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

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SUMMARY

A bioassay of the hypoglycemic drug tolazamide for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 35 rats and 35 mice of each sex were administered tolazamide at one of two doses, either 5,000 or 10,000 ppm, for 103 weeks. Matched controls consisted of 15 rats and 15 mice of each sex. All surviving rats and mice were killed at 104 or 105 weeks.

Survival rates for the dosed rats of each sex were higher than those for the matched controls, and were adequate for the development of late-appearing tumors. Survival rates for the mice were lower than those for the rats, particularly for the dosed females (matched controls 67%, low-dose 34%, high-dose 32%). However, a large number of these deaths in the dosed females occurred after 90 weeks on study, and survival of both males and females was adequate for the development of late-appearing tumors.

All observed tumors were of types commonly found in the strains of animals used, and there were no statistically significant increases in the incidence of tumors in the dosed animals as compared with controls.

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

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I. INTRODUCTION

Tolazamide (CAS 1156-19-0; NCI C03327) is an oral hypoglycemic agent of the arylsulfonylurea type, similar to tolbutamide, chlorpropamide, and acetohexamide. It is approximately five times more potent than tolbutamide in the human diabetic and was developed in an effort to achieve secondary responses in patients who relapsed following initial control by tolbutamide (The Upjohn Co., 1977). The hypoglycemic effects of the arylsulfonylureas are due to their ability to stimulate pancreatic secretion of insulin and are used, therefore, 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).

Tolazamide was selected for testing in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that are used extensively and for prolonged periods in humans.

II. MATERIALS AND METHODS

A. Chemical

Tolazamide (N-(p-toluenesulfonyl)-N′-hexamethyleniminourea) was obtained in two batches (Lot Nos. 555BD and 824BK) from the Upjohn Company, North Haven, Connecticut. Lot No. 555BD was used in the prechronic study; Lot No. 824BK was used in the chronic study. The purity and identity of Lot No. 824BK were established by analyses at Midwest Research Institute. The melting point was 171-172°C (literature: 170-173°C). Elemental analyses (C, H, N, S) were correct for $C_{14}H_{21}N_2O_3S$, the molecular formula of tolazamide. Nuclear magnetic resonance, infrared, and ultraviolet spectra were in agreement with the structure.

Lot No. 824BK was stored in the original cardboard container at 5°C; Lot No. 555BK was stored in the original container at 22°C.

B. Dietary Preparation

Test diets containing tolazamide were prepared every 2 weeks by mixing a known amount of sifted tolazamide 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 tolazamide in feed was tested at Midwest Research Institute by determining the concentrations of tolazamide in formulated diets stored at room temperature (25°C) for 2 weeks. The results of these analyses indicated that tolazamide mixed with animal meal is stable for 2 weeks at room temperature.

C. Animals

For the subchronic study, female Fischer 344 rats were obtained from Harlan Industries, Cumberland, Indiana, and female B6C3F1 mice from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts.

In the chronic study, male and female Fischer 344 rats were obtained from Charles River Breeding Laboratories and male and female B6C3F1 mice from A. R. Schmidt, Madison, Wisconsin, through a contract with the Division of Cancer Treatment, National Cancer Institute. On arrival at the laboratory, the animals were 30 days of age. All animals were quarantined (rats for 24 days, mice for 26 days). Animals with no visible signs of disease were assigned to control or treated groups, and then earmarked for individual identification.

D. Animal Maintenance

All 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 room 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 were available *ad libitum*.

Rats and mice 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 covered with filter bonnets during the second year of the study. Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 80°C once per week for the first year of the study and twice per week for the second year; and 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 tolazamide 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)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
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)
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)
reserpine (CAS 50-55-66)

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)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
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)
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)
reserpine (CAS 50-55-6)

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 [IPD] (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 tolazamide, on the basis of which low and high concentrations (hereinafter referred to as "low doses" and "high

doses") were determined for use in the chronic study. Tolazamide was administered in the diet at doses of 2,500, 5,000, 10,000, 20,000, or 40,000 ppm to female Fischer 344 rats and at doses of 2,000, 6,000, 12,000, 25,000, or 50,000 ppm to female B6C3F1 mice. Five animals were tested at each concentration, and 10 animals were maintained as untreated controls. Treated animals received the test diets 7 days per week for 45 days and were then observed for an additional 45 days.

In rats, 2/5 animals treated at 40,000 ppm died by week 3. No deaths occurred in any of the other groups. By the end of the period of treatment, the mean body weight of animals treated at 2,500 ppm was 81% of that of the controls, while weights of animals treated at 5,000 and 10,000 ppm were comparable to those of the controls. In animals treated at 20,000 ppm, the mean body weight was 91% of that of the controls, and in animals treated at 40,000 ppm, 67% of that of the controls. By the end of the study, weights of treated groups of animals were generally comparable to those of the controls, except at 2,500 ppm, where the final weight was low (81% of that of controls), and at 40,000 ppm, where the final weight was 90% of that of the controls. No gross abnormalities were noted at necropsy. The low and high doses were set at 5,000 and 10,000 ppm for the chronic study in both male and female rats.

In mice, death occurred during the final week of the study in one animal at 50,000 ppm and in one animal at 25,000 ppm. Mean body weights of the mice were essentially unaffected by the treatment with tolazamide. No gross abnormalities were detected at necropsy. The low and high doses were set at 5,000 and 10,000 ppm for the chronic study 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. Rats and mice were weighed individually every 2 weeks for about 47 weeks and once monthly 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

Table 1. Design of Tolazamide Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals[a]	Tolazamide in Diet[b] (ppm)	Time on Study	
			Treated[c] (weeks)	Untreated[d] (weeks)
Male				
Matched-Control	15	0		105
Low-Dose	35	5,000	103	2
High-Dose	35	10,000	103	1
Female				
Matched-Control	15	0		105
Low-Dose	35	5,000	103	2
High-Dose	35	10,000	103	1

[a]All animals were 54 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]Test diets were withdrawn for 3 days during week 25 and discontinued for 5 days during week 58.

[d]Observation period following the administration of the chemical.

Table 2. Design of Tolazamide Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals[a]	Tolazamide in Diet[b] (ppm)	Time on Study	
			Treated[c] (weeks)	Untreated[d] (weeks)
Male				
Matched-Control	15	0		104-105
Low-Dose	35	5,000	103	2
High-Dose	35	10,000	103	1
Female				
Matched-Control	15	0		105
Low-Dose	35	5,000	103	2
High-Dose	35	10,000	103	1

[a]All animals were 56 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]Test diets were withdrawn 3 days during week 27 and discontinued entirely during weeks 28 and 29, while animals were being treated with oxytetracycline for respiratory disease. They were also discontinued for 5 days during week 60 following a decrease in mean weights of both treated and control animals.

[d]Observation period following the administration of the chemical.

intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, 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 an animal died naturally or was 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

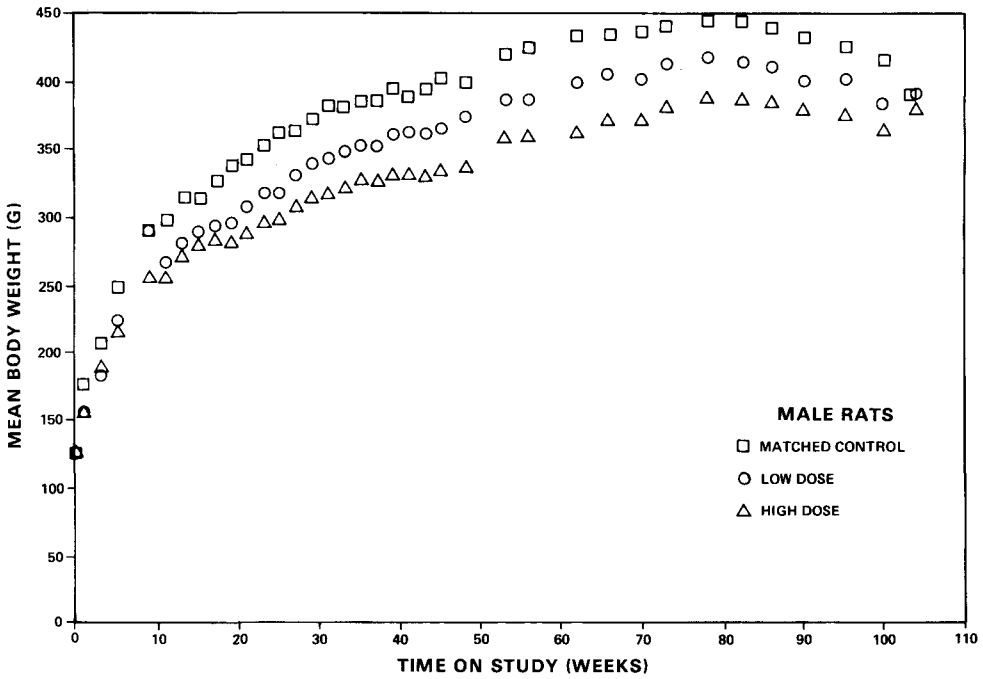
A. Body Weights and Clinical Signs (Rats)

Mean body weights of the tolazamide-treated rats were lower than those of the controls (figure 1). Body weights of the females were depressed more than those of the males during the second year on study. Fluctuations in the growth curve may have been due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other signs of drug-related toxicity were recorded in the rats. Rales were noted in several animals of both treated and control groups particularly during the second year of the study.

B. Survival (Rats)

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

In each sex, the Tarone test result for positive dose-related trend in mortality is not significant. In male rats, 31/35 (89%) of the high-dose group, 34/35 (97%) of the low-dose group, and 9/15 (60%) of the matched-control group lived to the end of the study. In females, the respective percentages of survival were



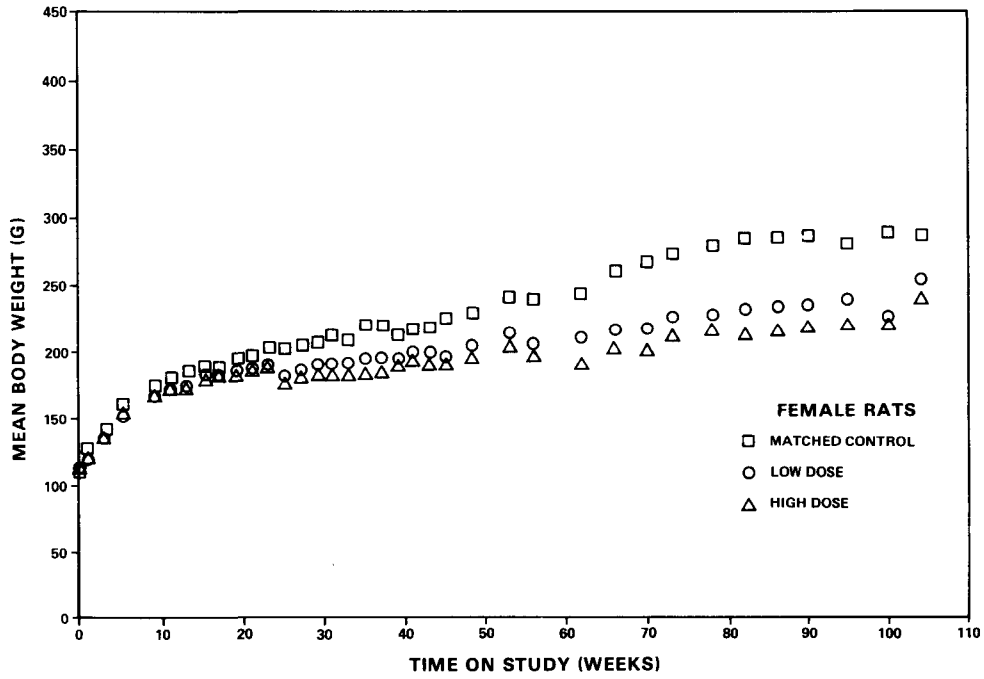
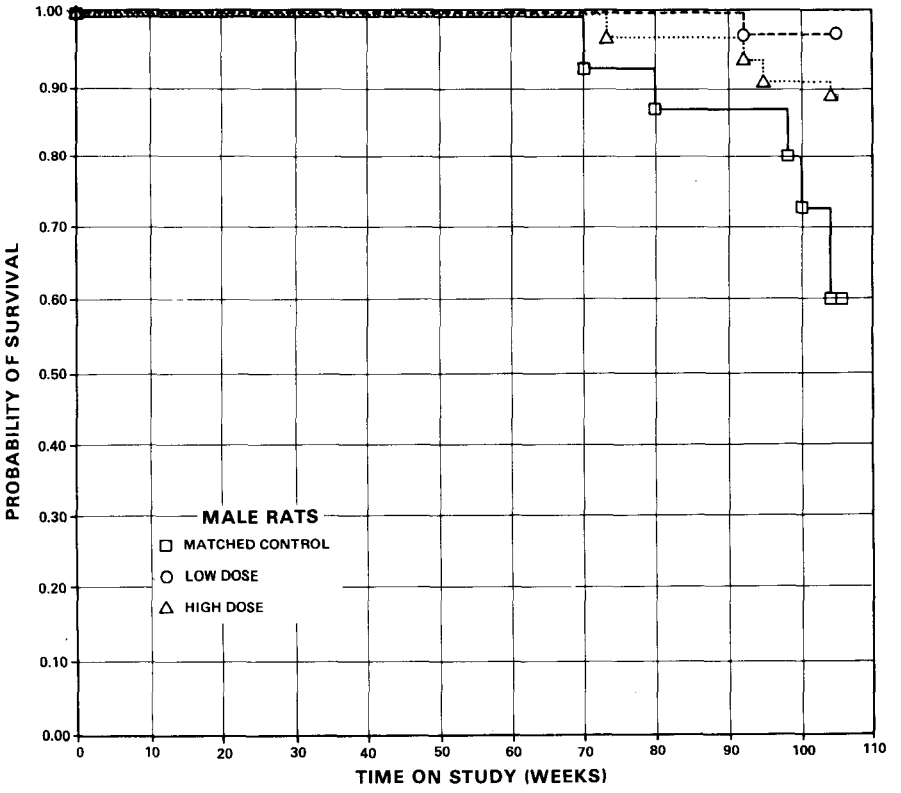


Figure 1. Growth Curves for Rats Fed Tolazamide in the Diet



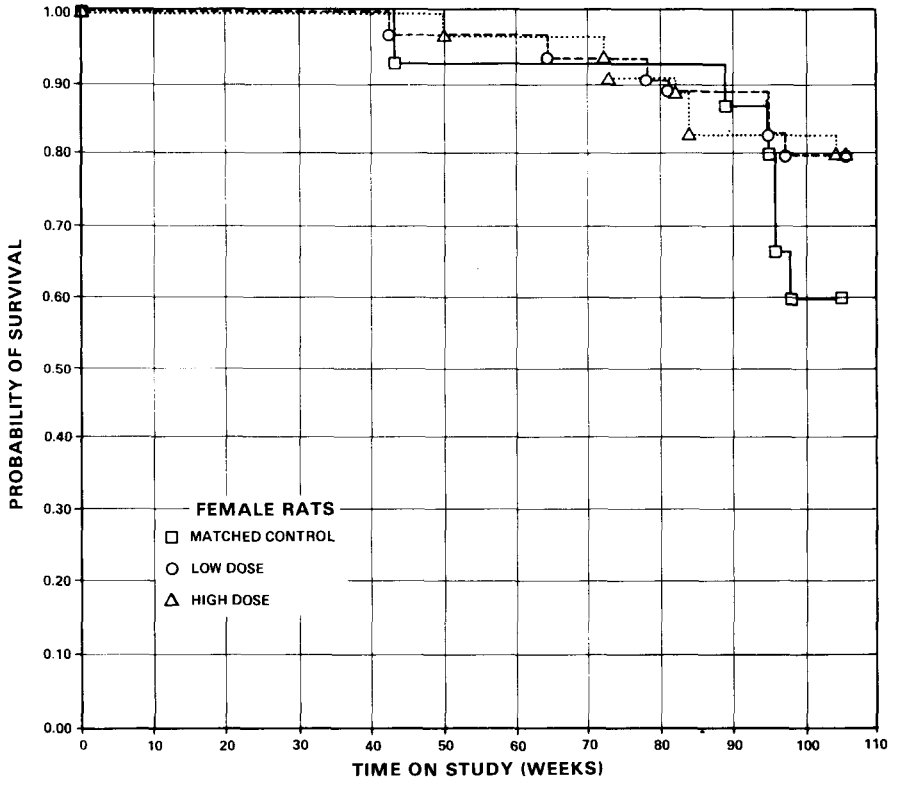


Figure 2. Survival Curves for Rats Fed Tolazamide in the Diet

28/35 (80%), 28/35 (80%), and 9/15 (60%). A sufficient number of rats of each sex was at risk for development of late-appearing 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 occurred both in the matched-control groups and in the treated groups. The neoplasms listed in Appendix A appeared with approximately equal frequency in treated and control rats, or appeared in insignificant numbers. These lesions are not uncommon in this strain of rat independent of any treatment. There was a slight increase in the number of thyroid tumors in the treated male and female rats. Although they occurred mainly in treated animals, the incidence of these neoplasms is within the range of that commonly observed among untreated Fischer 344 rats. The pancreas was examined, and no chemical-related lesions were observed.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in animals of the treated and control groups (Appendix C). These nonneoplastic lesions are commonly seen in aged rats.

In the judgment of the pathologists, tolazamide fed in the diet did not appear to be carcinogenic in Fischer 344 rats under the conditions of this bioassay.

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 and with an incidence of at least 5% in one or more of the 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. In each of the 95% confidence intervals of relative risk, shown in the tables, a value of one or of less than one is included, indicating the absence of positive significant results. It should also be noted that each of the intervals (except that for the incidence of fibroadenoma of the mammary gland in high-dose female rats) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by tolazamide, which could not be detected under the conditions of this test. Significant results in the negative direction were observed in

the incidence of fibroadenoma of the mammary gland and in the incidence of lymphoma or leukemia in the hematopoietic system in female rats.

IV. RESULTS - MICE

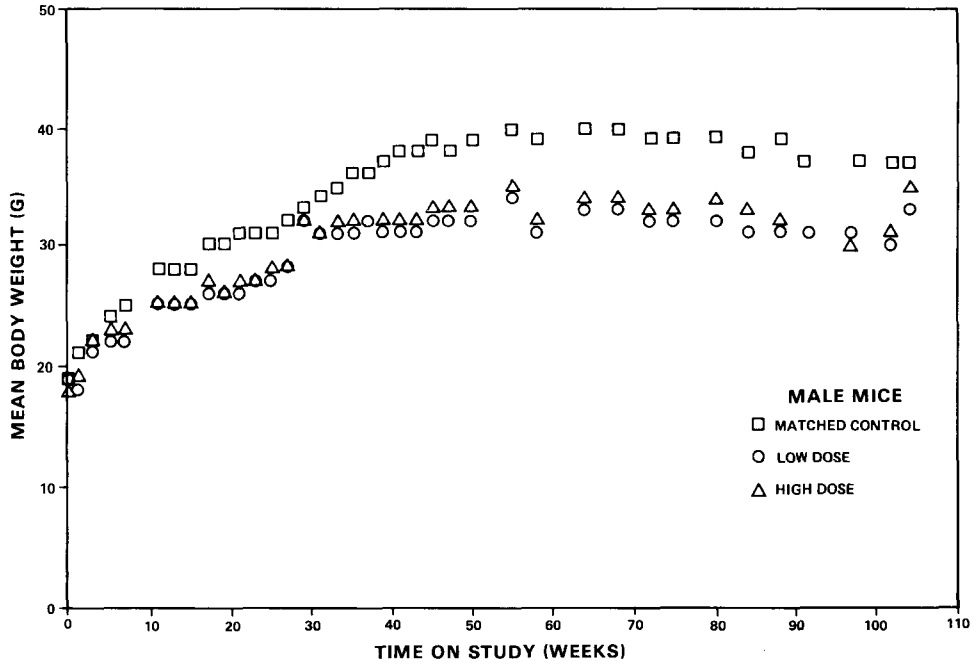
A. Body Weights and Clinical Signs (Mice)

Mean body weights of both male and female treated mice, when compared with those of the controls, were markedly depressed starting at approximately week 40 (figure 3). Fluctuations in the growth curve may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other signs of drug-related toxicity were noted in the mice. Since there was evidence of respiratory disease in some animals, oxytetracycline was administered in the drinking water at a dose of 0.6 mg/ml during week 28 and at 0.3 mg/ml during the following week. In an effort to decrease the transmission of bacteria, propylene glycol was vaporized in the animal rooms for about 2 months beginning at week 28.

B. Survival (Mice)

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

In each sex, the Tarone test result for positive dose-related trend in mortality is not significant. In male mice, 25/35 (71%)



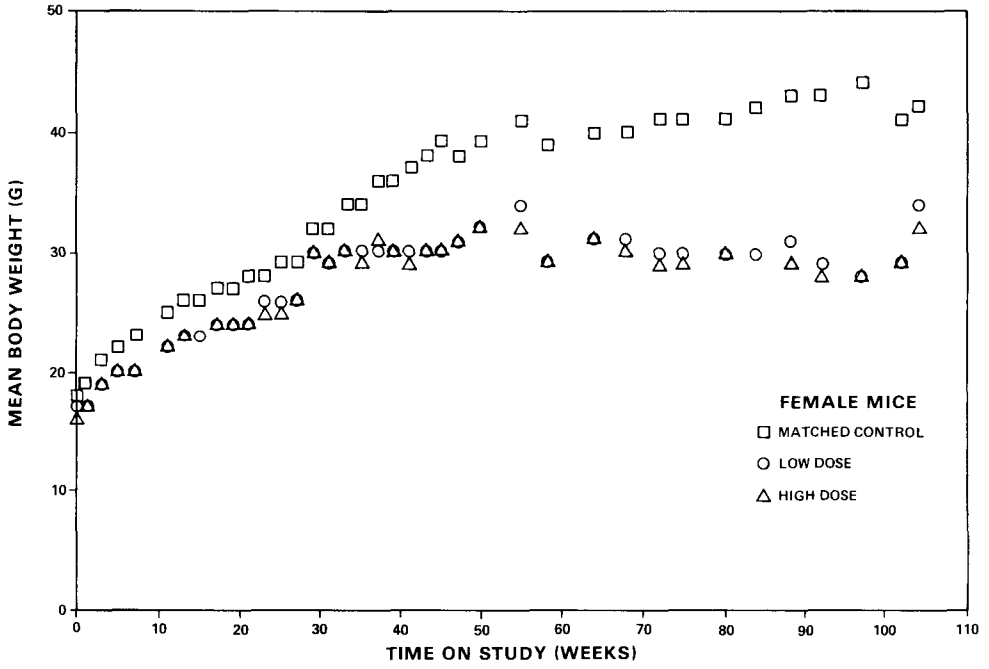
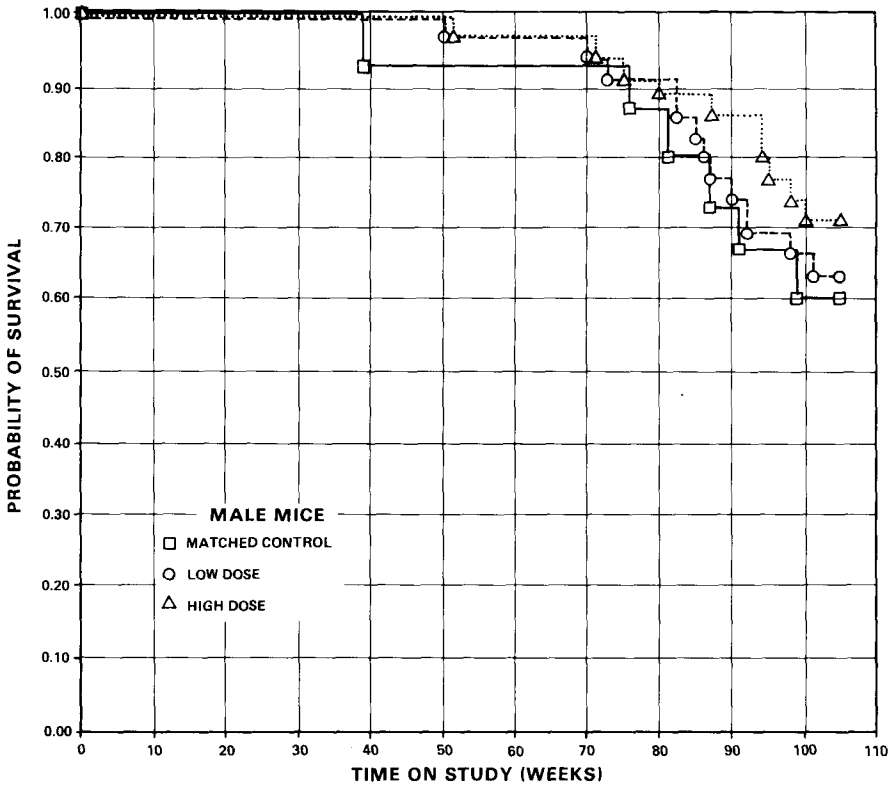


Figure 3. Growth Curves for Mice Fed Tolazamide in the Diet



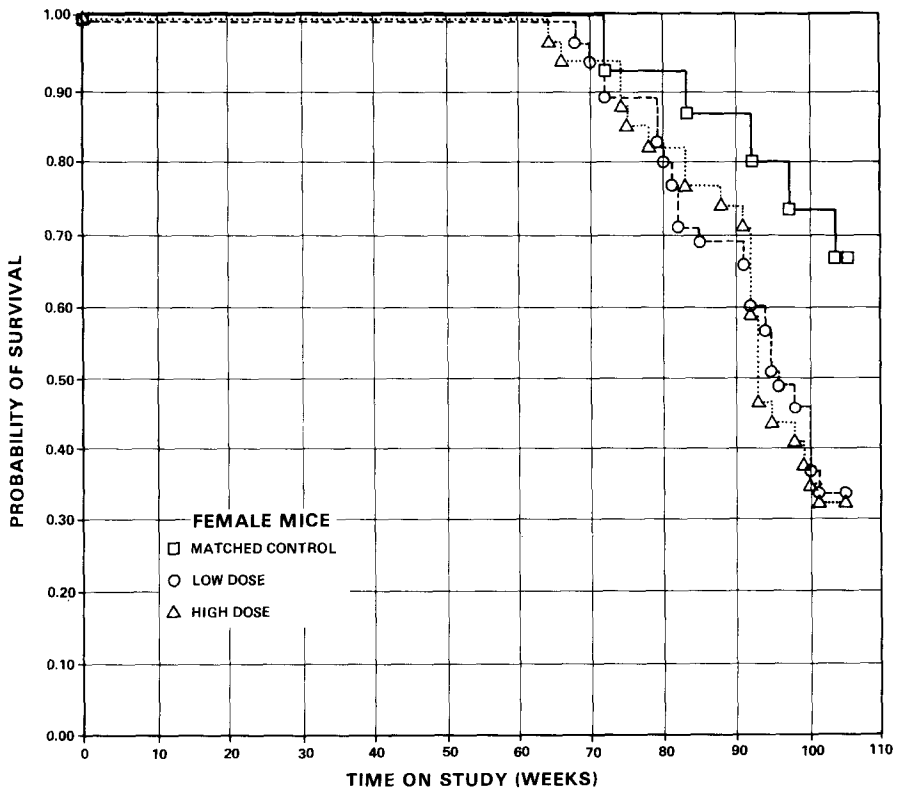


Figure 4. Survival Curves for Mice Fed Tolazamide in the Diet

of the high-dose group, 22/35 (63%) of the low-dose group, and 9/15 (60%) of the matched-control group lived to the end of the study, providing an adequate number of male mice at risk for development of late-appearing tumors. In females, although only 11/34 (32%) of the high-dose group and 12/35 (34%) of the low-dose group survived to termination of the study, all treated females lived beyond week 52 on study, and no tumor was found before this time. The percentage of survival in the matched-control group was 10/15 (67%).

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 occurred both in matched-control groups and in treated groups. As shown in Appendix B, some types of neoplasms occurred only, or with a greater frequency, in mice of treated groups compared with controls. These lesions, however, are not uncommon in this strain of mouse independent of any treatment. The pancreas was examined, and no chemical-related lesions were observed.

In addition to the neoplastic lesions, a number of degenerative, proliferative, and inflammatory changes were also encountered in

animals of the treated and control groups (Appendix D). Most of these nonneoplastic lesions are commonly seen in aged mice. The suppurative lesions involving the middle ear (otitis media) were associated with increased deaths or decreased life spans in the treated groups of female mice (matched controls 3/15 [20%], low-dose 11/33 [33%], high-dose 19/34 [56%]).

In the judgment of the pathologists, tolazamide fed in the diet did not appear to induce tumors in B6C3F1 mice under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals and with an incidence of at least 5% in one or more of the 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. In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or of less than one is included, indicating the absence of positive significant results. It should also be noted that each of the intervals (except those for

the incidence of lymphoma in low-dose female mice and in high-dose male mice) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by tolazamide, which could not be detected under the conditions of this test. In mice, a significant result in the negative direction occurs in the incidence of lymphoma, which was observed in 4/14 (29%) of the male control animals and 6/15 (40%) of the female control animals.

V. DISCUSSION

The dietary administration of tolazamide resulted in depression of the mean body weights of both rats and mice. No other clinical signs related to administration of the chemical were observed.

Survival rates for the dosed rats of both sexes were higher than those for the matched controls, and were adequate for the development of late-appearing tumors. Survival rates for the mice were lower than those for the rats, particularly for the dosed females (matched controls 67%, low-dose 34%, high-dose 32%). However, a large number of these deaths in the dosed females occurred after 90 weeks on study, and survival of both males and females was adequate for the development of late-appearing tumors.

In rats, the numbers of C-cell adenomas and carcinomas of the thyroid were slightly higher in the dosed male rats than in the matched controls (matched controls 1/15 [7%], low-dose 5/34 [15%], high-dose 6/35 [17%]). This incidence of tumors, however, was not statistically significant.

All observed tumors were of types commonly found in the strains of animals used and there were no statistically significant

increases in the incidence of tumors in the dosed animals as compared with controls.

Tolazamide is an oral hypoglycemic agent of the sulfonylurea type in common use in the United States. Long-term feeding studies conducted at the Upjohn Company in rats and dogs have been reported by Beckett and Donovan (1965). Rats administered tolazamide orally at 25 to 100 mg/kg for 1 year developed pancreatic islet cell hyperplasia, and dogs similarly administered with 12.5 to 50 mg/kg body weight for 6 months developed hypertrophy of the pancreatic islets.

It is concluded that under the conditions of this bioassay, tolazamide 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.

Beckett, A. G. and Donovan, R. J., Tolazamide (Tolanase) - A new sulphonylurea. Brit. J. Clin. Pract. 19(5):275-279, 1965.

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(2):187-220, 1972, pp. 48-52.

Cox, D. R., Analysis of Binary Data, Methuen & Co., Ltd., London, 1970.

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

Kaplan, E. L. and Meier, P., Nonparametric estimation from incomplete observations. J. Amer. 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. 1507 and 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.

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.

Shen, S. W. and Bressler, R., Medical Intelligence: Clinical pharmacology of oral antidiabetic agents (second of two parts). N. Engl. J. Med. 296(14):787-793.

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

The Upjohn Company, Tolanase® tablets. Physicians' Desk Reference, Medical Economics Co., Oradell, N. J., 1977, pp. 1652-1654.

APPENDIX A

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

TABLE A1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	35
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(35)	(35)
SQUAMOUS CELL PAPILLOMA			1 (3%)
*SUBCUT TISSUE	(15)	(35)	(35)
BASAL-CELL CARCINOMA			1 (3%)
FIBROMA			1 (3%)
LIPOMA		1 (3%)	
RESPIRATORY SYSTEM			
#TRACHEA	(15)	(35)	(35)
PAPILLOMA, NOS		1 (3%)	
#LUNG	(15)	(35)	(35)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (3%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (3%)
HEMATOPOIETIC SYSTEM			
*NERVOUS SYSTEM	(15)	(35)	(35)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
*MULTIPLE ORGANS	(15)	(35)	(35)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
UNDIFFERENTIATED LEUKEMIA	2 (13%)	1 (3%)	3 (9%)
#MANDIBULAR L. NODE	(15)	(35)	(35)
SQUAMOUS CELL CARCINOMA, METASTA	1 (7%)		
#CERVICAL LYMPH NODE	(15)	(35)	(35)
C-CELL CARCINOMA, METASTATIC		1 (3%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#PANCREAS	(15)	(35)	(35)
SARCOMA, NOS		1 (3%)	
#STOMACH	(15)	(34)	(35)
SQUAMOUS CELL PAPILLOMA			1 (3%)
#SMALL INTESTINE	(15)	(35)	(35)
SARCOMA, NOS			1 (3%)
*ANUS	(15)	(35)	(35)
FIBROSARCOMA	1 (7%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(15)	(33)	(30)
CHROMOPHOBE ADENOMA	1 (7%)	6 (18%)	4 (13%)
#ADRENAL	(15)	(35)	(35)
PHEOCHROMOCYTOMA	1 (7%)	2 (6%)	2 (6%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (3%)
#THYROID	(15)	(34)	(35)
FOLLICULAR-CELL ADENOMA		2 (6%)	
FOLLICULAR-CELL CARCINOMA		2 (6%)	1 (3%)
C-CELL ADENOMA		3 (9%)	3 (9%)
C-CELL CARCINOMA	1 (7%)	2 (6%)	3 (9%)
#PANCREATIC ISLETS	(15)	(35)	(35)
ISLET-CELL ADENOMA	1 (7%)	1 (3%)	1 (3%)
ISLET-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(35)	(35)
FIBROADENOMA	1 (7%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#TESTIS	(15)	(35)	(35)
INTERSTITIAL-CELL TUMOR	13 (87%)	32 (91%)	31 (89%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EAR CANAL	(15)	(35)	(35)
SQUAMOUS CELL CARCINOMA	1 (7%)		1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(15)	(35)	(35)
MESOTHELIOMA, NOS	1 (7%)		
*MESENTERY	(15)	(35)	(35)
SARCOMA, NOS	1 (7%)		
LIPOMA		1 (3%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(35)	(35)
SARCOMA, NOS	1 (7%)		
MESOTHELIOMA, NOS	1 (7%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@		1	1
MORIBUND SACRIFICE	6		3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	34	31
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	35	35
TOTAL PRIMARY TUMORS	26	56	59
TOTAL ANIMALS WITH BENIGN TUMORS	13	35	33
TOTAL BENIGN TUMORS	17	49	45
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	6	10
TOTAL MALIGNANT TUMORS	7	7	14
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2		
TOTAL UNCERTAIN TUMORS	2		
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 TOLAZAMIDE IN THE DIET

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	35
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(33)	(35)
SQUAMOUS CELL PAPILLOMA	1 (7%)		
RESPIRATORY SYSTEM			
#LUNG	(15)	(33)	(35)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)	1 (3%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (6%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(15)	(33)	(35)
UNDIFFERENTIATED LEUKEMIA	4 (27%)	3 (9%)	2 (6%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
HEPATOCELLULAR ADENOMA		1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(31)	(29)
CHROMOPHOBE ADENOMA	8 (57%)	13 (42%)	12 (41%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL	(15)	(33)	(35)
PHEOCHROMOCYTOMA		1 (3%)	1 (3%)
#THYROID	(15)	(33)	(35)
FOLLICULAR-CELL ADENOMA			1 (3%)
FOLLICULAR-CELL CARCINOMA		3 (9%)	
C-CELL ADENOMA	1 (7%)	2 (6%)	2 (6%)
C-CELL CARCINOMA	1 (7%)		2 (6%)
#PANCREATIC ISLETS	(15)	(33)	(35)
ISLET-CELL ADENOMA			1 (3%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(15)	(33)	(35)
FIBROADENOMA	4 (27%)	4 (12%)	1 (3%)
*PREPUTIAL GLAND	(15)	(33)	(35)
ADNEXAL ADENOMA		1 (3%)	
#UTERUS	(15)	(33)	(34)
ENDOMETRIAL STROMAL POLYP		1 (3%)	2 (6%)
ENDOMETRIAL STROMAL SARCOMA	2 (13%)	2 (6%)	2 (6%)
#UTERUS/ENDOMETRIUM	(15)	(33)	(34)
ADENOCARCINOMA, NOS		1 (3%)	
PAPILLARY ADENOMA	1 (7%)		
#OVARY	(15)	(33)	(34)
ADENOCARCINOMA, NOS		1 (3%)	
LIPOMA		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(33)	(35)
ADENOMA, NOS			1 (3%)
*EAR CANAL	(15)	(33)	(35)
SQUAMOUS CELL CARCINOMA			1 (3%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
LIPOMA			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	2	2	1
MORIBUND SACRIFICE	4	5	6
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	28	28
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	23	20
TOTAL PRIMARY TUMORS	23	37	29
TOTAL ANIMALS WITH BENIGN TUMORS	10	17	17
TOTAL BENIGN TUMORS	16	25	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	10	7
TOTAL MALIGNANT TUMORS	7	12	7
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 B

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

TABLE B1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(14)	(35)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (3%)	1 (3%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (3%)	
HEMATOPOIETIC SYSTEM			
*NERVOUS SYSTEM	(14)	(35)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (14%)	3 (9%)	
*MULTIPLE ORGANS	(14)	(35)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)	1 (3%)	1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (7%)		
#PEYERS PATCH	(14)	(35)	(34)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(35)	(34)
HEPATOCELLULAR ADENOMA	3 (21%)	1 (3%)	3 (9%)
HEPATOCELLULAR CARCINOMA		1 (3%)	
URINARY SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(28)	(26)
CARCINOMA,NOS			1 (4%)
CHROMOPHOBE ADENOMA		1 (4%)	
#THYROID	(14)	(34)	(31)
FOLLICULAR-CELL ADENOMA	1 (7%)		1 (3%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
*TRIGEMINAL GANGLION	(14)	(35)	(34)
SARCOMA, NOS		1 (3%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(14)	(35)	(34)
ADENOMA, NOS	1 (7%)	2 (6%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	3	4	2
MORIBUND SACRIFICE	3	9	8
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	9	22	25
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	11	5
TOTAL PRIMARY TUMORS	9	13	7
TOTAL ANIMALS WITH BENIGN TUMORS	5	5	4
TOTAL BENIGN TUMORS	5	5	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	8	2
TOTAL MALIGNANT TUMORS	4	8	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 B2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	@35
ANIMALS NECROPSIED	15	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	34
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(15)	(32)	(34)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (7%)		1 (3%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(14)	(32)	(33)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (3%)
*MULTIPLE ORGANS	(15)	(33)	(34)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		2 (6%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	4 (27%)		1 (3%)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (7%)		2 (6%)
#SPLEEN	(15)	(31)	(34)
HEMANGIOMA			1 (3%)
#OVARY	(15)	(33)	(34)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (7%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
HEPATOCELLULAR ADENOMA	1 (7%)		1 (3%)

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

@ 35 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HEPATOCELLULAR CARCINOMA		1 (3%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(13)	(27)	(26)
CHROMOPHOBE ADENOMA	1 (8%)		
#THYROID	(14)	(31)	(32)
FOLLICULAR-CELL CARCINOMA	1 (7%)		
#PANCREATIC ISLETS	(15)	(33)	(34)
ISLET-CELL CARCINOMA		1 (3%)	
REPRODUCTIVE SYSTEM			
*VAGINA	(15)	(33)	(34)
SQUAMOUS CELL CARCINOMA		1 (3%)	
#UTERUS	(15)	(33)	(34)
ENDOMETRIAL STROMAL POLYP		1 (3%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(15)	(33)	(34)
ADENOMA, NOS	1 (7%)		2 (6%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	15	35	35
NATURAL DEATH@	1	8	10
MORIBUND SACRIFICE	4	15	13
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	10	12	11
ANIMAL MISSING			
ANIMAL DELETED(WRONG SEX)			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	6	9
TOTAL PRIMARY TUMORS	11	6	9
TOTAL ANIMALS WITH BENIGN TUMORS	4	1	5
TOTAL BENIGN TUMORS	4	1	5
TOTAL ANIMALS WITH MALIGNANT TUMORS	7	5	4
TOTAL MALIGNANT TUMORS	7	5	4
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 TOLAZAMIDE IN THE DIET

TABLE C1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	35	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	35	35
INTEGUMENTARY SYSTEM			
*SKIN	(15)	(35)	(35)
EPIDERMAL INCLUSION CYST		1 (3%)	
RESPIRATORY SYSTEM			
#LUNG	(15)	(35)	(35)
PNEUMONIA, CHRONIC MURINE		3 (9%)	2 (6%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (7%)		
HEMATOPOIETIC SYSTEM			
NONE			
CIRCULATORY SYSTEM			
#MYOCARDIUM	(15)	(35)	(35)
INFLAMMATION, CHRONIC	4 (27%)	4 (11%)	1 (3%)
INFLAMMATION, CHRONIC FOCAL	1 (7%)		
DIGESTIVE SYSTEM			
#LIVER/CENTRILOBULAR	(14)	(34)	(35)
CYTOPLASMIC VACUOLIZATION		1 (3%)	
*BILE DUCT	(15)	(35)	(35)
HYPERPLASIA, NOS	1 (7%)		
#PANCREAS	(15)	(35)	(35)
INFLAMMATION, CHRONIC		4 (11%)	5 (14%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(15)	(35)	(35)
INFLAMMATION, DIFFUSE			1 (3%)
INFLAMMATION, SUPPURATIVE	1 (7%)		
INFLAMMATION, CHRONIC		4 (11%)	3 (9%)
INFLAMMATION, CHRONIC FOCAL		2 (6%)	
INFLAMMATION, CHRONIC DIFFUSE	11 (73%)	11 (31%)	5 (14%)
#URINARY BLADDER	(14)	(33)	(32)
ULCER, NOS	1 (7%)		
ENDOCRINE SYSTEM			
#THYROID	(15)	(34)	(35)
HYPERPLASIA, C-CELL		2 (6%)	4 (11%)
#PARATHYROID	(14)	(29)	(29)
HYPERPLASIA, NOS	1 (7%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(13)	(34)	(33)
INFLAMMATION, SUPPURATIVE	1 (8%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/RETINA	(15)	(35)	(35)
ATROPHY, NOS		1 (3%)	
*MIDDLE EAR	(15)	(35)	(35)
INFLAMMATION, SUPPURATIVE			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(15)	(35)	(35)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	15	33	35
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	35
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(15)	(33)	(35)
HYPERPLASIA, LYMPHOID			1 (3%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(15)	(33)	(35)
HYPERPLASIA, RETICULUM CELL	1 (7%)		
HEMATOPOIESIS	2 (13%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
NECROSIS, COAGULATIVE	1 (7%)		
CYTOPLASMIC VACUOLIZATION			2 (6%)
#PANCREAS	(15)	(33)	(35)
INFLAMMATION, CHRONIC	1 (7%)	2 (6%)	3 (9%)
URINARY SYSTEM			
#KIDNEY	(15)	(33)	(35)
INFLAMMATION, CHRONIC DIFFUSE	1 (7%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#URINARY BLADDER	(15)	(33)	(35)
HYPERPLASIA, EPITHELIAL	1 (7%)		
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(31)	(29)
HYPERPLASIA, CHROMOPHOBE-CELL			1 (3%)
#THYROID	(15)	(33)	(35)
HYPERPLASIA, C-CELL		1 (3%)	1 (3%)
REPRODUCTIVE SYSTEM			
#UTERUS/ENDOMETRIUM	(15)	(33)	(34)
INFLAMMATION, SUPPURATIVE	2 (13%)	2 (6%)	2 (6%)
HYPERPLASIA, EPITHELIAL		1 (3%)	
HYPERPLASIA, CYSTIC	2 (13%)	5 (15%)	3 (9%)
#OVARY/OVIDUCT	(15)	(33)	(34)
INFLAMMATION, SUPPURATIVE		1 (3%)	
#OVARY	(15)	(33)	(34)
INFLAMMATION, SUPPURATIVE		1 (3%)	1 (3%)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/RETINA	(15)	(33)	(35)
ATROPHY, NOS			1 (3%)
*MIDDLE EAR	(15)	(33)	(35)
INFLAMMATION, SUPPURATIVE			1 (3%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(33)	(35)
HYPERPLASIA, RETICULUM CELL			1 (3%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		6	12
AUTOLYSIS/NO NECROPSY		2	

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

APPENDIX D

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

TABLE D1.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	35
ANIMALS NECROPSIED	14	35	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	35	34
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(35)	(34)
INFLAMMATION, CHRONIC NECROTIZIN	1 (7%)		
RESPIRATORY SYSTEM			
#TRACHEA	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV			2 (6%)
#LUNG	(14)	(35)	(34)
BRONCHOPNEUMONIA SUPPURATIVE		12 (34%)	4 (12%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)		2 (6%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (7%)	1 (3%)	
HEMATOPOIETIC SYSTEM			
#MESENTERIC L. NODE	(14)	(34)	(34)
HYPERPLASIA, LYMPHOID			1 (3%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(35)	(34)
NECROSIS, COAGULATIVE	1 (7%)	1 (3%)	
HYPERPLASIA, FOCAL		2 (6%)	
URINARY SYSTEM			
#KIDNEY	(14)	(35)	(34)
INFLAMMATION, CHRONIC		1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL		1 (3%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
#PROSTATE	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV	1 (7%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
*MIDDLE EAR	(14)	(35)	(34)
INFLAMMATION, CHRONIC SUPPURATIV	4 (29%)	11 (31%)	11 (32%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	11	17

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
AUTOLYSIS/NO NECROPSY	1		1

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

TABLE D2.

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

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	35	@35
ANIMALS NECROPSIED	15	33	34
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	33	34
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(15)	(33)	(34)
HEMORRHAGE			1 (3%)
RESPIRATORY SYSTEM			
#LUNG	(15)	(32)	(34)
INFLAMMATION, INTERSTITIAL		1 (3%)	
BRONCHOPNEUMONIA SUPPURATIVE		3 (9%)	1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA		1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (3%)	
HEMATOPOIETIC SYSTEM			
#PANCREATIC L.NODE	(15)	(32)	(33)
NECROSIS, COAGULATIVE	1 (7%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(15)	(33)	(34)
NECROSIS, COAGULATIVE			1 (3%)
HYPERPLASIA, FOCAL	1 (7%)		
#PANCREAS	(15)	(33)	(34)
CYSTIC DUCTS	1 (7%)		
#PANCREATIC ACINUS	(15)	(33)	(34)
ATROPHY, NOS		1 (3%)	

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

@ 35 ANIMALS WERE INITIALLY IN THE STUDY, BUT ONE ANIMAL WAS FOUND TO BE A MALE IN A FEMALE GROUP.

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, DIFFUSE		1 (3%)	
#PEYERS PATCH	(15)	(33)	(34)
HYPERPLASIA, LYMPHOID		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(15)	(33)	(34)
HYDRONEPHROSIS		1 (3%)	
#URINARY BLADDER	(15)	(33)	(34)
INFLAMMATION, CHRONIC SUPPURATIV			1 (3%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*MIDDLE EAR	(15)	(33)	(34)
INFLAMMATION, CHRONIC SUPPURATIV	3 (20%)	11 (33%)	19 (56%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(15)	(33)	(34)
STEATITIS	1 (7%)	1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FAT	1 (7%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(15)	(33)	(34)
HYPERPLASIA, LYMPHOID		1 (3%)	1 (3%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	5	14	12
AUTOLYSIS/NO NECROPSY		2	

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/15 (0)	0/35 (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
Hematopoietic System: Leukemia[b]	2/15 (13)	1/35 (3)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.214	0.643
Lower Limit		0.004	0.085
Upper Limit		3.876	7.208
Weeks to First Observed Tumor	104	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	2/15 (13)	1/35 (3)	5/35 (14)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.214	1.071
Lower Limit		0.004	0.207
Upper Limit		3.876	10.495
Weeks to First Observed Tumor	104	105	104
Pituitary: Chromophobe Adenoma[b]	1/15 (7)	6/33 (18)	4/30 (13)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.727	2.000
Lower Limit		0.385	0.228
Upper Limit		121.009	94.875
Weeks to First Observed Tumor	105	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma[b]	1/15 (7)	2/35 (6)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.857	1.286
Lower Limit		0.050	0.117
Upper Limit		49.128	65.497
Weeks to First Observed Tumor	105	85	104
Thyroid: Follicular-cell Carcinoma[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	--	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/15 (0)	4/34 (12)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.437	0.024
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	104
Thyroid: C-cell Carcinoma[b]	1/15 (7)	2/34 (6)	3/35 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.882	1.286
Lower Limit		0.051	0.118
Upper Limit		50.522	64.499
Weeks to First Observed Tumor	105	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	1/15 (7)	5/34 (15)	6/35 (17)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		2.206	2.571
Lower Limit		0.286	0.363
Upper Limit		100.914	114.375
Weeks to First Observed Tumor	105	105	104
Pancreatic Islets: Islet-cell Adenoma or Carcinoma[b]	1/15 (7)	2/35 (6)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.857	0.429
Lower Limit		0.050	0.006
Upper Limit		49.128	32.715
Weeks to First Observed Tumor	104	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	13/15 (87)	32/35 (91)	31/35 (89)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.055	1.022
Lower Limit		0.881	0.852
Upper Limit		1.352	1.366
Weeks to First Observed Tumor	80	105	92

[a]Treated groups received doses of 5,000 or 10,000 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 Fischer 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 a control group.

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	0/15 (0)	2/33 (6)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.142	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/15 (7)	3/33 (9)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		1.364	0.000
Lower Limit		0.124	0.000
Upper Limit		69.321	7.949
Weeks to First Observed Tumor	105	105	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	4/15 (27)	3/33 (9)	2/35 (6)
P Values[c,d]	P = 0.040 (N)	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.341	0.214
Lower Limit		0.059	0.022
Upper Limit		1.805	1.347
Weeks to First Observed Tumor	89	81	73
Pituitary: Chromophobe Adenoma[b]	8/14 (57)	13/31 (42)	12/29 (41)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.734	0.724
Lower Limit		0.403	0.391
Upper Limit		1.615	1.613
Weeks to First Observed Tumor	95	78	72

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma[b]	0/15 (0)	3/33 (9)	0/35 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.036		
Relative Risk (Matched Control)[f]		Infinite	--
Lower Limit		0.290	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--
Thyroid: Follicular-cell Adenoma or Carcinoma[b]	0/15 (0)	3/33 (9)	1/35 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.290	0.024
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	1/15 (7)	0/33 (0)	2/35 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.857
Lower Limit		0.000	0.050
Upper Limit		8.417	49.128
Weeks to First Observed Tumor	105	--	104
Thyroid: C-cell Adenoma or Carcinoma[b]	1/15 (7)	2/33 (6)	4/35 (11)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.909	1.714
Lower Limit		0.053	0.196
Upper Limit		51.999	81.832
Weeks to First Observed Tumor	105	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	4/15 (27)	4/33 (12)	1/35 (3)
P Values[c,d]	P = 0.013 (N)	N.S.	P = 0.024 (N)
Relative Risk (Matched Control)[f]		0.455	0.107
Lower Limit		0.102	0.002
Upper Limit		2.174	0.986
Weeks to First Observed Tumor	98	95	105
Uterus: Endometrial Stromal Polyp[b]	0/15 (0)	1/33 (3)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.025	0.138
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Sarcoma[b]	2/15 (13)	2/33 (6)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.455	0.441
Lower Limit		0.037	0.036
Upper Limit		5.871	5.706
Weeks to First Observed Tumor	105	105	105
Uterus: Endometrial Stromal Polyp or Sarcoma[b]	2/15 (13)	3/33 (9)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.682	0.662
Lower Limit		0.090	0.087
Upper Limit		7.623	7.410
Weeks to First Observed Tumor	105	105	104

[a]Treated groups received doses of 5,000 or 10,000 ppm in feed.

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

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

(continued)

[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 Fischer 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 a control group.

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

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

APPENDIX F

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	0/14 (0)	2/35 (6)	1/34 (3)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		Infinite	Infinite
Lower Limit		0.125	0.023
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	104
Hematopoietic System: Lymphoma[b]	4/14 (29)	5/35 (14)	1/34 (3)
P Values[c,d]	P = 0.011 (N)	N.S.	P = 0.021 (N)
Relative Risk (Matched Control)[f]		0.500	0.103
Lower Limit		0.134	0.002
Upper Limit		2.235	0.942
Weeks to First Observed Tumor	81	98	104

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	3/14 (21)	2/35 (6)	3/34 (9)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.267	0.412
Lower Limit		0.026	0.065
Upper Limit		2.131	2.796
Weeks to First Observed Tumor	81	105	71
Harderian Gland: Adenoma, NOS (not otherwise specified)[b]	1/14 (7)	2/35 (6)	0/34 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.800	0.000
Lower Limit		0.047	0.000
Upper Limit		45.853	7.633
Weeks to First Observed Tumor	105	92	--

[a]Treated groups received doses of 5,000 or 10,000 ppm in feed.

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

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

(continued)

[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 Fischer 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 a control group.

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

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	6/15 (40)	2/33 (6)	4/34 (12)
P Values[c,d]	P = 0.036 (N)	P = 0.008 (N)	P = 0.033 (N)
Departure from Linear Trend[e]	P = 0.016		
Relative Risk (Matched Control)[f]		0.152	0.294
Lower Limit		0.018	0.077
Upper Limit		0.744	1.076
Weeks to First Observed Tumor	72	96	78
Harderian Gland: Adenoma, NOS[b]	1/15 (7)	0/33 (0)	2/34 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Matched Control)[f]		0.000	0.882
Lower Limit		0.000	0.051
Upper Limit		8.417	50.522
Weeks to First Observed Tumor	105	--	95

[a]Treated groups received doses of 5,000 or 10,000 ppm in feed.

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

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

(continued)

[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 Fischer 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 a control group.

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

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

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

March 6, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to 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 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 reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Tolazamide for carcinogenicity.

The primary reviewer agreed with the conclusion that, under the conditions of test, Tolazamide was not carcinogenic. He said that the pure grade compound was tested and that the study was well-conducted.

The secondary reviewer said that the high dose administered in both species achieved the maximum tolerated dose, based on an examination of weight loss. He noted that the treated animals survived longer than the associated controls. A staff member commented that an analysis of five oral hypoglycemic agents tested in bioassays, showed negative trends in mammary adenocarcinomas or endometrial stromal polyps in female rats. He suggested that the negative trends may be due to the weight loss rather than specifically to the action of the agents. Similarly, he suggested that increased longevity may be associated with the weight loss.

A Subgroup member said that an older manufacturing process of Tolazamide resulted in its contamination with

nitrosamines. He suggested that it would be worthwhile to determine if the supply used in the bioassay was manufactured under this old process.

One Subgroup member commented that the number of surviving animals at the end of the study was too few to allow any conclusion on the bioassay. Another Subgroup member disagreed, stating that there was still a sufficient number on which to evaluate the carcinogenicity of Tolazamide.

The primary reviewer moved that the report on the bioassay of Tolazamide be accepted as written. The motion was seconded and approved by all except Mr. Garfinkel, who opposed it.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
John Weisburger, American Health Foundation
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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