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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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CONTRIBUTORS: This report presents the results of the bioassay of chlorpropamide for possible carcinogenicity, conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI carcinogenesis bioassay program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and feeding of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[4]. The statistical analyses were performed by Dr. J. R. Joiner[5], using methods selected for the bioassay program by Dr. J. J. Gart[6]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[7], and the analytical results were reviewed by Dr. C. W. Jameson[5].

This report was prepared at Tracor Jitco under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. Marshall Steinberg[5], Director of the Bioassay Program; Drs. J. F. Robens[5] and R. W. Fogleman[5], toxicologists; Dr. R. L. Schueler[5], pathologist; Ms. L. A. Waitz[5] and Mr. W. D.

Reichardt[5], bioscience writers; and Dr. E. W. Gunberg[5], technical editor, assisted by Ms. Y. E. Presley[5] and Ms. P. J. Graboske[5].

The statistical analysis was reviewed by a member or members of the Mathematical Statistics and Applied Mathematics Section of NCI (Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone served as reviewers on an alternating basis).

The following scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

Dr. Kenneth C. Chu
Dr. Cipriano Cueto, Jr.
Dr. J. Fielding Douglas
Dr. Dawn G. Goodman
Dr. Richard A. Griesemer
Mr. Harry A. Milman
Dr. Thomas W. Orme
Dr. Robert A. Squire[8]
Dr. Jerrold M. Ward

---

[1]Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama.

[2]Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[3]Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammond House Road, Valhalla, New York.

[4]EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

[5]Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

[6]Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[7]Midwest Research Institute, 425 Volker Boulevard, Kansas City, Missouri.

[8]Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of chlorpropamide for possible carcinogenicity was conducted by administering the test material in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were administered chlorpropamide as follows: rats 5 days per week for 103 to 105 weeks at 3,000 or 6,000 ppm, and mice 5 days per week for 34 weeks at 5,000 or 10,000 ppm, followed by 70 weeks at 2,500 or 5,000 ppm. The time-weighted average doses for mice were 3,317 ppm for low-dose males and females, and 6,635 ppm for high-dose males and females. Matched controls consisted of groups of 15 untreated rats and 15 untreated mice of each sex. All surviving rats and mice were killed at 103 to 105 weeks.

Mean body weights of both low- and high-dose rats were lower than those of the matched controls throughout the study. In mice, doses were reduced at week 34, due to early deaths in the high-dose groups; following this adjustment the treated mice gained weight, but the weights never reached those of the controls. Survival of the treated rats and the low-dose mice was adequate for meaningful statistical analyses of the incidences of tumors.

In both rats and mice, the incidences of tumors among the treated groups were not significantly increased in comparison with matched controls.

It is concluded that under the conditions of this bioassay, chlorpropamide was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

Chlorpropamide (CAS 94-20-2; NCI C01752) is an oral hypoglycemic agent of the arylsulfonylurea type. Structurally, it is a derivative of tolbutamide in which chlorine is substituted for the methyl group on the aryl moiety, and n-propyl substituted for n-butyl on the urea moiety. These changes increase the duration of activity of chlorpropamide over that of other sulfonylurea hypoglycemic agents of this series (Goldstein et al., 1974). All of these compounds function by stimulating the secretion of insulin by the pancreas, and therefore, are used only in patients with at least minimal pancreatic function, as in maturity-onset diabetics (Larner and Haynes, 1975). Controlled studies have shown that the oral hypoglycemics may be no more effective than dietary modifications in controlling the symptoms of maturity-onset diabetes on a long-term basis, and may be associated with an increase in cardiovascular mortality (Shen and Bressler, 1977). Chlorpropamide was selected for testing in the carcinogenesis program because it is used extensively and for prolonged periods in humans.

II. MATERIALS AND METHODS

A. Chemical

Chlorpropamide (1-[(p-chlorophenyl)sulfonyl]-3-propylurea) was obtained in two batches (Lot Nos. 14603-17000 and 2Y419) from Pfizer Inc., New York, N.Y. According to the manufacturer, the purity of Lot No. 2Y419, which was used for the chronic study, met USP (U. S. Pharmacopeia, 1974) specifications and was > 99% pure. Analyses at Midwest Research Institute (melting point; elemental analysis; infrared, nuclear magnetic resonance, and ultraviolet spectrometry; and thin-layer chromatography) confirmed the identity of Lot No. 2Y419 and were consistent with the manufacturer's assay.

The batch used for the chronic study was stored in the original container at 5°C.

B. Dietary Preparation

Test diets containing chlorpropamide were prepared every 2 weeks by mixing a known amount of sifted chlorpropamide with a small amount of Wayne® Lab Blox animal meal (Allied Mills, Inc., Chicago, Ill.) in a portable mixer, then adding this mixture to the required amount of animal meal and mixing in a twin-shell blender for 10 minutes.

The prepared diets were stored at room temperature in sealed plastic containers.

The stability of chlorpropamide in feed was tested at the Midwest Research Institute by determining the concentration of chlorpropamide in formulated diets stored at room temperature (25°C) for a 2-week period. The test diets showed less than 5% change in concentration on standing at ambient temperature for this period.

C. Animals

For the subchronic study, female Sprague-Dawley rats and male Swiss mice were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

For the chronic study, Fischer 344 rats and B6C3F1 mice of both sexes, obtained through the Division of Cancer Treatment, National Cancer Institute, were supplied by Charles River Breeding Laboratories. The rats were 28 days of age and the mice were 34 days of age when received from the supplier. On arrival at the laboratory, all animals were quarantined for an acclimation period (rats for 11 days, mice for 16 days). Animals with no visible signs of disease were assigned to control and treated groups, and earmarked for individual identification.

D. Animal Maintenance

Animals were housed in temperature- and humidity-controlled rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. The air was changed 15 times per hour, and passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Food and water were supplied daily and available ad libitum.

All animals were housed five per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.), and cage tops were covered with disposable filter bonnets; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.) and cage tops were covered with disposable filter bonnets after 15 months on study. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with chlorpropamide were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
L-tryptophan (CAS 73-22-3)
N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea (tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)

estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
5-azacytidine (CAS 320-67-2)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR)
(CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester)
hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
(ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-
amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

Subchronic studies were conducted to estimate the maximum tolerated doses of chlorpropamide, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high doses") were determined for administration in the chronic studies. The drug was administered in feed to female Sprague-Dawley rats at doses of 1,200, 3,000, 6,000, 15,000, or 30,000 ppm, and to male Swiss mice at doses of 2,000, 5,000, 10,000, 25,000, or 50,000 ppm. Five animals of each species were tested at each dose, and 20 animals of each species were maintained as

untreated controls. Treated animals received the test diets 7 days per week for 45 days, and then were observed for an additional 45 days.

In rats, 4/5 animals that were treated at 30,000 ppm died by week 5. No deaths occurred in any of the other groups. By the end of the treatment period, body weight gain was markedly depressed in animals treated at 3,000 and 6,000 ppm, and body weights fell below pretreatment values in animals treated at 15,000 ppm. By the end of the study, body weight gains in all remaining treated groups were 78-88% of that of controls. No gross abnormalities were found in animals at necropsy. The low and high doses were set at 3,000 and 6,000 ppm for the chronic studies in both male and female rats.

In mice, all animals treated at 25,000 and 50,000 ppm died during the first week of the study. No animals receiving any lower dose died. Body weights in mice treated at 10,000 ppm showed no gain during the treatment period, but mean weight gain at the end of the study in this group was 88% of that of the untreated controls. At 2,000 and 5,000 ppm, weight gains in the treated groups were depressed during the treatment period, but increased to 81% and 88% of those of the controls, respectively, at the end of the study. No gross abnormalities were seen at necropsy. The

low and high doses were set at 5,000 and 10,000 ppm for the chronic studies in both male and female mice.

F. Designs of Chronic Studies

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

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and animals that were moribund were killed and necropsied, except for those dying prior to day 100, due, presumably, to toxicity of the test chemical. Rats and mice were weighed individually once every 2 weeks (rats for 63 weeks, mice for 62 weeks) and once every month for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and 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, mammary gland, prostate or uterus, testis or ovary,

Table 1. Design of Chlorpropamide Chronic Feeding Studies in Rats

Sex and Treatment Group	Initial No. of Animals[a]	Chlorpropamide in Diet[b] (ppm)	Time on Study	
			Treated (weeks)	Untreated (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	3,000	104-105	
High-Dose	35	6,000	103-104	
Female				
Matched-Control	15	0		105
Low-Dose	35	3,000	104-105	
High-Dose	35	6,000	104	

[a]All animals were 39 days of age when placed on study.

[b]The treated animals were fed test diets 5 days per week and control diets 2 days per week.

Table 2. Design of Chlorpropamide Chronic Feeding Studies in Mice

Sex and Treatment Group	Initial No. of Animals[a]	Chlorpropamide in Diet[b] (ppm)	Time On Study: Treated (weeks)	Time On Study: Untreated (weeks)	Time-Weighted Average Dose[c] (ppm)
Male					
Matched-Control	15	0		105	
Low-Dose	35	5,000	34		
		2,500[d]	70		3,317
High-Dose	35	10,000	34		
		5,000[d]	70		6,635
Female					
Matched-Control	15	0		105	
Low-Dose	35	5,000	34		
		2,500[d]	70		3,317
High-Dose	35	10,000	34		
		5,000[d]	70		6,635

[a]All animals were 50 days of age when placed on study.

[b]The treated animals were fed test diets 5 days per week and control diets 2 days per week.

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

[d]Doses were lowered at week 34 because of excessive mortality.

brain, and sensory organs. Peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances,

the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope

of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose 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 treated group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a

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

III. RESULTS - RATS

A. Clinical Signs and Body Weights (Rats)

A dose-related decrease in the mean body weights of both male and female rats, when compared with controls, was observed throughout the period of the bioassay (figure 1).

Rales were occasionally noted in a few rats of both sexes and in all groups, including the matched controls. During the second year, progressive weight loss, irritated eyelids, cutaneous erythema, alopecia, and paralysis of the hind legs were noted in one or more animals in the treated and control groups. Therefore, these observations were not considered to be related to treatment.

B. Survival (Rats)

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

In both sexes, the Tarone test results for positive dose-related trend are not significant; at least 80% of the rats of both sexes lived to the end of the study, providing sufficient numbers of

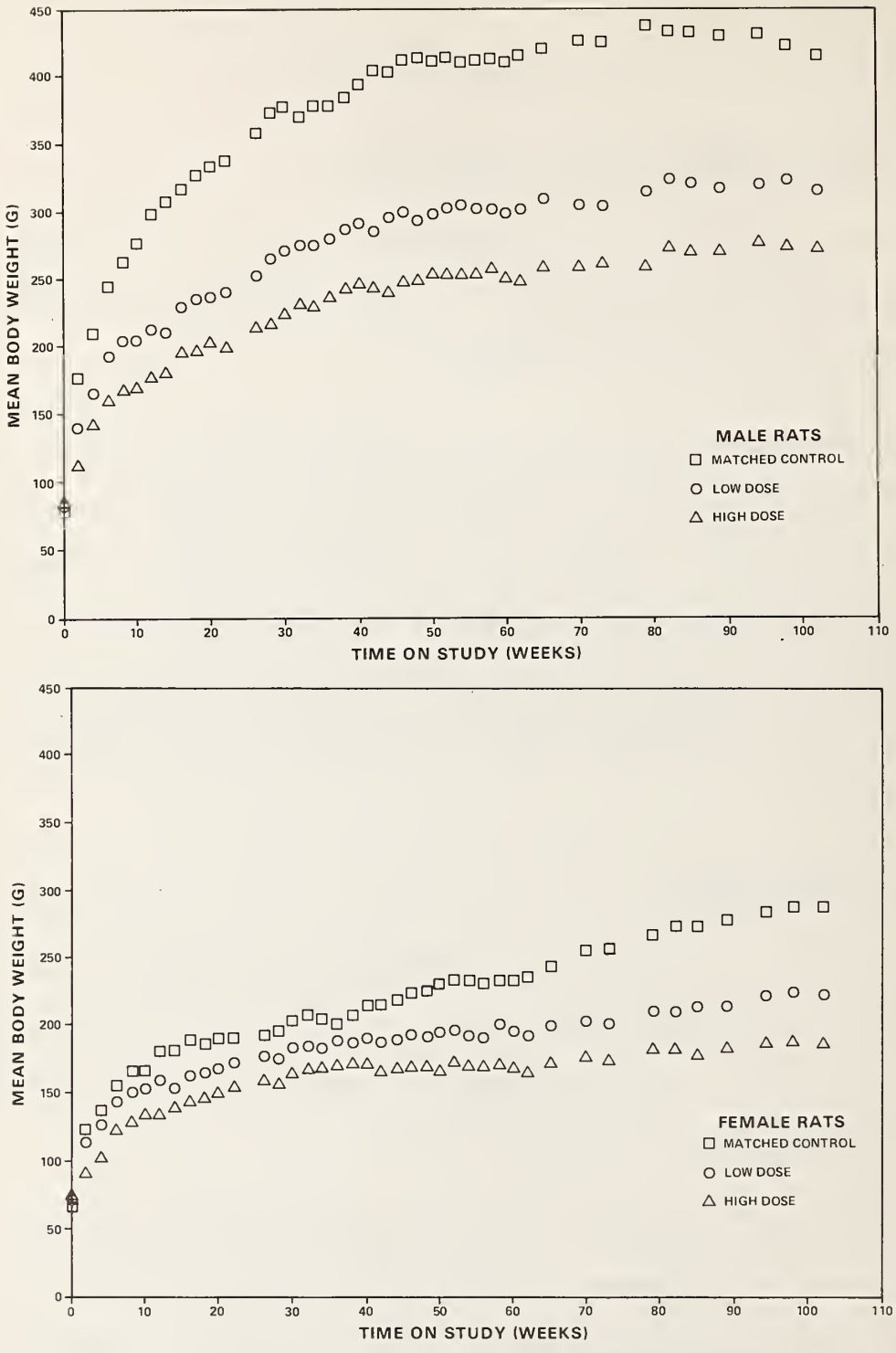
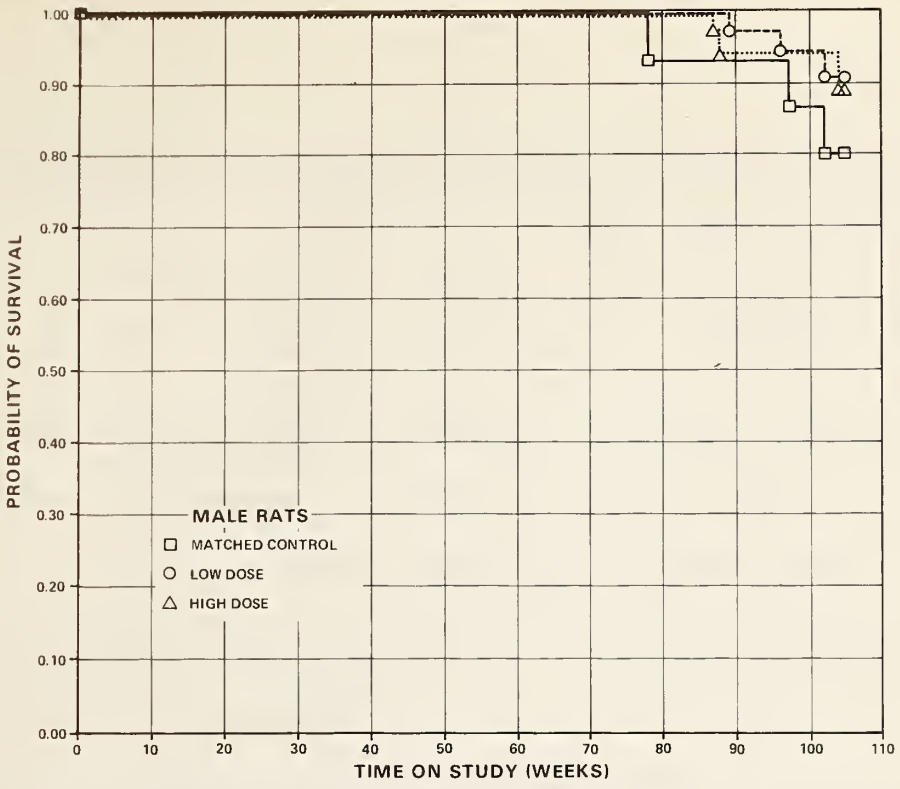


Figure 1. Growth Curves For Rats Fed Chlorpropamide In The Diet



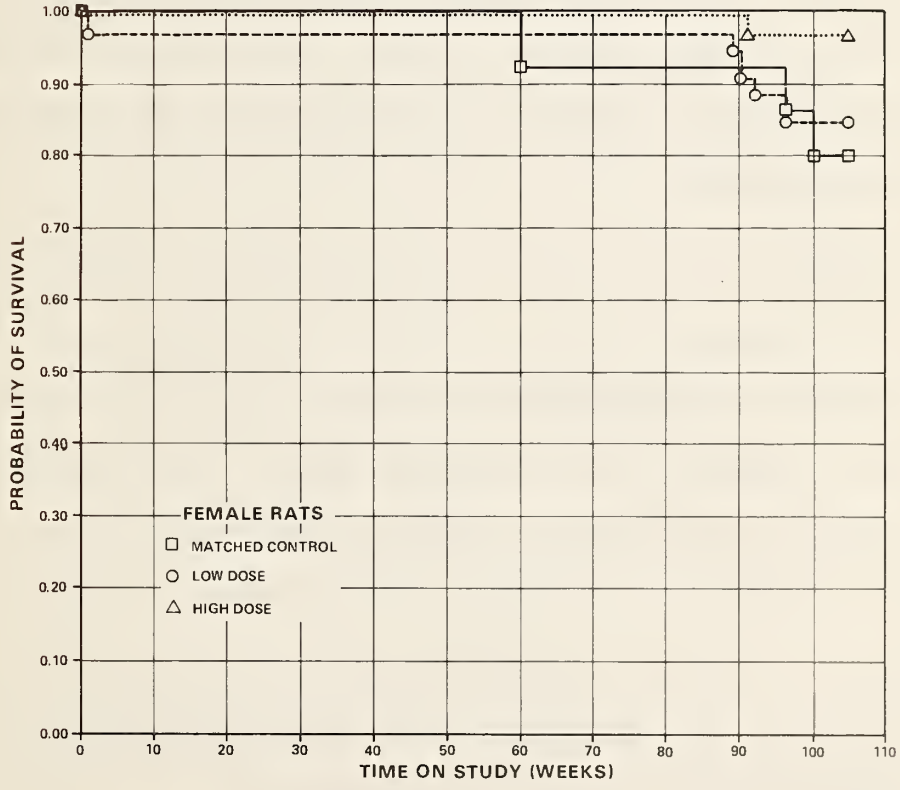


Figure 2. Survival Curves For Rats Fed Chlorpropamide In The Diet

animals for meaningful statistical analyses of the incidences of late-developing tumors.

C. Pathology (Rats)

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

A variety of neoplasms were observed in both the matched-control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in rats of treated groups as compared with controls. These lesions, however, either are not uncommon in this strain of rat independent of any treatment or occurred in insignificant numbers.

All neoplasms have been encountered previously as spontaneous lesions in Fischer 344 rats in this laboratory, with the exception of the endocardial sarcoma, NOS (not otherwise specified), observed in one low-dose female. The endocardial tumor consisted of pleomorphic spindle-shaped cells that had an elongated, basophilic nucleus and a moderate amount of eosinophilic cytoplasm. The tumor occupied most of the lumen of one ventricle. Few malignant tumors were observed, and tumor metastasis was observed in only one treated rat.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were encountered in animals of the treated and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged Fischer 344 rats. The pancreas was examined, and no lesions were found in this organ.

In the judgment of the pathologists, chlorpropamide did not appear to be carcinogenic in Fischer 344 rats under the conditions of this study.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more groups.

The results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of incidences between the matched-control group and each of the treated groups in the positive direction are not significant.

There are several indications of negative trend at tumor sites, in which the incidences in the control group exceed those observed in the treated groups. Such results are in the

incidence of interstitial-cell tumor of the testis in male rats and in the occurrences of tumors in the pituitary and mammary glands of female rats. These results cannot be accounted for on the basis of inadequate survival, since the survival rates of the treated and control groups are comparable.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or of a value less than one is included; this indicates the absence of significant positive results. It should also be noted that some of the intervals have an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by chlorpropamide, which could not be detected under the conditions of this test.

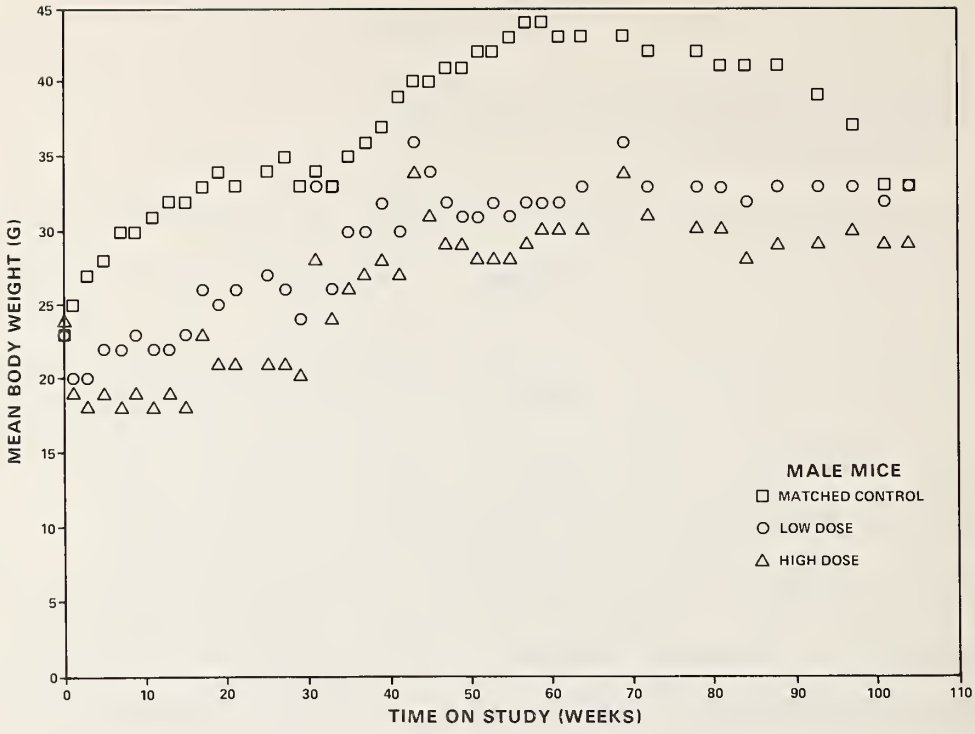
When the incidences of chromophobe adenoma or carcinoma of the pituitary are combined into a grouping for analysis, the incidences of carcinoma in both sexes are not included in tables E1 and E2, since the proportions in the treated groups of both sexes are less than 5%; however, a list of the incidences of each type of tumor is provided in tables A1 and A2 of Appendix A.

IV. RESULTS - MICE

A. Clinical Signs and Body Weights (Mice)

During the first 34 weeks of the study, both treated male and female mice failed to gain weight, and several deaths occurred, mostly among the high-dose animals (figure 3). After the doses of chlorpropamide were reduced, the treated animals gained weight, although their weights remained below those of the controls. Because of high mortality in the course of the bioassay, the mean body weights at any specific time do not represent a group of animals of the same composition as at earlier time periods. During the second year, progressive weight loss, alopecia, paralysis of the hind legs, dyspnea, cutaneous sores, and inactivity were noted in a few animals in all groups, including controls, but these observations were not considered to be related to treatment.

Piperazine at 4 g/liter was added to the drinking water for 3 or 4 days per week during weeks 29 to 31. In an effort to control respiratory disease, the animals were treated with oxytetracycline in the drinking water at 0.6 mg/ml for 5 days during week 42, followed by treatment for 5 days at 0.3 mg/ml. Beginning at week 41 and continuing for approximately 2 months, propylene



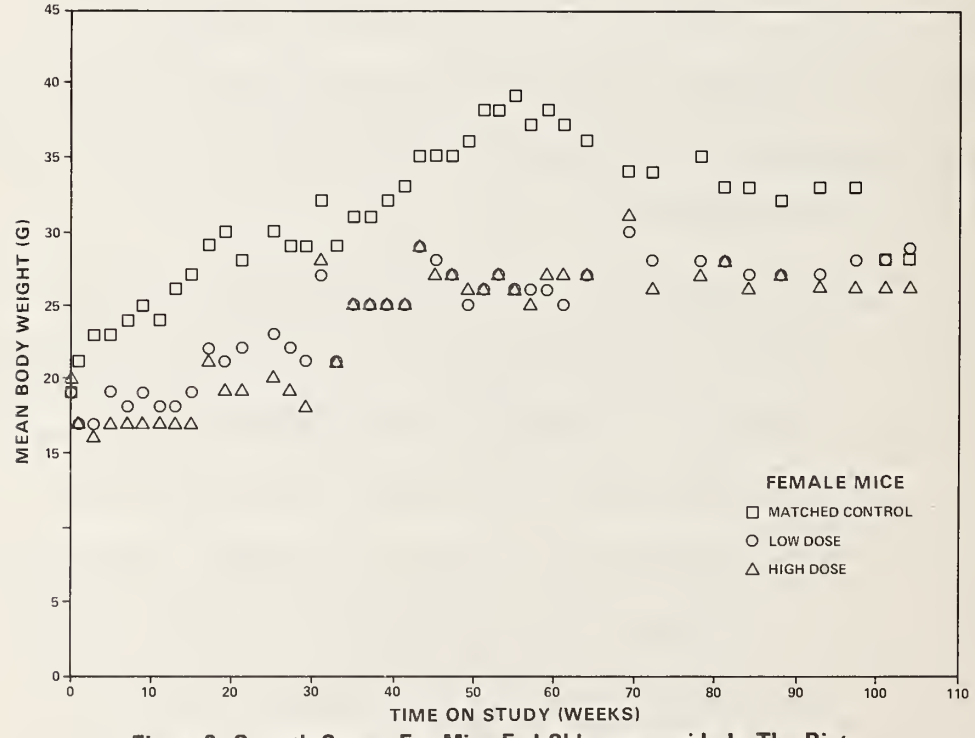


Figure 3. Growth Curves For Mice Fed Chlorpropamide In The Diet

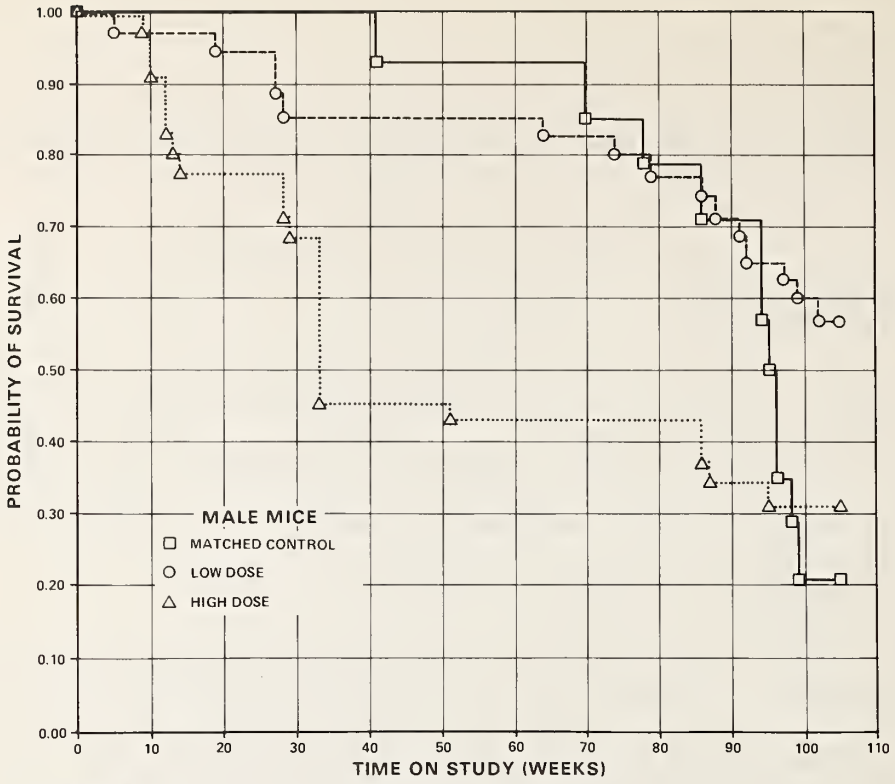
glycol vapor was also used in the mouse room to help control the disease.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice fed chlorpropamide in the diet at the doses of this experiment, together with those of the matched controls, are shown in figure 4.

In both sexes, the Tarone test results for positive dose-related linear trend over the three groups are not significant, but in male mice there is evidence of an increase in early mortality in the high-dose group, as evidenced by the difference between the low- and high-dose groups ($P = 0.043$). In male mice, 31% of the high-dose group, 57% of the low-dose group, and 21% of the matched controls lived to the end of the study. The median time on study for the high-dose male mice was 33 weeks, and 20/35 (57%) died during the first 52 weeks on study.

In female mice, 24% of the high-dose group, 60% of the low-dose group, and 27% of the matched controls lived to termination of the study. The median time on study for the high-dose female mice was 76 weeks; 7/35 (20%) died during the first 52 weeks on study.



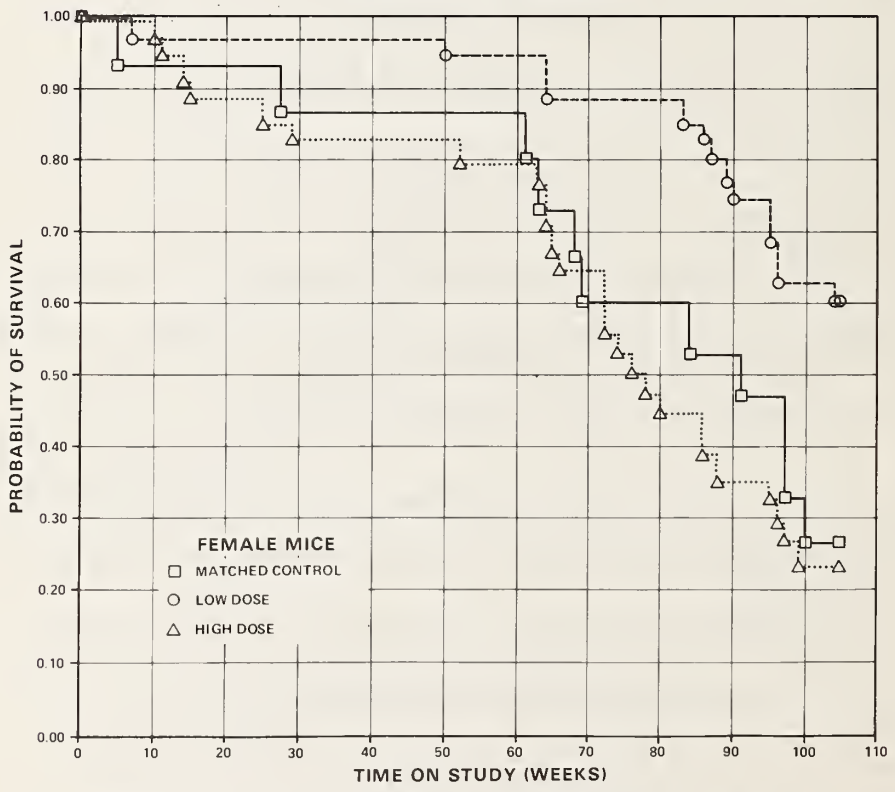


Figure 4. Survival Curves For Mice Fed Chlorpropamide In The Diet

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

A variety of neoplasms were observed in both the matched-control and treated groups. Some types of neoplasms occurred only, or with a greater frequency, in mice of treated groups as compared with controls. The lesions, however, either are not uncommon in this strain of mouse independent of any treatment or occurred in insignificant numbers.

The incidence of neoplasms in the treated mice was not greater than that in the control groups. All neoplasms have been encountered previously as spontaneous lesions in B6C3F1 mice, except for the testicular carcinoma, NOS, observed in one low-dose male. This lesion consisted of cells grouped into a lobular pattern, with the lobules being separated by a fine network of connective tissue and capillaries. The neoplastic cells had a large, vesicular to lightly basophilic nucleus, and cell boundaries could not be delineated. Tumor cells had infiltrated the vascular tunic, and multiple metastases were observed in the mesentery and an inguinal lymph node.

In addition to the neoplastic lesions, a number of degenerative,

proliferative, and inflammatory changes were encountered in animals of the treated and control groups (Appendix D). These nonneoplastic lesions are commonly seen in aged B6C3F1 mice; however, the severe chronic and subchronic suppurative bronchopneumonias were associated with decreased life spans and increased deaths in many mice. Bronchopneumonia was a problem especially in the high-dose females and both matched-control groups. The pancreas was examined, and no lesions were found in this organ.

Few histopathologic findings were observed in the high-dose male mice, due to early deaths which may have been related to chemical toxicity.

Chlorpropamide, in the judgment of the pathologists, did not appear to be carcinogenic when fed to B6C3F1 mice at the low dose (0.5 and 0.25%) for 24 months. The carcinogenic activity in the high-dose males (1.0 and 0.5%) could not be assessed, due to reduced life spans which probably resulted from chemical toxicity and suppurative bronchopneumonia.

D. Statistical Analyses of Results (Mice)

Tables F1-F3 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals in one group and with an incidence of at least 5% in one or more groups. Since there was a high mortality rate

among the high-dose male mice, time-adjusted analyses were performed, eliminating animals that died before week 52 on study (table F2).

The results of the Cochran-Armitage test for positive dose-related trend and of the Fisher exact test for direct comparison of incidences between the matched-control group and each of the treated groups in female and male mice (both time-adjusted and non-adjusted) are not significant.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by chlorpropamide, which could not be detected under the conditions of this test.

V. DISCUSSION

In this bioassay, mean body weights of both male and female treated rats were lower than those of the corresponding matched controls throughout the study. Survival was not affected by the treatment, however, and adequate numbers of rats survived for meaningful statistical analyses of the incidences of tumors.

In mice, the doses were sufficiently toxic to cause early deaths in the high-dose groups. Doses were reduced in both low- and high-dose groups at week 34; however, mean body weights continued to be depressed, indicating that the lower doses also were toxic. There was a significant ($P = 0.043$) difference between survival in the low- and high-dose groups, and survival at the low dose was satisfactory for meaningful statistical analyses of the incidences of tumors in these mice.

In both rats and mice, the incidences of tumors among the treated groups were comparable to those among the corresponding matched controls.

The acute oral LD_{50} of chlorpropamide was variously reported to be 0.9 or 2.4 g/kg in rats and 0.7 or 1.7 g/kg in mice (Root et al., 1959; Schneider et al., 1959). Chlorpropamide fed at up to 500 mg/kg for 1 year to rats reduced weight gain (Schneider et al., 1959). In humans, chlorpropamide is rapidly absorbed from

the gastrointestinal tract and slowly excreted unchanged (Larner et al., 1975).

It is concluded that under the conditions of this bioassay, chlorpropamide was not carcinogenic for Fischer 344 rats or B6C3F1 mice.

VI. BIBLIOGRAPHY

Armitage, P., Statistical Methods in Medical Research, John Wiley & Sons, Inc., New York, 1971, pp. 362-365.

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

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

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

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

Goldstein, A., Aronow, L., and Kalman, S. M., Methods of developing new drugs. In: Principles of Drug Action: The Basis of Pharmacology, John Wiley & Sons, New York, 1974, pp. 770-772.

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

Larner, J. and Haynes, R. C., Jr., Insulin and oral hypoglycemic drugs; glucagon. In: The Pharmacological Basis of Therapeutics, Goodman, L. S. and Gilman, A., eds., Macmillan Publishing Co., Inc., New York, 1975, pp. 1520-1523.

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

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

Root, M. A., Sigal, M. V., Jr., and Anderson, R. C., Pharmacology of 1-(p-chlorobenzenesulfonyl)-3-n-propylurea (chlorpropamide). Diabetes 8(1):7-13, 1959.

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

Schneider, J. A., Salgado, E. D., Jaeger, D., and Delahunt, C., The pharmacology of chlorpropamide. Ann. N. Y. Acad. Sci. 74(3):427-442, 1959.

Shen, S. W. and Bressler, R., Medical Intelligence; Clinical pharmacology of oral antidiabetic agents (first of two parts). New Engl. J. Med. 296(14):493-496, 1977.

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

U.S. Pharmacopeia, Chlorpropamide. United States Pharmacopeia XVIII, U. S. Pharmacopeial Convention, Inc., Rockville, Md., 1974, p. 127.

APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	35
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(35)	(35)
SQUAMOUS CELL PAPILLOMA		1 (3%)	
*SUBCUT TISSUE	(14)	(35)	(35)
FIBROMA	1 (7%)		
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(35)	(35)
UNDIFFERENTIATED LEUKEMIA	1 (7%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(31)	(32)
CHROMOPHOBE ADENOMA	1 (8%)		1 (3%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CHROMOPHOBE CARCINOMA	1 (8%)		
#ADRENAL	(14)	(35)	(35)
PHEOCHROMOCYTOMA	1 (7%)		
PHEOCHROMOCYTOMA, MALIGNANT	1 (7%)	1 (3%)	
#THYROID	(14)	(35)	(35)
FOLLICULAR-CELL CARCINOMA		1 (3%)	
C-CELL ADENOMA	1 (7%)	1 (3%)	2 (6%)
C-CELL CARCINOMA	1 (7%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(14)	(35)	(35)
INTERSTITIAL-CELL TUMOR	13 (93%)	33 (94%)	11 (31%)
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. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2		1
MORIBUND SACRIFICE	1	3	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	32	32
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	34	14
TOTAL PRIMARY TUMORS	21	37	14
TOTAL ANIMALS WITH BENIGN TUMORS	13	33	14
TOTAL BENIGN TUMORS	17	35	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	2	
TOTAL MALIGNANT TUMORS	4	2	
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	35
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(34)	(35)
SARCOMA, NOS		1 (3%)	
FIBROMA	1 (7%)		
FIBROSARCOMA		1 (3%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(34)	(35)
UNDIFFERENTIATED LEUKEMIA		2 (6%)	1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(34)	(35)
FIBROMA	1 (7%)		
#ENDOCARDIUM	(15)	(34)	(35)
SARCOMA, NOS		1 (3%)	
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(32)	(31)
CHROMOPHOBE ADENOMA	3 (30%)	4 (13%)	
CHROMOPHOBE CARCINOMA	2 (20%)	1 (3%)	
#ADRENAL	(15)	(34)	(35)
PHEOCHROMOCYTOMA			1 (3%)
#THYROID	(15)	(34)	(35)
FOLLICULAR-CELL CARCINOMA			2 (6%)
C-CELL ADENOMA	2 (13%)	5 (15%)	1 (3%)
C-CELL CARCINOMA		2 (6%)	3 (9%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(34)	(35)
FIBROADENOMA	5 (33%)	2 (6%)	
#UTERUS	(15)	(34)	(35)
ADENOCARCINOMA, NOS	1 (7%)	1 (3%)	
ENDOMETRIAL STROMAL POLYP	2 (13%)	7 (21%)	1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(34)	(35)
SARCOMA, NOS, METASTATIC		1 (3%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@		4	1
MORIBUND SACRIFICE	3	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	30	34
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	21	8
TOTAL PRIMARY TUMORS	17	27	9
TOTAL ANIMALS WITH BENIGN TUMORS	11	16	3
TOTAL BENIGN TUMORS	14	18	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	9	5
TOTAL MALIGNANT TUMORS	3	9	6
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

MICE FED CHLORPROPAMIDE IN THE DIET

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	13	34	25
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	34	25
INTEGUMENTARY SYSTEM			
*SKIN	(13)	(34)	(25)
SQUAMOUS CELL PAPILLOMA			1 (4%)
*SUBCUT TISSUE	(13)	(34)	(25)
FIBROSARCOMA			1 (4%)
RESPIRATORY SYSTEM			
#LUNG	(13)	(34)	(25)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
#BRAIN	(13)	(34)	(25)
MALIGNANT RETICULOSIS		1 (3%)	1 (4%)
*TRIGEMINAL GANGLION	(13)	(34)	(25)
MALIGNANT RETICULOSIS		1 (3%)	1 (4%)
*MULTIPLE ORGANS	(13)	(34)	(25)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (8%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(25)
HEPATOCELLULAR ADENOMA	1 (8%)	2 (6%)	1 (4%)
HEPATOCELLULAR CARCINOMA	1 (8%)	1 (3%)	1 (4%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#THYROID	(10)	(34)	(25)
•FOLLICULAR-CELL ADENOMA	1 (10%)		
REPRODUCTIVE SYSTEM			
#TESTIS	(13)	(33)	(24)
CARCINOMA,NOS		1 (3%)	
INTERSTITIAL-CELL TUMOR		1 (3%)	
INTERSTITIAL-CELL TUMOR, MALIGNA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(13)	(34)	(25)
CARCINOMA, NOS, METASTATIC		1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	6	7	11
MORIBUND SACRIFICE	5	8	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED	1		
TERMINAL SACRIFICE	3	20	11
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	8	5
TOTAL PRIMARY TUMORS	4	9	6
TOTAL ANIMALS WITH BENIGN TUMORS	2	4	2
TOTAL BENIGN TUMORS	2	4	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	4	3
TOTAL MALIGNANT TUMORS	2	5	4
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		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

TABLE B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING			1
ANIMALS NECROPSIED	14	33	28
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	33	28
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(31)	(28)
ALVEOLAR/BRONCHIOLAR ADENOMA		3 (10%)	2 (7%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(33)	(28)
HEPATOCELLULAR ADENOMA		4 (12%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(12)	(30)	(23)
CHROMOPHOBE ADENOMA	1 (8%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#UTERUS	(14)	(33)	(27)
HEMANGIOMA		1 (3%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(14)	(33)	(28)
SARCOMA, NOS		1 (3%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	4	9
MORIBUND SACRIFICE	8	10	17
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	4	21	8
ANIMAL MISSING			1

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	1	9	2
TOTAL PRIMARY TUMORS	1	9	2
TOTAL ANIMALS WITH BENIGN TUMORS	1	8	2
TOTAL BENIGN TUMORS	1	8	2
TOTAL ANIMALS WITH MALIGNANT TUMORS		1	
TOTAL MALIGNANT TUMORS		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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS FED CHLORPROPAMIDE IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	35
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(35)	(35)
INFLAMMATION, SUPPURATIVE	1 (7%)	11 (31%)	9 (26%)
INFLAMMATION, CHRONIC	1 (7%)	3 (9%)	1 (3%)
#LUNG	(14)	(35)	(35)
PNEUMONIA, CHRONIC MURINE		2 (6%)	2 (6%)
HYPERPLASIA, ALVEOLAR EPITHELIUM			1 (3%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(35)	(35)
CYTOPLASMIC VACUOLIZATION	1 (7%)		
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(35)
HEMORRHAGE, CHRONIC			1 (3%)
INFLAMMATION, CHRONIC	13 (93%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(14)	(35)	(35)
STEATITIS		1 (3%)	2 (6%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	16
AUTOLYSIS/NO NECROPSY	1		

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

TABLE C2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	34	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	34	35
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(34)	(35)
INFLAMMATION, SUPPURATIVE	3 (20%)	11 (32%)	2 (6%)
INFLAMMATION, CHRONIC	2 (13%)		
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#STOMACH	(15)	(34)	(35)
INFLAMMATION, SUPPURATIVE	1 (7%)		
URINARY SYSTEM			
#KIDNEY	(15)	(34)	(35)
HYDRONEPHROSIS	1 (7%)		
INFLAMMATION, CHRONIC	10 (67%)		
#URINARY BLADDER	(15)	(33)	(35)
INFLAMMATION, NECROTIZING		1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(10)	(32)	(31)
HEMORRHAGE		2 (6%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(15)	(34)	(35)
INFLAMMATION, CHRONIC		1 (3%)	
HYPERPLASIA, CYSTIC		1 (3%)	
#CERVIX UTERI	(15)	(34)	(35)
INFLAMMATION, SUPPURATIVE	1 (7%)		
#UTERUS/ENDOMETRIUM	(15)	(34)	(35)
INFLAMMATION, SUPPURATIVE	4 (27%)	1 (3%)	
INFLAMMATION, CHRONIC SUPPURATIV	2 (13%)		
#OVARY	(15)	(34)	(35)
INFLAMMATION, SUPPURATIVE	1 (7%)		
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
NERVOUS SYSTEM			
#BRAIN	(15)	(34)	(35)
HEMORRHAGE		1 (3%)	1 (3%)
MALACIA		1 (3%)	1 (3%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(15)	(34)	(35)
STEATITIS		1 (3%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(34)	(35)
HYPERPLASIA, LYMPHOID	1 (7%)		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	7	25
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX D

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

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	13	34	25
ANIMALS EXAMINED HISTOPATHOLOGICALLY	13	34	25
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(13)	(34)	(25)
INFLAMMATION, SUPPURATIVE	5 (38%)	2 (6%)	
#LUNG	(13)	(34)	(25)
INFLAMMATION, SUPPURATIVE			1 (4%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	9 (69%)	4 (12%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	2 (8%)
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#MYOCARDIUM	(13)	(34)	(24)
INFLAMMATION, INTERSTITIAL		1 (3%)	
*PULMONARY ARTERY	(13)	(34)	(25)
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)	1 (3%)	
DIGESTIVE SYSTEM			
#LIVER	(13)	(34)	(25)
INFLAMMATION, CHRONIC		1 (3%)	
NECROSIS, COAGULATIVE	1 (8%)		
HYPERPLASIA, NODULAR	2 (15%)		1 (4%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, FOCAL		1 (3%)	
HYPERPLASIA, DIFFUSE	1 (8%)		
URINARY SYSTEM			
#URINARY BLADDER	(13)	(33)	(25)
INFLAMMATION, FOCAL GRANULOMATOU		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(13)	(34)	(25)
INFLAMMATION, CHRONIC SUPPURATIV	1 (8%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		17	16
ACCIDENTAL DEATH	1		
NO NECROPSY PERFORMED		1	8
AUTOLYSIS/NO NECROPSY	1		2

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

TABLE D2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS MISSING			1
ANIMALS NECROPSIED	14	33	28
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	33	28
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(33)	(28)
INFLAMMATION, SUPPURATIVE	4 (29%)		4 (14%)
#LUNG	(14)	(31)	(28)
BRONCHOPNEUMONIA SUPPURATIVE			1 (4%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	9 (64%)		10 (36%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		2 (6%)	
HEMATOPOIFTIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(33)	(28)
HEMORRHAGE		1 (3%)	
INFLAMMATION, SUPPURATIVE		1 (3%)	
NECROSIS, COAGULATIVE		2 (6%)	
URINARY SYSTEM			
#KIDNEY	(14)	(32)	(28)
HYDRONEPHROSIS			1 (4%)
INFLAMMATION, CHRONIC			1 (4%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
*THYROID	(14)	(32)	(28)
HYPERPLASIA, FOLLICULAR-CELL	1 (7%)		
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(14)	(33)	(28)
INFLAMMATION, SUPPURATIVE			2 (7%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(14)	(33)	(28)
STEATITIS		1 (3%)	
NECROSIS, FOCAL		1 (3%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(14)	(33)	(28)
HYPERPLASIA, GRANULOCYTIC	1 (7%)		
HYPERPLASIA, LYMPHOID			1 (4%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	20	13
ANIMAL MISSING/NO NECROPSY			1
NO NECROPSY PERFORMED		1	2
AUTOLYSIS/NO NECROPSY	1	1	4

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma[b]	1/13 (8)	0/31 (0)	0/32 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		7.754	7.520
Weeks to First Observed Tumor	102	--	--
Pituitary: Chromophobe Adenoma or Carcinoma[b]	2/13 (15)	0/31 (0)	1/32 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.050		
Relative Risk (Matched Control)[f]		0.000	0.203
Lower Limit		0.000	0.004
Upper Limit		1.383	3.648
Weeks to First Observed Tumor	102	--	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/14 (7)	0/35 (0)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		7.421	7.421
Weeks to First Observed Tumor	105	--	--
Thyroid: C-cell Adenoma or Carcinoma[b]	2/14 (14)	1/35 (3)	2/35 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.200	0.400
Lower Limit		0.004	0.033
Upper Limit		3.612	5.172
Weeks to First Observed Tumor	105	103	103

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	13/14 (93)	33/35 (94)	11/35 (31)
P Values[c,d]	P < 0.001(N)	N.S.	P < 0.001(N)
Departure from Linear Trend[e]	P = 0.003		
Relative Risk (Matched Control)[f]		1.015	0.338
Lower Limit		0.917	0.287
Upper Limit		1.226	0.591
Weeks to First Observed Tumor	97	102	87

[a]Treated groups received doses of 3,000 or 6,000 ppm in feed.

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

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

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

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Leukemia[b]	0/15 (0)	2/34 (6)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.138	0.024
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	90	91
Pituitary: Chromophobe Carcinoma[b]	2/10 (20)	1/32 (3)	0/31 (0)
P Values[c,d]	P = 0.023(N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.156	0.000
Lower Limit		0.003	0.000
Upper Limit		2.784	1.060
Weeks to First Observed Tumor	96	89	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma[b]	5/10 (50)	5/32 (16)	0/31 (0)
P Values[c,d]	P < 0.001(N)	P = 0.040(N)	P < 0.001(N)
Relative Risk (Matched Control)[f]		0.313	0.000
Lower Limit		0.107	0.000
Upper Limit		1.136	0.241
Weeks to First Observed Tumor	96	89	--
Thyroid: C-cell Carcinoma[b]	0/15 (0)	2/34 (6)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.138	0.273
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	2/15 (13)	7/34 (21)	4/35 (11)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.544	0.857
Lower Limit		0.350	0.144
Upper Limit		14.128	8.856
Weeks to First Observed Tumor	100	105	104
Thyroid: Follicular-cell Carcinoma[b]	0/15 (0)	0/34 (0)	2/35 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		--	Infinite
Lower Limit		--	0.135
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	5/15 (33)	2/34 (6)	0/35 (0)
P Values[c,d]	P = 0.001(N)	P = 0.022(N)	P = 0.001(N)
Relative Risk (Matched Control)[f]		0.176	0.000
Lower Limit		0.020	0.000
Upper Limit		0.960	0.329
Weeks to First Observed Tumor	105	105	--
Uterus: Endometrial Stromal Polyp[b]	2/15 (13)	7/34 (21)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.544	0.214
Lower Limit		0.350	0.004
Upper Limit		14.128	3.876
Weeks to First Observed Tumor	105	89	104

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

(continued)

[a]Treated groups received doses of 3,000 or 6,000 ppm in feed.

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

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

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

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

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

APPENDIX F

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[ab]	1/13 (8)	1/34 (3)	1/25 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.382	0.520
Lower Limit		0.005	0.007
Upper Limit		29.166	39.218
Weeks to First Observed Tumor	96	86	104
Liver: Hepatocellular Adenoma or Carcinoma[b]	2/13 (15)	3/34 (9)	2/25 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.574	0.520
Lower Limit		0.077	0.044
Upper Limit		6.399	6.591
Weeks to First Observed Tumor	94	86	104

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

(continued)

[a]Treated groups received doses of 3,317 or 6,635 ppm in feed.

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

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

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

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

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

Table F2. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Fed Chlorpropamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma[b]	1/12 (8)	1/30 (3)	1/14 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.400	0.857
Lower Limit		0.006	0.012
Upper Limit		30.386	62.334
Weeks to First Observed Tumor	96	86	104
Liver: Hepatocellular Adenoma or Carcinoma[b]	2/12 (17)	3/30 (10)	2/14 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.600	0.857
Lower Limit		0.083	0.073
Upper Limit		6.635	10.246
Weeks to First Observed Tumor	94	86	104

Table F2. Time-adjusted Analyses of the Incidence of Primary Tumors in Male Mice Fed Chlorpropamide in the Diet[a]

(continued)

[a]Treated groups received doses of 3,317 or 6,635 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (percent), based upon animals that lived at least as long as 52 weeks on study.

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

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

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

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

Table F3. Analyses of the Incidence of Primary Tumors in Female Mice Fed Chlorpropamide in the Diet[a]

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/14 (0)	3/31 (10)	2/28 (7)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.292	0.157
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	104
Liver: Hepatocellular Adenoma[b]	0/14 (0)	4/33 (12)	0/28 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.024		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.424	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

Table F3. Analyses of the Incidence of Primary Tumors in Female Mice Fed Chlorpropamide in the Diet[a]

(continued)

[a]Treated groups received doses of 3,317 or 6,635 ppm in feed.

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

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

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

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

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

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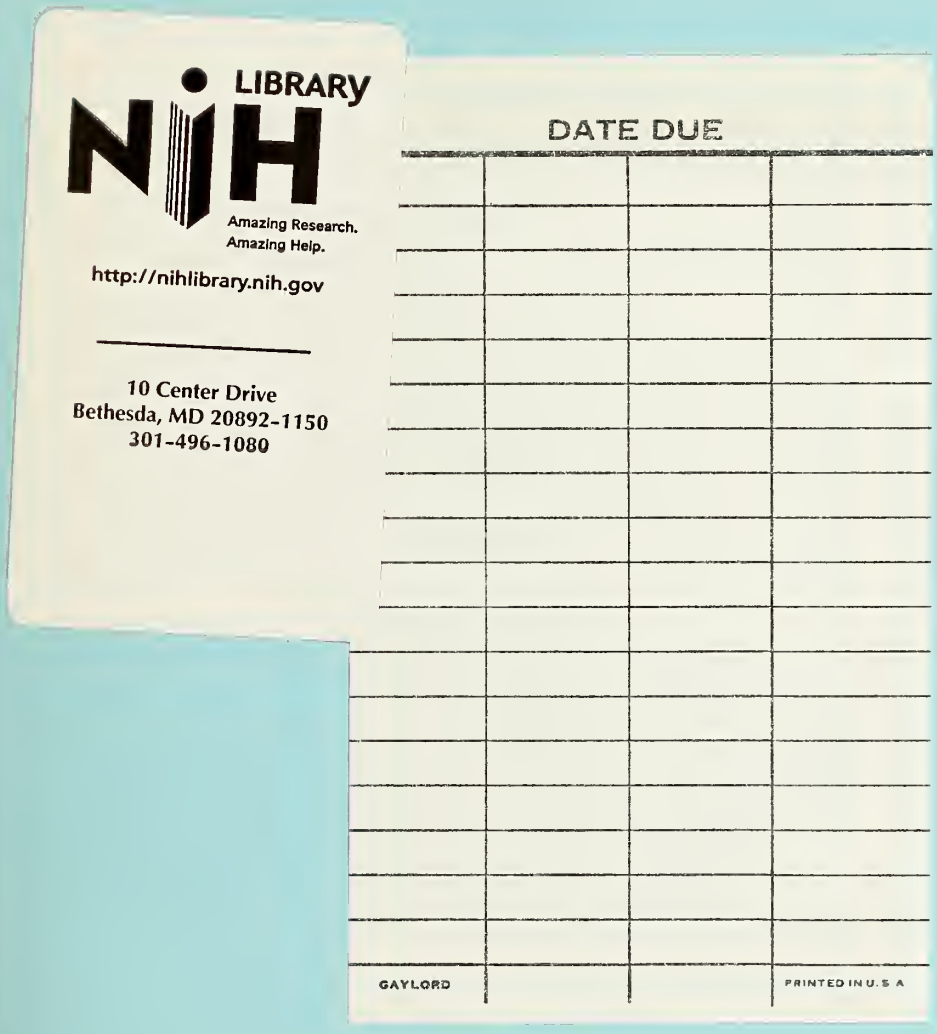

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