)	(19)	(49)	(48)
LEIOMYOSARCOMA, METASTATIC	1 (5%)			
#MESENTERIC L. NODE	(20)	(19)	(46)	(44)
LEIOMYOSARCOMA, METASTATIC	1 (5%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(20)	(19)	(49)	(48)
HEPATOCELLULAR CARCINOMA	1 (5%)			
#STOMACH	(20)	(19)	(48)	(48)
SQUAMOUS CELL PAPILLOMA			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#PITUITARY	(17)	(18)	(44)	(32)
CHROMOPHOBE ADENOMA	1 (6%)			
#THYROID	(16)	(16)	(44)	(36)
FOLLICULAR-CELL ADENOMA				1 (3%)
REPRODUCTIVE SYSTEM				
#UTERUS	(20)	(19)	(49)	(47)
ENDOMETRIAL STROMAL POLYP			1 (2%)	
#OVARY	(20)	(19)	(49)	(47)
CYSTADENOMA, NOS		1 (5%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(20)	(19)	(50)	(48)
ADENOMA, NOS		1 (5%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

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

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	20	20	50	50
NATURAL DEATH@	3	2	7	32
MORIBUND SACRIFICE				
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED	1		1	
TERMINAL SACRIFICE	16	18	42	18
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	7	4	9	10
TOTAL PRIMARY TUMORS	7	4	9	10
TOTAL ANIMALS WITH BENIGN TUMORS	2	3	5	7
TOTAL BENIGN TUMORS	2	3	5	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	1	4	3
TOTAL MALIGNANT TUMORS	5	1	4	3
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			
TOTAL SECONDARY TUMORS	2			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH CHLOROPICRIN

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		3 (6%)	1 (2%)
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)	1 (2%)
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	16 (80%)	18 (90%)	36 (72%)	29 (58%)
CALCIUM DEPOSIT	1 (5%)			
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(50)	(49)
HEMATOPOIESIS	1 (5%)	2 (10%)	1 (2%)	1 (2%)
#CERVICAL LYMPH NODE	(19)	(20)	(50)	(47)
INFLAMMATION, NOS	1 (5%)	1 (5%)		
#MESENTERIC L. NODE	(19)	(20)	(50)	(47)
INFLAMMATION, NOS		2 (10%)		
CIRCULATORY SYSTEM				
#HEART	(20)	(20)	(50)	(50)
CALCIUM DEPOSIT	1 (5%)		1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	2 (10%)			
FIBROSIS		1 (5%)	1 (2%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
DEGENERATION, NOS	1 (5%)			
CALCIFICATION, NOS		1 (5%)		
#ENDOCARDIUM	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)			1 (2%)
*AORTA	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	2 (10%)	2 (10%)	2 (4%)	1 (2%)
*CORONARY ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION		1 (5%)		
*MESENTERIC ARTERY	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)	1 (5%)	1 (2%)	
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(50)	(49)
INFLAMMATION, NOS	1 (5%)			
CIRRHOSIS, NOS				1 (2%)
PELIOSIS HEPATIS			1 (2%)	
METAMORPHOSIS FATTY	2 (10%)		1 (2%)	
ANGIECTASIS	3 (15%)	1 (5%)		
*GALLBLADDER	(20)	(20)	(50)	(50)
PIGMENTATION, NOS				1 (2%)
*BILE DUCT	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS	4 (20%)			1 (2%)
#PANCREAS	(20)	(20)	(50)	(48)
PERIARTERITIS	4 (20%)	4 (20%)	3 (6%)	1 (2%)
#STOMACH	(20)	(20)	(49)	(49)
ULCER, FOCAL			1 (2%)	2 (4%)
CALCIUM DEPOSIT	2 (10%)	2 (10%)	2 (4%)	1 (2%)
HYPERKERATOSIS		1 (5%)	1 (2%)	8 (16%)
ACANTHOSIS			1 (2%)	3 (6%)
#COLON	(19)	(20)	(50)	(49)
NEMATODIASIS	1 (5%)		1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(49)	(49)
PYELONEPHRITIS, NOS	1 (5%)	1 (5%)	1 (2%)	3 (6%)

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
INFLAMMATION, CHRONIC	15 (75%)	8 (40%)	14 (29%)	10 (20%)
CALCIUM DEPOSIT	1 (5%)	2 (10%)	1 (2%)	1 (2%)
#URINARY BLADDER	(19)	(20)	(48)	(49)
INFLAMMATION, NOS	1 (5%)			1 (2%)
HYPERPLASIA, EPITHELIAL				1 (2%)
POLYP				1 (2%)
ENDOCRINE SYSTEM				
#PITUITARY	(20)	(20)	(50)	(49)
ANGIECTASIS	1 (5%)			
#ADRENAL	(20)	(20)	(49)	(49)
CALCIUM DEPOSIT			1 (2%)	
#ADRENAL CORTEX	(20)	(20)	(49)	(49)
DEGENERATION, NOS			1 (2%)	
ANGIECTASIS	1 (5%)		2 (4%)	
#THYROID	(19)	(20)	(49)	(47)
ULTIMOBRANCHIAL CYST	2 (11%)			
HYPERPLASIA, C-CELL	1 (5%)			
HYPERPLASIA, FOLLICULAR-CELL	1 (5%)	1 (5%)		
#PARATHYROID	(3)	(16)	(26)	(33)
HYPERPLASIA, NOS	2 (67%)	4 (25%)	2 (8%)	
REPRODUCTIVE SYSTEM				
#PROSTATE	(20)	(17)	(30)	(24)
INFLAMMATION, NOS	5 (25%)	1 (6%)		1 (4%)
*SEMINAL VESICLE	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
#TESTIS	(20)	(20)	(50)	(49)
GRANULOMA, SPERMATIC	1 (5%)			
ATROPHY, NOS	11 (55%)	12 (60%)	10 (20%)	9 (18%)
*EPIDIDYMIS	(20)	(20)	(50)	(50)
NECROSIS, FAT	1 (5%)			1 (2%)
ATROPHY, NOS	3 (15%)			
NERVOUS SYSTEM				
NONE				

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

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 01-031M	CONTROL (VEH) 01-041M	LOW DOSE 01-042M	HIGH DOSE 01-043M
SPECIAL SENSE ORGANS				
*EYE/CORNEA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		
*EYE/LACRIMAL GLAND	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	1 (5%)			
*PERICARDIUM	(20)	(20)	(50)	(50)
INFLAMMATION, NOS	2 (10%)			
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS	4 (20%)	3 (15%)	4 (8%)	1 (2%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED		1	14	15

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

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	20	50	50
INTEGUMENTARY SYSTEM				
*SKIN	(20)	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (5%)		
INFLAMMATION, NOS		1 (5%)		
*SUBCUT TISSUE	(20)	(20)	(50)	(50)
ABSCESS, NOS		1 (5%)		
RESPIRATORY SYSTEM				
#LUNG	(20)	(20)	(50)	(50)
PNEUMONIA, CHRONIC MURINE	18 (90%)	20 (100%)	31 (62%)	30 (60%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(20)	(50)	(50)
HEMATOPOIESIS		4 (20%)	2 (4%)	1 (2%)
#CERVICAL LYMPH NODE	(20)	(18)	(48)	(48)
INFLAMMATION, NOS		1 (6%)		
CIRCULATORY SYSTEM				
#MYOCARDIUM	(20)	(20)	(49)	(50)
FIBROSIS	1 (5%)			1 (2%)
#ENDOCARDIUM	(20)	(20)	(49)	(50)
HYPERPLASIA, NOS			1 (2%)	1 (2%)
*AORTA	(20)	(20)	(50)	(50)
MEDIAL CALCIFICATION	1 (5%)			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
DIGESTIVE SYSTEM				
#LIVER	(20)	(20)	(49)	(50)
METAMORPHOSIS FATTY	1 (5%)			2 (4%)
FOCAL CELLULAR CHANGE		1 (5%)		
#LIVER/CENTRILOBULAR	(20)	(20)	(49)	(50)
NECROSIS, NOS	1 (5%)			
*BILE DUCT	(20)	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)	
#PANCREAS	(20)	(20)	(50)	(50)
PERIARTERITIS		1 (5%)		
#STOMACH	(20)	(20)	(50)	(49)
INFLAMMATION, NOS	1 (5%)			
ULCER, FOCAL		1 (5%)	1 (2%)	
CALCIUM DEPOSIT				1 (2%)
HYPERKERATOSIS	1 (5%)		2 (4%)	1 (2%)
ACANTHOSIS	1 (5%)		2 (4%)	1 (2%)
#COLON	(19)	(20)	(50)	(50)
NEMATODIASIS		1 (5%)		
URINARY SYSTEM				
#KIDNEY	(20)	(20)	(49)	(50)
PYELONEPHRITIS, NOS		1 (5%)		
INFLAMMATION, CHRONIC	9 (45%)	5 (25%)	4 (8%)	6 (12%)
CALCIUM DEPOSIT	1 (5%)		1 (2%)	1 (2%)
#URINARY BLADDER	(19)	(20)	(48)	(49)
INFLAMMATION, NOS			1 (2%)	
ENDOCRINE SYSTEM				
#PITUITARY	(19)	(20)	(48)	(50)
CYST, NOS		1 (5%)	1 (2%)	
#ADRENAL	(20)	(20)	(49)	(50)
CYST, NOS			1 (2%)	
#ADRENAL CORTEX	(20)	(20)	(49)	(50)
DEGENERATION, NOS		1 (5%)	1 (2%)	

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

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
ANGIECTASIS	3 (15%)	3 (15%)	4 (8%)	6 (12%)
#THYROID	(20)	(20)	(49)	(46)
FOLLICULAR CYST, NOS		1 (5%)		
HYPERPLASIA, C-CELL	4 (20%)			
#PARATHYROID	(1)	(11)	(32)	(22)
HYPERPLASIA, NOS	1 (100%)			
REPRODUCTIVE SYSTEM				
*VAGINA	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		1 (2%)
#UTERUS	(20)	(20)	(49)	(50)
HYDROMETRA	4 (20%)	2 (10%)	5 (10%)	4 (8%)
#UTERUS/ENDOMETRIUM	(20)	(20)	(49)	(50)
INFLAMMATION, NOS	1 (5%)		1 (2%)	
HYPERPLASIA, CYSTIC	1 (5%)			
#OVARY	(20)	(19)	(49)	(50)
CYST, NOS		1 (5%)		2 (4%)
INFLAMMATION, NOS				1 (2%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*HARDERIAN GLAND	(20)	(20)	(50)	(50)
INFLAMMATION, NOS		1 (5%)		
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(20)	(20)	(50)	(50)
PERIARTERITIS		1 (5%)		

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 01-031F	CONTROL (VEH) 01-041F	LOW DOSE 01-044F	HIGH DOSE 01-045F
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED			16	18

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH CHLOROPICRIN

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS MISSING			1	
ANIMALS NECROPSIED	18	20	46	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	15	20	46	47
INTEGUMENTARY SYSTEM				
*SKIN	(18)	(20)	(46)	(48)
INFLAMMATION, NOS		2 (10%)	1 (2%)	
*SUBCUT TISSUE	(18)	(20)	(46)	(48)
ABSCESS, NOS	1 (6%)	1 (5%)	5 (11%)	
RESPIRATORY SYSTEM				
#LUNG	(15)	(20)	(46)	(48)
PNEUMONIA, CHRONIC MURINE			6 (13%)	3 (6%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(15)	(20)	(46)	(46)
AMYLOIDOSIS	7 (47%)	2 (10%)	5 (11%)	
HEMATOPOIESIS		3 (15%)		
#LYMPH NODE	(15)	(19)	(46)	(47)
INFLAMMATION, NOS				1 (2%)
#CERVICAL LYMPH NODE	(15)	(19)	(46)	(47)
INFLAMMATION, NOS		1 (5%)	1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)	
#MESENTERIC L. NODE	(15)	(19)	(46)	(47)
INFLAMMATION, NOS	1 (7%)	7 (37%)	1 (2%)	5 (11%)
CIRCULATORY SYSTEM				
*AORTA	(18)	(20)	(46)	(48)
INFLAMMATION, NOS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
DIGESTIVE SYSTEM				
#LIVER	(15)	(20)	(46)	(48)
AMYLOIDOSIS			3 (7%)	
METAMORPHOSIS FATTY			1 (2%)	
#PANCREAS	(14)	(20)	(46)	(46)
INFLAMMATION, NOS	1 (7%)			
#STOMACH	(15)	(20)	(46)	(48)
HYPERKERATOSIS			3 (7%)	1 (2%)
ACANTHOSIS			3 (7%)	1 (2%)
URINARY SYSTEM				
#KIDNEY	(15)	(20)	(46)	(48)
HYDRONEPHROSIS			1 (2%)	
CYST, NOS		1 (5%)		
PYELONEPHRITIS, NOS		2 (10%)		1 (2%)
INFLAMMATION, CHRONIC	12 (80%)	2 (10%)	6 (13%)	
AMYLOIDOSIS	7 (47%)	2 (10%)	5 (11%)	
CALCIUM DEPOSIT				1 (2%)
#URINARY BLADDER	(15)	(19)	(46)	(47)
INFLAMMATION, NOS		2 (11%)	1 (2%)	1 (2%)
ENDOCRINE SYSTEM				
#THYROID	(10)	(20)	(35)	(40)
FOLLICULAR CYST, NOS	1 (10%)	1 (5%)		
REPRODUCTIVE SYSTEM				
#PROSTATE	(15)	(12)	(46)	(45)
INFLAMMATION, NOS				2 (4%)
#TESTIS	(15)	(20)	(46)	(46)
ATROPHY, NOS	1 (7%)		1 (2%)	
NERVOUS SYSTEM				
NONE				

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

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 02-M041	CONTROL (VEH) 02-M031	LOW DOSE 02-M042	HIGH DOSE 02-M043
SPECIAL SENSE ORGANS				
*EYE/LACRIMAL GLAND	(18)	(20)	(46)	(48)
HYPERPLASIA, NOS			2 (4%)	
*HARDERIAN GLAND	(18)	(20)	(46)	(48)
HYPERPLASIA, NOS				1 (2%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*ABDOMINAL CAVITY	(18)	(20)	(46)	(48)
NECROSIS, FAT			1 (2%)	
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	3	7	18	31
ANIMAL MISSING/NO NECROPSY			1	
AUTO/NECROPSY/NO HISTO	3			1
AUTOLYSIS/NO NECROPSY	2		3	2

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH CHLOROPICRIN

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
ANIMALS INITIALLY IN STUDY	20	20	50	50
ANIMALS NECROPSIED	20	19	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	19	49	48
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(20)	(19)	(49)	(48)
PNEUMONIA, CHRONIC MURINE	1 (5%)	1 (5%)	2 (4%)	15 (31%)
HEMATOPOIETIC SYSTEM				
#SPLEEN	(20)	(19)	(49)	(48)
AMYLOIDOSIS				1 (2%)
HEMATOPOIESIS				3 (6%)
#LYMPH NODE	(20)	(19)	(46)	(44)
INFLAMMATION, NOS	1 (5%)			
#CERVICAL LYMPH NODE	(20)	(19)	(46)	(44)
INFLAMMATION, NOS				3 (7%)
#MESENTERIC L. NODE	(20)	(19)	(46)	(44)
INFLAMMATION, NOS		1 (5%)		1 (2%)
ANGIECTASIS		1 (5%)		
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(20)	(19)	(49)	(48)
PERIOSIS HEPATIS			1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
AMYLOIDOSIS				1 (2%)
LEUKEMOID REACTION	1 (5%)			
*BILE DUCT	(20)	(19)	(50)	(48)
DILATATION, NOS	1 (5%)			
HYPERPLASIA, NOS		1 (5%)		
#PANCREAS	(20)	(19)	(47)	(48)
CYST, NOS		1 (5%)	1 (2%)	1 (2%)
INFLAMMATION, NOS		1 (5%)		
#STOMACH	(20)	(19)	(48)	(48)
ULCER, NOS			1 (2%)	
HYPERKERATOSIS			10 (21%)	9 (19%)
ACANTHOSIS			10 (21%)	9 (19%)
#PEYERS PATCH	(20)	(19)	(47)	(45)
HYPERPLASIA, LYMPHOID			1 (2%)	
#LARGE INTESTINE	(19)	(19)	(43)	(46)
HEMORRHAGIC CYST			1 (2%)	
URINARY SYSTEM				
#KIDNEY	(20)	(19)	(49)	(48)
PYELONEPHRITIS, NOS				1 (2%)
INFLAMMATION, CHRONIC				1 (2%)
AMYLOIDOSIS				1 (2%)
ENDOCRINE SYSTEM				
#THYROID	(16)	(16)	(44)	(36)
FOLLICULAR CYST, NOS	1 (6%)			
HYPERPLASIA, FOLLICULAR-CELL	1 (6%)			
REPRODUCTIVE SYSTEM				
*VAGINA	(20)	(19)	(50)	(48)
INFLAMMATION, NOS		1 (5%)		
#UTERUS	(20)	(19)	(49)	(47)
HYDROMETRA	5 (25%)	2 (11%)	6 (12%)	5 (11%)
#UTERUS/ENDOMETRIUM	(20)	(19)	(49)	(47)
INFLAMMATION, NOS	3 (15%)	2 (11%)	3 (6%)	2 (4%)

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 02-F041	CONTROL (VEH) 02-F031	LOW DOSE 02-F044	HIGH DOSE 02-F045
HYPERPLASIA, CYSTIC	6 (30%)	9 (47%)	16 (33%)	7 (15%)
#OVARY/OVIDUCT	(20)	(19)	(49)	(47)
INFLAMMATION, NOS		1 (5%)		
#OVARY	(20)	(19)	(49)	(47)
CYST, NOS	12 (60%)	4 (21%)	9 (18%)	8 (17%)
INFLAMMATION, NOS	5 (25%)	4 (21%)	4 (8%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EYE	(20)	(19)	(50)	(48)
PHTHISIS BULBI				1 (2%)
*HARDERIAN GLAND	(20)	(19)	(50)	(48)
HYPERPLASIA, NOS			1 (2%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(20)	(19)	(50)	(48)
INFLAMMATION, NOS		1 (5%)		1 (2%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	1	3	11	11
NECROPSY PERF/NO HISTO PERFORMED			1	
AUTOLYSIS/NO NECROPSY		1		2

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

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