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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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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 PENTACHLORONITROBENZENE
FOR POSSIBLE CARCINOGENICITY

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

CONTRIBUTORS: This report presents the results of the bioassay of pentachloronitrobenzene conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay 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 Bioassay 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 EG&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).

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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).

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SUMMARY

A bioassay of technical-grade pentachloronitrobenzene (PCNB) for possible carcinogenicity was conducted using Osborne-Mendel rats and B6C3F1 mice. PCNB was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The time-weighted average dietary concentrations of PCNB were, respectively, 10,064 and 5417 ppm for male rats, 14,635 and 7875 ppm for female rats, 5213 and 2606 ppm for male mice, and 8187 and 4093 ppm for female mice. After a 78-week period of compound administration, observation of the rats continued for an additional 33 to 35 weeks and observation of the mice continued for 14 or 15 additional weeks.

For each species, 20 animals of each sex were placed on test as controls and fed only the basal diet.

No rare or unusual tumors were observed during the histopathologic examinations and no statistically significant positive associations were demonstrated between chemical administration and the incidence of neoplasms in either sex of either species.

It is concluded that under the conditions of this bioassay PCNB was not carcinogenic in either Osborne-Mendel rats or B6C3F1 mice.

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

LIST OF TABLES

LIST OF TABLES (Concluded)

I. INTRODUCTION

Pentachloronitrobenzene (PCNB) (NCI No. C00419), a halogenated benzene derivative and agricultural pesticide, was selected for bioassay by the National Cancer Institute following its classification as a tumorigenic agent by The Secretary's Commission on Pesticides and Their Relationship to Environmental Health (U.S. Department of Health, Education, and Welfare, 1969).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is pentachloronitrobenzene.* It is also known as quintozene (the common name approved by The International Standards Organization [International Agency For Research on Cancer, 1974]), terrachlor, and PCNB.

PCNB was introduced as a fungicide in Germany in the 1930s but did not achieve commercial importance in the United States until the early 1960s (International Agency for Research on Cancer, 1974). PCNB is presently approved in the United States for use as a soil fungicide on fruit (bananas) and on a wide variety of vegetables (e.g., cabbages, potatoes, and tomatoes), field crops (e.g., cotton and soybeans) and ornamentals (e.g., carnations, lilies, roses, and grasses); it may also be applied as a seed protectant fungicide for crops such as barley, corn, cotton, oats, rice and wheat (International Agency for Research on Cancer, 1974).

*The CAS registry number is 82-68-8.

Recent production statistics for PCNB are considered proprietary and are therefore not available; however, the listing of PCNB in the 1976 Directory of Chemical Producers, U.S.A. (Stanford Research Institute, 1976) implies that the compound is presently manufactured in commercial quantities (greater than 1000 pounds or $1000 in value) and 1971 production was estimated to be in excess of 3 million pounds (Johnson, 1972; as cited in International Agency for Research on Cancer, 1974).

Occupational exposure to PCNB may occur at pesticide production and formulating facilities and among agricultural workers engaged in the treatment of soil or seeds with the fungicide. Exposure of the general population may occur either through ingestion of residues accumulating in food crops grown in PCNB-treated soils, or through ingestion of milk from cows fed contaminated feed. PCNB residues were detected in endive leaves and roots (0.06 to 83 ppm), in a fruit sample from a total diet residue study (0.003 ppm), and in potatoes (as high as 0.1 ppm in the peel following treatment of the soil at a rate of 25 pounds per acre, a representative application rate for vegetables of this type) (International Agency for Research on Cancer, 1974). Trace levels of PCNB (0.001 to 0.01 ppm) were also detected in cows' milk from animals fed milking chow found to be contaminated with 0.002 to 0.006 ppm of the chemical (Borzelleca et al., 1971).

Although data concerning human exposure are limited, PCNB is generally considered to be of a very low order of toxicity (Courtney et al., 1976).

PCNB was found to be mutagenic in a tryptophan-requiring strain of E. coli, causing a tenfold increase in the number of revertant colonies over that expected as a result of spontaneous reversion (Clarke, 1971). The fungicide, however, did not exhibit mutagenic activity in the sex-linked lethal test in Drosophila melanogaster (Vogel and Chandler, 1974) or in the host-mediated bioassay in mice using several test organisms (Buselmaier et al., 1973).

Purified PCNB exhibited some teratogenic activity in CD-1 mice, producing cleft palate in an average of 8 percent of each litter following oral administration of 500 mg/kg/day on days 7 through 16 of gestation (Courtney et al., 1976). PCNB was not, however, teratogenic in either CD or Wistar rats (Courtney et al., 1976; Khera and Villeneuve, 1975; Jordan et al., 1975).

II. MATERIALS AND METHODS

A. Chemicals

Two batches of technical-grade pentachloronitrobenzene (PCNB) were purchased from the Olin Mathieson Chemical Corporation. The manufacturer's stated assay for PCNB is 98 percent. Analysis was performed by Hazleton Laboratories America, Inc., Vienna, Virginia. The wide range observed in the melting point (134° to 145°C) indicated the presence of impurities even though the values were close to that reported in the literature (146°C).

Total area analysis by gas-liquid chromatography suggested a purity of approximately 97 percent, with 12 impurities present. Similar results after 12 and 24 months suggested little or no change in composition. The four impurities eluted from the column before PCNB were identified by the manufacturer as pentachlorobenzene, chloranil, tetrachloronitrobenzene, and hexachloronitrobenzene.

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

B. Dietary Preparation

The basal laboratory diet consisted of Wayne Lab-Blox® (Allied Mills, Inc.) plus 2 percent Duke's® corn oil (S.F. Sauer Company) by weight. Fresh mixtures of PCNB in corn oil were prepared each week and stored in the dark. The PCNB mixtures were incorporated into the appropriate amount of laboratory diet in a twin-shell blender fitted

with an accelerator bar so that the final concentrations of PCNB in the diet varied from 1075 to 22,000 ppm.

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 10 to 15 complete changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle. Rats were individually

housed in suspended galvanized-steel wire-mesh cages with perforated floors, while mice were housed by sex in groups of ten in solid-bottom polypropylene cages with filter tops. Sanitized cages with fresh bedding (Sanichips®, Shurfire) 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, while fresh heat-sterilized glass water bottles were provided three times a week. Food (Wayne Lab-Blox®) and water were available ad libitum.

The rats treated with PCNB and their controls were housed in the same room as other rats receiving diets treated with* clonitralid (1420-04-8) and amitrole (61-82-5). All mice used in this study were housed in the same room as other mice receiving diets treated with trifluralin (1582-09-8); dioxathion (78-34-2); sulfallate (95-06-7); p,p'-DDT (50-29-3); methoxychlor (72-43-5); p,p'-DDE (72-55-9); p,p'-TDE (72-54-8); dicofol (115-32-2); chlorobenzilate (510-15-6); clonitralid (1420-04-8); nitrofen (1836-75-5); endosulfan (115-29-7); mexacarbate (315-18-4); amitrole (61-82-5); acetylaminofluorene (53-96-3); and safrole (94-59-7).

E. Selection of Initial Concentrations

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

*CAS registry numbers are given in parentheses.

Animals of each species were distributed among six groups, each consisting of five males and five females. PCNB was premixed with a small amount of corn oil. This mixture was then incorporated into the laboratory diet and fed ad libitum to five of the six rat groups and five of the six mouse groups in concentrations of 2150, 4640, 10,000, 21,500 and 46,400 ppm. The sixth group of each species served as a control group, receiving only the mixture of corn oil and laboratory chow. The dosed dietary preparations were administered for a period of 6 weeks, followed by a 2-week observation period during which all animals were fed the basal diet.

A concentration inducing no mortality and resulting in a depression of mean group body weight of approximately 20 percent relative to controls was to be selected as the initial high concentration for the chronic study. When weight gain criteria were not applicable, mortality data alone were utilized.

All of the male rats and one of the female rats receiving 46,400 ppm PCNB died before the 6-week period of compound administration was over. In males, mean body weight depression was 7 and 42 percent at 10,000 and 21,500 ppm, respectively. In females, mean body weight depression was 16 percent at 21,500 ppm. The initial high concentrations selected for male and female rats in the chronic bioassay were 15,000 and 22,000 ppm, respectively.

At a concentration of 2150 ppm, none of the mice died during the 8-week study. However, four of the five males receiving 4640 ppm

died by week 4. Mean body weight depression was only 3 percent in the males receiving 2150 ppm but, due to the severe mortality observed at 4640 ppm, 2150 ppm was selected as the initial high concentration for the male mice. Mean body weight depression was only 10 percent in females treated with 4640 ppm. However, two of the five females receiving 10,000 ppm died. The initial high concentration selected for the chronic bioassay was 4640 ppm for the female mice.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, duration of treated and untreated observation periods, and the time-weighted average concentrations) are summarized in Tables 1 and 2.

The high dose, low dose, and control rats were all approximately 6 weeks old when the bioassay began. The high and low concentrations of PCNB initially utilized for males were 15,000 and 7500 ppm, respectively. During week 14 of the experiment, when the male rats were approximately 20 weeks old, the high and low concentrations were decreased to 10,000 and 5000 ppm, respectively, in response to adverse clinical reactions observed in the treated animals. In week 53 administration of PCNB to the high dose males ceased for 1 week, followed by 4 weeks of feeding at the previous concentration of 10,000 ppm. This cyclic pattern of dose administration, an effort to reduce total chemical intake, was continued for the remainder of the 78-week period.

TABLE 1

DESIGN SUMMARY FOR OSBORNE-MENDEL RATS
PCNB FEEDING EXPERIMENT

	INITIAL GROUP SIZE	PCNB CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION OVER A 78-WEEK PERIOD[b]
MALE					
CONTROL	20	0		111	0
LOW DOSE	50	7,500	13		5,417
		5,000	65		
		0		33	
HIGH DOSE	50	15,000	13		10,064
		10,000	39		
		10,000[c]	20	6	
		0		34	
FEMALE					
CONTROL	20	0		113	0
LOW DOSE	50	11,000	13		7,875
		7,250	65		
		0		35	
HIGH DOSE	50	22,000	13		14,635
		14,500	35		
		14,500[c]	24	6	
		0		33	

[a] Concentrations given in parts per million

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

[c] These concentrations were cyclically administered with a pattern of 1 treatment-free week followed by 4 weeks of treatment at the level indicated.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
PCNB FEEDING EXPERIMENT

	INITIAL GROUP SIZE	PCNB CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)	TIME-WEIGHTED AVERAGE CONCENTRATION[b]
MALE					
CONTROL	20	0		91	0
LOW DOSE	50	1075	4		2606
		1500	4		
		2000	10		
		2500	14		
		3000	46		
		0		14	
HIGH DOSE	50	2150	4		5213
		3000	4		
		4000	10		
		5000	14		
		6000	46		
		0		14	
FEMALE					
CONTROL	20	0		92	0
LOW DOSE	50	2320	4		4093
		3000	4		
		3500	10		
		4000	14		
		4500	46		
		0		15	
HIGH DOSE	50	4640	4		8187
		6000	4		
		7000	10		
		8000	14		
		9000	46		
		0		15	

[a] Concentrations given in parts per million

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

For female rats, the initial high and low concentrations of PCNB used were 22,000 and 11,000 ppm, respectively. During week 14, when the female rats were approximately 20 weeks old, the high and low levels were decreased to 14,500 and 7250 ppm, respectively, due to the observation of adverse clinical reactions in the treated animals. In week 49, administration of PCNB to the high dose females ceased for 1 week, followed by 4 weeks of feeding at the previous concentration of 14,500 ppm in an effort to reduce total chemical intake. This cyclic pattern of dose administration was continued for the remainder of the 78-week study period.

The high dose, low dose, and control mice were all approximately 5 weeks old when compound administration began. The high and low concentrations initially administered to male mice were 2150 and 1075 ppm, respectively. Because the treated animals exhibited no adverse reaction to the PCNB, the concentrations were increased four times over the duration of the study. In week 5 the high and low concentrations were increased to 3000 and 1500 ppm, respectively. Concentrations were again raised in week 9 of the experiment, to 4000 and 2000 ppm, respectively, in week 19 to 5000 and 2500 ppm, and in week 33 to 6000 and 3000 ppm.

The high and low concentrations of PCNB initially administered to female mice were 4640 and 2320 ppm, respectively. Because the treated animals exhibited no adverse reaction to the chemical, the concentrations were increased four times over the course of the

bioassay. In week 5 of the study the high and low concentrations were raised to 6000 and 3000 ppm, respectively, in week 9 to 7000 and 3500 ppm, 10 weeks later to 8000 and 4000 ppm, and finally, in week 33, to 9000 and 4500 ppm, respectively.

G. 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 and food consumption and data concerning appearance, behavior, signs of toxic effects, and incidence, size, and location of tissue masses were recorded once weekly for the first 10 weeks and monthly thereafter. Incidence of tissue masses was determined by observation and palpation of each animal.

During the course of this bioassay several pathology protocols were in effect, each for different periods of time. The minimum protocol required that, if possible, certain tissues were to be taken and examined histopathologically from all control animals, from any animal in which a tumor was observed during gross examination, and from at least 10 grossly normal males and 10 grossly normal females from each treated group. In addition, any tissues showing gross abnormalities were to be taken and examined histopathologically. Under later protocols, some tissues were taken from additional dosed animals. The number of animals in each group from which a tissue was examined is indicated in Appendices A through D.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at termination

of the bioassay. The animals were euthanized by exsanguination under sodium pentobarbital anesthesia, and were immediately necropsied. The histopathologic examination consisted of gross examination of all major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Slides were prepared from the following tissues from selected animals: skin, 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, pancreatic islets, testis, prostate, brain, muscle, 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.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum,

1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and 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.

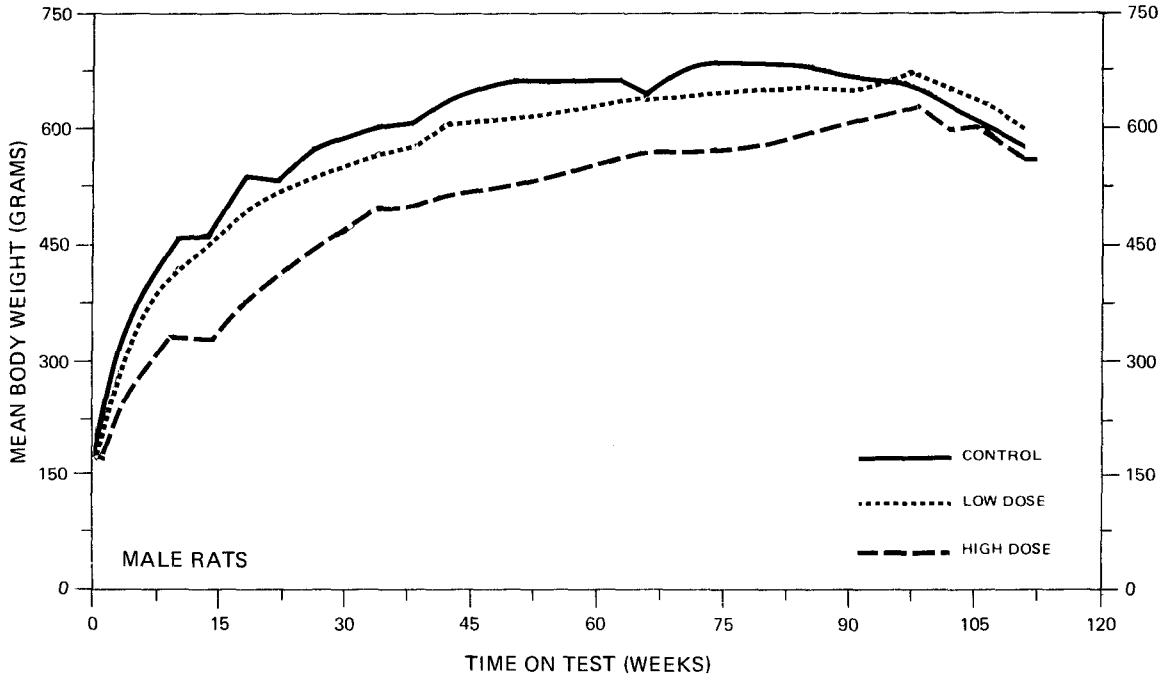
III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

Distinct, dose-related mean body weight depression was evident in both male and female rats throughout the bioassay (Figure 1).

Clinical signs were observed in the PCNB-treated groups as early as week 1 of the study. The predominant clinical signs were urine staining in the abdominal area and a hunched or thin appearance indicative of body weight effects. Beginning in week 1, approximately 40 percent of the high dose males, 70 percent of the high dose females, and a few low dose females showed abdominal urine stains. Concomitant thin or hunched appearance was displayed by about 25 percent of the animals in these groups and was noted in increasing numbers from week 1 until cessation of compound administration in week 78. Thereafter, until termination of the study, a comparable number of treated and control animals appeared hunched. Abdominal urine stains were persistently observed in most of the treated groups except in the low dose males. Abdominal urine staining was infrequently observed in this group and in the controls throughout the study.

Respiratory signs characterized by labored or difficult respiration, wheezing, and/or nasal discharge were observed at a low incidence in all groups including controls during the first part of the second year. The incidence increased gradually in all groups during the last six months of the study.



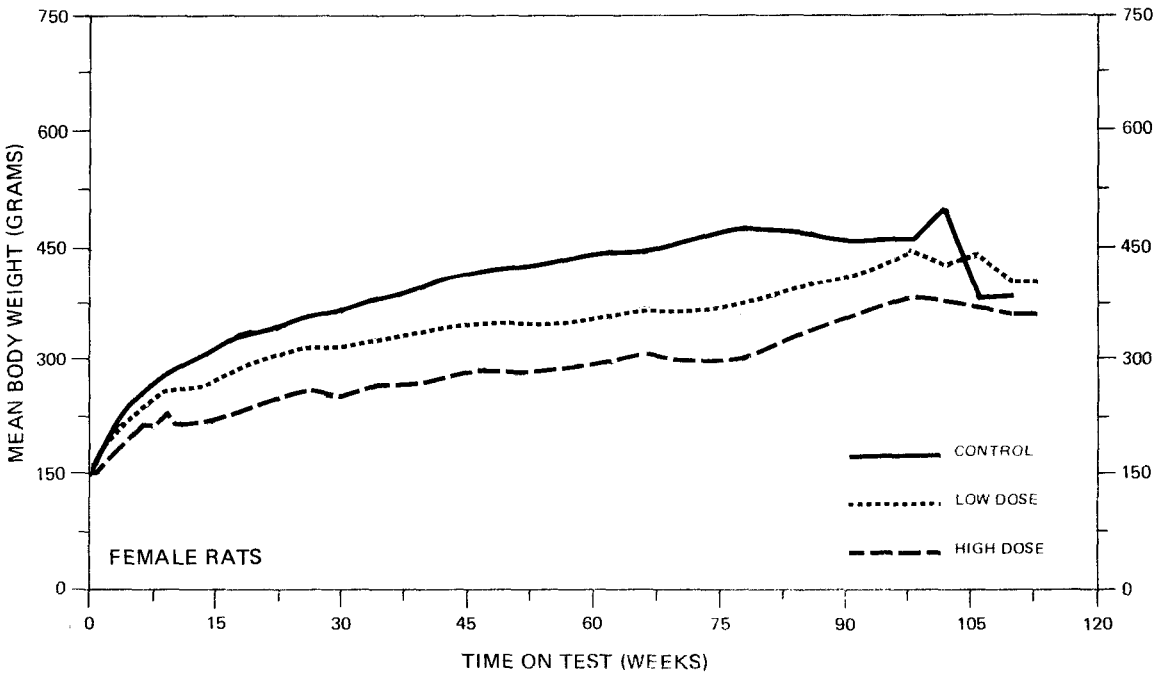


FIGURE 1
GROWTH CURVES FOR PCNB CHRONIC STUDY RATS

Other signs associated with aging were observed in comparable numbers of control and treated animals during the second year. These signs included rough or stained fur, alopecia, sores on parts of the body, reddish discharge or crust around body orifices, palpable masses, wart-like growths, and nodules. Isolated, apparently spontaneous signs noted in one to three animals in each group included tremors, head tilt or circling, small-appearing testes, and partial limb paralysis.

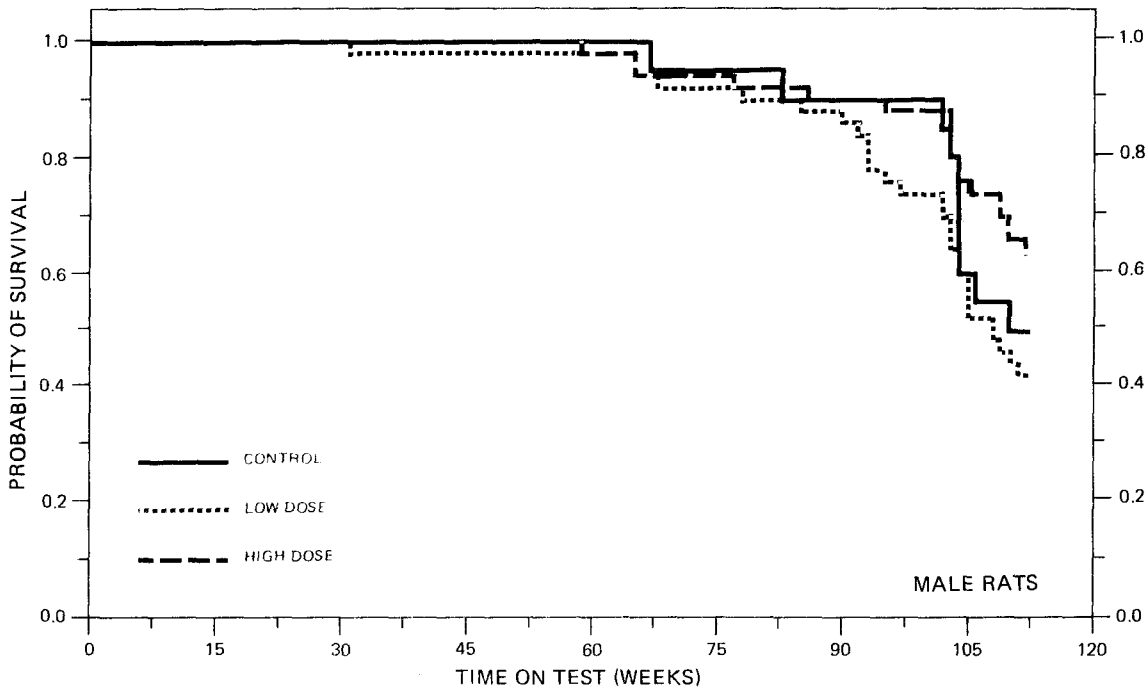
B. Survival

The estimated probabilities of survival for male and female rats in the control and PCNB-treated groups are shown in Figure 2.

For both male and female rats the Tarone test did not indicate a significant positive association between dosage and mortality. For males 64 percent (32/50) of the high dose, 42 percent (21/50) of the low dose, and 50 percent (10/20) of the control group survived until the end of the study. For females 74 percent (37/50) of the high dose, 80 percent (40/50) of the low dose, and 80 percent (16/20) of the control group survived at least 101 weeks. Thus in both sexes survival was adequate for meaningful statistical analyses of tumor incidence.

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).



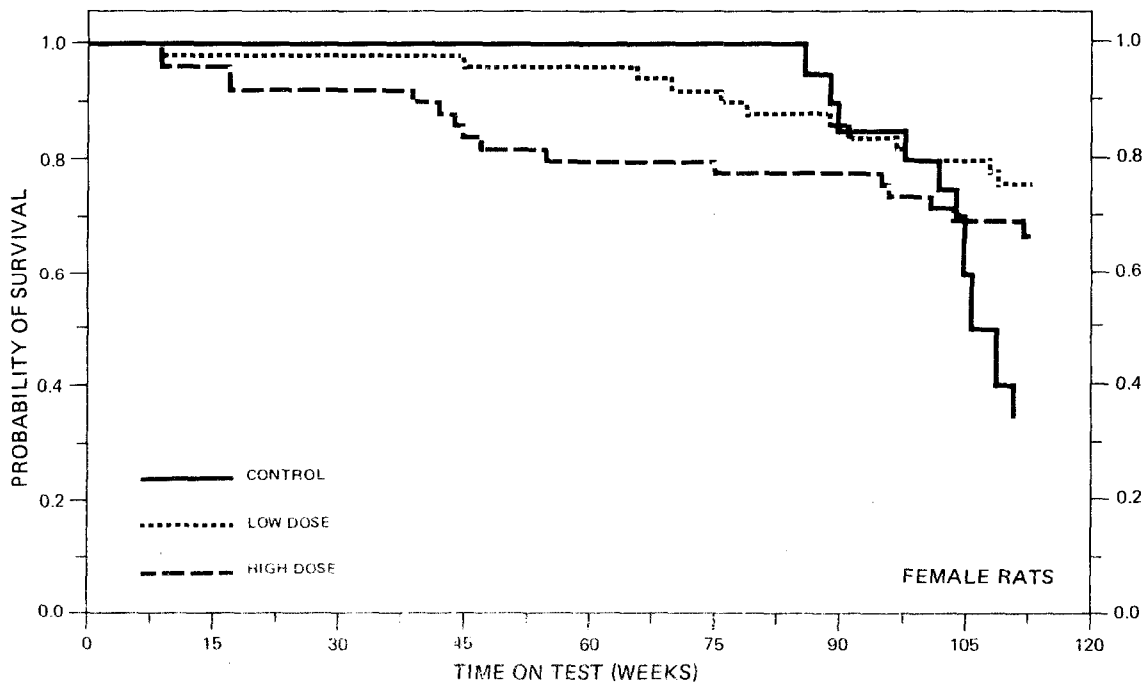


FIGURE 2
SURVIVAL COMPARISONS OF PCNB CHRONIC STUDY RATS

A variety of neoplasms were observed among both treated and control rats. Each type of tumor observed has been encountered previously as a spontaneous lesion in Osborne-Mendel rats. No appreciable difference in the incidence of neoplasia was noted between the control and treated rats in this study.

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

The results of this histopathologic examination did not indicate evidence of carcinogenicity in Osborne-Mendel rats of either sex.

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 PCNB-dosed groups of either sex is included.

For both male and female rats, no statistical tests showed a significant positive association between dosage and tumor incidence.

The possibility of a negative association between compound administration and the incidence of mammary fibroadenomas was noted for the male rats. The Fisher exact tests, however, were not significant.

To provide additional insight, 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

TABLE 3

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibroma[b]	1/20(0.05)	3/48(0.06)	2/49(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.250	0.816
Lower Limit	---	0.111	0.046
Upper Limit	---	64.251	47.195
Weeks to First Observed Tumor	104	110	112
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/20(0.05)	2/48(0.04)	4/49(0.08)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.833	1.633
Lower Limit	---	0.047	0.179
Upper Limit	---	48.155	78.704
Weeks to First Observed Tumor	106	85	95
Circulatory System: Hemangiosarcoma[b]	1/20(0.05)	1/48(0.02)	3/49(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.417	1.224
Lower Limit	---	0.006	0.107
Upper Limit	---	32.057	62.958
Weeks to First Observed Tumor	83	105	112

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Kidney: Mixed Tumor Malignant[b]	0/20(0.00)	3/43(0.07)	1/37(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.291	0.030
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	68	112
Pituitary: Chromophobe Adenoma[b]	2/20(0.10)	3/42(0.07)	7/37(0.19)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.714	1.892
Lower Limit	---	0.091	0.411
Upper Limit	---	8.119	17.507
Weeks to First Observed Tumor	106	111	86
Thyroid: Follicular-Cell Adenoma or Follicular-Cell Carcinoma[b]	2/20(0.10)	2/43(0.05)	2/37(0.05)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.465	0.541
Lower Limit	---	0.037	0.043
Upper Limit	---	6.107	7.057
Weeks to First Observed Tumor	104	68	103

TABLE 3 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	2/20(0.10)	4/48(0.08)	0/49(0.00)
P Values[c]	P = 0.043(N)	N.S.	N.S.
Relative Risk (Control)[d]	---	0.833	0.000
Lower Limit	---	0.134	0.000
Upper Limit	---	8.776	1.372
Weeks to First Observed Tumor	104	104	---

[a]Treated groups received time-weighted average doses of 5417 or 10,064 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the 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.

TABLE 4

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Carcinoma[b]	0/20(0.00)	3/40(0.08)	0/36(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.041	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.313	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	112	---
Thyroid: C-Cell Adenoma or C-Cell Carcinoma[b]	0/20(0.00)	4/40(0.10)	0/36(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.018	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.484	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	112	---
Mammary Gland: Fibroadenoma[b]	7/20(0.35)	9/50(0.18)	8/48(0.17)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.514	0.476
Lower Limit	---	0.208	0.183
Upper Limit	---	1.436	1.365
Weeks to First Observed Tumor	86	112	113

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Circulatory System: Hemangiosarcoma[b]	1/20(0.05)	1/50(0.02)	1/47(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	0.426
Lower Limit	---	0.005	0.006
Upper Limit	---	30.802	32.057
Weeks to First Observed Tumor	104	89	113
Pituitary: Chromophobe Adenoma[b]	7/20(0.35)	20/40(0.50)	8/35(0.23)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.038	---	---
Relative Risk (Control)[d]	---	1.429	0.653
Lower Limit	---	0.726	0.253
Upper Limit	---	3.333	1.829
Weeks to First Observed Tumor	90	91	101
Uterus: Endometrial Stromal Polyp[b]	1/19(0.05)	2/39(0.05)	1/37(0.03)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.974	0.514
Lower Limit	---	0.055	0.007
Upper Limit	---	56.013	39.250
Weeks to First Observed Tumor	109	112	113

TABLE 4 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 7875 or 14,635 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the 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 $P < 0.05$.

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 a significantly increased rate of tumor incidence induced in rats by PCNB that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

No consistent dose-related mean body weight depression was evident among male mice. A slight dose-related depression of mean body weight became apparent for female mice after week 35 (Figure 3).

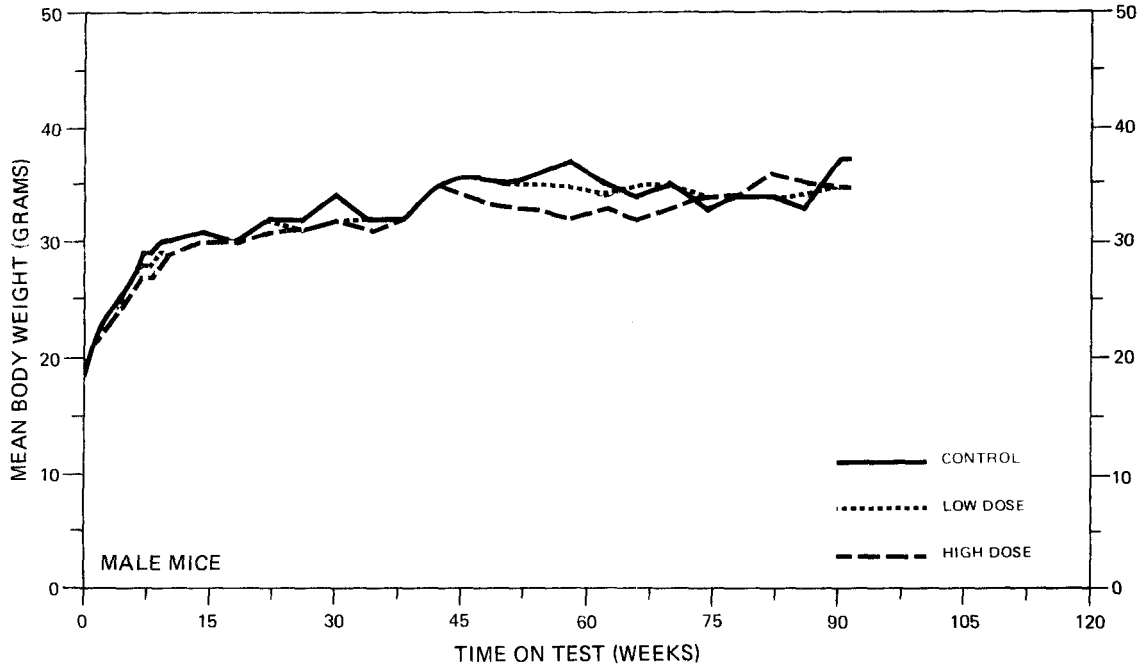
During the first 6 months of the study, treated and control mice showed comparable patterns of appearance and behavior. Clinical signs often observed in group-housed laboratory mice, particularly in males, were observed in a comparable number of treated and control mice. Such signs included body sores (mostly from fighting), localized alopecia, abdominal urine stains, penile/anal/vulvar irritation, bloating, eyes showing cloudiness, redness and/or discharge, swollen areas on the body or extremities, and palpable nodules.

In week 34 a hunched appearance was evident in approximately 27 percent of the low dose males and 70 percent of the high dose males. This sign persisted in the survivors to termination of the study in week 90.

B. Survival

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

For both male and female mice the Tarone test did not indicate a significant positive association between dosage and mortality. For males 64 percent (32/50) of the high dose, 70 percent (33/50) of the low dose, and 75 percent (15/20) of the control group survived at



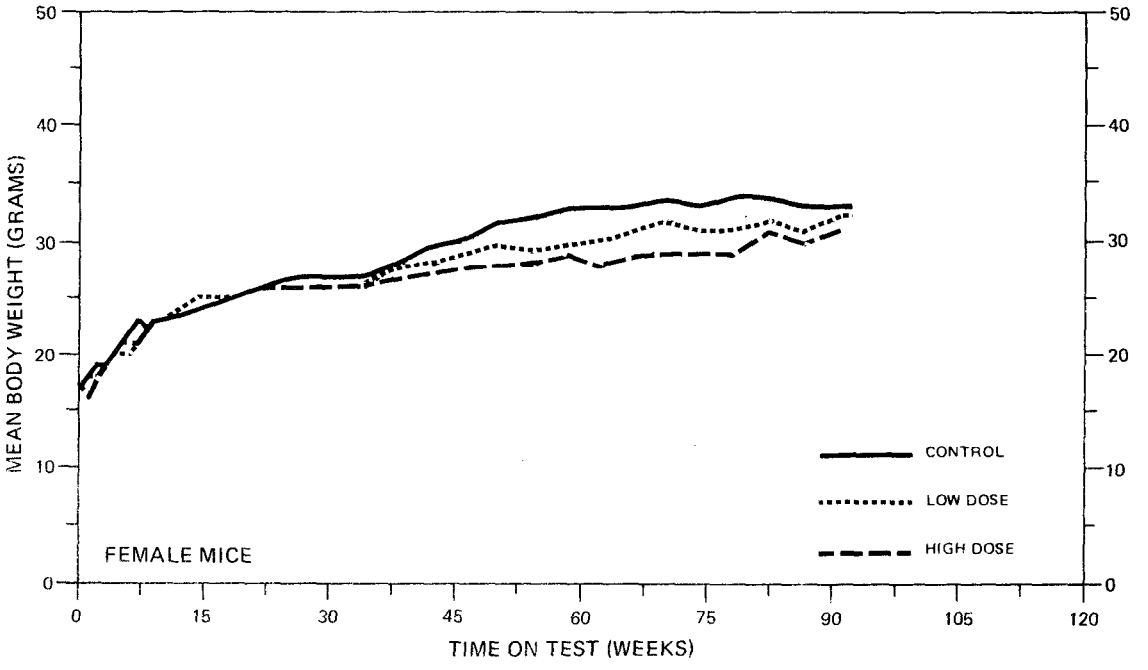


FIGURE 3
GROWTH CURVES FOR PCNB CHRONIC STUDY MICE

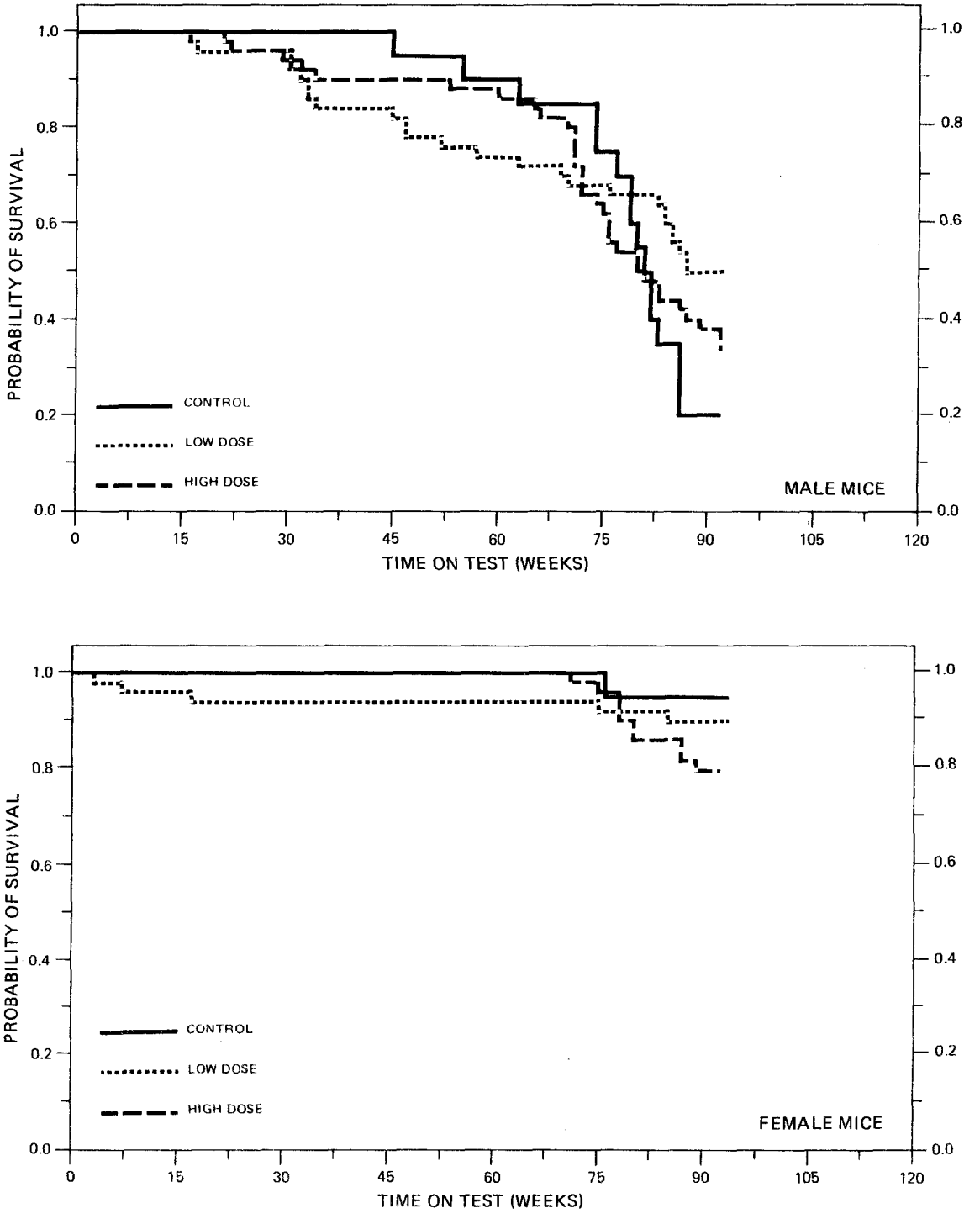


FIGURE 4
SURVIVAL COMPARISONS OF PCNB CHRONIC STUDY MICE

least 75 weeks. After 75 weeks, mortality of males was greatly accelerated so that by week 90, only 25 low dose, 17 high dose, and 4 control mice were available for terminal sacrifice. For females survival was adequate to permit development of possible late-occurring tumors as 78 percent (39/50) of the high dose, 86 percent (43/50) of the low dose, and 95 percent (19/20) of the control group survived until the end of the study.

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).

A variety of neoplasms were represented among both the treated and control mice. Each type of tumor represented has been encountered previously as a naturally occurring lesion in B6C3F1 mice and was without apparent relationship to administration of the chemical.

The relative low incidence of hepatocellular carcinomas in male mice and the lack of a relationship of these lesions to dosage, suggest that the increased incidence in the low dose group was not significant. The results of this histopathologic examination did not indicate evidence of carcinogenicity in B6C3F1 mice of either sex.

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

TABLE 5

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Subcutaneous Tissue: Fibrosarcoma[b]	2/20(0.10)	3/45(0.07)	5/48(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.667	1.042
Lower Limit	---	0.085	0.191
Upper Limit	---	7.596	10.410
Weeks to First Observed Tumor	63	87	72
Hematopoietic System: Malignant Lymphoma[b]	2/20(0.10)	1/45(0.02)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.222	0.417
Lower Limit	---	0.004	0.033
Upper Limit	---	4.070	5.490
Weeks to First Observed Tumor	55	76	77
Liver: Hepatocellular Carcinoma[b]	2/20(0.10)	8/35(0.23)	4/42(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.286	0.952
Lower Limit	---	0.525	0.154
Upper Limit	---	20.614	9.980
Weeks to First Observed Tumor	79	86	65

TABLE 5 (CONCLUDED)

[a]Treated groups received time-weighted average doses of 2606 or 5213 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the 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.

TABLE 6

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

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Malignant Lymphoma[b]	1/20(0.05)	1/47(0.02)	5/46(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.426	2.174
Lower Limit	---	0.006	0.271
Upper Limit	---	32.720	100.415
Weeks to First Observed Tumor	91	75	80
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	0/14(0.00)	3/20(0.15)
P Values[c]	P = 0.043	N.S.	N.S.
Relative Risk (Control)[d]	---	---	Infinite
Lower Limit	---	---	0.630
Upper Limit	---	---	Infinite
Weeks to First Observed Tumor	---	---	71

[a]Treated groups received time-weighted average doses of 4093 or 8187 ppm in feed.

[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 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 control group is given beneath the incidence of tumors in the 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.

of tumor that was observed in more than 5 percent of any of the PCNB-dosed groups of either sex is included.

For female mice the Cochran-Armitage test indicated a significant ($P = 0.043$) positive association between dosage and the incidence of hepatocellular carcinomas. The Fisher exact tests did not support this finding, but a small number of tissues (20) were examined microscopically. In the historical data compiled by this laboratory for the NCI Bioassay Program 3/380 (1 percent) of the untreated female B6C3F1 mice had this tumor.

No other statistical tests for any site in mice of either sex indicated a significant positive association between the administration of PCNB and tumor incidence. Thus, at the dose levels used in this experiment there was not adequate evidence to conclude that PCNB was a carcinogen in B6C3F1 mice.

To provide additional insight, 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 a significantly increased rate of tumor incidence induced in mice by PCNB that could not be established under the conditions of this test.

V. DISCUSSION

Survival among male rats, female rats, and female mice was adequate for meaningful statistical analyses of possible late-developing tumors. Although more than half of each male mouse group survived the 78-week period of compound administration, mortality of male mice was high during the observation period following compound administration with only 25 low dose mice, 17 high dose mice, and 4 control mice remaining alive at termination of the mouse bioassay in week 90. There is a possibility that if more male mice had survived the observation period, a higher incidence of tumors would have been observed in treated or control groups at terminal sacrifice.

It appears that maximum tolerated doses were received by treated groups. Dose-related depression of mean body weight was observed for male and female rats. Abdominal urine staining in all treated rat groups except low dose males was further indication that rats were given a maximum tolerated dose. From about week 34 until termination of the study, a slight dose-related mean body weight depression was observed among female mice and a dose-related incidence of hunched body posture was observed among male mice.

Among rats, chromophobe adenomas of the pituitary were observed in 2/20 (10 percent), 3/42 (7 percent), and 7/37 (19 percent) of the control, low dose, and high dose males, respectively, and in 7/20 (35 percent), 20/40 (50 percent), and 8/35 (23 percent) of the control, low dose, and high dose females, respectively. As there was

no consistent relationship between PCNB concentration received and incidences of lesions observed, and as these tumors occur spontaneously with similar incidences in untreated Osborne-Mendel rats, no significance was attributed to the incidences of these neoplasms in treated animals. In addition, statistical analyses revealed no significant positive associations between compound administration and the incidence of pituitary chromophobe adenoma or any of the other neoplasms that were observed in rats.

In mice, hepatocellular carcinomas were found in 2/20 (10 percent), 8/35 (23 percent), and 4/42 (10 percent) of the control, low dose, and high dose males, respectively, and in 0/20, 0/14, and 3/20 (15 percent) of the control, low dose, and high dose females, respectively. As the incidence was low and as there was no consistent relationship between PCNB concentration received and incidences observed, these neoplasms were not considered to be compound-related. In addition, the increased incidences observed in the low dose male mice and the high dose female mice were not statistically significant when compared to controls.

Under the series of pathology protocols in effect during the course of this bioassay, not all grossly normal tissues were examined histopathologically. The incidences presented in this discussion and the statistical analyses presented in Tables 3, 4, 5, and 6 are based on the number of tissues histopathologically examined. According to

these statistical analyses, no significant increases in tumor incidence were observed in treated rats or mice, although sometimes the sample sizes were quite low. If it is assumed that no tumors ocurred in the grossly normal tissues which were not examined histopathologically, then the statistical analyses can be based on the total number of tissues necropsied. This lowers the percentage of animals with tumors in each group, but increases the sample size. Statistical analyses performed on the basis of total number of animals necropsied indicated no significant increase in tumor incidence related to PCNB administration.

In an earlier study (Innes et al., 1969), orally administered PCNB was found to be tumorigenic in male B6C3F1 mice, producing an increased incidence of hepatomas. The dosage level giving positive results in the Innes et al. study, 1206 ppm in the diet, was considerably less than the dosage levels used in this bioassay (time-weighted average low and high concentrations of, respectively, 2606 and 5213 ppm for males and 4093 and 8187 ppm for females). The reasons that results of the Innes et al. study differed from results of this bioassay are not readily apparent. A possible cause of the difference is that PCNB treatment began at an earlier age in the Innes et al. study. Dosing by Innes et al. began at the age of 7 days and was performed by gastric intubation (464 mg/kg body weight/day) until the mice were weaned at the age of 4 weeks at which time PCNB was administered in the diet. In this bioassay PCNB was only administered in

the diet, beginning at 6 weeks of age. In the Innes et al. study, the term "hepatoma" was used for all hepatic tumors except for those rare cases where the hepatic tumor was accompanied by unmistakable pulmonary metastases (U.S. Department of Health, Education and Welfare, 1969). The fact that these NCI analyses were based on hepatocellular carcinomas would not account for differences in the final study, since no hepatocellular tumors except carcinomas were reported in this study. Other carcinogenicity studies, including a feeding study in rats (Finnegan et al., 1958) and a skin painting study in mice (Searle, 1966) were either inadequately reported or inconclusive (International Agency for Research on Cancer, 1974).

It is concluded that under the conditions of this bioassay PCNB was not demonstrated to be carcinogenic in either Osborne-Mendel rats or B6C3F1 mice.

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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, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Pentachloronitrobenzene was reviewed.

The primary reviewer commented that adjustments in exposure levels had to be made during the chronic phase due to overt toxicity. He concurred with the conclusion in the report that Pentachloronitrobenzene (PCNB) was not carcinogenic in rats or mice, under the conditions of test. There was no observation of rare or unusual tumors or statistically significant associations between the incidence of neoplasms and treatment.

The secondary reviewer also agreed with the conclusion given in the report. He pointed out, however, that the survival among the male mice was relatively poor and the size of the control groups were too small.

It was moved that the bioassay report be accepted as written. The motion was seconded and approved unanimously.

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 K. 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 PCNB

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

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	43	37
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(48)	(49)
FIBROMA	1 (5%)	3 (6%)	2 (4%)
FIBROSARCOMA		1 (2%)	
LIPOMA		1 (2%)	
HEMANGIOSARCOMA	1 (5%)	1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(48)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	1 (2%)
GRANULOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(20)	(42)	(35)
HEMANGIOSARCOMA			2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		1 (3%)
#KIDNEY	(20)	(43)	(37)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(43)	(36)
HEPATOCELLULAR CARCINOMA		1 (2%)	

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
#PANCREAS	(20)	(43)	(36)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(43)	(37)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
MIXED TUMOR, MALIGNANT		3 (7%)	1 (3%)
HEMANGIOSARCOMA			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(42)	(37)
CHROMOPHOBE ADENOMA	2 (10%)	3 (7%)	7 (19%)
#ADRENAL	(20)	(43)	(37)
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(20)	(43)	(37)
FOLLICULAR-CELL ADENOMA	1 (5%)	2 (5%)	2 (5%)
FOLLICULAR-CELL CARCINOMA	1 (5%)		
C-CELL ADENOMA	1 (5%)	1 (2%)	
C-CELL CARCINOMA		1 (2%)	1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(48)	(49)
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS		1 (2%)	2 (4%)
FIBROADENOMA	2 (10%)	4 (8%)	
*EPIDIDYMIS	(20)	(48)	(49)
MESOTHELIOMA, METASTATIC	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A1 (CONTINUED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(48)	(49)
LIPOMA		1 (2%)	1 (2%)
*MESENTERY	(20)	(48)	(49)
MESOTHELIOMA, MALIGNANT	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	10	28	18
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	21	32
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	20	18
TOTAL PRIMARY TUMORS	11	27	23
TOTAL ANIMALS WITH BENIGN TUMORS	5	13	10
TOTAL BENIGN TUMORS	7	17	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	10	11
TOTAL MALIGNANT TUMORS	4	10	11
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	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

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

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	40	34
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(48)
SQUAMOUS CELL CARCINOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(48)
FIBROMA	1 (5%)	2 (4%)	
FIBROSARCOMA		1 (2%)	
LIPOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(40)	(37)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	
FIBROSARCOMA, METASTATIC			1 (3%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#SPLEEN	(20)	(40)	(36)
HEMANGIOSARCOMA	1 (5%)	1 (3%)	1 (3%)
#LUNG	(20)	(40)	(37)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
URINARY SYSTEM			
#KIDNEY	(20)	(39)	(37)
ADENOCARCINOMA, NOS, METASTATIC		1 (3%)	
MIXED TUMOR, MALIGNANT			1 (3%)
#URINARY BLADDER	(19)		(33)
PAPILLOMA, NOS			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(40)	(35)
CHROMOPHOBE ADENOMA	7 (35%)	20 (50%)	8 (23%)
#ADRENAL	(20)	(39)	(36)
CORTICAL ADENOMA	1 (5%)		
#THYROID	(20)	(40)	(36)
FOLLICULAR-CELL CARCINOMA		1 (3%)	1 (3%)
C-CELL ADENOMA		1 (3%)	
C-CELL CARCINOMA		3 (8%)	
#PANCREATIC ISLETS	(20)	(40)	(36)
ISLET-CELL ADENOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROADENOMA	7 (35%)	9 (18%)	8 (17%)
#UTERUS	(19)	(39)	(37)
ADENOCARCINOMA, NOS		2 (5%)	
ENDOMETRIAL STROMAL POLYP	1 (5%)	2 (5%)	1 (3%)
#OVARY	(20)	(40)	(37)
CYSTADENOMA, NOS		1 (3%)	
SERTOLI-CELL TUMOR		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE A2 (CONTINUED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
MUSCULOSKELETAL SYSTEM			
*MUSCLE HIP/THIGH	(20)	(50)	(48)
FIBROSARCOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(48)
LIPOMA		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	13	11	16
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	7	38	33
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	34	20
TOTAL PRIMARY TUMORS	19	51	23
TOTAL ANIMALS WITH BENIGN TUMORS	13	29	16
TOTAL BENIGN TUMORS	17	40	18
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	10	5
TOTAL MALIGNANT TUMORS	2	11	5
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH PCNB

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

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-M039
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	45	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	33	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(45)	(48)
FIBROMA			1 (2%)
FIBROSARCOMA	2 (10%)	3 (6%)	5 (10%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(45)	(48)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		2 (4%)
#MESENTERIC L. NODE	(13)	(20)	(34)
HEMANGIOSARCOMA	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(35)	(42)
HEPATOCELLULAR CARCINOMA	2 (10%)	8 (23%)	4 (10%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
NONE			

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

TABLE B1 (CONTINUED)

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-M039
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
#BRAIN	(20)	(35)	(41)
EPENDYMOMA		1 (3%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	16	24	33
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	4	25	17
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1 (CONCLUDED)

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-M039
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	11	12
TOTAL PRIMARY TUMORS	7	14	12
TOTAL ANIMALS WITH BENIGN TUMORS			1
TOTAL BENIGN TUMORS			1
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	11	11
TOTAL MALIGNANT TUMORS	7	14	11
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 B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH PCNB

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	1
ANIMALS NECROPSIED	20	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	23	21
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(47)	(46)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(23)	(20)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (5%)
OSTEOSARCOMA, METASTATIC			1 (5%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		5 (11%)
#SPLEEN	(20)	(23)	(20)
HEMANGIOSARCOMA	1 (5%)	1 (4%)	1 (5%)
#MESENTERIC L. NODE	(20)	(20)	(19)
FIBROSARCOMA, METASTATIC			1 (5%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(14)	(20)
HEPATOCELLULAR CARCINOMA			3 (15%)
URINARY SYSTEM			
NONE			

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

TABLE B2 (CONTINUED)

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(47)	(46)
ADENOMA, NOS		2 (4%)	
ADENOCARCINOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	5	10
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	43	39
ANIMAL MISSING		2	1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2 (CONCLUDED)

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	5	10
TOTAL PRIMARY TUMORS	2	5	11
TOTAL ANIMALS WITH BENIGN TUMORS		2	1
TOTAL BENIGN TUMORS		2	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	3	9
TOTAL MALIGNANT TUMORS	2	3	10
TOTAL ANIMALS WITH SECONDARY TUMORS#			2
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 PCNB

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

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	43	37
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(48)	(49)
ABSCESS, NOS	1 (5%)		
RESPIRATORY SYSTEM			
*ACCESSORY SINUS	(20)	(48)	(49)
INFLAMMATION, NOS		1 (2%)	
#LUNG	(20)	(43)	(37)
PNEUMONIA, CHRONIC MURINE	8 (40%)	11 (26%)	9 (24%)
HYPERPLASIA, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(43)	(37)
METAMORPHOSIS FATTY		1 (2%)	
*SPLEEN	(20)	(42)	(35)
HEMATOPOIESIS	1 (5%)	1 (2%)	
#MESENTERIC L. NODE	(19)	(42)	(36)
INFLAMMATION, NOS			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(20)	(43)	(37)
THROMBOSIS, NOS	1 (5%)		
CALCIUM DEPOSIT	3 (15%)	2 (5%)	

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
HYPERPLASIA, NOS		1 (2%)	
#MYOCARDIUM	(20)	(43)	(37)
INFLAMMATION, NOS			3 (8%)
DEGENERATION, NOS	3 (15%)	2 (5%)	3 (8%)
#ENDOCARDIUM	(20)	(43)	(37)
INFLAMMATION, NOS			1 (3%)
*AORTA	(20)	(48)	(49)
THROMBOSIS, NOS	1 (5%)		
ARTERIOSCLEROSIS, NOS	2 (10%)	5 (10%)	
CALCIUM DEPOSIT	1 (5%)		
*MESENTERIC ARTERY	(20)	(48)	(49)
THROMBOSIS, NOS		2 (4%)	
CALCIUM DEPOSIT	1 (5%)		
DIGESTIVE SYSTEM			
#LIVER	(20)	(43)	(36)
THROMBUS, ORGANIZED			1 (3%)
INFLAMMATION, NOS		2 (5%)	1 (3%)
PELIOSIS HEPATIS		1 (2%)	
METAMORPHOSIS FATTY		4 (9%)	
*BILE DUCT	(20)	(48)	(49)
HYPERPLASIA, NOS		1 (2%)	
#STOMACH	(20)	(43)	(37)
ULCER, FOCAL		2 (5%)	2 (5%)
CALCIUM DEPOSIT	2 (10%)	5 (12%)	
#COLON	(20)	(42)	(37)
PARASITISM		1 (2%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(20)	(43)	(37)
CYST, NOS		2 (5%)	
PYELONEPHRITIS, NOS	1 (5%)		
INFLAMMATION, CHRONIC	10 (50%)	27 (63%)	15 (41%)
CALCIUM DEPOSIT	2 (10%)	5 (12%)	1 (3%)

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

TABLE C1 (CONTINUED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(42)	(37)
CYST, NOS	1 (5%)	3 (7%)	1 (3%)
#THYROID	(20)	(43)	(37)
CYST, NOS	1 (5%)	2 (5%)	
FOLLICULAR CYST, NOS	1 (5%)		
HYPERPLASIA, C-CELL	1 (5%)		
HYPERPLASIA, FOLLICULAR-CELL	3 (15%)	1 (2%)	
#PARATHYROID	(1)	(6)	
HYPERPLASIA, NOS	1 (100%)	6 (100%)	
REPRODUCTIVE SYSTEM			
#PROSTATE	(17)	(38)	(33)
INFLAMMATION, NOS		3 (8%)	2 (6%)
#TESTIS	(20)	(43)	(37)
CALCIUM DEPOSIT		1 (2%)	
ATROPHY, NOS	7 (35%)	15 (35%)	8 (22%)
HYPERPLASIA, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(43)	(37)
INFLAMMATION, NOS			1 (3%)
SPECIAL SENSE ORGANS			
*EYE	(20)	(48)	(49)
INFLAMMATION, NOS	1 (5%)		
CATARACT		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(48)	(49)
INFLAMMATION, NOS			1 (2%)

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

TABLE C1 (CONCLUDED)

	CONTROL (VEH) 01-M041	LOW DOSE 01-M042	HIGH DOSE 01-M043
*PERICARDIUM	(20)	(48)	(49)
INFLAMMATION, NOS			1 (2%)
*MESENTERY	(20)	(48)	(49)
PERIARTERITIS		5 (10%)	2 (4%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	3	5
NECROPSY PERF/NO HISTO PERFORMED		4	11
AUTO/NECROPSY/NO HISTO		1	1
AUTOLYSIS/NO NECROPSY		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 TREATED WITH PCNB

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	40	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*ACCESSORY SINUS	(20)	(50)	(48)
INFLAMMATION, NOS		2 (4%)	
#LUNG	(20)	(40)	(37)
PNEUMONIA, CHRONIC MURINE	2 (10%)	2 (5%)	3 (8%)
CALCIUM DEPOSIT	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(40)	(37)
METAMORPHOSIS FATTY		3 (8%)	2 (5%)
#SPLEEN	(20)	(40)	(36)
ANGIECTASIS		1 (3%)	
HEMATOPOIESIS	1 (5%)	7 (18%)	
#CERVICAL LYMPH NODE	(20)	(38)	(31)
INFLAMMATION, NOS		1 (3%)	
CIRCULATORY SYSTEM			
#HEART	(20)	(40)	(37)
THROMBUS, ORGANIZED			2 (5%)
CALCIUM DEPOSIT	1 (5%)	1 (3%)	2 (5%)
#MYOCARDIUM	(20)	(40)	(37)
DEGENERATION, NOS	1 (5%)	1 (3%)	

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
*AORTA	(20)	(50)	(48)
ARTERIOSCLEROSIS, NOS	2 (10%)		
DIGESTIVE SYSTEM			
*GUM	(20)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
#LIVER	(20)	(40)	(37)
INFLAMMATION, NOS		1 (3%)	
INFLAMMATION, FOCAL		1 (3%)	
METAMORPHOSIS FATTY		4 (10%)	1 (3%)
FOCAL CELLULAR CHANGE		1 (3%)	
#STOMACH	(20)	(40)	(37)
ULCER, NOS		1 (3%)	
CALCIUM DEPOSIT	2 (10%)	1 (3%)	
#COLON	(20)	(40)	(37)
PARASITISM		2 (5%)	
URINARY SYSTEM			
#KIDNEY	(20)	(39)	(37)
INFLAMMATION, CHRONIC	4 (20%)	5 (13%)	5 (14%)
CALCIUM DEPOSIT		1 (3%)	1 (3%)
#URINARY BLADDER	(19)		(33)
INFLAMMATION, CHRONIC			1 (3%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(40)	(35)
CYST, NOS	1 (5%)		
#ADRENAL	(20)	(39)	(36)
CYST, NOS		2 (5%)	
ANGIECTASIS	1 (5%)	3 (8%)	
#PARATHYROID	(1)	(1)	
HYPERPLASIA, NOS	1 (100%)	1 (100%)	

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

TABLE C2 (CONTINUED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(48)
GALACTOCELE		1 (2%)	
*VAGINA	(20)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
#UTERUS	(19)	(39)	(37)
HYDROMETRA		2 (5%)	5 (14%)
INFLAMMATION, NOS	1 (5%)	4 (10%)	2 (5%)
#UTERUS/ENDOMETRIUM	(19)	(39)	(37)
HYPERPLASIA, CYSTIC			1 (3%)
#OVARY	(20)	(40)	(37)
CYST, NOS			1 (3%)
INFLAMMATION, NOS	1 (5%)		
ABSCESS, NOS		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(20)	(50)	(48)
INFLAMMATION, NOS	1 (5%)		
MUSCULOSKELETAL SYSTEM			
*MUSCLE HIP/THIGH	(20)	(50)	(48)
CALCIUM DEPOSIT		1 (2%)	
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(48)
INFLAMMATION, NOS		1 (2%)	
*MESENTERY	(20)	(50)	(48)
PERIARTERITIS	2 (10%)		2 (4%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (VEH) 01-F041	LOW DOSE 01-F044	HIGH DOSE 01-F045
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	1	8
ANIMAL MISSING/NO NECROPSY			1
NECROPSY PERF/NO HISTO PERFORMED		10	11
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO			3
AUTOLYSIS/NO NECROPSY			1

APPENDIX D

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

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

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-M039
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	45	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	33	42
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(45)	(48)
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(35)	(41)
PNEUMONIA, CHRONIC MURINE	1 (5%)	1 (3%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(33)	(39)
INFLAMMATION, NOS			1 (3%)
AMYLOIDOSIS	9 (45%)	3 (9%)	11 (28%)
HEMATOPOIESIS		5 (15%)	
#MESENTERIC L. NODE	(13)	(20)	(34)
INFLAMMATION, NOS		1 (5%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(35)	(41)
INFLAMMATION, NOS	2 (10%)	1 (3%)	
#ENDOCARDIUM	(20)	(35)	(41)
INFLAMMATION, NOS	2 (10%)		
DIGESTIVE SYSTEM			
#LIVER	(20)	(35)	(42)
CYST, NOS	1 (5%)		

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

TABLE D1 (CONTINUED)

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-MC39
INFLAMMATION, NOS		1 (3%)	
AMYLCIDOSIS	2 (10%)	1 (3%)	
METAMORPHOSIS FATTY	1 (5%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)
#PANCREATIC DUCT	(20)	(30)	(38)
CYST, NOS			1 (3%)
#COLON	(18)	(33)	(36)
PARASITISM	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(20)	(35)	(42)
HYDRONEPHROSIS	1 (5%)		
CYST, NOS	1 (5%)		1 (2%)
POLYCYSTIC KIDNEY	1 (5%)		
PYELONEPHRITIS, NOS	2 (10%)	1 (3%)	2 (5%)
INFLAMMATION, CHRONIC	4 (20%)	3 (9%)	12 (29%)
AMYLCIDOSIS	4 (20%)	1 (3%)	7 (17%)
CALCIUM DEPOSIT	1 (5%)		1 (2%)
#URINARY BLADDER	(20)	(35)	(42)
CYST, NOS	1 (5%)		
INFLAMMATION, NOS	1 (5%)		
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(18)	(31)	(34)
INFLAMMATION, NOS	1 (6%)		
*SEMINAL VESICLE	(20)	(45)	(48)
INFLAMMATION, NOS	1 (5%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL (VEH) 02-M037	LOW DOSE 02-M038	HIGH DOSE 02-M039
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	12	13
ACCIDENTAL DEATH		1	
NECROPSY PERF/NO HISTO PERFORMED		11	6
AUTO/NECROPSY/HISTO PERF		3	2
AUTO/NECROPSY/NO HISTO		1	
AUTOLYSIS/NO NECROPSY		4	2

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

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	1
ANIMALS NECROPSIED	20	47	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	20	23	21
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(23)	(20)
PNEUMONIA, CHRONIC MURINE		1 (4%)	2 (10%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(23)	(20)
AMYLOIDOSIS			1 (5%)
HEMATOPOIESIS	2 (10%)	2 (9%)	1 (5%)
#LYMPH NODE	(20)	(20)	(19)
INFLAMMATION, NOS			1 (5%)
#PANCREATIC L.NODE	(20)	(20)	(19)
INFLAMMATION, NOS	1 (5%)		
#MESENTERIC L. NODE	(20)	(20)	(19)
HYPERPLASIA, NOS		1 (5%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(23)	(20)
INFLAMMATION, NOS			1 (5%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(14)	(20)
INFLAMMATION, NOS			1 (5%)

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

TABLE D2 (CONTINUED)

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
*GALLBLADDER	(20)	(47)	(46)
INFLAMMATION, NOS			1 (2%)
#PANCREAS	(19)	(21)	(20)
INFLAMMATION, NOS			1 (5%)
#COLON	(20)	(20)	(18)
PARASITISM	1 (5%)		
URINARY SYSTEM			
#KIDNEY	(20)	(23)	(20)
PYELONEPHRITIS, NOS			1 (5%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(21)	(19)
HYDROMETRA	3 (15%)	1 (5%)	2 (11%)
INFLAMMATION, NOS	6 (30%)	7 (33%)	4 (21%)
#UTERUS/ENDOMETRIUM	(20)	(21)	(19)
HYPERPLASIA, CYSTIC	5 (25%)	4 (19%)	5 (26%)
#OVARY/OVIDUCT	(20)	(21)	(19)
INFLAMMATION, NOS	2 (10%)		
#OVARY	(20)	(21)	(18)
CYST, NOS	4 (20%)	1 (5%)	5 (28%)
INFLAMMATION, NOS	3 (15%)	4 (19%)	2 (11%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE	(20)	(47)	(46)
INFLAMMATION, NOS			1 (2%)

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

TABLE D2 (CONCLUDED)

	CONTROL (VEH) 02-F037	LOW DOSE 02-F040	HIGH DOSE 02-F041
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(47)	(46)
INFLAMMATION, NOS	2 (10%)	1 (2%)	3 (7%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	7	2
ANIMAL MISSING/NO NECROPSY		2	1
NECROPSY PERF/NO HISTO PERFORMED		24	25
AUTOLYSIS/NO NECROPSY		1	3

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

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