National Cancer Institute
CARCINOGENESIS
Technical Report Series
NO. 83
1978

BIOASSAY OF DAMINOZIDE FOR POSSIBLE CARCINOGENICITY

CAS No. 1596-84-5

NCI-CG-TR-83

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

BIOASSAY OF

DAMINOZIDE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

DHEW Publication No. (NIH) 78-1333

BIOASSAY OF
DAMINOZIDE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

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

CONTRIBUTORS: This bioassay of daminozide was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were chosen by Drs. E. K. Weisburger[1], J. H. Weisburger[1,2], N. P. Page[1,3] and F. M. Garner[4]. The administration of the test chemical and the observation of the animals were supervised by Dr. F. M. Garner[4], with the technical assistance of Mr. R. Cypher[4], Mr. H. D. Thornett[4], and Mr. D. J. Howard[4].

Histologic examinations of rats were performed by Drs. H. Seibold[4], B. C. Zook[4], and N. J. Wosu[4] and those of mice by Drs. R. J. Montali[4], F. M. Garner[4], N. J. Wosu[4], and H. Seibold[4]. Histologic sections of all tumors and hyperplasias were reexamined by Dr. Montali, who also reviewed all diagnoses and prepared the initial interpretative pathology summary.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. The statistical analyses were performed by Dr. J. R. Joiner[6] and Ms. P. L. Yong[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed under the direction of Dr. E. Murrill[8], feed samples were analyzed by Mr. H. Paulin[4], and the results of the analyses were reviewed by Dr. S. S. Olin[6].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Drs. J. F. Robens and C. H. Williams, toxicologists; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Waitz, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI were responsible for evaluating the bioassay, 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, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Robert A. Squire[9], Dr. Jerrold M. Ward.

---

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

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

[3]Now with the National Institute of Occupational Safety and Health, Rockville, Maryland.

[4]Litton Bionetics, Inc., 5516 Nicholson Lane, Kensington, Maryland.

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

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

[7]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.

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

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

SUMMARY

A bioassay of daminozide, a plant growth regulator, for possible carcinogenicity was conducted by administering the test chemical in the diet to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered daminozide at one of two doses, either 5,000 or 10,000 ppm, for 104 weeks, then observed for an additional week. Matched controls consisted of 20 untreated males and 20 untreated females of each species. All surviving rats and mice were killed at 105 weeks.

Mean body weights of the high-dose female mice were appreciably lower than those of the corresponding controls, while mean body weights of all other dosed groups of rats and mice were essentially unaffected. No other clinical signs related to administration of daminozide were observed. Sufficient numbers of animals in all groups of rats and mice were at risk for development of late-appearing tumors.

In the male rats, no tumors occurred at incidences that were significantly higher in dosed groups than in controls, except for interstitial-cell tumors of the testis (controls 13/20, low-dose 49/50, high-dose 47/50). These tumors occurred, however, at a high spontaneous rate (182/220) in historical-control male rats; thus, the association of the interstitial-cell tumors with administration of the chemical is doubtful.

In the female rats, adenocarcinomas of the endometrium and leiomyosarcomas of the uterus occurred only in the dosed groups (adenocarcinomas: controls 0/19, low-dose 5/50, high-dose 3/50; leiomyosarcomas: controls 0/19, low-dose 1/50, high-dose 3/50). The incidences in the dosed groups were too low to be statistically significant; however, the low incidence of these tumors in historical-control female rats (2/220 adenocarcinoma and 0/220 leiomyosarcoma) indicate that the occurrence of these tumors in

the dosed female rats was associated with the administration of daminozide.

In the male mice, there was a dose-related trend (P = 0.008) in the incidence of hepatocellular carcinomas; also, the incidence in the high-dose group was significant (P = 0.020) compared with that in the controls (controls 0/14, low-dose 7/50, high-dose 13/46). The incidence of these tumors in the historical-control male mice was, however, 21/216; thus, the association of the hepatocellular carcinomas with administration of daminozide is not clear. In the female mice, only three such tumors occurred.

It is concluded that under the conditions of this bioassay, daminozide was not carcinogenic in the male Fischer 344 rats or in the female B6C3F1 mice. In male B6C3F1 mice, the induction of hepatocellular carcinomas may have been associated with the administration of the test chemical. Daminozide was carcinogenic in female Fischer 344 rats, inducing adenocarcinomas of the endometrium of the uterus and leiomyosarcomas of the uterus.

TABLE OF CONTENTS

APPENDIXES

Page

TABLES

FIGURES

I. INTRODUCTION

Daminozide (CAS 1596-84-5; NCI C03827) is a hydrazine compound that has been used as a plant growth regulator since 1962 (Riddell et al., 1962). It retards stem growth, protects against heat, drought, and frost, and induces the development of multiple flowers (Thomson, 1976). The Environmental Protection Agency (1976) has established residue tolerances of 1-55 ppm on a variety of crops which include cherries, plums, apples, nectarines, peaches, pears, grapes, melons, tomatoes, brussel sprouts, peppers, and peanuts; residues of 0.02-2 ppm are allowed in the meat or milk of animals exposed to the herbicide.

Daminozide was selected for testing for carcinogenic activity because other hydrazine compounds had been shown to be carcinogenic, as reviewed by Toth (1975), and because of the potential for long-term human exposure to this chemical during agricultural application or from residues in food products.

II. MATERIALS AND METHODS

A. Chemical

Daminozide, which is the common name for succinic acid mono-2,2-dimethylhydrazide, was obtained from Aldrich Chemical Co., Milwaukee, Wisconsin, in three lots (Lot Nos. 101927, 121327, and 071637). The identity and purity of each lot was confirmed by analysis. Elemental analyses (C, H, N) were consistent with $C_6H_{12}N_2O_3$, the molecular formula for daminozide. Titration with sodium hydroxide indicated purities close to 100% (100.0, 100.2, and 99.3, respectively). Thin-layer chromatography showed a single component. Melting point and infrared and nuclear magnetic resonance spectra were consistent with the structure.

The chemical was stored at 4°C in the original container.

B. Dietary Preparation

A 6-kg diet was prepared twice per week for mice and three times per week for rats. To obtain each dietary concentration, the appropriate weight of daminozide was mixed with a small portion of Wayne® Lab Blox animal feed (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. This premix was then added to the remaining weight of feed and mixed in a twin-shell blender

for at least 15 minutes. Feed preparations containing the test chemical were stored at 1°C for no longer than 1 week.

The stability of the chemical in feed was checked by triplicate analyses at 5,000 and 10,000 ppm. The recovery of test chemical from feed decreased about 20% after the feed was stored for 10 days at 25°C; thus, some decomposition of the test chemical appeared to occur in the feed hoppers under the conditions of this bioassay.

Ground Wayne® Lab Blox animal meal not containing the test chemical was used as the diet for the control groups of animals.

C. Animals

Rats and mice of each sex, obtained through contracts of the Division of Cancer Treatment, National Cancer Institute, were used in these bioassays. The rats were of the Fischer 344 strain obtained from A. R. Schmidt/Sprague-Dawley, Madison, Wisconsin, and the mice were B6C3F1 hybrids obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. On arrival at the laboratory, the animals were approximately 4 weeks of age. All animals were quarantined for 2 weeks. After the period of quarantine, all animals were weighed individually and segregated into equal weight groups. Cage assignments were made

by selecting one animal from each group so that the total weights of animals in each cage were about the same.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled rooms. The temperature was maintained at 21-25°C and the relative humidity at 45-55%. There were 15 changes of room air per hour, and the air was passed through both incoming and exhaust HEPA (High Efficiency Particulate Air) filters. The animal rooms were positively pressurized with respect to the exit hall and negatively pressurized with respect to the entrance hall. Cool white fluorescent lighting was provided 8 hours per day. Test and control diets were available ad libitum and were replenished three times per week. Tap water, acidified with HCl to pH 2.5, also was available ad libitum.

Rats were housed four per cage and mice five per cage in solid polycarbonate cages. Each cage was covered with a wire mesh screen and a sheet of filter paper. Heat-treated hardwood chip bedding (Absorb-Dri®, Lab Products, Garfield, N.J.) was used in the cages. Cages and water bottles were sanitized twice per week and feed hoppers once per week at approximately 82°C; bedding was replaced twice per week.

Rats and mice were housed in separate rooms. Control animals and

dosed animals were housed in the same room. Animals administered daminozide were housed in the same room as animals of the same species administered the following chemicals:

RATS

hydroxytriphenylstannane (CAS 76-87-9)
2-nitroethenylbenzene (CAS 102-96-5)
N-(aminocarbonyl)-2-bromo-2-ethylbutanamide (CAS 77-65-6)

MICE

hydroxytriphenylstannane (CAS 76-87-9)
4,4'-diisocyanato-3,3'-dimethoxy-1,1'-biphenyl (CAS 91-93-0)
N-(aminocarbonyl)-2-bromo-2-ethylbutanamide (CAS 77-65-6)
N,N'-diethylthiourea (CAS 105-55-5)
iodomethanesulfonic acid, sodium salt (CAS 126-31-8)
2,5-cyclohexadiene-1,4-dione, dioxime (CAS 105-11-3)
3-hydroxy-(3 alpha, 5 beta) cholan-24-oic acid (CAS 434-13-9)
4-amino-2-nitrophenol (CAS 119-34-6)
ethylenediaminetetraacetic acid, trisodium salt trihydrate (EDTA) (CAS 150-38-9)

All of these compounds were administered in the diet, with the exception of 2-nitroethenylbenzene and 3-hydroxy-(3 alpha, 5 beta)cholan-24-oic acid, which were administered by gavage, and iodomethanesulfonic acid, sodium salt, which was administered by intraperitoneal injection.

E. Subchronic Studies

Subchronic feeding studies were conducted with rats and mice to estimate the maximum tolerated doses of daminozide, on the basis of which low and high concentrations (hereinafter referred to as

"low doses" and "high doses") were determined for administration in the chronic studies. In the subchronic studies, daminozide was added to the animal feed in concentrations of 10,000, 20,000, 30,000, 40,000, or 50,000 ppm. Feed containing the chemical was provided to groups of five male and female animals of each species for 6 weeks, and the standard commercial diet was similarly provided to groups of five male and female animals of each species. All surviving animals were killed and necropsied at week 8.

In dosed male and female rats, soft stools and diarrhea were noted at doses of 20,000 ppm and above. After week 7, there were no effects on mean body weight gain in rats, except for a slight weight depression in the males administered 50,000 ppm. Water consumption also increased in this group after week 3 of the study.

There were no effects on mean body weight gain in the mice administered the different doses; however, during week 2, two female mice died that were administered 30,000 ppm. There were no gross pathologic changes noted in any of the dosed animals.

The low and high doses for the chronic studies using rats and mice were set at 5,000 and 10,000 ppm.

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 at regular intervals. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of all major tissues, organs, or gross lesions from killed animals and from animals found dead. The following tissues and organs were routinely subjected to microscopic examination: skin, lymph nodes, mammary gland, salivary gland, bone marrow, trachea, lungs and bronchi, heart, thyroid, parathyroid, esophagus, stomach, small intestine, large intestine, liver, gallbladder (mice), pancreas, spleen, kidney, adrenal, urinary bladder, prostate or uterus, testis or ovary, brain, and pituitary. 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.

Table 1. Design of Chronic Feeding Studies of Daminozide in Rats

Sex and Test Group	Initial No. of Animals[a]	Daminozide in Diet (ppm)[b]	Time on Study	
			Dosed (Weeks)	Observed (weeks)
Male				
Matched-Control	20	0		105
Low-Dose	50	5,000	104	1
High-Dose	50	10,000	104	1
Female				
Matched-Control	20	0		105
Low-Dose	50	5,000	104	1
High-Dose	50	10,000	104	1

[a]Animals were approximately 42 days of age when placed on study.

[b]Test diets were made available ad libitum, 7 days per week.

Table 2. Design of Chronic Feeding Studies of Daminozide in Mice

Sex and Test Group	Initial No. of Animals[a]	Daminozide in Diet (ppm)[b]	Time on Study	
			Dosed (Weeks)	Observed (weeks)
Male				
Matched-Control	20	0		105
Low-Dose	50	5,000	104	1
High-Dose	50	10,000	104	1
Female				
Matched-Control	20	0		105
Low-Dose	50	5,000	104	1
High-Dose	50	10,000	104	1

[a]Animals were approximately 42 days of age when placed on study.

[b]Test diets were made available ad libitum, 7 days per week.

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 dosed animals at each dose level. When results for a number of dosed 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 dosed group compared with 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 dosed 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 dosed 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 dosed 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 dosed 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

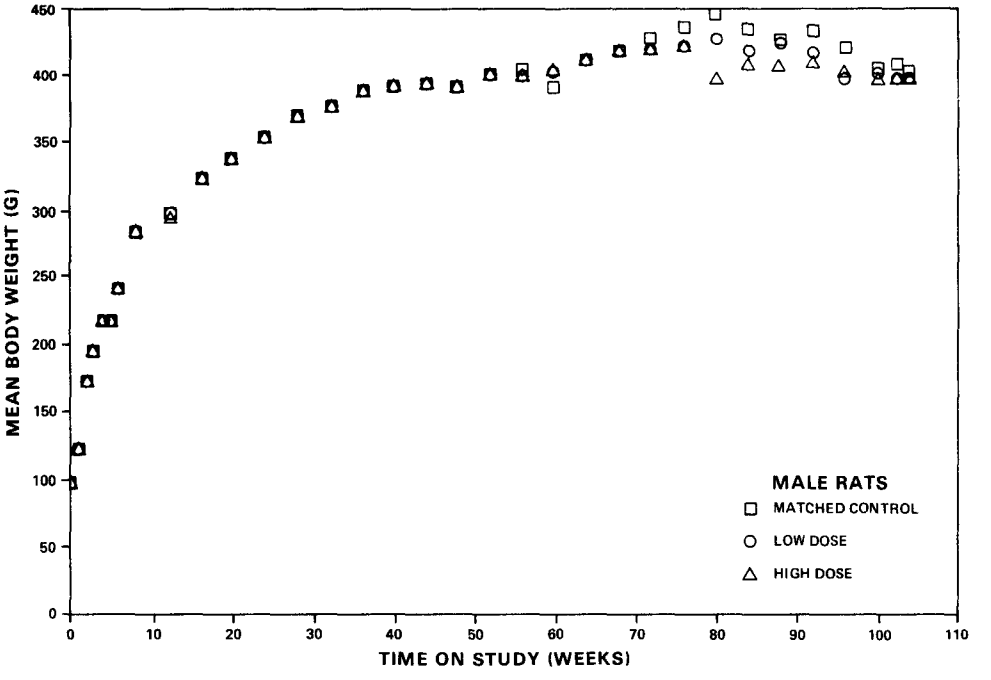
A. Body Weights and Clinical Signs (Rats)

Mean body weights of all groups of dosed rats were generally comparable with those of the matched controls, except toward the end of the study, when the mean weights of the high-dose males were slightly depressed (figure 1). Fluctuation in the growth curve for the male rats may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. No other clinical signs related to administration of daminozide were reported.

B. Survival (Rats)

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

The results of the Tarone test for positive dose-related trend in mortality are not significant in either sex. At least 70% of the animals lived to the end of the study (44/50 of the high-dose males, 37/50 of the low-dose males, 14/20 of the control males; 39/50 of the high-dose females, 35/50 of the low-dose females, and all 20 of the control females; one female control died in the



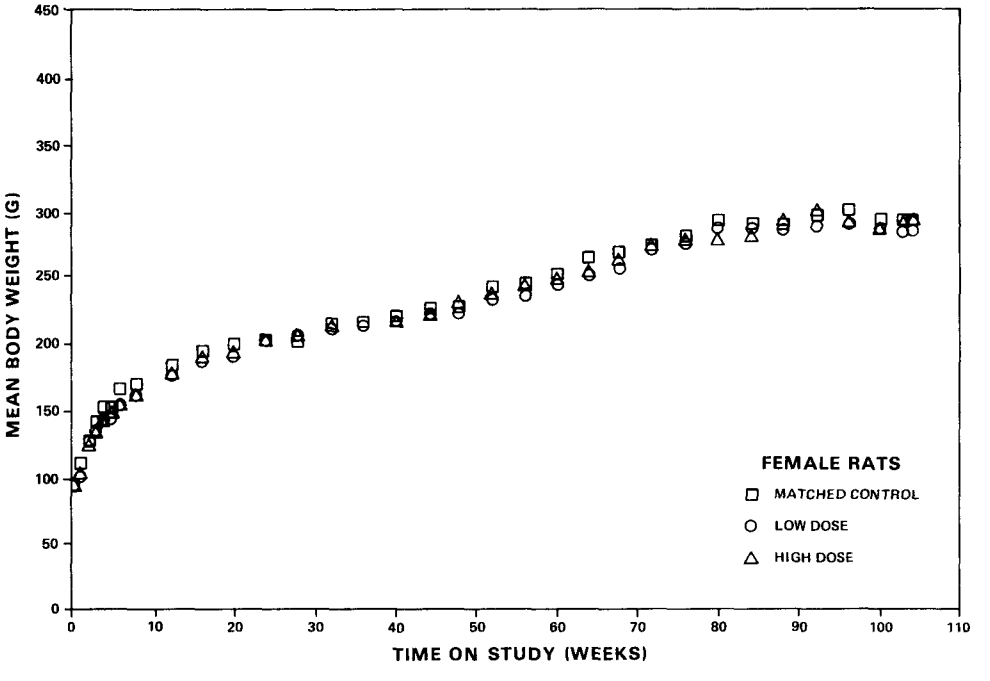


Figure 1. Growth Curves For Rats Fed Daminozide in the Diet

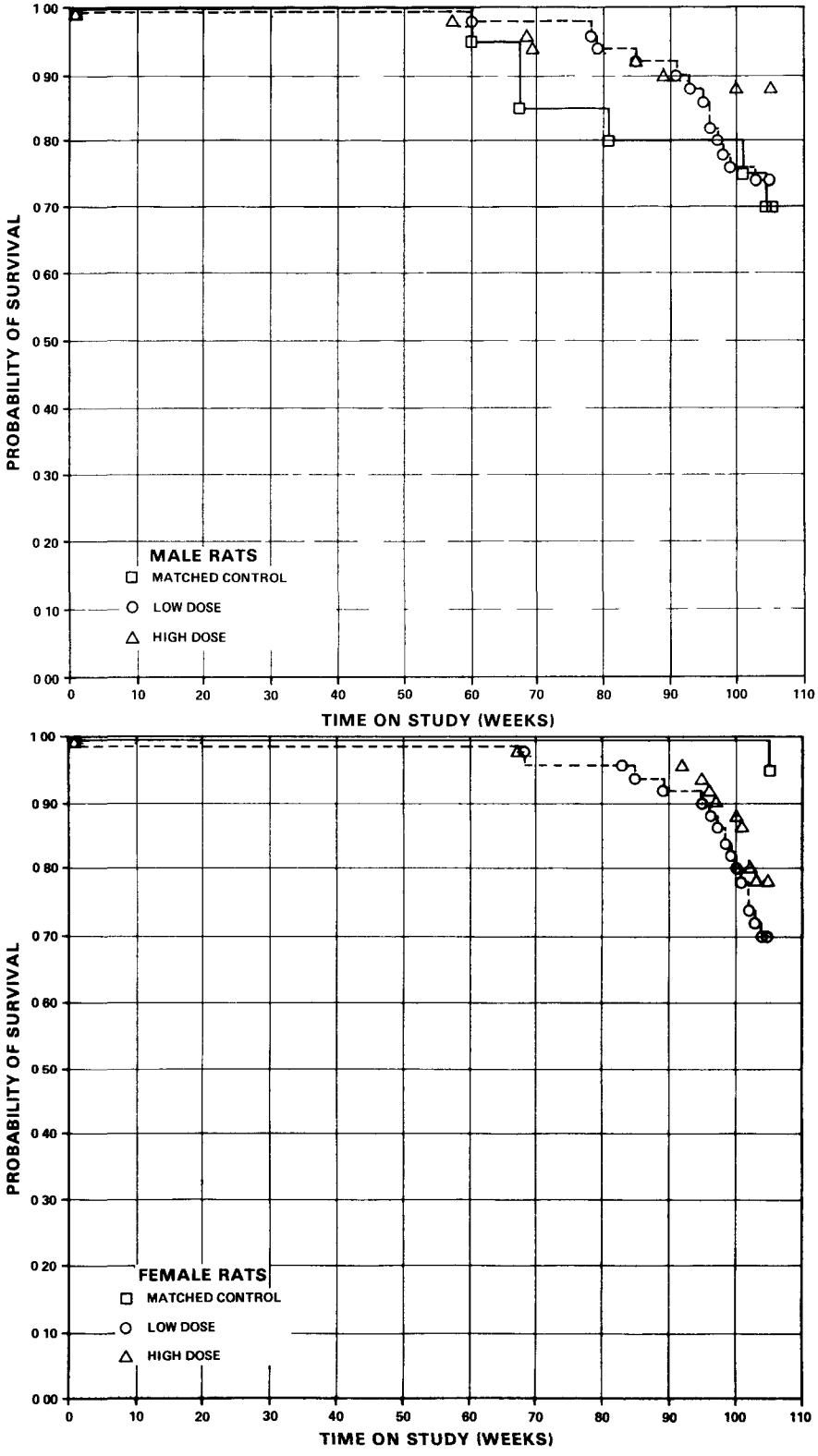


Figure 2. Survival Curves For Rats Fed Daminozide in the Diet

last week of the study). Sufficient numbers of rats of each sex were at risk for development of late-appearing tumors. In male rats, the control group had an increased incidence in mortality compared with that of the dosed groups from week 60 to the end of the study.

C. Pathology (Rats)

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

A variety of neoplastic and nonneoplastic changes were observed in the low- and high-dose groups of rats administered daminozide and in the control groups.

The most significant finding in this bioassay was the increased incidence of malignant tumors of the uterus in the rats administered the test chemical. The incidences were as follows:

	Matched Control	Low Dose	High Dose
Number of animals with tissues examined microscopically	19	50	50
Uterus/Endometrium			
Endometrial Adenocarcinoma	0	5(10%)	3(6%)
Uterus			
Leiomyosarcoma	0	1(2%)	3(6%)

The endometrial adenocarcinomas were characterized by irregular acini of pleomorphic, hyperchromatic epithelial cells that invaded the myometrium and in many cases extended to the serosa and mesometrium. Most of the adenocarcinomas were scirrhous; two metastasized to the lungs and one to the bladder wall. Several appeared to arise from endometrial stromal polyps, which also occurred in 6/50 (12%) low-dose and 4/50 (8%) high-dose female rats that did not have adenocarcinomas. The leiomyosarcomas were rather poorly differentiated, with the exception of one which occurred in a high-dose female. They consisted of spindle cells, often with abundant eosinophilic cytoplasm and elongated pleomorphic nuclei. The cells formed irregular, interwoven patterns which obliterated the endometrium and extended through the entire uterine wall. Giant spindle cells and bizarre nuclear forms were evident in some areas. In one high-dose animal, a leiomyosarcoma occurred simultaneously with a noninvasive endometrial adenocarcinoma.

Other tumors and hyperplasias that occurred in the control and dosed rats were of the expected types and incidences for this age group of Fischer 344 rats. A few more sebaceous gland carcinomas of the ear (Zymbal's gland tumors) and adenomas of the preputial glands and lungs were encountered in the dosed groups when compared with controls. Although these tumors are not common in

aging Fischer 344 rats, the differences are not considered great enough in magnitude to be meaningful.

All of the nonneoplastic lesions were considered to be spontaneous changes that occurred with similar frequency and intensity in control and dosed rats.

Based on the histopathologic examination, it is concluded that under the conditions of this bioassay administration of daminozide was associated with low numbers of endometrial adenocarcinomas, as well as of leiomyosarcomas, of the uterus.

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 occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In the male rats, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of interstitial-cell tumors of the testis are significant ($P = 0.003$), but an indicated departure from linear trend is observed ($P = 0.042$), because of the relatively steep increase in incidence observed in the dosed groups. The results of the Fisher exact test show that the incidences in both the low- and high-dose groups are signifi-

cantly higher than that in the matched controls (P = 0.009 and P = 0.004, respectively). Data compiled to date on the historical-control male rats at this laboratory show, however, an incidence of 182/220 (83%). The matched-control group of male rats had a higher incidence of mortality from week 60 to the end of the study than did the dosed groups, and this may account for the apparent significance of the incidence of interstitial-cell tumors of the testis in the dosed groups.

In the female rats, the results of the Cochran-Armitage test for the incidence of lung tumors are significant (P = 0.036), but the results of the Fisher exact test are not significant. A negative linear trend (P = 0.021) is observed in the combined incidence of C-cell adenomas and carcinomas of the thyroid, and the results of the Fisher exact test for the comparison of the incidence in the high-dose group with that in the matched controls is 0.032 in the negative direction; this P value of 0.032 is above that of 0.025 required for statistical significance when the Bonferroni criterion for multiple comparisons is considered. While the difference in survivals of the high-dose and control groups is not statistically significant (P > 0.05), some increase in mortality in the dosed group is observed, and this may account for the incidence of the thyroid tumors in the negative direction.

Although the results of the Cochran-Armitage and Fisher exact tests on the incidences of adenocarcinomas of the uterus/endometrium and of leiomyosarcomas of the uterus in female rats were not significant, these tumors are rarely observed in female untreated-control rats. Data compiled to date from all laboratories in the bioassay program show that the incidence of adenocarcinomas of the uterus/endometrium is 4/1,659 (0.24%) and that the incidence of leiomyosarcomas of the uterus is 1/1,659 (0.06%). Of the 220 female untreated-control rats observed to date in the present laboratory, no leiomyosarcomas of the uterus and only two adenocarcinomas of the uterus (0.9%) were found.

Assuming that these adenocarcinomas (2/220 [0.9%]) follow a binomial distribution, the probability of occurrence of 3 or more tumors in 50 high-dose animals is less than 0.002, while the occurrence of 5 or more tumors in 50 low-dose animals is less than 0.001. Assuming that the leiomyosarcomas with assumed incidence of 1/221 (0.5%) follow a binomial distribution, the probability of the occurrence of 3 or more tumors in 50 high-dose animals is less than 0.0001.

In each of the 95% confidence intervals of relative risk, shown in the tables, except that for the incidence of tumors of the testis, a 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 daminozide, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

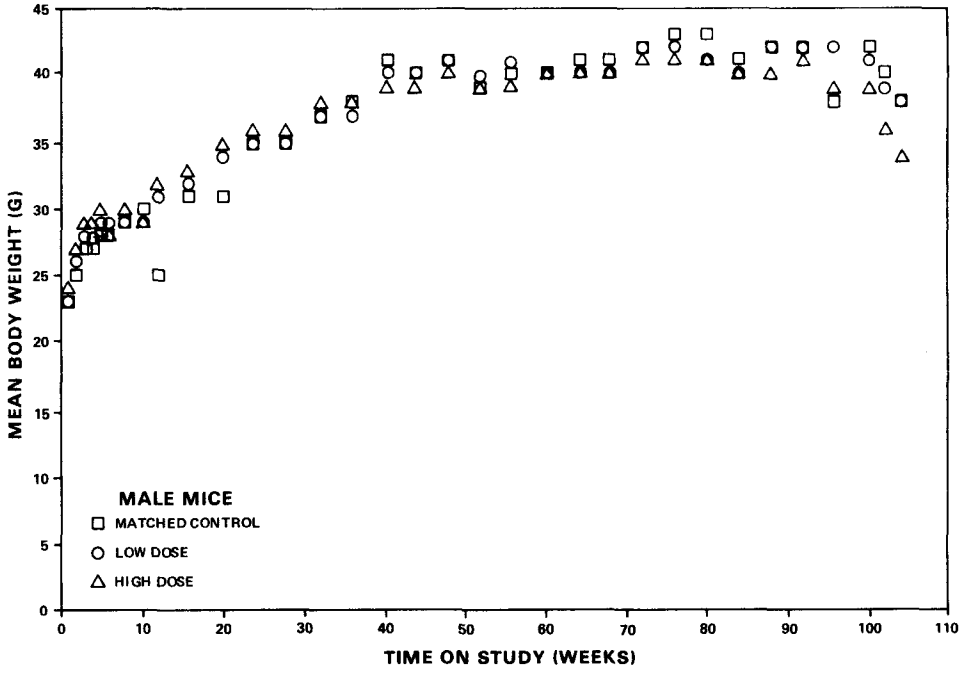
A. Body Weights and Clinical Signs (Mice)

Mean body weights of the high-dose female mice were lower than those of the matched controls throughout the bioassay, while mean body weights of the other dosed groups were essentially unaffected (figure 3). Fluctuation 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 clinical signs related to administration of daminozide were observed.

B. Survival (Mice)

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

In the male mice, the results of the Tarone test for positive dose-related trend in mortality are not significant. There were 32/50 (64%) of the high-dose group, 38/50 (76%) of the low-dose group, and 11/20 (55%) of the controls alive at the end of the bioassay. Five of the male controls and four of the male high-dose group were reported missing during the study. In the females, the results of the Tarone test are significant (P =



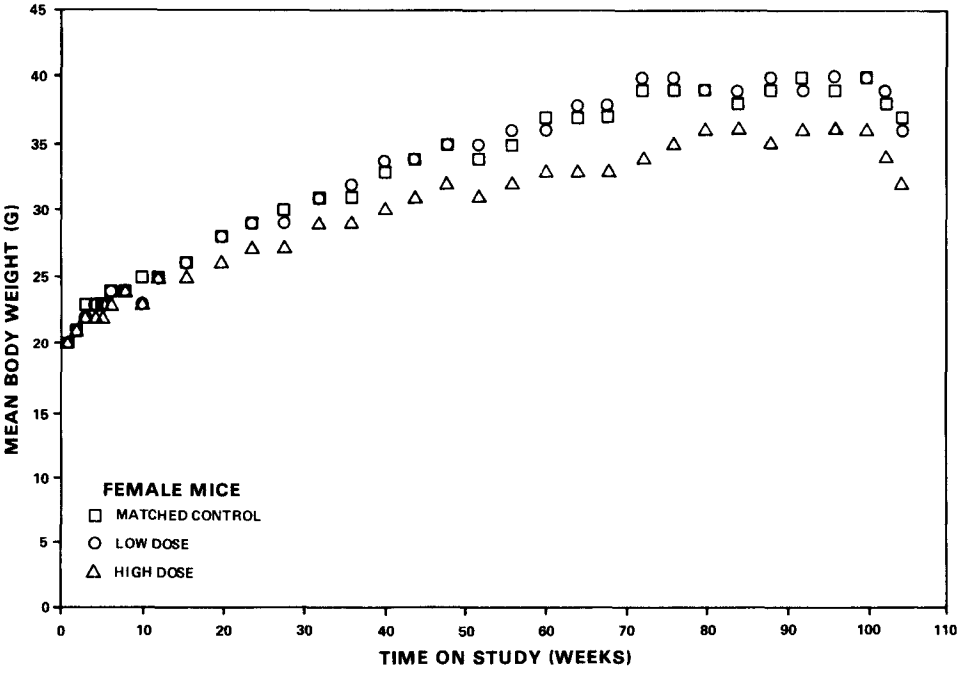


Figure 3. Growth Curves For Mice Fed Daminozide in the Diet

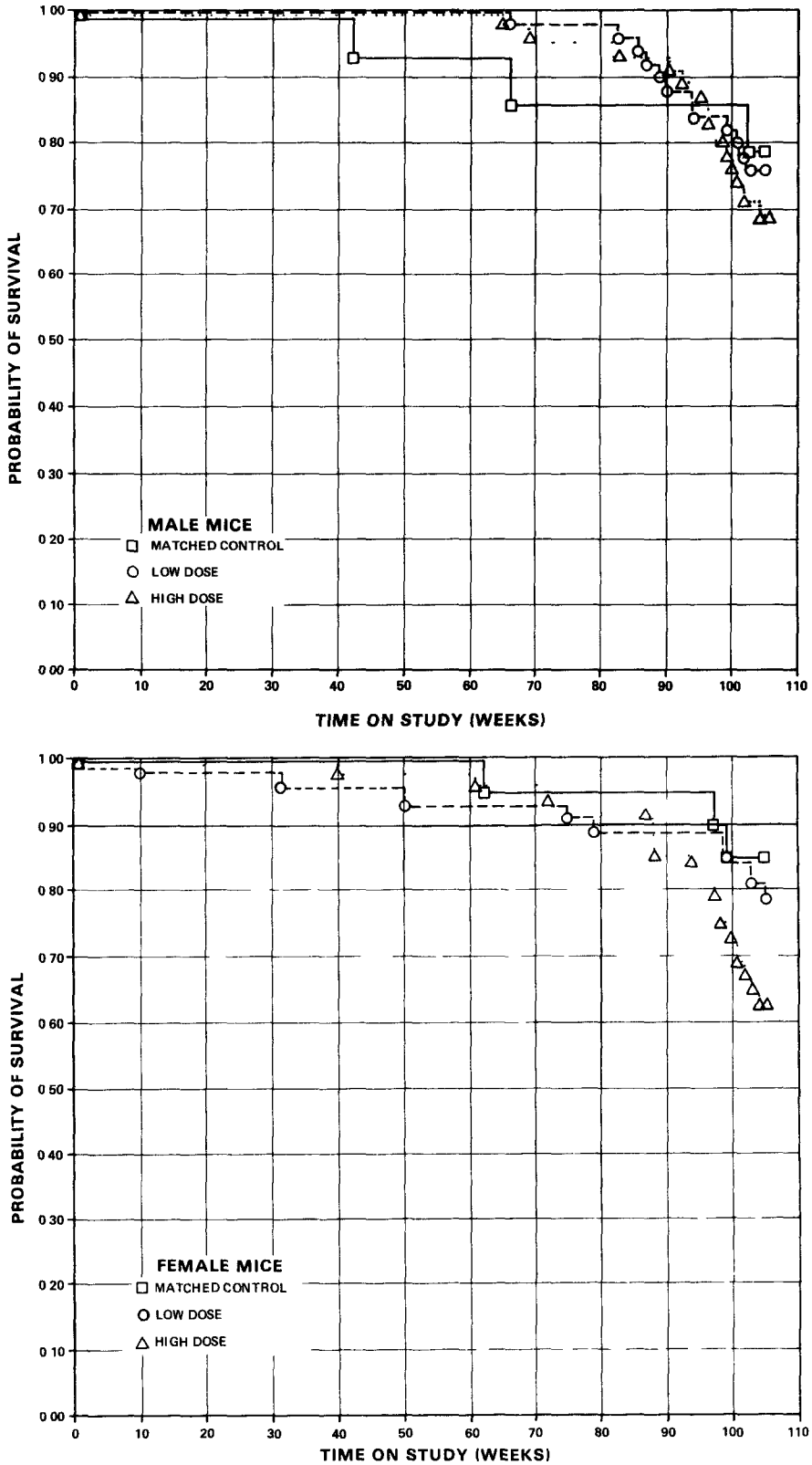


Figure 4. Survival Curves For Mice Fed Daminozide in the Diet

0.044). There were 30/50 (60%) of the high-dose group, 32/50 (64%) of the low-dose group, and 17/20 (85%) of the controls alive at the end of the bioassay. Sufficient numbers of mice of each sex were at risk for development of late-appearing tumors.

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.

There was a relatively high incidence of hepatocellular carcinomas observed in the dosed mice. Hepatocellular carcinomas were observed in 7/50 (14%) low-dose and 13/46 (28%) high-dose male mice and in 3/40 (8%) low-dose female mice. No hepatocellular carcinomas were observed in the high-dose female mice or in the control mice of either sex. Grossly, the liver tumors were multinodular friable masses that usually measured 1 cm or greater in diameter. Some had necrotic or hemorrhagic areas. Microscopically, the liver tumors had a variety of patterns including disorganized plates, irregular trabeculae, and acinar-like structures of hepatocyte-like cells that were often hyperchromatic and pleomorphic, with basophilic or vacuolated cytoplasm. There was a loss of lobular architecture in the neoplastic areas that compressed and invaded normal surrounding hepatic

parenchyma. Cords of neoplastic hepatic cells often traversed blood-filled spaces that lay in some of the affected zones. The hepatocellular carcinomas metastasized to the lungs of one low-dose and two high-dose male mice and to the adrenal of one high-dose male mouse. An angioma was observed in the liver of a high-dose male mouse, and an angiosarcoma was observed in the liver of a low-dose female.

Other tumors and hyperplasias that occurred in the control and dosed groups were of the expected types and incidences for aged B6C3F1 mice.

All of the nonneoplastic lesions were considered to be spontaneous changes, and most have been previously documented by long-term experiments in B6C3F1 mice. These changes occurred with similar incidences in control and dosed groups.

Based on the histopathologic examination, a relationship may exist between the numbers of hepatocellular carcinomas observed in the dosed mice and the administration of daminozide under the conditions of this bioassay. However, the incidences seen are within the range expected for this strain of mice.

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 occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In the male mice, the results of the Cochran-Armitage test for positive dose-related trend in the incidence of hepatocellular carcinomas are significant (P = 0.008), and the results of the Fisher exact test show that the incidence in the high-dose group is significantly higher (P = 0.020) than that in the controls. Data compiled to date on the historical-control male mice at this laboratory show, however, an incidence of 21/216 (10%), and data compiled to date on the historical-control male mice of the entire bioassay program show an incidence of 266/2,182 (12.2%). The incidence of hepatocellular carcinomas in the female mice is not significant using either statistical test. When the combined incidence of adenomas and carcinomas of the liver in each sex is analysed, the results of the Fisher exact test are not significant for either the low- or high-dose groups. The results of the Cochran-Armitage test for the combined incidence show a probability level of 0.031 in males, whereas in females it is above the 0.05 level.

Significant trends in the negative direction are observed in the incidences of lipomas of the peritoneum and of the mesentery in the female mice. This significance in the negative direction may

be accounted for by the increased mortality in the dosed groups of animals when compared with that in the control group. The results of the Fisher exact test are not significant.

In each of the 95% confidence intervals of relative risk, shown in the tables (except that for the incidence of hepatocellular carcinoma of the liver in high-dose males), a 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 daminozide, which could not be detected under the conditions of this test.

V. DISCUSSION

Under the conditions of this bioassay, daminozide was only slightly toxic to female B6C3F1 mice. Mean body weights of the high-dose female mice were lower than those of the controls; mean body weights of all other dosed groups of rats and mice were essentially unaffected, and these animals may have been able to tolerate a higher dose. No other clinical signs related to administration of daminozide were recorded, and sufficient numbers of rats and mice of each sex were at risk for development of late-appearing tumors.

In the male rats, no tumors occurred at incidences that were significantly higher in dosed groups than in controls, except for interstitial-cell tumors of the testis (controls 13/20, low-dose 49/50, high-dose 47/50). These tumors occurred, however, at a high spontaneous rate (182/220) in historical-control male rats; thus, the association of the interstitial-cell tumors with administration of the chemical is doubtful.

In the female rats, adenocarcinomas of the endometrium and leiomyosarcomas of the uterus occurred only in the dosed groups (adenocarcinomas: controls 0/19, low-dose 5/50, high-dose 3/50; leiomyosarcomas: controls 0/19, low-dose 1/50, high-dose 3/50). In historical-control female rats in this laboratory, 2/220

adenocarcinomas and 0/220 leiomyosarcomas of the uterus were found. Assuming binomial distributions for these tumors, the probability of 3/50 leiomyosarcomas in high-dose animals and 5/50 adenocarcinomas in low-dose animals is less than 0.001. Thus, the occurrence of these tumors in the dosed animals was associated with the administration of daminozide.

In the male mice, there was a dose-related trend ($P = 0.008$) in the incidence of hepatocellular carcinomas; furthermore, the incidence in the high-dose group was significant ($P = 0.020$) compared with that in the controls (controls 0/14, low-dose 7/50, high-dose 13/46). The incidence of these tumors in the historical-control male mice was, however, 21/216; thus, the association of the hepatocellular carcinomas with administration of daminozide is not clear. In the female mice, only three such tumors occurred.

Alveolar/bronchiolar adenomas or carcinomas occurred at higher incidences in the dosed groups of both male and female mice (males: controls 4/14, low-dose 15/50, high-dose 18/46; females: controls 1/20, low-dose 8/39, high-dose 10/48), but none of these incidences were significantly higher than those in the matched controls. The incidence of alveolar/bronchiolar adenomas and carcinomas in historical-control male mice at the laboratory was 39/216 and that of female controls was 12/217. Since these

incidences were also lower than those of the dosed groups, they suggest that, at least in the dosed females, there may have been an increase in the incidence of these tumors of the lung.

Daminozide has a low acute toxicity, having an acute oral LD_{50} of 8,400 mg/kg for rats (Farm Chemicals Handbook, 1977). One long-term feeding study has been reported (Farm Chemicals Handbook, 1977) in which no effects were noted after rats and dogs were fed the compound at doses up to 3,000 ppm for 2 years.

Daminozide was previously reported to induce angiomas and angiosarcomas of the blood vessels, adenomas and adenocarcinomas of the lungs, and adenomas of the kidneys in Swiss albino mice during lifetime administration of the chemical at a concentration of 2% in drinking water (Toth et al., 1977). None of these types of tumors were observed at incidences in the present bioassay which could be clearly associated with administration of the test chemical; however, alveolar/bronchiolar adenomas or carcinomas occurred at increased incidences in both sexes of mice and in the high-dose female rats. The differences in results may have been due to differences in the species and strains of animals used or in the route of administration of the test chemical. 1,1-Dimethylhydrazine, produced by hydrolysis of the daminozide, may have contributed to the carcinogenicity of the test chemical, as previously suggested (Toth et al., 1977). 1,1-Dimethylhydra-

zine has been shown to induce alveolar/bronchiolar adenomas or adenocarcinomas in Swiss albino mice administered the chemical by gavage (Roe et al., 1967) and also vascular angiosarcomas in the same strain of mice administered the chemical in the drinking water (Toth, 1973).

It is concluded that under the conditions of this bioassay, daminozide was not carcinogenic in the male Fischer 344 rats or in the female B6C3F1 mice. In male B6C3F1 mice, the induction of hepatocellular carcinomas may have been associated with the administration of the test chemical. Daminozide was carcinogenic in female Fischer 344 rats, inducing adenocarcinomas of the endometrium of the uterus and leiomyosarcomas of the uterus.

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.

Environmental Protection Agency, Title 40-Protection of Environment, Code of Federal Regulations, 180.246, 1976.

Farm Chemicals Handbook, Meister Publishing Co., Willoughby, Ohio, 1977, p. D9.

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. Am. Statist. Assoc. 53:457-481, 1958.

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.

Riddell, J. A., Hageman, H. A., J'Anthony, C. M., and Hubbard, W. L., Retardation of plant growth by a new group of chemicals. Science 136:391, 1962.

Roe, F. J. C., Grant, G. A., Millican, D. M., Carcinogenicity of hydrazine and 1,1-dimethylhydrazine for mouse lung, Nature 216:375-376, 1967.

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.

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

Thomson, W. T., Fumigants, growth regulators, repellents and rodenticides. Agricultural Chemicals - Book III: Miscellaneous Chemicals, Thomson Publications, Fresno, Calif., 1976, pp. 46-47.

Toth, B., Wallcave, W., Patie, K., Schmeltz, I., and Hoffmann, D., Induction of tumors in mice with the herbicide succinic acid 2,2-dimethylhydrazine. Cancer Res. 37:3497-3500, 1977.

Toth, B., Synthetic and naturally occurring hydrazines as possible cancer causative agents. Cancer Res. 35:3693-3697, 1975.

Toth, B., 1,1-Dimethylhydrazine (unsymmetrical) carcinogenesis in mice. Light microscopic and utrastructural studies on neoplastic blood vessels, J. Natl. Cancer Inst. 50:181-194, 1973.

APPENDIX A

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

TABLE A1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
SQUAMOUS CELL PAPILLOMA	1 (5%)		
SQUAMOUS CELL CARCINOMA		1 (2%)	
FIBROMA		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA		1 (2%)	
FIBROSARCOMA		2 (4%)	2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	
THYMOMA, METASTATIC	1 (5%)		
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
LEUKEMIA,NOS	1 (5%)		
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
#THYMUS	(1)	(2)	(1)
THYMOMA, MALIGNANT	1 (100%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*LIP	(20)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR CARCINOMA		1 (2%)	
#STOMACH	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
#SMALL INTESTINE	(19)	(50)	(50)
LEIOMYOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
TUBULAR-CELL ADENOCARCINOMA		1 (2%)	
THYMOMA, METASTATIC	1 (5%)		
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(43)	(49)
CHROMOPHOBE ADENOMA		2 (5%)	2 (4%)
#ADRENAL	(20)	(50)	(50)
PHEOCHROMOCYTOMA	2 (10%)	2 (4%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(16)	(38)	(43)
FOLLICULAR-CELL CARCINOMA	1 (6%)		
C-CELL ADENOMA	2 (13%)	3 (8%)	
C-CELL CARCINOMA			2 (5%)
#PARATHYROID	(12)	(22)	(22)
ADENOMA, NOS		1 (5%)	
#PANCREATIC ISLETS	(20)	(48)	(49)
ISLET-CELL ADENOMA	1 (5%)		
REPRODUCTIVE SYSTEM			
*PREPUCE	(20)	(50)	(50)
TRICHOEPITHELIOMA			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(20)	(50)	(50)
CARCINOMA,NOS	1 (5%)		
ADENOMA, NOS	1 (5%)	1 (2%)	4 (8%)
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	13 (65%)	46 (92%)	47 (94%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(19)	(50)	(50)
MESOTHELIOMA, METASTATIC	1 (5%)		
#BRAIN	(19)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
THYMOMA, METASTATIC	1 (5%)		
MEDULLOBLASTOMA			1 (2%)
SPECIAL SENSE ORGANS			
*EYELID	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
*ZYMBAL'S GLAND	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
*VERTEBRA	(20)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
LEIOMYOMA			1 (2%)
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)
MESOTHELIOMA, MALIGNANT	1 (5%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	3	3
MORIBUND SACRIFICE	2	10	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	37	44
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	16	50	49
TOTAL PRIMARY TUMORS	26	70	69
TOTAL ANIMALS WITH BENIGN TUMORS	13	48	47
TOTAL BENIGN TUMORS	20	60	58
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	8	10
TOTAL MALIGNANT TUMORS	6	9	10
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	2	
TOTAL SECONDARY TUMORS	4	3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
TRICHOEPITHELIOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA	1 (5%)		
*SUBCUT TISSUE	(20)	(50)	(50)
LIPOMA		2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
ADENOCARCINOMA, NOS, METASTATIC		3 (6%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			4 (8%)
SEBACEOUS ADENOCARCINOMA, METAST	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
LEUKEMIA,NOS		1 (2%)	
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
LYMPHOCYTIC LEUKEMIA			1 (2%)
GRANULOCYTIC LEUKEMIA		2 (4%)	
#LYMPH NODE	(20)	(49)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(48)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
#LIVER	(19)	(50)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	1 (2%)
URINARY SYSTEM			
#URINARY BLADDER	(19)	(43)	(43)
PAPILLOMATOSIS		1 (2%)	
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(45)	(43)
CHROMOPHOBE ADENOMA	3 (16%)	7 (16%)	8 (19%)
CHROMOPHOBE CARCINOMA		3 (7%)	
#ADRENAL	(20)	(48)	(48)
CORTICAL ADENOMA	1 (5%)		
PHEOCHROMOCYTOMA, MALIGNANT	1 (5%)		
#THYROID	(15)	(38)	(44)
C-CELL ADENOMA	2 (13%)	2 (5%)	2 (5%)
C-CELL CARCINOMA	2 (13%)	1 (3%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	
ADENOCARCINOMA, NOS			1 (2%)
PAPILLARY ADENOCARCINOMA		1 (2%)	
ACINAR-CELL ADENOMA		1 (2%)	
FIBROADENOMA	3 (15%)	9 (18%)	2 (4%)
*MAMMARY DUCT	(20)	(50)	(50)
ADENOMA, NOS			1 (2%)
*PREPUTIAL GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#UTERUS	(19)	(50)	(50)
LEIOMYOSARCOMA		1 (2%)	3 (6%)
ENDOMETRIAL STROMAL POLYP		6 (12%)	4 (8%)
HEMANGIOMA			1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(50)	(50)
ADENOCARCINOMA, NOS		5 (10%)	3 (6%)
#OVARY	(19)	(50)	(49)
GRANULOSA-CELL TUMOR		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
CHROMOPHOBE CARCINOMA, INVASIVE		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
LIPOMA			2 (4%)
HEMANGIOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@		7	6
MORIBUND SACRIFICE	1	8	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	35	39
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	32	28
TOTAL PRIMARY TUMORS	13	51	36
TOTAL ANIMALS WITH BENIGN TUMORS	8	24	19
TOTAL BENIGN TUMORS	9	32	26
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	15	9
TOTAL MALIGNANT TUMORS	4	18	10
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	4	
TOTAL SECONDARY TUMORS	1	5	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

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

TABLE B1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	6		4
ANIMALS NECROPSIED	14	50	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	50	46
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(50)	(46)
FIBROMA	1 (7%)		
NEUROFIBROSARCOMA	1 (7%)		
RESPIRATORY SYSTEM			
#LUNG	(14)	(50)	(46)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (14%)	7 (14%)	6 (13%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (14%)	9 (18%)	12 (26%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(50)	(46)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE			5 (11%)
LEUKEMIA,NOS			2 (4%)
LYMPHOCYTIC LEUKEMIA			1 (2%)
#SPLEEN	(13)	(40)	(43)
FIBROSARCOMA			1 (2%)
HEMANGIOMA	1 (8%)	1 (3%)	
ANGIOMA	1 (8%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (8%)		
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#MESENTERIC L. NODE	(9)	(42)	(43)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
#SMALL INTESTINE	(13)	(48)	(44)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PEYERS PATCH	(13)	(48)	(44)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (8%)	1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(14)	(50)	(46)
HEPATOCELLULAR ADENOMA	1 (7%)	2 (4%)	1 (2%)
HEPATOCELLULAR CARCINOMA		7 (14%)	13 (28%)
SARCOMA, NOS			1 (2%)
ANGIOMA			1 (2%)
*BILE DUCT	(14)	(50)	(46)
BILE DUCT CARCINOMA		1 (2%)	
#PANCREAS	(12)	(47)	(42)
BILE DUCT CARCINOMA, METASTATIC		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(8)	(35)	(38)
HEPATOCELLULAR CARCINOMA, METAST			1 (3%)
PHEOCHROMOCYTOMA		1 (3%)	
#THYROID	(12)	(39)	(35)
FOLLICULAR-CELL ADENOMA*		1 (3%)	1 (3%)
#PANCREATIC ISLETS	(12)	(47)	(42)
ISLET-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(14)	(50)	(46)
FIBROSARCOMA			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(14)	(50)	(46)
NEOPLASM, NOS			1 (2%)
LIPOMA		1 (2%)	1 (2%)
*PERITONEUM	(14)	(50)	(46)
LIPOMA		1 (2%)	
*MESENTERY	(14)	(50)	(46)
NEOPLASM, NOS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	9	10
MORIBUND SACRIFICE	1	3	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	11	38	32
ANIMAL MISSING	6		4

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	28	37
TOTAL PRIMARY TUMORS	11	38	51
TOTAL ANIMALS WITH BENIGN TUMORS	5	10	11
TOTAL BENIGN TUMORS	6	14	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	22	33
TOTAL MALIGNANT TUMORS	5	23	39
TOTAL ANIMALS WITH SECONDARY TUMORS#		2	2
TOTAL SECONDARY TUMORS		2	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	1
TOTAL UNCERTAIN TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		9	2
ANIMALS NECROPSIED	20	41	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	41	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(41)	(48)
HEMANGIOMA		1 (2%)	
RESPIRATORY SYSTEM			
*LUNG	(20)	(39)	(48)
CARCINOMA, NOS, METASTATIC		1 (3%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		4 (10%)	8 (17%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	4 (10%)	2 (4%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(41)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	2 (4%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)	5 (12%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		5 (12%)	
PLASMA-CELL TUMOR			1 (2%)
LEUKEMIA,NOS	1 (5%)		1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (5%)	1 (2%)	1 (2%)
PLASMACYTIC LEUKEMIA		1 (2%)	
GRANULOCYTIC LEUKEMIA			1 (2%)
*ABDOMINAL CAVITY	(20)	(41)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#SPLEEN	(17)	(34)	(42)
HEMANGIOMA		1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (6%)		1 (2%)
ERYTHROCYTIC LEUKEMIA		1 (3%)	
#MANDIBULAR L. NODE	(20)	(37)	(40)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#MEDIASTINAL L.NODE	(20)	(37)	(40)
MALIGNANT LYMPHOMA, NOS			1 (3%)
#MESENTERIC L. NODE	(20)	(37)	(40)
HEMANGIOMA		1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (5%)		
MALIGNANT LYMPHOMA, MIXED TYPE			1 (3%)
#SMALL INTESTINE	(20)	(38)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(40)	(48)
HEPATOCELLULAR ADENOMA	1 (5%)	1 (3%)	
HEPATOCELLULAR CARCINOMA		3 (8%)	
ANGIOSARCOMA		1 (3%)	
HAMARTOMA		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(20)	(41)	(48)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(19)	(14)
CHROMOPHOBE ADENOMA	2 (14%)	1 (5%)	
#ADRENAL	(17)	(31)	(38)
CORTICAL CARCINOMA		1 (3%)	
#THYROID	(13)	(28)	(38)
FOLLICULAR-CELL ADENOMA	1 (8%)		
#PANCREATIC ISLETS	(16)	(36)	(44)
ISLET-CELL ADENOMA			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(37)	(45)
ADENOCA/SQUAMOUS METAPLASIA			1 (2%)
ENDOMETRIAL STROMAL POLYP		3 (8%)	
HEMANGIOMA		1 (3%)	
NEURILEMOMA	1 (5%)		
#UTERUS/ENDOMETRIUM	(20)	(37)	(45)
CARCINOMA,NOS		1 (3%)	
#OVARY	(13)	(14)	(23)
LUTEOMA	1 (8%)		
GRANULOSA-CELL TUMOR	1 (8%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(41)	(48)
LEIOMYOMA			1 (2%)
*PERITONEUM	(20)	(41)	(48)
LIPOMA	2 (10%)		
*MESENTERY	(20)	(41)	(48)
LIPOMA	2 (10%)	1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	8	13
MORIBUND SACRIFICE		1	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	32	30
ANIMAL MISSING		9	2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	27	25
TOTAL PRIMARY TUMORS	17	41	28
TOTAL ANIMALS WITH BENIGN TUMORS	8	13	10
TOTAL BENIGN TUMORS	10	15	10
TOTAL ANIMALS WITH MALIGNANT TUMORS	6	19	15
TOTAL MALIGNANT TUMORS	6	26	17
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1		1
TOTAL UNCERTAIN TUMORS	1		1
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

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

TABLE C1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		2 (4%)	
ABSCESS, NOS		1 (2%)	
*SUBCUT TISSUE	(20)	(50)	(50)
HEMORRHAGIC CYST	1 (5%)		
ABSCESS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(20)	(50)	(50)
BRONCHIECTASIS		1 (2%)	
#LUNG	(20)	(50)	(50)
PNEUMONIA, ASPIRATION	1 (5%)	1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	7 (35%)	7 (14%)	16 (32%)
PNEUMONIA INTERSTITIAL CHRONIC		1 (2%)	
HYPERPLASIA, ADENOMATOUS		2 (4%)	3 (6%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(48)	(48)
HEMOSIDEROSIS	1 (5%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HEMATOPOIESIS		1 (2%)	1 (2%)
#THYMUS	(1)	(2)	(1)
HYPERPLASIA, EPITHELIAL			1 (100%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(18)	(50)	(49)
INFLAMMATION, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS	2 (11%)	10 (20%)	12 (24%)
FIBROSIS, FOCAL		4 (8%)	4 (8%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(49)	(46)
ATROPHY, NOS		1 (2%)	
#LIVER	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
BASOPHILIC CYTO CHANGE	3 (15%)	5 (10%)	4 (8%)
HEMATOPOIESIS		1 (2%)	
*BILE DUCT	(20)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
#PANCREAS	(20)	(48)	(49)
FIBROSIS		3 (6%)	
FIBROSIS, FOCAL		5 (10%)	3 (6%)
#PANCREATIC ACINUS	(20)	(48)	(49)
ATROPHY, NOS			3 (6%)
ATROPHY, FOCAL			1 (2%)
#LARGE INTESTINE	(16)	(49)	(48)
NEMATODIASIS	5 (31%)	17 (35%)	13 (27%)
#COLONIC MUCOUS MEMBR	(16)	(49)	(48)
ULCER, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC	11 (55%)	30 (60%)	32 (64%)
HYPERTROPHY, NOS			1 (2%)
#KIDNEY/PELVIS	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#URINARY BLADDER	(19)	(45)	(42)
INFLAMMATION, ACUTE HEMORRHAGIC			1 (2%)

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

TABLE C1. MALE RATS: NONNEPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(43)	(49)
CYST, NOS			1 (2%)
HEMORRHAGIC CYST	1 (6%)		
#ADRENAL	(20)	(50)	(50)
DEGENERATION, LIPOID	1 (5%)		
NECROSIS, NOS			1 (2%)
METAMORPHOSIS FATTY			1 (2%)
#ADRENAL MEDULLA	(20)	(50)	(50)
DEGENERATION, HYALINE			1 (2%)
#THYROID	(16)	(38)	(43)
FOLLICULAR CYST, NOS	1 (6%)		
HYPERPLASIA, C-CELL	1 (6%)	2 (5%)	7 (16%)
#PARATHYROID	(12)	(22)	(22)
HYPERPLASIA, NOS			2 (9%)
#PANCREATIC ISLETS	(20)	(48)	(49)
HYPERPLASIA, NOS		3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GYNECOMASTIA		1 (2%)	
#PROSTATE	(19)	(40)	(47)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
*SEMINAL VESICLE	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (5%)	1 (2%)	1 (2%)
#TESTIS	(20)	(50)	(50)
ATROPHY, NOS			3 (6%)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(19)	(50)	(50)
HEMORRHAGE		1 (2%)	

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

TABLE C1. MALE RATS: NONNEPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
PERIARTERITIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		

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

TABLE C2.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
CONGESTION, NOS		1 (2%)	
EDEMA, NOS		1 (2%)	
PNEUMONIA, LIPID		2 (4%)	
PNEUMONIA, ASPIRATION	1 (5%)		
PNEUMONIA, CHRONIC MURINE	5 (25%)	13 (26%)	9 (19%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(46)	(48)
FIBROSIS, FOCAL	1 (5%)		
#SPLEEN	(19)	(50)	(48)
HEMOSIDEROSIS		1 (2%)	
CYTOMEGALY			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIESIS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(50)	(50)
FIBROSIS		4 (8%)	4 (8%)
FIBROSIS, FOCAL		1 (2%)	
CALCIFICATION, FOCAL			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
*GUM OF MANDIBLE	(20)	(50)	(50)
ABSCESS, NOS			1 (2%)
#LIVER	(19)	(50)	(50)
INFLAMMATION, CHRONIC			2 (4%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	2 (11%)	1 (2%)	3 (6%)
NECROSIS, CENTRAL			1 (2%)
METAMORPHOSIS FATTY		3 (6%)	2 (4%)
BASOPHILIC CYTO CHANGE	5 (26%)	7 (14%)	11 (22%)
HEMATOPOIESIS			1 (2%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(48)	(49)
FIBROSIS, FOCAL	1 (5%)	2 (4%)	
NECROSIS, NOS		1 (2%)	
#ESOPHAGUS	(20)	(49)	(47)
INFLAMMATION, NECROTIZING			1 (2%)
#LARGE INTESTINE	(17)	(44)	(48)
NEMATODIASIS	8 (47%)	8 (18%)	12 (25%)
#COLON	(17)	(44)	(48)
NEMATODIASIS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(48)
HYDRONEPHROSIS		2 (4%)	
PYELONEPHRITIS, NOS			1 (2%)
INFLAMMATION, CHRONIC	2 (10%)	9 (18%)	5 (10%)
NEPHROSIS, CHOLEMIC		1 (2%)	
INFARCT, NOS	1 (5%)		
INFARCT, HEALED			1 (2%)
CALCIFICATION, FOCAL		1 (2%)	1 (2%)
#URINARY BLADDER	(19)	(43)	(43)
INFLAMMATION, SUPPURATIVE			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#U. BLADDER/MUCOSA	(19)	(43)	(43)
ATYPIA, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(45)	(43)
CYST, NOS		1 (2%)	
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	
#ADRENAL	(20)	(48)	(48)
DEGENERATION, LIPOID		1 (2%)	
NECROSIS, FOCAL			1 (2%)
#ADRENAL CORTEX	(20)	(48)	(48)
LIPOIDOSIS	1 (5%)		
#THYROID	(15)	(38)	(44)
HYPERPLASIA, C-CELL			3 (7%)
#PANCREATIC ISLETS	(19)	(48)	(49)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
GALACTOCELE			1 (2%)
CYST, NOS		1 (2%)	
HYPERPLASIA, CYSTIC		1 (2%)	
*VAGINA	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#UTERUS	(19)	(50)	(50)
CYST, NOS		1 (2%)	
PYOMETRA	3 (16%)	4 (8%)	3 (6%)
ABSCESS, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL		1 (2%)	1 (2%)
POLYP, INFLAMMATORY	2 (11%)	1 (2%)	2 (4%)
#UTERUS/ENDOMETRIUM	(19)	(50)	(50)
INFLAMMATION, NOS	1 (5%)		
INFLAMMATION, SUPPURATIVE	4 (21%)	9 (18%)	7 (14%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		2 (4%)	
HYPERPLASIA, CYSTIC		1 (2%)	1 (2%)
HYPERPLASIA, STROMAL		1 (2%)	
#OVARY/OVIDUCT	(19)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (5%)	1 (2%)	3 (6%)
ABSCESS, NOS		1 (2%)	
#OVARY	(19)	(50)	(49)
CYST, NOS	4 (21%)	9 (18%)	8 (16%)
ABSCESS, NOS	1 (5%)	1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
COMPRESSION			1 (2%)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		3

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

APPENDIX D

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

TABLE D1.

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

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	6		4
ANIMALS NECROPSIED	14	50	46
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	50	46
INTEGUMENTARY SYSTEM			
*SKIN	(14)	(50)	(46)
CYST, NOS	2 (14%)	1 (2%)	1 (2%)
*SUBCUT TISSUE	(14)	(50)	(46)
GRANULOMA, NOS	1 (7%)		
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(14)	(50)	(46)
INFLAMMATION, NOS	1 (7%)	1 (2%)	
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC	4 (29%)	2 (4%)	1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#LUNG	(14)	(50)	(46)
CONGESTION, NOS	1 (7%)	3 (6%)	
HEMORRHAGE		2 (4%)	3 (7%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE		3 (6%)	
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
GRANULOMA, NOS	1 (7%)		
NODULE		1 (2%)	
PERIVASCULITIS	5 (36%)	4 (8%)	7 (15%)
HYPERPLASIA, ADENOMATOUS		3 (6%)	1 (2%)
LEUKEMOID REACTION	1 (7%)		
#LUNG/ALVEOLI	(14)	(50)	(46)
HISTIOCYTOSIS		3 (6%)	3 (7%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(14)	(50)	(46)
MYELOPROLIFERATIVE DISORDER			1 (2%)
#BONE MARROW	(14)	(45)	(43)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
#SPLEEN	(13)	(40)	(43)
INFLAMMATION, FOCAL GRANULOMATOU		1 (3%)	
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID		3 (8%)	1 (2%)
HEMATOPOIESIS	1 (8%)	5 (13%)	1 (2%)
#LYMPH NODE	(9)	(42)	(43)
HYPERPLASIA, RETICULUM CELL			1 (2%)
#MANDIBULAR L. NODE	(9)	(42)	(43)
HYPERPLASIA, NOS		2 (5%)	5 (12%)
HISTIOCYTOSIS		1 (2%)	
PLASMACYTOSIS		1 (2%)	2 (5%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#BRONCHIAL LYMPH NODE	(9)	(42)	(43)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(9)	(42)	(43)
DILATATION, NOS			1 (2%)
LYMPHANGIECTASIS		7 (17%)	2 (5%)
CONGESTION, CHRONIC			1 (2%)
HEMORRHAGE	1 (11%)	3 (7%)	1 (2%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
HYPERPLASIA, NOS		2 (5%)	
HYPERPLASIA, FOCAL		1 (2%)	
HISTIOCYTOSIS	1 (11%)	1 (2%)	
PLASMACYTOSIS			1 (2%)
MEGAKARYOCYTOSIS	1 (11%)		
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HEMATOPOIESIS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(14)	(48)	(45)
ENDOCARDITIS, BACTERIAL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CALCIFICATION, FOCAL			1 (2%)
#MYOCARDIUM	(14)	(48)	(45)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
*CORONARY ARTERY	(14)	(50)	(46)
ARTERIOSCLEROSIS, NOS		1 (2%)	
*INGUINAL ARTERY	(14)	(50)	(46)
INFLAMMATION, NOS		1 (2%)	
ARTERIOSCLEROSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(12)	(47)	(43)
PERIVASCULITIS	1 (8%)	6 (13%)	2 (5%)
#LIVER	(14)	(50)	(46)
MULTILOCULAR CYST		3 (6%)	
THROMBUS, ORGANIZED			1 (2%)
CONGESTION, CHRONIC PASSIVE			5 (11%)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION ACTIVE CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (7%)	2 (4%)	2 (4%)
CIRRHOSIS, NOS		3 (6%)	
PERIVASCULITIS	1 (7%)	1 (2%)	
DEGENERATION, HYDROPIC			1 (2%)
NECROSIS, FOCAL			1 (2%)
INFARCT, NOS			2 (4%)
METAMORPHOSIS FATTY		2 (4%)	
HEMOSIDEROSIS			4 (9%)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
BASOPHILIC CYTO CHANGE	1 (7%)		
HYPERPLASIA, NOS	1 (7%)		1 (2%)
ANGIECTASIS		1 (2%)	1 (2%)
HEMATOPOIESIS		1 (2%)	1 (2%)
*GALLBLADDER	(14)	(50)	(46)
CALCULUS, NOS			1 (2%)
*BILE DUCT	(14)	(50)	(46)
DILATATION, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NOS			2 (4%)
#PANCREAS	(12)	(47)	(42)
CYST, NOS		1 (2%)	
CYSTIC DUCTS		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
PERIVASCULITIS			1 (2%)
METAMORPHOSIS FATTY		1 (2%)	
ATROPHY, NOS			1 (2%)
#PANCREATIC DUCT	(12)	(47)	(42)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
PARASITISM	1 (8%)		
#PANCREATIC ACINUS	(12)	(47)	(42)
ATROPHY, NOS		2 (4%)	
#STOMACH	(13)	(48)	(42)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
HEMOSIDEROSIS			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#PEYERS PATCH	(13)	(48)	(44)
HYPERPLASIA, NOS	4 (31%)	9 (19%)	9 (20%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#COLON	(13)	(39)	(41)
NEMATODIASIS			1 (2%)
PARASITISM	3 (23%)	7 (18%)	16 (39%)
#COLONIC SUBMUCOSA	(13)	(39)	(41)
INFLAMMATION, ACUTE		1 (3%)	1 (2%)
INFLAMMATION, CHRONIC		1 (3%)	
INFLAMMATION, GRANULOMATOUS		1 (3%)	
URINARY SYSTEM			
#KIDNEY	(14)	(50)	(46)
INFLAMMATION, CHRONIC	2 (14%)	1 (2%)	3 (7%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
PERIVASCULITIS	2 (14%)	7 (14%)	6 (13%)
INFARCT, NOS			1 (2%)
INFARCT, HEALED		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CYTOPLASMIC VACUOLIZATION	1 (7%)		
HYPERPLASIA, TUBULAR CELL	2 (14%)	2 (4%)	1 (2%)
#KIDNEY/CORTEX	(14)	(50)	(46)
CYST, NOS			1 (2%)
SCAR			1 (2%)
#KIDNEY/TUBULE	(14)	(50)	(46)
DEGENERATION, NOS			1 (2%)
LIPOIDOSIS	3 (21%)	1 (2%)	2 (4%)
#KIDNEY/PELVIS	(14)	(50)	(46)
INFLAMMATION, CHRONIC	2 (14%)		
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#URINARY BLADDER	(10)	(47)	(39)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (10%)	1 (2%)	1 (3%)
INFLAMMATION, CHRONIC FOCAL			1 (3%)
ENDOCRINE SYSTEM			
#ADRENAL	(8)	(35)	(38)
CONGESTION, NOS			1 (3%)
FIBROSIS, FOCAL			1 (3%)
#THYROID	(12)	(39)	(35)
FOLLICULAR CYST, NOS			1 (3%)
PERIVASCULITIS		1 (3%)	
#THYROID FOLLICLE	(12)	(39)	(35)
ECTOPIA		1 (3%)	
#PANCREATIC ISLETS	(12)	(47)	(42)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(14)	(50)	(46)
INFLAMMATION, NOS	1 (7%)		
INFLAMMATION, CHRONIC		1 (2%)	
CHOLESTEROL DEPOSIT	1 (7%)		
CYTOMEGALY	1 (7%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(14)	(49)	(45)
HEMORRHAGE		1 (2%)	
CORPORA AMYLACEA		1 (2%)	1 (2%)
CALCIFICATION, NOS			1 (2%)
CALCIFICATION, FOCAL	5 (36%)	15 (31%)	13 (29%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(14)	(50)	(46)
FIBROUS OSTEODYSTROPHY		1 (2%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(14)	(50)	(46)
NECROSIS, NOS			1 (2%)
*PERITONEUM	(14)	(50)	(46)
NECROSIS, NOS		1 (2%)	
*MESENTERY	(14)	(50)	(46)
NECROSIS, FOCAL			1 (2%)
ALL OTHER SYSTEMS			
THORAX			
NODULE			1
DIAPHRAGM			
INFLAMMATION, ACUTE NECROTIZING		1	
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		6	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY	6		4
AUTO/NECROPSY/HISTO PERF	1	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 DAMINOZIDE IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		9	2
ANIMALS NECROPSIED	20	41	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	41	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#TRACHEA	(16)	(31)	(43)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#LUNG/BRONCHUS	(20)	(39)	(48)
INFLAMMATION, NOS	2 (10%)	3 (8%)	6 (13%)
INFLAMMATION, FOCAL		1 (3%)	
INFLAMMATION ACTIVE CHRONIC			1 (2%)
INFLAMMATION, CHRONIC	2 (10%)	3 (8%)	4 (8%)
#LUNG	(20)	(39)	(48)
EMPHYSEMA, NOS		1 (3%)	
CONGESTION, NOS			6 (13%)
HEMORRHAGE	1 (5%)		1 (2%)
INFLAMMATION, NOS			3 (6%)
INFLAMMATION, FOCAL	1 (5%)	2 (5%)	
INFLAMMATION, ACUTE			1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (5%)		
INFLAMMATION, CHRONIC		1 (3%)	
SCLEROSIS			1 (2%)
NODULE		1 (3%)	
PERIVASCULITIS	8 (40%)	12 (31%)	13 (27%)
HYPERTROPHY, NOS		1 (3%)	
HYPERPLASIA, ADENOMATOUS		2 (5%)	3 (6%)
LEUKEMOID REACTION			2 (4%)
RETICULOENDOTHELIOSIS	1 (5%)		
#LUNG/ALVEOLI	(20)	(39)	(48)
HISTIOCYTOSIS		3 (8%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(18)	(34)	(41)
HYPERPLASIA, HEMATOPOIETIC		1 (3%)	1 (2%)
#SPLEEN	(17)	(34)	(42)
INFLAMMATION, GRANULOMATOUS			1 (2%)
AMYLOID, NOS			1 (2%)
AMYLOIDOSIS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
LEUKEMOID REACTION			1 (2%)
HYPERPLASIA, LYMPHOID	2 (12%)	6 (18%)	
HEMATOPOIESIS	1 (6%)	3 (9%)	2 (5%)
#LYMPH NODE	(20)	(37)	(40)
INFLAMMATION, NOS		1 (3%)	
HYPERPLASIA, NOS			1 (3%)
HYPERPLASIA, DIFFUSE		1 (3%)	
#MANDIBULAR L. NODE	(20)	(37)	(40)
HYPERPLASIA, NOS		4 (11%)	5 (13%)
HISTIOCYTOSIS			1 (3%)
#BRONCHIAL LYMPH NODE	(20)	(37)	(40)
HYPERPLASIA, NOS	1 (5%)	1 (3%)	1 (3%)
HISTIOCYTOSIS			1 (3%)
PLASMACYTOSIS		1 (3%)	
#MESENTERIC L. NODE	(20)	(37)	(40)
DILATATION, NOS			1 (3%)
LYMPHANGIECTASIS		1 (3%)	1 (3%)
HEMORRHAGE		1 (3%)	1 (3%)
INFLAMMATION, NOS		1 (3%)	
INFLAMMATION ACUTE AND CHRONIC			1 (3%)
HEMOSIDEROSIS			1 (3%)
HYPERPLASIA, NOS			2 (5%)
HYPERPLASIA, FOCAL			1 (3%)
HISTIOCYTOSIS			1 (3%)
PLASMACYTOSIS		1 (3%)	
MEGAKARYOCYTOSIS			1 (3%)
HEMATOPOIESIS			1 (3%)
#RENAL LYMPH NODE	(20)	(37)	(40)
HYPERPLASIA, NOS		1 (3%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID			1 (3%)
CIRCULATORY SYSTEM			
#HEART	(20)	(41)	(47)
INFARCT, HEALED			1 (2%)
CALCIFICATION, FOCAL			1 (2%)
#MYOCARDIUM	(20)	(41)	(47)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
FIBROSIS, FOCAL		2 (5%)	
*CORONARY ARTERY	(20)	(41)	(48)
ARTERIOSCLEROSIS, NOS		1 (2%)	
*PULMONARY ARTERY	(20)	(41)	(48)
HYPERTROPHY, NOS	1 (5%)	1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (5%)	1 (2%)	1 (2%)
*ANTERIOR MENINGEAL A	(20)	(41)	(48)
ARTERIOSCLEROSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(37)	(42)
INFLAMMATION, CHRONIC FOCAL	1 (6%)		
PERIVASCULITIS	1 (6%)	3 (8%)	4 (10%)
METAMORPHOSIS FATTY			1 (2%)
#LIVER	(20)	(40)	(48)
CYST, NOS	1 (5%)		
CONGESTION, NOS		1 (3%)	
CONGESTION, CHRONIC PASSIVE			1 (2%)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	1 (3%)	
FIBROSIS, FOCAL			1 (2%)
PERIVASCULITIS			4 (8%)
DEGENERATION, HYDROPIC	5 (25%)		
NECROSIS, COAGULATIVE		1 (3%)	
INFARCT, NOS		1 (3%)	1 (2%)
AMYLOIDOSIS			1 (2%)
METAMORPHOSIS FATTY		1 (3%)	3 (6%)
ANGIECTASIS			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID		1 (3%)	
HEMATOPOIESIS		1 (3%)	
#LIVER/CENTRILOBULAR	(20)	(40)	(48)
CONGESTION, NOS	1 (5%)		
METAMORPHOSIS FATTY	1 (5%)		
*GALLBLADDER	(20)	(41)	(48)
INFLAMMATION, ACUTE			1 (2%)
*BILE DUCT	(20)	(41)	(48)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	2 (4%)
INFLAMMATION, CHRONIC FOCAL	1 (5%)	1 (2%)	1 (2%)
GRANULOMA, NOS			1 (2%)
FIBROSIS			1 (2%)
LYMPHOID DEPLETION	1 (5%)		
#PANCREAS	(16)	(36)	(44)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ATROPHY, FOCAL			1 (2%)
#ESOPHAGUS	(19)	(36)	(45)
INFLAMMATION ACTIVE CHRONIC		1 (3%)	
#STOMACH	(20)	(39)	(45)
ULCER, NOS			1 (2%)
INFLAMMATION, HEMORRHAGIC			1 (2%)
INFLAMMATION, ACUTE FOCAL		1 (3%)	
CALCIFICATION, NOS		1 (3%)	
#SMALL INTESTINE	(20)	(38)	(46)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#PEYERS PATCH	(20)	(38)	(46)
HYPERPLASIA, NOS	2 (10%)	4 (11%)	6 (13%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
#DUODENUM	(20)	(38)	(46)
HYPERPLASIA, LYMPHOID		1 (3%)	
#LARGE INTESTINE	(17)	(37)	(31)
CONGESTION, NOS			1 (3%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#COLON	(17)	(37)	(31)
PARASITISM	1 (6%)	3 (8%)	1 (3%)
#COLONIC SUBMUCOSA	(17)	(37)	(31)
INFLAMMATION, GRANULOMATOUS	1 (6%)		
URINARY SYSTEM			
#KIDNEY	(20)	(41)	(48)
HYDRONEPHROSIS	1 (5%)		
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC			2 (4%)
GLOMERULONEPHRITIS, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	3 (15%)		
PERIVASCULITIS	5 (25%)	5 (12%)	5 (10%)
NECROSIS, MEDULLARY			1 (2%)
AMYLOID, NOS			1 (2%)
AMYLOIDOSIS			3 (6%)
HYPERPLASIA, TUBULAR CELL			1 (2%)
PLASMACYTOSIS		1 (2%)	
#KIDNEY/TUBULE	(20)	(41)	(48)
LIPOIDOSIS			1 (2%)
#KIDNEY/PELVIS	(20)	(41)	(48)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)
*URETER	(20)	(41)	(48)
PERIARTERITIS			1 (2%)
#URINARY BLADDER	(15)	(31)	(33)
LYMPHOCYTIC INFLAMMATORY INFILTR	7 (47%)	7 (23%)	9 (27%)
INFLAMMATION, CHRONIC		1 (3%)	
PLASMA-CELL INFILTRATE		1 (3%)	
NODULE		1 (3%)	
PERIVASCULITIS		1 (3%)	
ANGIECTASIS		1 (3%)	
#U. BLADDER/MUCOSA	(15)	(31)	(33)
NODULE	1 (7%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#ADRENAL	(17)	(31)	(38)
CONGESTION, NOS	1 (6%)		
INFLAMMATION, CHRONIC		1 (3%)	
DEGENERATION, NOS		1 (3%)	
AMYLOIDOSIS			1 (3%)
CYTOLOGIC DEGENERATION			1 (3%)
#ADRENAL MEDULLA	(17)	(31)	(38)
CONGESTION, NOS		1 (3%)	
#THYROID	(13)	(28)	(38)
INFLAMMATION ACTIVE CHRONIC		1 (4%)	
HYPERPLASIA, ADENOMATOUS		1 (4%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(37)	(45)
HYDROMETRA		1 (3%)	
CYST, NOS		2 (5%)	4 (9%)
HEMORRHAGIC CYST			1 (2%)
INFLAMMATION, NOS		1 (3%)	1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
INFLAMMATION, VESICULAR			2 (4%)
INFLAMMATION ACTIVE CHRONIC		1 (3%)	
SCLEROSIS			1 (2%)
METAPLASIA, SQUAMOUS			3 (7%)
#CERVIX UTERI	(20)	(37)	(45)
INFLAMMATION, ACUTE		2 (5%)	5 (11%)
#UTERUS/ENDOMETRIUM	(20)	(37)	(45)
CYST, NOS	5 (25%)	6 (16%)	1 (2%)
INFLAMMATION, NOS			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR			1 (2%)
INFLAMMATION, VESICULAR	2 (10%)	1 (3%)	1 (2%)
ABSCESS, NOS			1 (2%)
HYPERPLASIA, CYSTIC	5 (25%)	12 (32%)	22 (49%)
METAPLASIA, SQUAMOUS			1 (2%)
#UTERUS/MYOMETRIUM	(20)	(37)	(45)
INFLAMMATION, ACUTE			2 (4%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE VESICULAR			1 (2%)
#OVARY/OVIDUCT	(20)	(37)	(45)
INFLAMMATION ACTIVE CHRONIC		1 (3%)	
#OVARY	(13)	(14)	(23)
CYST, NOS	6 (46%)	3 (21%)	6 (26%)
PAROVARIAN CYST		1 (7%)	
HEMORRHAGIC CYST		1 (7%)	
ATROPHY, NOS			1 (4%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(19)	(40)	(46)
INFLAMMATION, CHRONIC	1 (5%)		
PERIARTERITIS	1 (5%)		
#BRAIN	(19)	(40)	(46)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
PERIVASCULITIS	1 (5%)	1 (3%)	
CORPORA AMYLACEA		1 (3%)	
CALCIFICATION, FOCAL	4 (21%)	12 (30%)	10 (22%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(20)	(41)	(48)
FIBROUS OSTEODYSTROPHY	13 (65%)	18 (44%)	21 (44%)
OSTEOSCLEROSIS		1 (2%)	
*SKELETAL MUSCLE	(20)	(41)	(48)
PARASITISM		1 (2%)	
BODY CAVITIES			
*MEDIASTINUM	(20)	(41)	(48)
THROMBUS, ORGANIZED		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
PERIARTERITIS		1 (2%)	
*PERITONEUM	(20)	(41)	(48)
INFLAMMATION, FOCAL		1 (2%)	
*MESENTERY	(20)	(41)	(48)
STEATITIS		1 (2%)	
NECROSIS, FAT		2 (5%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(41)	(48)
RUSSELL BODY			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY		9	2
AUTO/NECROPSY/HISTO PERF		2	

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

APPENDIX E

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	1/20 (5)	3/50 (6)	0/50 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.200	0.000
Lower Limit		0.106	0.000
Upper Limit		61.724	7.475
Weeks to First Observed Tumor	105	105	--
Pituitary: Chromophobe Adenoma[b]	0/18 (0)	2/43 (5)	2/49 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.129	0.113
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	96	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant[b]	2/20 (10)	2/50 (4)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.400	0.400
Lower Limit		0.032	0.032
Upper Limit		5.277	5.277
Weeks to First Observed Tumor	105	105	48
Thyroid: C-cell Carcinoma[b]	0/16 (0)	0/38 (0)	2/43 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.116
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma or Carcinoma[b]	2/16 (13)	3/38 (8)	2/43 (5)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.632	0.372
Lower Limit		0.082	0.030
Upper Limit		7.118	4.864
Weeks to First Observed Tumor	104	105	105
Preputial Gland: Adenoma or Carcinoma, NOS[b]	2/20 (10)	1/50 (2)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.200	0.800
Lower Limit		0.004	0.128
Upper Limit		3.681	8.436
Weeks to First Observed Tumor	67	99	85

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor[b]	13/20 (65)	46/50 (92)	47/50 (94)
P Values[c,d]	P = 0.003	P = 0.009	P = 0.004
Departure from Linear Trend[e]	P = 0.042		
Relative Risk[f]		1.415	1.446
Lower Limit		1.043	1.074
Upper Limit		1.873	1.830
Weeks to First Observed Tumor	101	78	85

[a]Dosed groups received 5,000 or 10,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-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 each dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed 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 dosed group and the control group.

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma[b]	0/20 (0)	0/50 (0)	4/48 (8)
P Values[c,d]	P = 0.036	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.402
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	102
Hematopoietic System: Leukemia[b]	0/20 (0)	4/50 (8)	1/50 (2)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.386	0.022
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	83	102

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Chromophobe Carcinoma[b]	0/19 (0)	3/45 (7)	0/43 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.046		
Relative Risk[f]		Infinite	--
Lower Limit		0.265	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	96	--
Pituitary: Chromophobe Adenoma or Carcinoma[b]	3/19 (16)	10/45 (22)	8/43 (19)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.407	1.178
Lower Limit		0.424	0.330
Upper Limit		7.336	6.361
Weeks to First Observed Tumor	105	89	92

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma[b]	2/15 (13)	1/38 (3)	0/44 (0)
P Values[c,d]	P = 0.026 (N)	N.S.	N.S.
Relative Risk[f]		0.197	0.000
Lower Limit		0.004	0.000
Upper Limit		3.581	1.140
Weeks to First Observed Tumor	105	99	105
Thyroid: C-cell Adenoma or Carcinoma[b]	4/15 (27)	3/38 (8)	2/44 (5)
P Values[c,d]	P = 0.021 (N)	N.S.	P = 0.032 (N)
Relative Risk[f]		0.296	0.170
Lower Limit		0.051	0.018
Upper Limit		1.578	1.085
Weeks to First Observed Tumor	105	99	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Fibroadenoma[b]	3/20 (15)	9/50 (18)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.200	0.267
Lower Limit		0.346	0.024
Upper Limit		6.408	2.190
Weeks to First Observed Tumor	105	89	105
Uterus: Leiomyosarcoma[b]	0/19 (0)	1/50 (2)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.021	0.238
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	98	96

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	0/19 (0)	6/50 (12)	4/50 (8)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.636	0.368
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105
Uterus/Endometrium: Adenocarcinoma, NOS[b]	0/19 (0)	5/50 (10)	3/50 (6)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.501	0.238
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	83	105

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mesentery: Lipoma[b]	0/20 (0)	0/50 (0)	2/50 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		--	Infinite
Lower Limit		--	0.123
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	92

[a]Dosed groups received 5,000 or 10,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-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 each dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed 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 dosed group and the control group.

APPENDIX F

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

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	2/14 (14)	9/50 (18)	12/46 (26)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.260	1.826
Lower Limit		0.314	0.493
Upper Limit		11.287	15.708
Weeks to First Observed Tumor	66	89	92
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	4/14 (29)	15/50 (30)	18/46 (39)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.050	1.370
Lower Limit		0.425	0.576
Upper Limit		3.858	4.875
Weeks to First Observed Tumor	66	83	92

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia[b]	2/14 (14)	6/50 (12)	11/46 (24)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.840	1.674
Lower Limit		0.179	0.442
Upper Limit		8.038	14.549
Weeks to First Observed Tumor	103	90	83
Liver: Hepatocellular Carcinoma[b]	0/14 (0)	7/50 (14)	13/46 (28)
P Values[c,d]	P = 0.008	N.S.	P = 0.020
Relative Risk[f]		Infinite	Infinite
Lower Limit		0.583	1.314
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	86	65

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/14 (7)	9/50 (18)	14/46 (30)
P Values[c,d]	P = 0.031	N.S.	N.S.
Relative Risk[f]		2.520	4.261
Lower Limit		0.411	0.770
Upper Limit		107.898	174.781
Weeks to First Observed Tumor	105	86	65

[a]Dosed groups received 5,000 or 10,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-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 each dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed 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 dosed group and the control group.

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

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma[b]	1/20 (5)	4/39 (10)	2/48 (4)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.051	0.833
Lower Limit		0.225	0.047
Upper Limit		98.244	48.155
Weeks to First Observed Tumor	105	105	72
Lung: Alveolar/Brochiolar Adenoma or Carcinoma[b]	1/20 (5)	8/39 (21)	10/48 (21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		4.103	4.167
Lower Limit		0.621	0.669
Upper Limit		176.419	176.294
Weeks to First Observed Tumor	105	105	72

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: All Neoplasms[b]	5/20 (25)	16/41 (39)	14/48 (29)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.561	1.167
Lower Limit		0.661	0.476
Upper Limit		4.762	3.693
Weeks to First Observed Tumor	97	75	72
Hematopoietic System: Malignant Lymphoma, Lymphocytic Leukemia, or Leukemia, NOS[b]	5/20 (25)	14/41 (34)	13/48 (27)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		1.336	1.083
Lower Limit		0.560	0.434
Upper Limit		4.262	3.470
Weeks to First Observed Tumor	97	75	72

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
All Sites: Hemangioma[b]	0/20 (0)	4/41 (10)	0/48 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[b]	P = 0.012		
Relative Risk[f]		Infinite	--
Lower Limit		0.471	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--
Liver: Hepatocellular Carcinoma[b]	0/20 (0)	3/40 (8)	0/48 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.027		
Relative Risk[f]		Infinite	--
Lower Limit		0.313	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma[b]	1/20 (5)	4/40 (10)	0/48 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		2.000	0.000
Lower Limit		0.220	0.000
Upper Limit		95.888	7.780
Weeks to First Observed Tumor	105	105	--
Pituitary: Chromophobe Adenoma[b]	2/14 (14)	1/19 (5)	0/14 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk[f]		0.368	0.000
Lower Limit		0.007	0.000
Upper Limit		6.421	3.151
Weeks to First Observed Tumor	62	105	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp[b]	0/20 (0)	3/37 (8)	0/45 (0)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.024		
Relative Risk[f]		Infinite	--
Lower Limit		0.338	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--
Peritoneum: Lipoma[b]	2/20 (10)	0/41 (0)	0/48 (0)
P Values[c,d]	P = 0.028 (N)	N.S.	N.S.
Relative Risk[f]		0.000	0.000
Lower Limit		0.000	0.000
Upper Limit		1.632	1.400
Weeks to First Observed Tumor	105	--	--

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

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mesentery: Lipoma[b]	2/20 (10)	1/41 (2)	0/48 (0)
P Values[c,d]	P = 0.038 (N)	N.S.	N.S.
Relative Risk[f]		0.244	0.000
Lower Limit		0.004	0.000
Upper Limit		4.462	1.400
Weeks to First Observed Tumor	105	105	--

[a]Dosed groups received 5,000 or 10,000 ppm.

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

[c]Beneath the incidence of tumors in the matched-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 each dosed group is the probability level for the Fisher exact test for the comparison of that dosed 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 dosed 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 dosed group and the control group.

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

March 7, 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 Daminozide for carcinogenicity.

The primary reviewer said that Daminozide was, at most, a borderline carcinogen in female rats, inducing adenocarcinomas of the endometrium and leiomyosarcomas of the uterus. In male mice, Daminozide induced a dose-related incidence of hepatocellular carcinomas. He briefly described the experimental design and conditions under which Daminozide was tested. In his critique, the primary reviewer made the following points: the number of matched controls was too small; the degradation products of Daminozide and their concentrations were not determined; other chemicals were tested in the same room as Daminozide; and the use of historical controls diminished the significance of certain tumors observed in treated animals.

The secondary reviewer agreed with the conclusion in the report that Daminozide was carcinogenic in female rats and was associated with the induction of hepatocellular carcinomas in male mice. He pointed out the increased incidence of alveolar/bronchiolar carcinomas and malignant

lymphomas in treated mice. With respect to rats, the secondary reviewer noted the incidence of C-cell hyperplasia of the thyroid in treated females. Given the carcinogenicity of the drug in rats and mice, he concluded that Daminozide poses a potential carcinogenic risk to humans.

A motion was made that the report on the bioassay of Daminozide be accepted as written. The motion was seconded and approved unanimously.

In further discussion, the inconsistency was noted between the primary reviewer's evaluation that Daminozide is, at most, a borderline carcinogen and the secondary reviewer's statement that it poses a potential carcinogenic risk to humans. A Program staff pathologist said that the finding of relatively rare tumors in the treated animals should take on greater significance, despite their equivocal statistical significance. A Subgroup member added that it was valid to use historical control animals to assess the significance of rare tumors. It was noted that from among 318 historical controls, not a single adenocarcinoma of the endometrium or leiomyosarcoma of the uterus was observed. The probability of getting eight such tumors by chance in the treated female rats would be remote.

Based on the additional discussion, it was moved that the report on the bioassay of Daminozide be accepted as written. The motion was seconded and approved unanimously. Another motion was made that the data available were insufficient to assess the potential carcinogenic risk of Daminozide to humans. All the members were in favor of the motion except Dr. Highland, who opposed it.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold Brown, Mayo Clinic
E. Cuyler Hammond, American Cancer Society
Joseph Highland, Environmental Defense Fund
Henry Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Michael Shimkin, University of California at San Diego

---

* 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.

*U.S. GOVERNMENT PRINTING OFFICE: 1978 260-899/3110 1-3

DHEW Publication No. (NIH) 78-1333