NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 221

CARCINOGENESIS BIOASSAY OF LOCUST BEAN GUM

(CAS NO. 9000-40-2)

IN F344 RATS AND B6C3F$_1$ MICE

(FEED STUDY)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
National Institutes of Health

NATIONAL TOXICOLOGY PROGRAM

The National Toxicology Program (NTP), established in 1978, develops and evaluates scientific information about potentially toxic and hazardous chemicals. This knowledge can be used for protecting the health of the American people and for the primary prevention of chemically induced disease. By bringing together the relevant programs, staff, and resources from the U.S. Public Health Service, DHHS, the National Toxicology Program has centralized and strengthened activities relating to toxicology research, testing and test development/validation efforts, and the dissemination of toxicological information to the public and scientific communities and to the research and regulatory agencies.

The NTP is comprised of four charter DHHS agencies: the National Cancer Institute, National Institutes of Health; the National Institute of Environmental Health Sciences, National Institutes of Health; the National Center for Toxicological Research, Food and Drug Administration; and the National Institute for Occupational Safety and Health, Centers for Disease Control. In June 1981, the Carcinogenesis Bioassay Testing Program, NCI, was transferred to the NIEHS.

NTP Technical Report

on the

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NATIONAL TOXICOLOGY PROGRAM
Research Triangle Park
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NOTE TO THE READER

This is one in a series of experiments designed to determine whether selected chemicals produce cancer in animals. Chemicals selected for testing in the NTP carcinogenesis bioassay program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

This study was initiated by the National Cancer Institute's Carcinogenesis Testing Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program.

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650).

Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to the National Toxicology Program, located at Room A-306, Landow Building, Bethesda, MD 20205 (301-496-1152) or at Research Triangle Park, NC 27709 (919-541-3991).

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to communicate any mistakes to the Deputy Director, NTP (P.O. Box 12233, Research Triangle Park, NC 27709), so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP.

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ABSTRACT

A carcinogenesis bioassay of locust bean gum, a widely used food stabilizer, was conducted by feeding diets containing 25,000 or 50,000 ppm of the test substance to 50 F344 rats and 50 B6C3F1 mice of either sex for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

Mean body weights of high- and low-dose rats of either sex, of low-dose male mice, and of high- and low-dose female mice were comparable with those of the controls; mean body weights of high-dose male mice were slightly lower than those of controls. No other compound-related clinical signs or effects on survival were observed. Although the rats and mice might have been able to tolerate higher doses, 50,000 ppm (5%) is the recommended maximum concentration of a test chemical mixed in feed according to the guidelines of the Bioassay Program.

Although alveolar/bronchiolar adenomas occurred in low-dose male mice at a significantly (P=0.017) higher incidence than that in the controls (7/50, 17/50, 11/50), no significant statistical results were obtained when the combined incidence of animals with either alveolar/bronchiolar adenomas or carcinomas was analyzed (14/50, 21/50, 14/50). Cortical adenomas in the adrenal gland of female rats occurred with a statistically significant (P=0.042) positive trend (1/50, 4/50, 6/50), but comparisons between test groups and the control group were not statistically different.

Under the conditions of this bioassay, locust bean gum was not carcinogenic for male or female F344 rats or B6C3F1 mice.

CONTRIBUTORS

The bioassay of locust bean gum was conducted at EG&G Mason Research Institute, Worcester, Massachusetts, from October 1977 to November 1979 under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI Carcinogenesis Testing Program.

The bioassay was conducted under the supervision of Drs. H. Lilja (1) and E. Massaro (1,2), principal investigators. Doses of the test chemical were selected by Drs. J. Robens (3,4) and C. Cueto (5). The program manager was Ms. R. Monson (1). Ms. A. Good (1) supervised the technicians in charge of animal care, and Ms. E. Zepp (1) supervised the preparation of the feed mixtures and collected samples of the diets for analysis. Ms. D. Bouthot (1) kept all daily records of the test. Drs. D. S. Wyand (1) and R. W. Fleischman (1) pathologists, directed the necropsies and performed the histopathologic evaluations. The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described in Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group, with final approval by the NCI Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute, Rockville, Maryland (7). The statistical analyses were performed by Dr. J. R. Joiner (3) and Mr. J. Warner (3), using methods selected for the bioassay program by Dr. J. J. Gart (8).

Chemicals used in this study were analyzed at Midwest Research Institute (9), and dosed feed mixtures were analyzed by Dr. M. Hagopian (1).

This report was prepared at Tracor Jitco (3) and reviewed by NTP. Those responsible for the report at Tracor Jitco were Dr. C. Cueto, Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. W. D. Theriault, reports manager; Dr. A. C. Jacobs, bioscience writer; and Ms. M. W. Glasser, technical editor.

The following scientists at NTP (6) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Charles K. Grieshaber. Dr. Larry Hart, Dr. Joseph Haseman, Dr. James Huff, Dr. C. W. Jameson, Dr. Eugene E. McConnell, Dr. John A. Moore, Dr. R. Tennant, and Dr. Jerrold M. Ward.

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PEER REVIEW PANEL AND COMMENTS

On October 15, 1980 this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Conference Room 6, Building 31C, National Institutes of Health, Bethesda, Maryland. Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, Thomas Shepard, and Alice Whittemore. Members of the Panel are: Drs. Norman Breslow, Joseph Highland, Charles Irving, Frank Mirer, Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams. Drs. Highland, Irving, Whittemore, and Williams were unable to attend the review.

Dr. Shore, as the primary reviewer for the report on the bioassay of locust bean gum, agreed with the conclusion in the report that the compound was not carcinogenic for male or female F344 rats or B6C3F1 mice under the conditions of this carcinogenesis bioassay. He stressed that a maximum tolerated dose may not have been attained. He noted, however, that the high dose (50,000 ppm) was in keeping with the upper limit established for the bioassay program (five percent concentration in the feed). Also the actual concentration of locust bean gum could not be measured with sufficient accuracy to verify the dose levels. He commented on a statistically significant negative trend for lymphomas of the hematopoietic system in female mice.

As the secondary reviewer, Dr. Mirer also agreed with the conclusions that locust bean gum was not carcinogenic. He reported that there were small increases in several tumor types but the biological significance of each was questionable because they were statistically marginal, or did not follow an increasing trend with dose, or were within the range of historical controls, and in each case were found only in one sex of one species.

Dr. Shore moved that the report on the bioassay of locust bean gum be accepted. Dr. Nielsen seconded the motion, and the report was approved unanimously by the Peer Review Panel.

I. INTRODUCTION

Locust bean gum (CAS No. 9000-40-2) is a neutral galactomannan polymer consisting of a main chain of D-mannose units and a side chain of D-galactose on every fourth or fifth unit (Furia, 1972).

Locust bean gum -- also known as carob seed gum -- is produced by milling the endosperm of the fruit pod of the carob tree, Ceratonia siliqua. Widely used in the food industry as a stabilizer in ice cream, sauces, salad dressings, pie fillings, jams, and jellies and as a binder in processed meat products, locust bean gum is also used in the manufacture of pharmaceuticals and cosmetics, textiles, paper, ceramics, paints, and gun powder (LSRO, 1972; Kirk-Othmer, 1966). It is on the list of food additives "generally recognized as safe" by the U.S. Food and Drug Administration (CFR, 1974) and is approved for use in foods when it contains not less than 73% galactomannans and not more than 15% water, 8% protein, 5% insoluble material, and 1.2% ash (Food Chemicals Codex, 1972). In 1970, 4.9 million kilograms of locust bean gum were imported to the United States, primarily from the Mediterranean area (Furia, 1972; LSRO, 1972).

The oral LD_{50} of locust bean gum in rats and mice was reported as 13.0 g/kg body weight (Bailey and Morgareidge, 1976).

Locust bean gum was tested without S-9 activation in several short term mutagenicity assay systems, including Salmonella typhimurium TA 1530 and G-46, and Saccharomyces cerevisiae D-3 (Green, 1977), and was found to be non-mutagenic.

Locust bean gum was tested for potential carcinogenicity by the Bioassay Program because of its widespread use as a food additive for human consumption.

II. MATERIALS AND METHODS

A. Chemical

Locust bean gum (CAS No. 9000-40-2) was obtained in two batches from Stein Hall Company (Louisville, KY), a division of the Celanese Corporation Lot No. CN-361 was used for the subchronic studies and the first 15 weeks of the chronic studies, and Lot No. 265-76 was used for the remainder of the chronic studies. Both lots were food grade material.

Purity and identity analyses were performed at Midwest Research Institute (Kansas City, MO). Results of titration by periodate oxidation indicated that both lots contained more than the 73% minimum of galactomannans specified in the Food Chemicals Codex (1972) -- Lot No. CN-361 contained 77.2% and Lot No. 265-76 contained 88.0%. Results of Karl Fisher titration indicated 5.7% water in each of the batches. Results of thin-layer chromatography of the hydrolysis products of locust bean gum indicated that D-mannose is the major component and D-galactose is a minor component. The infrared spectra of both batches were consistent with the literature spectra (Appendixes E and F).

Locust bean gum was stored in the dark in its original paperboard drum at 4°C.

B. Dietary Preparation

Each test diet was prepared by mixing the chemical and an aliquot of Wayne® Lab Blox animal meal with a mortar and pestle and then adding this premix to the rest of the feed and mixing in a Patterson-Kelly® twin-shell V-blender for 20 minutes. Test diets were sealed in plastic bags and stored at 4°C for no longer than 14 days.

Due to the chemical similarity of the test compound and the feed, the quantitative method available could not measure chronic dose levels reproducibly within ± 10%. Thus formulated diets were not analyzed for concentrations of locust bean gum during the study.

C. Animals

Four-week old F344 rats and B6C3F1 mice were obtained from the NCI Frederick Cancer Research Center (Frederick, Maryland), observed for the presence of parasites and other diseases for 8 days (mice) or 9 days (rats), and then assigned to control or dosed groups according to a table of random numbers.

D. Animal Maintenance

Rats and mice were housed five per cage in suspended polycarbonate cages equipped with disposable nonwoven fiber filter sheets (Table 1). Hardwood chip bedding and cages were changed twice weekly, and cage racks were changed every 2 weeks. Water was supplied by an Edstrom automatic watering system, and Wayne Lab Blox diet in stainless-steel, gang-style hoppers was available *ad libitum*.

The temperature in the animal rooms was 19°-32°C and relative humidity was uncontrolled (0%-66%). Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters, with 10 to 12 changes of air per hour. Fluorescent lighting was provided 12 hours per day. Rats and mice were housed by species in rooms in which chronic feed studies were also being conducted on gum arabic (CAS No. 9000-01-5).

E. Range Finding and Repeated-Dose Studies

Range finding and repeated-dose feed studies were conducted using F344 rats and B6C3F1 mice to determine the concentrations of locust bean gum to be used in the subchronic studies.

In the range finding study, groups of five males and five females of each species were administered single doses of 0.3, 0.77, or 1.09 g/kg locust bean gum by gavage. All survived to the end of the test period at day 15. No compound-related effects were observed.

In the repeated-dose study, groups of five males and five females of each species were administered 0, 6,300, 12,500, 25,000, 50,000, or 100,000

Table 1. Source and Descriptions of Materials Used for Animal Maintenance

Item	Description	Source
Animal Feed	Wayne® Lab Blox meal	Allied Mills (Chicago, IL)
Feed Hoppers	Stainless steel, gang style	Scientific Cages, Inc. (Bryan, TX)
Cages	Polycarbonate	Lab Products, Inc. (Garfield, NJ)
Filter Sheets	Disposable, nonwoven fiber	Lab Products, Inc. (Rochelle Park, NJ)
Bedding	Hardwood chips: Aspen bed®	American Excelsior (Baltimore, MD)
	Betta Chips®	Agway Corp. (Syracuse, NY)

ppm locust bean gum in feed for 2 weeks and then killed. No compound-related effects were observed. Two male mice died, one that received 25,000 ppm and one that received 100,000 ppm.

F. Subchronic Studies

Subchronic studies were conducted to determine the concentrations to be used in the chronic studies. Diets containing 0, 6,300, 12,500, 25,000, 50,000, or 100,000 ppm locust bean gum were fed for 13 weeks to groups of 10 males and 10 females of each species (Tables 2 and 3). Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day study, survivors were killed, necropsies were performed on all animals, and tissues were taken for histopathologic analysis.

Rats: One female rat receiving 12,500 ppm died. Weight gain depression was 10% or less for all dosed groups. No compound-related effects were detected. Doses selected for the rats for the chronic study were 25,000 and 50,000 ppm locust bean gum in the diet, since the upper limit recommended for chronic feeding studies is 50,000 ppm (NCI, 1976).

Mice: Two male mice (one that received 100,000 ppm and one control) and two female mice (one that received 50,000 and one that received 100,000 ppm) died from accidental causes. No compound-related weight gain depression was observed. Doses selected for the mice for the chronic study were 25,000 and 50,000 ppm.

G. Design of Chronic Studies

The number of animals in test groups, doses administered, and durations of the chronic studies are shown in Table 4.

H. Clinical Examinations and Pathology

Animals were inspected twice daily and weighed monthly. Animals that were moribund and those that survived to the end of the study were killed using CO_2 and necropsied.

Table 2. Dosage, Survival, and Mean Body Weights of Rats Fed Diets Containing Locust Bean Gum for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (Percent)
MALE					
0	10/10	88.0	371.1	283.1	
6,300	10/10	88.7	369.1	280.4	-1.0
12,500	10/10	88.7	374.4	285.7	+0.9
25,000	10/10	88.3	367.2	278.9	-1.5
50,000	10/10	88.3	356.2	267.9	-5.4
100,000	10/10	88.4	343.3	254.9	-10.0
FEMALE					
0	10/10	68.8	194.7	125.9	
6,300	10/10	70.8	202.1	131.3	+4.3
12,500	9/10	68.6	196.6	128.0	+1.7
25,000	10/10	71.0	199.9	128.9	+2.4
50,000	10/10	73.3	197.7	124.4	-1.2
100,000	10/10	71.4	196.8	125.4	-0.4

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 3. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing Locust Bean Gum for 13 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (Percent)
MALE					
0	9/10 (c)	20.1	33.9	13.8	
6,300	10/10	20.8	32.7	11.9	-13.8
12,500	10/10	20.6	34.3	13.7	-0.7
25,000	10/10	20.5	34.9	14.4	+4.3
50,000	10/10	20.6	32.9	12.3	-10.9
100,000	9/10 (c)	20.1	32.6	12.5	-9.4
FEMALE					
0	10/10	16.3	26.2	9.9	
6,300	10/10	16.2	25.4	9.2	-7.1
12,500	10/10	16.2	25.5	9.3	-6.1
25,000	10/10	15.9	25.0	9.1	-8.1
50,000	9/10 (c)	16.2	25.1	8.9	-10.1
100,000	9/10 (c)	15.9	25.5	9.6	-3.0

(a) Number surviving/number per group

(b) Weight Change Relative to Controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

(c) Deaths were accidental.

Table 4. Experimental Design of Chronic Feeding Studies with Locust Bean Gum in Rats and Mice

Test Group	Initial No. of Animals	Dose (ppm)	Time on Study	
			Dosed (weeks)	Observed (weeks)
Male Rats				
Control	50	0	0	105
Low-dose	50	25,000	103	2
High-dose	50	50,000	103	2
Female Rats				
Control	50	0	0	106
Low-dose	50	25,000	103	2
High-dose	50	50,000	103	2
Male Mice				
Control	50	0	0	105
Low-dose	50	25,000	103	2
High-dose	50	50,000	103	1
Female Mice				
Control	50	0	0	105
Low-dose	50	25,000	103	2
High-dose	50	50,000	103	1

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph nodes, heart, salivary gland, liver, pancreas, stomach, small intestine, large intestine, kidneys, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate and seminal vesicles or uterus, testis or ovary, brain, thymus, larynx, and esophagus.

Necropsies were performed on all animals found dead unless precluded in whole or in part by autolysis or cannibalization. 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.

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in 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).

Probablilities 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 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) extension 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 reported only 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 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 level. When results for two dosed groups are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 is made. The Bonferroni inequality criterion (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.025. When this correction was used, it is discussed in the narrative section. It is not 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. 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 an 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.

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 killed was entered as the time point of tumor observation. The methods of Cox and of Tarone were used for the statistical tests of the groups. The statistical tests were one-tailed.

The approximate 95% 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 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 has occurred (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.025 when the control incidence is zero). 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 there is a theoretical possiblity 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 dosed and control rats of either sex were comparable throughout the study (Figure 1). No compound-related clinical signs were reported.

B. Survival (Rats)

Estimates of the probabilities of survival of male and female rats administered locust bean gum in the diet at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 2. No significant differences were found between any of the groups of either sex.

In male rats, 34/50 (68%) of the control group, 36/50 (72%) of the low-dose group, and 33/50 (66%) of the high-dose group lived to the end of the study at 105 weeks. In female rats, 42/50 (84%) of the control group, 38/50 (76%) of the low-dose group, and 39/50 (78%) of the high-dose group lived to the end of the study at 105-106 weeks.

C. Pathology (Rats)

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

The tumors encountered were those commonly found in aging rats of this strain. They occurred in comparable numbers in test animals and controls and were not considered to be related to administration of the test compound.

The degenerative and inflammatory lesions encountered are often found in F344 rats of this age group and were not associated with exposure to locust bean gum.

The results of histopathologic examination indicated that locust bean gum was not carcinogenic when fed to F344 rats, under the conditions of this bioassay.

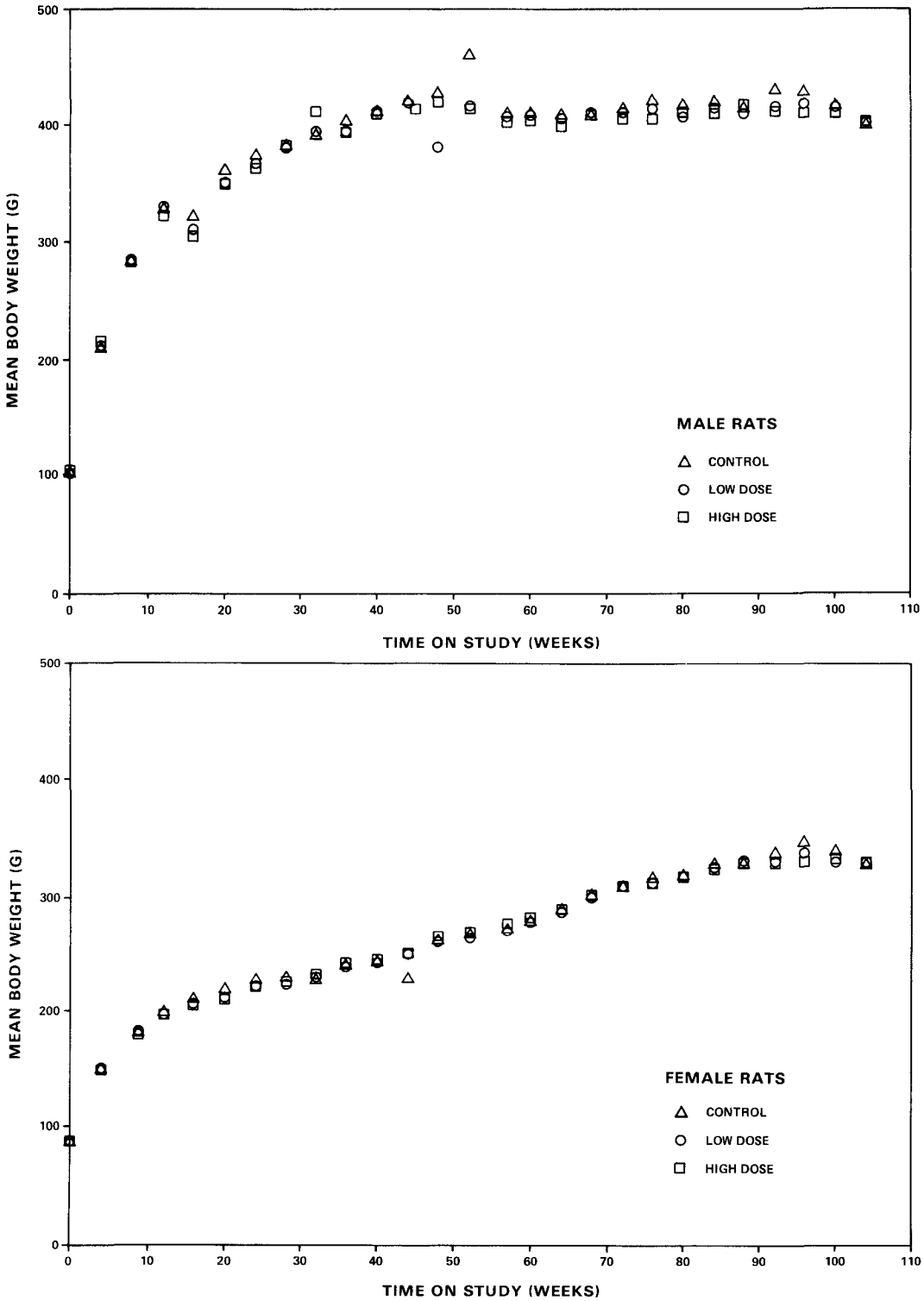


Figure 1. Growth Curves for Rats Fed Diets Containing Locust Bean Gum

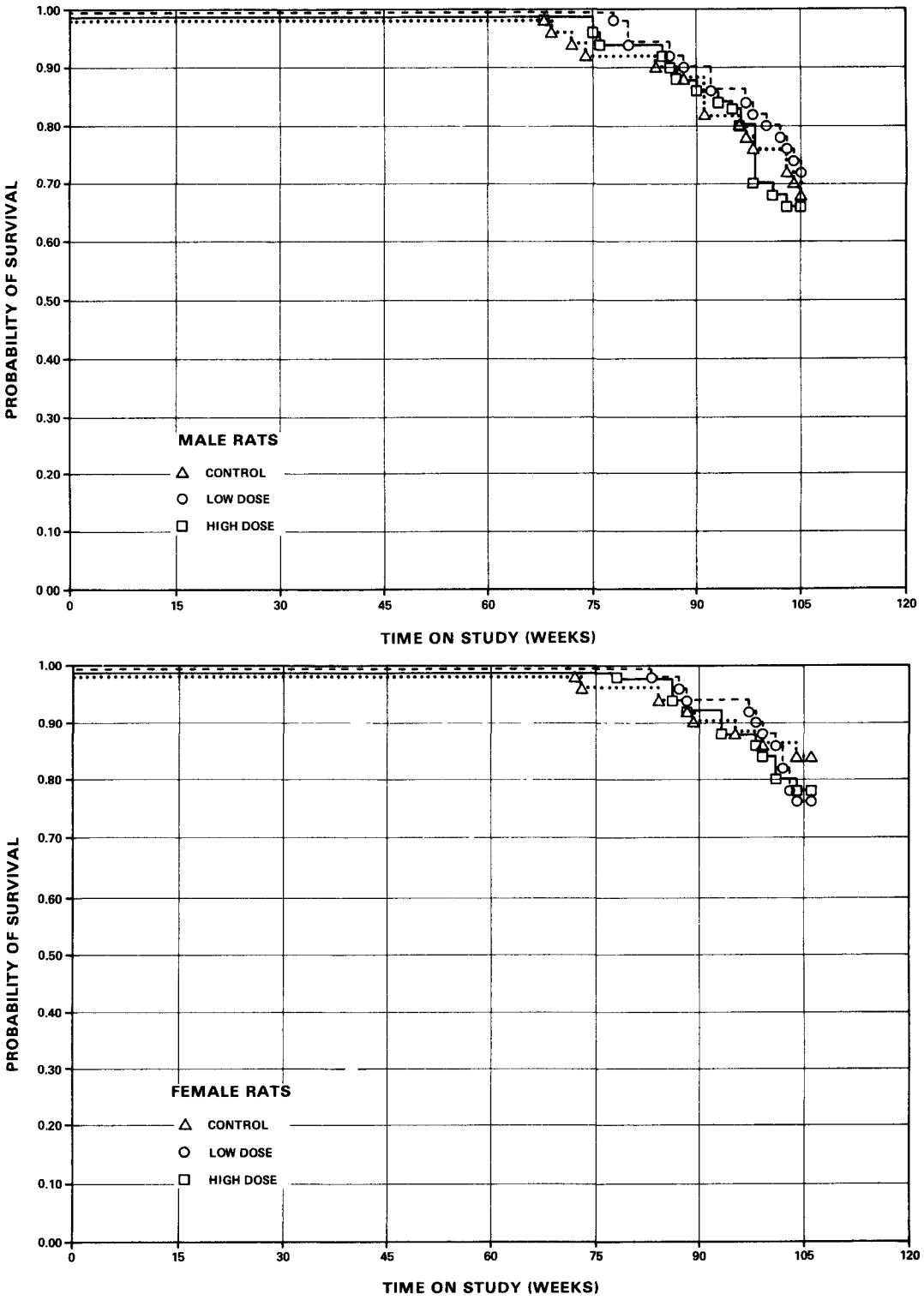


Figure 2. Survival Curves for Rats Fed Diets Containing Locust Bean Gum

D. Statistical Analyses of Results (Rats)

Tables 5 and 6 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Cortical adenomas of the adrenal gland in female rats were observed in increasing incidence (1/50, 2% in the controls; 4/50, 8% in the low-dose; 6/50, 12% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.042) but the Fisher exact tests were not significant. The incidence of control animals with cortical adenomas under this contract at this laboratory is 8/346(2.3%). In male rats, this tumor was not observed in statistically significant proportions.

Carcinomas of the pituitary in female rats were observed in decreased incidence in the low-dose group compared with the other two groups. The Cochran-Armitage test for linear trend was not significant. The Fisher exact test between the low-dose group and the control group was significant (P=0.049) but the value of P=0.049 is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. No significant incidence was observed in the high-dose group. When the incidence of female rats with either adenomas or carcinomas in the pituitary is analyzed, the result is not significant. This tumor was not observed in a statistically significant proportion in males.

Analyses of the time to observation of tumors by life table methods and analyses by the time-adjusted tests, eliminating those animals that died prior to 52 weeks, did not materially alter the significance of test results in Tables 5 and 6.

Statistically, there was no site at which an increase in tumor incidence could be associated unequivocally with administration of the chemical.

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Locust Bean Gum (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Fibroma (b)	0/50(0)	3/50(6)	1/50(2)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.601	0.054
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	78	105
Hematopoietic System:			
Myelomonocytic Leukemia (b)	21/50(42)	13/50(26)	15/50(30)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.619	0.714
Lower Limit		0.325	0.393
Upper Limit		1.141	1.274
Weeks to First Observed Tumor	68	86	75
Hematopoietic System:	21/50(42)	13/50(26)	16/50(32)
Lymphoma or Leukemia (b)			
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.619	0.762
Lower Limit		0.325	0.427
Upper Limit		1.141	1.340
Weeks to First Observed Tumor	68	86	68

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	5/47(11)	6/46(13)	8/45(18)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.226	1.671
Lower Limit		0.335	0.523
Upper Limit		4.735	6.020
Weeks to First Observed Tumor	105	105	90
Pituitary: Carcinoma, NOS (b)	3/47(6)	0/46(0)	1/45(2)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	0.348
Lower Limit		0.000	0.007
Upper Limit		1.695	4.143
Weeks to First Observed Tumor	72	--	98
Pituitary: Adenoma or Carcinoma (b)	8/47(17)	6/46(13)	9/45(20)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.766	1.175
Lower Limit		0.237	0.442
Upper Limit		2.315	3.187
Weeks to First Observed Tumor	72	105	90

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	4/50(8)	10/50(20)	7/49(14)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.500	1.786
Lower Limit		0.779	0.486
Upper Limit		10.246	7.830
Weeks to First Observed Tumor	103	100	105
Thyroid: C-Cell Carcinoma (b)	4/49(8)	1/50(2)	1/47(2)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.245	0.261
Lower Limit		0.005	0.005
Upper Limit		2.362	2.507
Weeks to First Observed Tumor	105	105	98
Thyroid: C-Cell Adenoma or Carcinoma (b)	5/49(10)	1/50(2)	2/47(4)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.196	0.417
Lower Limit		0.004	0.041
Upper Limit		1.665	2.405
Weeks to First Observed Tumor	105	105	98

Table 5. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Preputial Gland:			
Carcinoma, NOS (b)	3/50(6)	4/50(8)	0/50(0)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.333	0.000
Lower Limit		0.238	0.000
Upper Limit		8.684	1.663
Weeks to First Observed Tumor	96	88	--
Testis: Interstitial-Cell			
Tumor (b)	46/50(92)	50/50(100)	47/48(98)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.087	1.064
Lower Limit		0.985	0.954
Upper Limit		1.087	1.110
Weeks to First Observed Tumor	74	78	68

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval for the relative risk between each dosed group and the control group.

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Locust Bean Gum (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Myelomonocytic Leukemia (b)	9/50(18)	15/50(30)	9/50(18)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.667	1.000
Lower Limit		0.758	0.384
Upper Limit		3.901	2.603
Weeks to First Observed Tumor	72	83	86
Pituitary: Adenoma, NOS (b)	20/49(41)	27/48(56)	22/49(45)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.378	1.100
Lower Limit		0.876	0.666
Upper Limit		2.173	1.822
Weeks to First Observed Tumor	84	87	86
Pituitary: Carcinoma, NOS (b)	8/49(16)	2/48(4)	4/49(8)
P Values (c), (d)	N.S.	P=0.049(N)	N.S.
Relative Risk (Control) (e)		0.255	0.500
Lower Limit		0.028	0.117
Upper Limit		1.197	1.735
Weeks to First Observed Tumor	89	105	93

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma or Carcinoma (b)	28/49(57)	29/48(60)	26/49(53)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.057	0.929
Lower Limit		0.734	0.629
Upper Limit		1.517	1.372
Weeks to First Observed Tumor	84	87	86
Adrenal: Cortical Adenoma (b)	1/50(2)	4/50(8)	6/50(12)
P Values (c), (d)	P=0.042	N.S.	N.S.
Relative Risk (Control) (e)		4.000	6.000
Lower Limit		0.415	0.768
Upper Limit		192.805	269.891
Weeks to First Observed Tumor	106	103	93
Thyroid: C-Cell Carcinoma (b)	5/50(10)	2/46(4)	3/46(7)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.435	0.652
Lower Limit		0.043	0.106
Upper Limit		2.506	3.152
Weeks to First Observed Tumor	95	105	105

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma or Carcinoma (b)	6/50(12)	3/46(7)	3/46(7)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.543	0.543
Lower Limit		0.093	0.093
Upper Limit		2.383	2.383
Weeks to First Observed Tumor	95	105	105
Mammary Gland: Fibroadenoma (b)	16/50(32)	11/50(22)	15/50(30)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.688	0.938
Lower Limit		0.323	0.488
Upper Limit		1.411	1.793
Weeks to First Observed Tumor	89	99	93
Uterus: Endometrial Stromal Polyp (b)	12/50(24)	7/50(14)	6/50(12)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.583	0.500
Lower Limit		0.212	0.167
Upper Limit		1.467	1.318
Weeks to First Observed Tumor	84	83	101

Table 6. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Locust Bean Gum (a)

(Continued)

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Throughout the second year of the study, the mean body weight of high-dose male mice was slightly lower than that of the controls. Mean body weights of low-dose male mice and dosed female mice were comparable with those of controls (Figure 3). No other compound-related clinical signs were reported.

B. Survival (Mice)

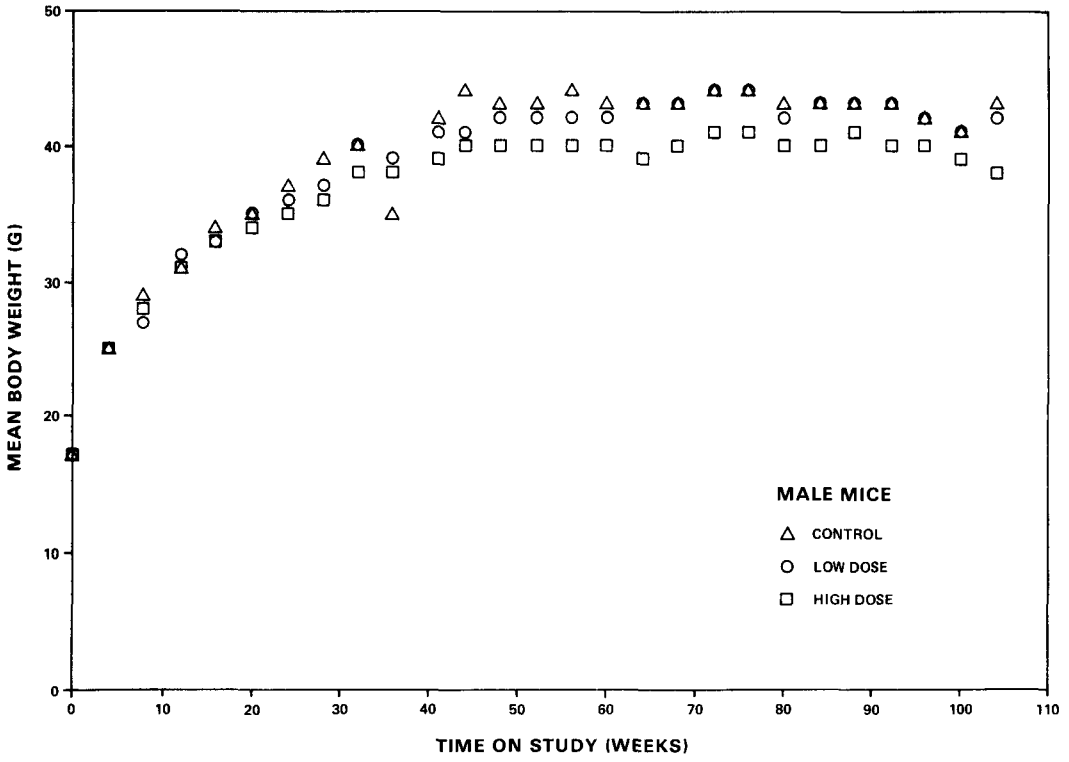
Estimates of the probabilities of survival of male and female mice administered locust bean gum in the diet at the concentrations of this bioassay, together with those of the control group, are shown by the Kaplan and Meier curves in Figure 4. No significant differences were observed between any of the groups for either males or females.

In female mice, 39/50 (78%) of the control group, 41/50 (82%) of the low-dose group, and 44/50 (88%) of the high-dose group lived to the end of the study at 105 weeks. In male mice, 35/50 (70%) of the control group, 34/50 (68%) of the low-dose group, and 41/50 (82%) of the high-dose group lived to the end of the study at 105 weeks.

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.

Neoplastic, proliferative, degenerative, inflammatory, and developmental lesions observed in the dosed mice were considered to be unrelated to administration of the test compound and to be within the normal incidence limits in historical B6C3F1 control mice, with the possible exception of lung tumors



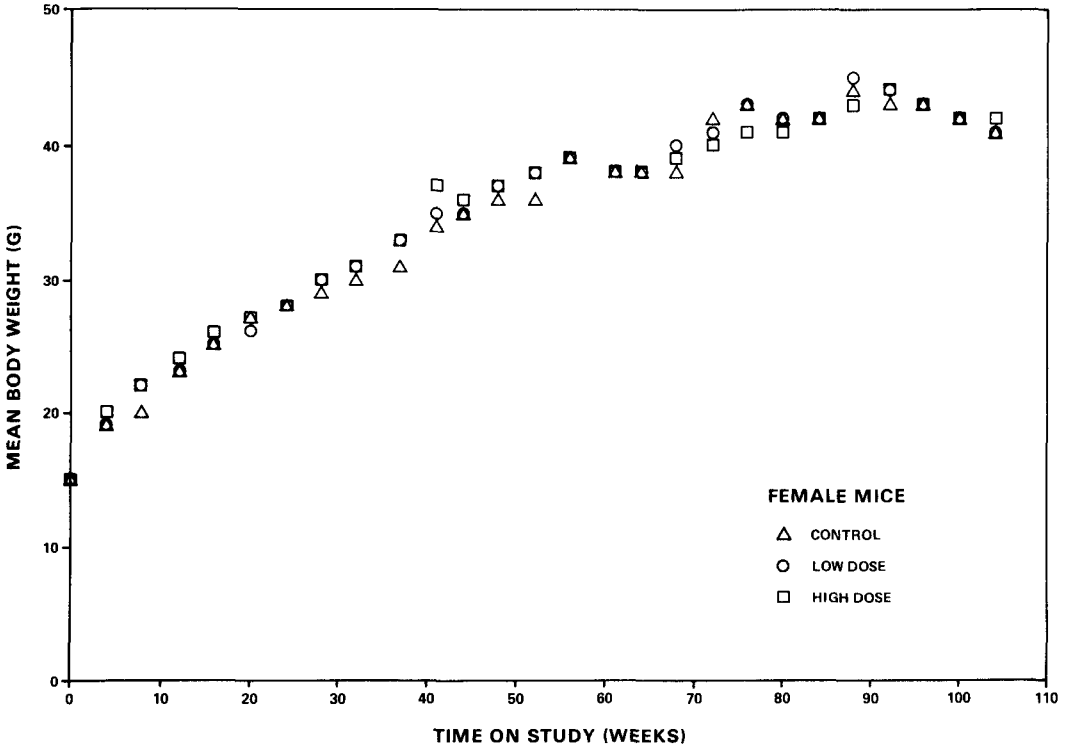


Figure 3. Growth Curves for Mice Fed Diets Containing Locust Bean Gum

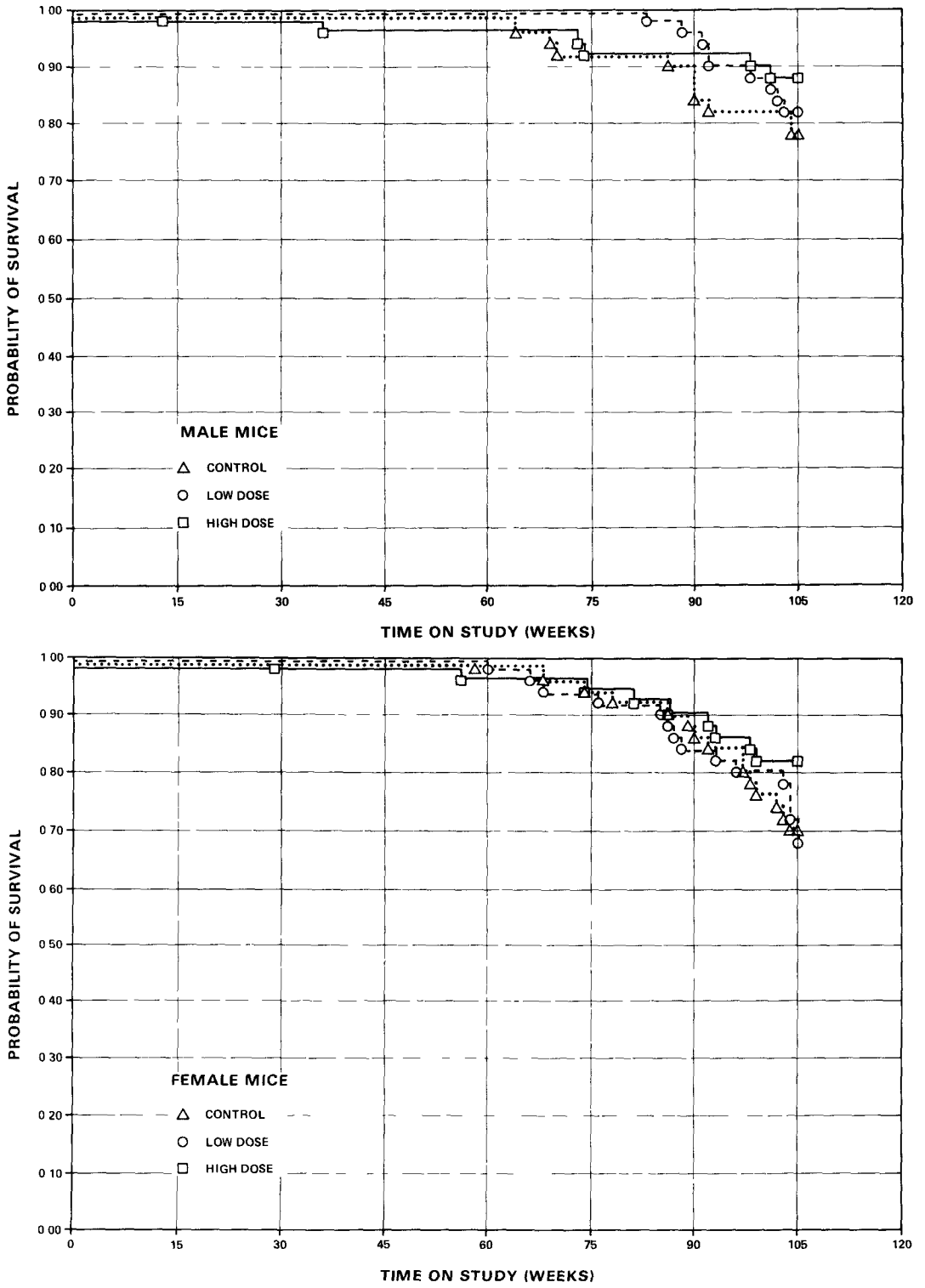


Figure 4. Survival Curves for Mice Fed Diets Containing Locust Bean Gum

in males. The incidence of alveolar/bronchiolar adenomas or carcinomas was 14/50 in the controls, 21/50 in the low-dose group, and 14/50 in the high-dose group. There was no difference in the number of mice with multiple lung tumors in each group.

D. Statistical Analyses of Results (Mice)

Tables 7 and 8 contain the statistical analyses of those primary tumors that occurred in at least two animals of one group and with an incidence of at least 5% in one or more groups.

Alveolar/bronchiolar adenomas of the lung in male mice were observed in a statistically significant positive association (P=0.017) in the low-dose group compared with the control (7/50, 14%, controls; 17/50, 34%, low-dose; 11/50, 22%, high-dose) but no significant incidence was observed in the high-dose group. The Cochran-Armitage test for linear trend was not significant, but there was a departure from linear trend due to the sharp increase of the incidence in the low-dose group compared with the other two groups. The historical incidence for this tumor type in untreated control male mice is 289/3,543 (8.1%). The incidence in control groups at this laboratory has ranged up to 13/50 (26%). In female mice, this tumor was not observed in statistically significant proportions. The lack of significant results in the high-dose group, taken together with the relatively high variation in the background rate of this tumor, precludes a clear decision as to the effect of locust bean gum at this site. Moreover, when the incidence of male mice with adenomas or carcinomas is analyzed, there are no significant increases in the dosed groups (14/50, 28%, controls; 21/50, 42%, low-dose; 14/50, 28%, high-dose).

Lymphomas of the hematopoietic system in female mice were observed in a statistically significant negative relation (31/50, 62%, controls; 23/50, 46%, low-dose; 14/50, 28%, high-dose). The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.001). The Fisher exact test between the high-dose group and the control group was significant (P=0.001). No significant incidence was observed in the low-dose group; however, this tumor occurred in decreased incidence in the low-dose group compared with the control group.

Adenomas of the pituitary in female mice were observed in increased incidence in the low-dose group (4/36, 11%) compared with the other two groups (controls, 0/39, 0%; high-dose 1/41, 2%). The Cochran-Armitage test of linear trend was not significant, but there was a departure from linear trend due to the increased incidence in the low-dose group compared with the other two groups. The Fisher exact test between the low-dose group and the control group was significant (P=0.048), but this value of P=0.048 is above the value of P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dose groups are compared with a common control group. No significant incidence was observed in the high-dose group. Historical records of seven control groups at this laboratory show a combined incidence of this tumor of 21/289 (7%), with the highest incidence being 8/39 (21%). This tumor was not observed in statistically significant proportions in male mice.

Endometrial stromal polyps of the uterus in female mice were observed in increased incidence in the high-dose group (0/45, 0% in the controls; 0/49, 0% in the low-dose; 3/49, 6% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.041). The Fisher exact tests were not significant. The historical incidence of control female mice with endometrial stromal polyps at this laboratory and under this contract is 4/335(1.2%), and the maximum incidence seen in a control group is 2/48(4.2%).

Analyses of the time to observation of tumors by life table methods and analyses by the time-adjusted test, eliminating those animals that died prior to week 52, did not materially alter the significance of the test results reported in Tables 7 and 8.

Statistically, there was no site at which an increase in tumor incidence could be associated unequivocally with administration of the chemical.

Table 7. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Locust Bean Gum (a)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	7/50(14)	17/50(34)	11/50(22)
P Values (c)	N.S.	P=0.017	N.S.
Departure from Linear Trend (e)	P=0.029		
Relative Risk (Control) (d)		2.429	1.571
Lower Limit		1.059	0.608
Upper Limit		6.285	4.394
Weeks to First Observed Tumor	90	83	74
Lung: Alveolar/Bronchiolar Carcinoma (b)	8/50(16)	5/50(10)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.625	0.500
Lower Limit		0.172	0.117
Upper Limit		2.011	1.737
Weeks to First Observed Tumor	64	91	74
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	14/50(28)	21/50(42)	14/50(28)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.500	1.000
Lower Limit		0.828	0.496
Upper Limit		2.789	2.018
Weeks to First Observed Tumor	64	83	74

Table 7. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Malignant Lymphoma, NOS (b)	12/50(24)	13/50(26)	11/50(22)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.083	0.917
Lower Limit		0.507	0.406
Upper Limit		2.334	2.049
Weeks to First Observed Tumor	92	101	104
Hematopoietic System:			
Lymphoma (b)	12/50(24)	14/50(28)	11/50(22)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.167	0.917
Lower Limit		0.559	0.406
Upper Limit		2.475	2.049
Weeks to First Observed Tumor	92	101	104
Liver: Hepatocellular			
Adenoma (b)	6/50(12)	7/49(14)	5/49(10)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.190	0.850
Lower Limit		0.369	0.219
Upper Limit		3.987	3.123
Weeks to First Observed Tumor	105	105	104

Table 7. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	15/50(30)	10/49(20)	9/49(18)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.680	0.612
Lower Limit		0.304	0.262
Upper Limit		1.453	1.344
Weeks to First Observed Tumor	90	92	101
Liver: Hepatocellular Adenoma or Carcinoma (b)	18/50(36)	16/49(33)	14/49(29)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.907	0.794
Lower Limit		0.493	0.414
Upper Limit		1.654	1.490
Weeks to First Observed Tumor	90	92	101

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 8. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Locust Bean Gum (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Sarcoma, NOS (b)	0/50(0)	3/50(6)	0/50(0)
P Values (c), (d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.014		
Relative Risk (Control) (e)		Infinite	--
Lower Limit		0.601	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	103	--
Lung: Alveolar/Bronchiolar			
Adenoma (b)	2/50(4)	1/50(2)	4/49(8)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	2.041
Lower Limit		0.009	0.308
Upper Limit		9.290	21.737
Weeks to First Observed Tumor	105	105	93
Lung: Alveolar/Bronchiolar			
Carcinoma (b)	3/50(6)	1/50(2)	0/49(0)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	0.000
Lower Limit		0.006	0.000
Upper Limit		3.983	1.696
Weeks to First Observed Tumor	86	105	--

Table 8. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	5/50(10)	2/50(4)	4/49(8)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.400	0.816
Lower Limit		0.040	0.171
Upper Limit		2.313	3.567
Weeks to First Observed Tumor	86	105	93
Hematopoietic System: Malignant Lymphoma, NOS (b)	30/50(60)	20/50(40)	13/50(26)
P Values (c), (d)	P=0.001(N)	P=0.036(N)	P=0.001(N)
Relative Risk (Control) (e)		0.667	0.433
Lower Limit		0.430	0.245
Upper Limit		1.032	0.740
Weeks to First Observed Tumor	78	93	104
Hematopoietic System: Lymphoma (b)	31/50(62)	23/50(46)	14/50(28)
P Values (c), (d)	P=0.001(N)	N.S.	P=0.001(N)
Relative Risk (Control) (e)		0.742	0.452
Lower Limit		0.499	0.265
Upper Limit		1.106	0.752
Weeks to First Observed Tumor	58	68	74

Table 8. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	2/49(4)	1/49(2)	1/49(2)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	0.500
Lower Limit		0.009	0.009
Upper Limit		9.284	9.284
Weeks to First Observed Tumor	97	105	105
Liver: Hepatocellular Adenoma or Carcinoma (b)	3/49(6)	2/49(4)	2/49(4)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	0.667
Lower Limit		0.058	0.058
Upper Limit		5.565	5.565
Weeks to First Observed Tumor	97	105	104
Pituitary: Adenoma, NOS (b)	0/39(0)	4/36(11)	1/41(2)
P Values (c), (d)	N.S.	P=0.048	N.S.
Departure from Linear Trend (f)	P=0.015		
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		1.014	0.051
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	105

Table 8. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing Locust Bean Gum (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	0/45(0)	0/49(0)	3/49(6)
P Values (c), (d)	P=0.041	N.S.	N.S.
Relative Risk (Control) (e)		--	Infinite
Lower Limit		--	0.554
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	104

(a) Dosed groups received doses of 25,000 or 50,000 ppm in the diet.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise, not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

V. DISCUSSION

Throughout the study, mean body weights of dosed rats of either sex and of low-dose male and dosed female mice were comparable with those of the controls; those of the high-dose male mice were slightly lower. No other compound-related clinical signs or effects on survival were observed in the chronic study at doses of 50,000 ppm. No compound-related histopathology, effects on survival, or consistent mean body weight gain depressions were noted in the subchronic studies, even at doses as high as 100,000 ppm. Although the results of both the subchronic and chronic studies suggest that the rats and mice might have been able to tolerate higher doses of locust bean gum than 50,000 ppm, this 5% level is the recommended maximum concentration of a test chemical in feed in the Bioassay Program.

Tumors commonly occurring in these strains were seen in both control and dosed animals, but none of these tumors was considered compound related. Although alveolar/bronchiolar adenomas occurred in low-dose male mice at an incidence significantly higher (P=0.017) than that in the controls, when the combined incidence of male mice with either alveolar/bronchiolar adenomas or carcinomas was analyzed, no significant statistical results were obtained.

Cortical adenomas in the adrenal gland of female rats occurred with a statistically significant positive trend (P = 0.042), but the Fisher exact tests were not significant.

Adenomas of the pituitary in low-dose female mice were observed at a probability level of P=0.048 when compared with the controls, but this value is above the P=0.025 required by the Bonferroni inequality criterion for an overall significance of P=0.05 when two dosed groups are compared with a common control group. The incidence in the high-dose group was not significant.

A significant linear trend was observed (P=0.041) in the incidence of female mice with endometrial stromal polyps of the uterus; however, the Fisher exact tests were not significant.

Besides locust bean gum, four other "gums' have been tested recently by the NCI/NTP bioassay program; each was added to the diet (2.5% and 5.0%) and fed for 104 weeks to F344 rats and B6C3F1 mice of each sex. Under these

test conditions, all were considered not carcinogenic (agar, NTP 1982a; gum arabic, NTP 1982b; guar gum, NTP 1982c; and tara gum, NTP 1982d).

VI. CONCLUSION

Under the conditions of this bioassay, locust bean gum was not carcinogenic for male or female F344 rats or B6C3F1 mice.

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APPENDIX A

Summary of the Incidence of Neoplasms in Rats Fed Diets Containing Locust Bean Gum

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		1 (2%)
TRICHOEPITHELIOMA			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
FIBROMA		3 (6%)	1 (2%)
FIBROSARCOMA	1 (2%)	1 (2%)	2 (4%)
LIPOMA	1 (2%)		
HIBERNOMA		1 (2%)	
RESPIRATORY SYSTEM			
*NOSE	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MYELOMONOCYTIC LEUKEMIA	21 (42%)	13 (26%)	15 (30%)
#SPLEEN	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)		
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA	1 (2%)	1 (2%)	1 (2%)
#FORESTOMACH	(50)	(50)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#ILEUM	(49)	(50)	(48)
MUCINOUS CYSTADENOCARCINOMA	1 (2%)		
URINARY SYSTEM			
#URINARY BLADDER	(48)	(48)	(45)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(46)	(45)
CARCINOMA,NOS	3 (6%)		1 (2%)
ADENOMA, NOS	5 (11%)	6 (13%)	8 (18%)
#ADRENAL	(50)	(50)	(49)
CORTICAL ADENOMA	1 (2%)		
PHEOCHROMOCYTOMA	4 (8%)	10 (20%)	7 (14%)
#THYROID	(49)	(50)	(47)
C-CELL ADENOMA	1 (2%)		1 (2%)
C-CELL CARCINOMA	4 (8%)	1 (2%)	1 (2%)
#THYROID FOLLICLE	(49)	(50)	(47)
PAPILLARY CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(49)	(49)	(49)
ISLET-CELL ADENOMA	1 (2%)		1 (2%)
ISLET-CELL CARCINOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROADENOMA	1 (2%)	2 (4%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	3 (6%)	4 (8%)	
#TESTIS	(50)	(50)	(48)
INTERSTITIAL-CELL TUMOR	46 (92%)	50 (100%)	47 (98%)
*SCROTUM	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EXTERNAL EAR	(50)	(50)	(50)
FIBROSARCOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)	1 (2%)	
TAIL			
SQUAMOUS CELL PAPILLOMA			1
FIBROSARCOMA	1		
OMENTUM			
MESOTHELIOMA, NOS			1

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHS	8	7	12
MORIBUND SACRIFICE	8	7	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	34	36	33
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	50	48
TOTAL PRIMARY TUMORS	102	96	95
TOTAL ANIMALS WITH BENIGN TUMORS	46	50	48
TOTAL BENIGN TUMORS	65	72	68
TOTAL ANIMALS WITH MALIGNANT TUMORS	32	21	23
TOTAL MALIGNANT TUMORS	36	22	25
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	2
TOTAL UNCERTAIN TUMORS	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

S INCLUDES AUTOLYZED ANIMALS

* 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 DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)	1 (2%)
FIBROMA	1 (2%)	1 (2%)	
FIBROSARCOMA	1 (2%)		
FIBROADENOMA	1 (2%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA	9 (18%)	14 (28%)	9 (18%)
#LIVER	(50)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#FORESTOMACH	(49)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#JEJUNUM	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
UNDIFFERENTIATED CARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(48)	(49)
CARCINOMA,NOS	8 (16%)	2 (4%)	4 (8%)
ADENOMA, NOS	20 (41%)	27 (56%)	22 (45%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	1 (2%)	4 (8%)	6 (12%)
PHEOCHROMOCYTOMA	1 (2%)		1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT			1 (2%)
#THYROID	(50)	(46)	(46)
C-CELL ADENOMA	1 (2%)	1 (2%)	
C-CELL CARCINOMA	5 (10%)	2 (4%)	3 (7%)
#THYROID FOLLICLE	(50)	(46)	(46)
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
#PANCREATIC ISLETS	(49)	(48)	(50)
ISLET-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	1 (2%)
PAPILLARY ADENOCARCINOMA			1 (2%)
CYSTADENOMA, NOS		2 (4%)	
FIBROADENOMA	16 (32%)	11 (22%)	15 (30%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)	1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS	1 (2%)	1 (2%)	
#UTERUS	(50)	(50)	(50)
ENDOMETRIAL STROMAL POLYP	12 (24%)	7 (14%)	6 (12%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#CEREBRUM	(50)	(50)	(50)
ASTROCYTOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*EAR	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH∂	2	7	6
MORIBUND SACRIFICE	6	5	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	38	39
ANIMAL MISSING			

∂ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	44	43	42
TOTAL PRIMARY TUMORS	82	80	74
TOTAL ANIMALS WITH BENIGN TUMORS	35	37	36
TOTAL BENIGN TUMORS	56	57	52
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	22	18
TOTAL MALIGNANT TUMORS	26	23	22
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

Summary of the Incidence of Neoplasms in Mice Fed Diets Containing Locust Bean Gum

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	2 (4%)		
FIBROMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)	1 (2%)	1 (2%)
FIBROMA		1 (2%)	
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
CARCINOMA, NOS, METASTATIC	1 (2%)		
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)	1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	7 (14%)	17 (34%)	11 (22%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	8 (16%)	5 (10%)	4 (8%)
OSTEOSARCOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	8 (16%)	10 (20%)	10 (20%)
#SPLEEN	(47)	(46)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#BRONCHIAL LYMPH NODE	(41)	(40)	(44)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (3%)	
#MESENTERIC L. NODE	(41)	(40)	(44)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MALIGNANT LYMPHOMA, NOS	3 (7%)	1 (3%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (3%)	
#PEYER'S PATCH	(48)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)	1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
#SPLEEN	(47)	(46)	(49)
ANGIOSARCOMA	1 (2%)		
#MEDIASTINAL L.NODE	(41)	(40)	(44)
HEMANGIOMA	1 (2%)		
#MESENTERIC L. NODE	(41)	(40)	(44)
HEMANGIOSARCOMA	1 (2%)		
*MUSCLE OF LEG	(50)	(50)	(50)
ANGIOSARCOMA			1 (2%)
#HEART	(50)	(50)	(50)
ANGIOSARCOMA		1 (2%)	
#LIVER	(50)	(49)	(49)
ANGIOSARCOMA		1 (2%)	1 (2%)
#KIDNEY	(47)	(48)	(50)
ANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
BILE DUCT ADENOMA		1 (2%)	
HEPATOCELLULAR ADENOMA	6 (12%)	7 (14%)	5 (10%)
HEPATOCELLULAR CARCINOMA	15 (30%)	10 (20%)	9 (18%)
#PANCREAS	(45)	(48)	(48)
ACINAR-CELL ADENOMA			1 (2%)
#PERIESOPHAGEAL TISSU	(39)	(39)	(47)
ALVEOLAR/BRONCHIOLAR CA, INVASIV		1 (3%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#STOMACH	(47)	(48)	(47)
ADENOMATOUS POLYP, NOS		1 (2%)	
#FORESTOMACH	(47)	(48)	(47)
PAPILLOMA, NOS		1 (2%)	
#JEJUNUM	(48)	(50)	(49)
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
#COLON	(43)	(44)	(47)
ADENOCARCINOMA, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(47)	(48)	(50)
OSTEOSARCOMA, METASTATIC	1 (2%)		
ENDOCRINE SYSTEM			
#THYROID	(46)	(45)	(49)
ADENOMA, NOS		1 (2%)	
FOLLICULAR-CELL ADENOMA	1 (2%)		
#PANCREATIC ISLETS	(45)	(48)	(48)
ISLET-CELL ADENOMA	2 (4%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
#TESTIS	(48)	(48)	(50)
INTERSTITIAL-CELL TUMOR	1 (2%)		1 (2%)
NERVOUS SYSTEM			
#BRAIN	(45)	(41)	(45)
ALVEOLAR/BRONCHIOLAR CA, METASTA		1 (2%)	
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
CARCINOMA,NOS	1 (2%)		

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ADENOMA, NOS	2 (4%)	1 (2%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
*INTERCOSTAL MUSCLE	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV		1 (2%)	
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SARCOMA, NOS, METASTATIC		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATHS@	9	7	4
MORIBUND SACRIFICE	2	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	39	41	44
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	35	41	38
TOTAL PRIMARY TUMORS	64	65	50
TOTAL ANIMALS WITH BENIGN TUMORS	16	24	21
TOTAL BENIGN TUMORS	20	31	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	29	30	26
TOTAL MALIGNANT TUMORS	44	34	29
TOTAL ANIMALS WITH SECONDARY TUMORS#	4	3	3
TOTAL SECONDARY TUMORS	5	6	3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS		3 (6%)	
SARCOMA, NOS, METASTATIC		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	1 (2%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	1 (2%)	
PAPILLARY ADENOCARCINOMA, METAST	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	28 (56%)	17 (34%)	12 (24%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE		2 (4%)	
*HEMATOPOIETIC SYSTEM	(50)	(50)	(50)
NEOPLASM, NOS	2 (4%)	1 (2%)	
#SPLEEN	(50)	(50)	(47)
MALIGNANT LYMPHOMA, NOS	2 (4%)	3 (6%)	1 (2%)
#LYMPH NODE	(41)	(43)	(41)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#BRONCHIAL LYMPH NODE	(41)	(43)	(41)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (2%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGHDOSE
#STOMACH	(50)	(48)	(47)
MAST-CELL SARCOMA	1 (2%)		
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)		
#SPLEEN	(50)	(50)	(47)
ANGIOSARCOMA		2 (4%)	
#LIVER	(49)	(49)	(49)
HEMANGIOSARCOMA	1 (2%)		
ANGIOSARCOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(49)
HEPATOCELLULAR ADENOMA	1 (2%)	1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA	2 (4%)	1 (2%)	1 (2%)
#STOMACH	(50)	(48)	(47)
PAPILLOMA, NOS	1 (2%)	1 (2%)	
#FORESTOMACH	(50)	(48)	(47)
PAPILLOMA, NOS			1 (2%)
#CECUM	(46)	(47)	(47)
LEIOMYOSARCOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(36)	(41)
ADENOMA, NOS		4 (11%)	1 (2%)
#ADRENAL	(45)	(46)	(46)
PHEOCHROMOCYTOMA	1 (2%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(47)	(50)	(41)
FOLLICULAR-CELL ADENOMA	1 (2%)		
FOLLICULAR-CELL CARCINOMA		1 (2%)	
#PANCREATIC ISLETS	(45)	(46)	(47)
ISLET-CELL ADENOMA	1 (2%)		1 (2%)
ISLET-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
PAPILLARY ADENOCARCINOMA	1 (2%)		
FIBROADENOMA		1 (2%)	
#UTERUS	(45)	(49)	(49)
NEOPLASM, NOS			1 (2%)
LEIOMYOMA		1 (2%)	
LEIOMYOSARCOMA			2 (4%)
ENDOMETRIAL STROMAL POLYP			3 (6%)
#OVARY/OVIDUCT	(45)	(49)	(49)
PAPILLARY ADENOMA	1 (2%)		
#OVARY	(47)	(48)	(45)
CYSTADENOCARCINOMA, NOS		1 (2%)	
PAPILLARY CYSTADENOMA, NOS		1 (2%)	
TERATOMA, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(48)	(48)	(45)
MENINGIOMA	2 (4%)		
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA	1 (2%)		
*PERITONEUM	(50)	(50)	(50)
SARCOMA, NOS, METASTATIC		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SARCOMA, NOS, METASTATIC		1 (2%)	
OMENTUM			
CYSTADENOCARCINOMA, METASTATIC		1	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	14	10	8
MORIBUND SACRIFICE	1	6	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	34	41
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	45	36	30
TOTAL PRIMARY TUMORS	56	44	33
TOTAL ANIMALS WITH BENIGN TUMORS	9	8	11
TOTAL BENIGN TUMORS	11	10	12
TOTAL ANIMALS WITH MALIGNANT TUMORS	38	30	18
TOTAL MALIGNANT TUMORS	43	33	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	4	
TOTAL SECONDARY TUMORS	3	5	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	1	2
TOTAL UNCERTAIN TUMORS	2	1	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Diets Containing Locust Bean Gum

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)		
HYPERKERATOSIS	2 (4%)	1 (2%)	1 (2%)
ACANTHOSIS		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
STEATITIS	1 (2%)		
PUS	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, NOS		1 (2%)	
NECROSIS, FAT	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
EDEMA, NOS			1 (2%)
HEMORRHAGE			1 (2%)
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
DEGENERATION, HYALINE		1 (2%)	
HEMOSIDEROSIS	1 (2%)		
HEMATOPOIESIS		1 (2%)	
ERYTHROPOIESIS	1 (2%)		
#SPLENIC FOLLICLES	(50)	(50)	(50)
ATROPHY, NOS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(46)	(47)	(49)
CYST, NOS	1 (2%)		
CONGESTION, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(49)	(50)	(50)
FIBROSIS	2 (4%)		
FIBROSIS, DIFFUSE	1 (2%)		
PERIARTERITIS	1 (2%)		
#HEART/ATRIUM	(49)	(50)	(50)
THROMBUS, MURAL		1 (2%)	3 (6%)
#LEFT VENTRICLE	(49)	(50)	(50)
FIBROSIS			2 (4%)
#MYOCARDIUM	(49)	(50)	(50)
FIBROSIS		1 (2%)	
*PANCREATIC ARTERY,	(50)	(50)	(50)
MEDIAL CALCIFICATION			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#PANCREAS	(49)	(49)	(49)
PERIARTERITIS	1 (2%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
INFLAMMATION ACTIVE CHRONIC			1 (2%)
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
METAMORPHOSIS FATTY	1 (2%)		2 (4%)
BASOPHILIC CYTO CHANGE	3 (6%)	1 (2%)	3 (6%)
GROUND-GLASS CYTO CHANGE			1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
CLEAR-CELL CHANGE			1 (2%)
ATROPHY, NOS		1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
NECROSIS, NOS			2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
METAMORPHOSIS FATTY		1 (2%)	
#LIVER/KUPFFER CELL	(50)	(50)	(50)
HEMOSIDEROSIS			1 (2%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	26 (52%)	30 (60%)	24 (48%)
#PANCREAS	(49)	(49)	(49)
INFLAMMATION, INTERSTITIAL	1 (2%)		
#GASTRIC MUCOSA	(50)	(50)	(49)
ULCER, NOS			1 (2%)
CALCIFICATION, NOS	1 (2%)		
#FORESTOMACH	(50)	(50)	(49)
INFLAMMATION, ACUTE			1 (2%)
#COLON	(46)	(50)	(48)
NEMATODIASIS	2 (4%)	6 (12%)	4 (8%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
PYELONEPHRITIS, ACUTE	1 (2%)		
NEPHROSIS, NOS	46 (92%)	45 (90%)	48 (96%)
CALCIFICATION, NOS		1 (2%)	
HEMOSIDEROSIS		2 (4%)	1 (2%)
#KIDNEY/CORTEX	(50)	(50)	(50)
HAMARTOMA			1 (2%)
CYST, NOS		1 (2%)	
#RENAL PAPILLA	(50)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(50)
NECROSIS, CORTICAL	1 (2%)		
HEMOSIDEROSIS	1 (2%)		
#KIDNEY/PELVIS	(50)	(50)	(50)
HYPERPLASIA, PAPILLARY		1 (2%)	
#URINARY BLADDER	(48)	(48)	(45)
PARASITISM			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(47)	(46)	(45)
CYST, NOS		1 (2%)	
HEMORRHAGIC CYST	1 (2%)		
#ADRENAL	(50)	(50)	(49)
HYPERPLASTIC NODULE	1 (2%)		
HYPERPLASIA, FOCAL	1 (2%)		
#ADRENAL MEDULLA	(50)	(50)	(49)
HYPERPLASIA, FOCAL	2 (4%)	1 (2%)	3 (6%)
#THYROID	(49)	(50)	(47)
HYPERPLASIA, C-CELL	2 (4%)	1 (2%)	
#THYROID FOLLICLE	(49)	(50)	(47)
HYPERPLASIA, PAPILLARY			1 (2%)
#PARATHYROID	(23)	(23)	(11)
HYPERPLASIA, NOS	1 (4%)		
#PANCREATIC ISLETS	(49)	(49)	(49)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
HYPERPLASIA, CYSTIC	1 (2%)		
*PREPUTIAL GLAND	(50)	(50)	(50)
PUS	4 (8%)	4 (8%)	1 (2%)
INFLAMMATION, ACUTE	2 (4%)		
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, NOS	5 (10%)	4 (8%)	1 (2%)
#PROSTATE	(49)	(49)	(46)
PUS			1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)	3 (6%)	2 (4%)
INFLAMMATION, CHRONIC		1 (2%)	
#TESTIS	(50)	(50)	(48)
NECROSIS, NOS	1 (2%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#TESTIS/TUBULE	(50)	(50)	(48)
DEGENERATION, NOS	2 (4%)	1 (2%)	
*SCROTUM	(50)	(50)	(50)
STEATITIS			1 (2%)
NECROSIS, FAT			1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(49)	(50)
CYST, NOS	1 (2%)		
#CEREBRAL CORTEX	(50)	(49)	(50)
MALACIA			1 (2%)
#CEREBELLUM	(50)	(49)	(50)
HEMORRHAGE	1 (2%)	1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
STEATITIS	3 (6%)	4 (8%)	1 (2%)
NECROSIS, FAT	3 (6%)	1 (2%)	1 (2%)
CALCIFICATION, NOS		2 (4%)	
ALL OTHER SYSTEMS			
TAIL			
EPIDERMAL INCLUSION CYST			1
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC	1		1

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FAT	1		
OMENTUM			
STEATITIS		1	
NECROSIS, FAT			1

SPECIAL MORPHOLOGY SUMMARY

NONE

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS			2 (4%)
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(50)
NECROSIS, DIFFUSE		1 (2%)	
HEMATOPOIESIS	1 (2%)		
#MESENTERIC L. NODE	(48)	(48)	(49)
FIBROSIS			1 (2%)
#LIVER	(50)	(50)	(50)
HYPERPLASIA, BASOPHILIC		2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATITIS, TOXIC			1 (2%)
METAMORPHOSIS FATTY	3 (6%)	2 (4%)	1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CYTOPLASMIC CHANGE, NOS	1 (2%)		1 (2%)
BASOPHILIC CYTO CHANGE	35 (70%)	29 (58%)	36 (72%)
ANGIECTASIS		1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
CONGESTION, NOS			1 (2%)
NECROSIS, FAT			1 (2%)
#BILE DUCT	(50)	(50)	(50)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS	10 (20%)	4 (8%)	7 (14%)
#STOMACH	(49)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
#FORESTOMACH	(49)	(50)	(50)
EDEMA, NOS		1 (2%)	
INFLAMMATION, ACUTE	1 (2%)	2 (4%)	
#COLON	(49)	(50)	(50)
NEMATODIASIS	5 (10%)	4 (8%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
NEPHROSIS, NOS	35 (70%)	34 (68%)	22 (44%)
HEMOSIDEROSIS		1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(50)
CALCIFICATION, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(48)	(49)
CYST, NOS		1 (2%)	
HEMORRHAGIC CYST			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
ANGIECTASIS		1 (2%)	
#ADRENAL	(50)	(50)	(50)
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	
#ADRENAL CORTEX	(50)	(50)	(50)
METAMORPHOSIS FATTY	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CALCIFICATION, NOS		1 (2%)	
#THYROID	(50)	(46)	(46)
HYPERPLASIA, C-CELL			2 (4%)
#THYROID FOLLICLE	(50)	(46)	(46)
HYPERPLASIA, CYSTIC		1 (2%)	
#PANCREATIC ISLETS	(49)	(48)	(50)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
DILATATION/DUCTS	2 (4%)		1 (2%)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	6 (12%)
HYPERPLASIA, CYSTIC	1 (2%)	4 (8%)	1 (2%)
LACTATION			1 (2%)
*MAMMARY LOBULE	(50)	(50)	(50)
HYPERPLASIA, NOS	3 (6%)		
*CLITORAL GLAND	(50)	(50)	(50)
PUS		2 (4%)	
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV			1 (2%)
HYPERPLASIA, NOS		1 (2%)	3 (6%)
#UTERUS	(50)	(50)	(50)
HYDROMETRA		2 (4%)	1 (2%)
HEMORRHAGE	1 (2%)		
HEMATOMA, NOS			1 (2%)
FIBROSIS	1 (2%)		
NECROSIS, NOS	1 (2%)	1 (2%)	1 (2%)
INFARCT, NOS	1 (2%)		
HEMOSIDEROSIS	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC		1 (2%)	2 (4%)
#ENDOMETRIAL GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#OVARY	(48)	(50)	(49)
CYST, NOS	1 (2%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CYSTIC FOLLICLES		1 (2%)	
FOLLICULAR CYST, NOS	1 (2%)	1 (2%)	
PAROVARIAN CYST	1 (2%)		
NERVOUS SYSTEM			
#CEREBRUM	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
MALACIA		1 (2%)	
CALCIFICATION, FOCAL		1 (2%)	
#BRAIN	(50)	(50)	(50)
INFLAMMATION WITH CAVITATION			1 (2%)
HEMOSIDEROSIS			1 (2%)
#CEREBELLUM	(50)	(50)	(50)
MALACIA		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
STEATITIS		1 (2%)	
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

APPENDIX D

Summary of the Incidence of Nonneoplastic Lesions in Mice Fed Diets Containing Locust Bean Gum

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ULCER, FOCAL	1 (2%)		
ABSCESS, NOS	1 (2%)	1 (2%)	1 (2%)
FIBROSIS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
MINERALIZATION	1 (2%)		
CONGESTION, NOS	1 (2%)		1 (2%)
EDEMA, NOS			1 (2%)
HEMORRHAGE	1 (2%)		
INFLAMMATION, NOS	3 (6%)		1 (2%)
INFLAMMATION, FOCAL	1 (2%)	1 (2%)	
INFLAMMATION, HEMORRHAGIC		1 (2%)	
BRONCHOPNEUMONIA, ACUTE	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(46)	(49)
HYPERPLASIA, HEMATOPOIETIC		1 (2%)	
HYPERPLASIA, LYMPHOID	8 (17%)	6 (13%)	4 (8%)
HEMATOPOIESIS	1 (2%)		
#SPLENIC FOLLICLES	(47)	(46)	(49)
ATROPHY, NOS		2 (4%)	
#LYMPH NODE	(41)	(40)	(44)
NECROSIS, FOCAL			1 (2%)
#PANCREATIC L.NODE	(41)	(40)	(44)
HEMORRHAGE		1 (3%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#MESENTERIC L. NODE	(41)	(40)	(44)
HEMORRHAGE		1 (3%)	
INFLAMMATION, NOS			1 (2%)
ANGIECTASIS	10 (24%)	7 (18%)	7 (16%)
HYPERPLASIA, RETICULUM CELL	2 (5%)		2 (5%)
HYPERPLASIA, LYMPHOID	3 (7%)	1 (3%)	2 (5%)
MYELOID METAPLASIA			1 (2%)
#PEYER'S PATCH	(48)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		2 (4%)
#THYMUS	(22)	(21)	(14)
CYST, NOS	1 (5%)		
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
PERIARTERITIS	1 (2%)		
#HEART	(50)	(50)	(50)
DEGENERATION, NOS			1 (2%)
#MYOCARDIUM	(50)	(50)	(50)
CALCIFICATION, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(49)
NECROSIS, FOCAL			2 (4%)
METAMORPHOSIS FATTY	1 (2%)		1 (2%)
PIGMENTATION, NOS			1 (2%)
NUCLEAR ENLARGEMENT			1 (2%)
CLEAR-CELL CHANGE			1 (2%)
#BILE DUCT	(50)	(49)	(49)
CYST, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
#PANCREAS	(45)	(48)	(48)
CYST, NOS	1 (2%)		
CYSTIC DUCTS	1 (2%)		
ABSCESS, NOS	1 (2%)		

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU	1 (2%)		
#PANCREATIC ACINUS	(45)	(48)	(48)
CYTOLOGIC DEGENERATION			1 (2%)
ATROPHY, NOS	2 (4%)		
#STOMACH	(47)	(48)	(47)
HYPERPLASIA, BASAL CELL			1 (2%)
#FORESTOMACH	(47)	(48)	(47)
METAPLASIA, SQUAMOUS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(47)	(48)	(50)
INFLAMMATION, INTERSTITIAL	1 (2%)		
NEPHROPATHY			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
#KIDNEY/TUBULE	(47)	(48)	(50)
MINERALIZATION			1 (2%)
#U.BLADDER/SUBMUCOSA	(46)	(50)	(49)
FIBROSIS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(46)	(44)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(46)	(45)	(49)
CYST, NOS	1 (2%)		
FOLLICULAR CYST, NOS	1 (2%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	
HYPERPLASIA, EPITHELIAL		1 (2%)	
#PANCREATIC ISLETS	(45)	(48)	(48)
HYPERPLASIA, NOS	1 (2%)	3 (6%)	
REPRODUCTIVE SYSTEM			
*PREPUCE	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ABSCESS, CHRONIC			1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CYST, NOS	1 (2%)		
EPIDERMAL INCLUSION CYST		1 (2%)	
ABSCESS, NOS	2 (4%)		1 (2%)
HYPERPLASIA, NOS	1 (2%)		
#TESTIS	(48)	(48)	(50)
MINERALIZATION	1 (2%)		3 (6%)
DEGENERATION, NOS		2 (4%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
*MESENTERY	(50)	(50)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
HEMORRHAGE			1
NECROSIS, FAT	2		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	6	4

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF	2		

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING LOCUST BEAN GUM

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROSIS			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(49)
HEMORRHAGE			2 (4%)
INFLAMMATION, FOCAL	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#SPLEEN	(50)	(50)	(47)
HEMORRHAGE	1 (2%)		
ANGIECTASIS	1 (2%)		
HYPERPLASIA, GRANULOCYTIC	1 (2%)	1 (2%)	2 (4%)
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	6 (12%)	11 (22%)	8 (17%)
#SPLENIC FOLLICLES	(50)	(50)	(47)
ATROPHY, NOS		1 (2%)	
#LUMBAR LYMPH NODE	(41)	(43)	(41)
HEMORRHAGE		1 (2%)	
#MESENTERIC L. NODE	(41)	(43)	(41)
HEMATOMA, NOS		1 (2%)	
ANGIECTASIS	2 (5%)		1 (2%)
HYPERPLASIA, RETICULUM CELL			1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, LYMPHOID			1 (2%)
#PEYER'S PATCH	(46)	(49)	(47)
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(25)	(17)	(24)
CYST, NOS	1 (4%)		
NECROSIS, NOS			1 (4%)
CIRCULATORY SYSTEM			
#MESENTERIC L. NODE	(41)	(43)	(41)
LYMPHANGIECTASIS	1 (2%)		
#LUNG	(50)	(50)	(49)
PERIVASCULITIS		1 (2%)	
#HEART	(47)	(50)	(47)
MINERALIZATION	1 (2%)		
PERIARTERITIS			1 (2%)
DEGENERATION, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(47)	(48)	(46)
FIBROSIS, DIFFUSE	1 (2%)		
#LIVER	(49)	(49)	(49)
ABSCESS, NOS			1 (2%)
NECROSIS, NOS	2 (4%)		
NECROSIS, FOCAL	1 (2%)	2 (4%)	
METAMORPHOSIS FATTY	1 (2%)		2 (4%)
BASOPHILIC CYTO CHANGE		1 (2%)	
CLEAR-CELL CHANGE			1 (2%)
#BILE DUCT	(49)	(49)	(49)
CYST, NOS	1 (2%)		
#PANCREAS	(45)	(46)	(47)
DILATATION/DUCTS	2 (4%)		
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, ACUTE			1 (2%)
ABSCESS, NOS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, NOS	1 (2%)		
#PANCREATIC ACINUS	(45)	(46)	(47)
CYTOLOGIC DEGENERATION			1 (2%)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(50)	(48)	(47)
INFLAMMATION, ACUTE FOCAL	1 (2%)	1 (2%)	
PIGMENTATION, NOS		1 (2%)	
HYPERPLASIA, BASAL CELL		1 (2%)	
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		
#GASTRIC MUCOSA	(50)	(48)	(47)
METAPLASIA, SQUAMOUS			1 (2%)
#FORESTOMACH	(50)	(48)	(47)
ULCER, FOCAL		1 (2%)	
INFLAMMATION, ACUTE FOCAL	1 (2%)		1 (2%)
HYPERPLASIA, BASAL CELL		2 (4%)	1 (2%)
ACANTHOSIS	1 (2%)	1 (2%)	
#ILEUM	(46)	(49)	(47)
CONGESTION, NOS		1 (2%)	
#COLON	(46)	(47)	(47)
NEMATODIASIS			1 (2%)
#CECUM	(46)	(47)	(47)
EDEMA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(48)	(50)	(49)
INFLAMMATION, INTERSTITIAL	1 (2%)	1 (2%)	1 (2%)
GLOMERULOSCLEROSIS, NOS	2 (4%)		
INFARCT, HEALED			1 (2%)
#KIDNEY/TUBULE	(48)	(50)	(49)
NECROSIS, FOCAL	1 (2%)		
METAMORPHOSIS FATTY		1 (2%)	
PIGMENTATION, NOS		1 (2%)	
REGENERATION, NOS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(36)	(41)
CYST, NOS		1 (3%)	1 (2%)
HYPERPLASIA, FOCAL	1 (3%)		
#THYROID	(47)	(50)	(41)
CYST, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
#UTERUS	(45)	(49)	(49)
HYDROMETRA		4 (8%)	5 (10%)
ABSCESS, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(45)	(49)	(49)
INFLAMMATION, NECROTIZING	2 (4%)		
INFLAMMATION, ACUTE	2 (4%)	2 (4%)	2 (4%)
HYPERPLASIA, NOS		1 (2%)	2 (4%)
HYPERPLASIA, CYSTIC	30 (67%)	32 (65%)	28 (57%)
#OVARY	(47)	(48)	(45)
MINERALIZATION		2 (4%)	
CYST, NOS	8 (17%)	3 (6%)	5 (11%)
PAROVARIAN CYST		1 (2%)	
HEMORRHAGIC CYST	3 (6%)	3 (6%)	1 (2%)
ABSCESS, NOS	1 (2%)		
HYPERPLASIA, GRANULOSA-CELL	1 (2%)		
HYPERPLASIA, CYSTIC			1 (2%)
NERVOUS SYSTEM			
#BRAIN	(48)	(48)	(45)
MINERALIZATION	1 (2%)		1 (2%)
MALACIA	1 (2%)		
#CEREBELLUM	(48)	(48)	(45)
GLIOSIS	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)		2 (4%)
*MESENTERY	(50)	(50)	(50)
CYST, NOS		1 (2%)	
NECROSIS, FAT		1 (2%)	
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT	2	2	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTO/NECROPSY/HISTO PERF		1	

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

APPENDIX E

Analyses of Locust Bean Gum

(Lot No. CN-361)

Midwest Research Institute

APPENDIX E

Analyses of Locust Bean Gum (Lot No. CN-361)
Midwest Research Institute

A. MELTING POINT

Determined	Literature Values
m.p.: 210°-300°C, decomp. (visual, capillary) Exotherm beginning at 302°C, decomp. (DuPont 900 DTA)	No literature value found

B. THIN-LAYER CHROMATOGRAPHY (of hydrolysis products after reaction with H_2SO_4, neutralization with $BaCO_3$, and filtration)

Plates Silica Gel 60 F-254

Amount Spotted: 42 μg

Ref. Standard: D-Galactose and D-Mannose

Visualization: 0.5% Potassium permanganate in 1N sodium hydroxide

System 1:	System 2:
n-Butanol:acetic acid:water (63:12:25)	n-Butanol:pyridine:water (46:31:23)
R_f: 0.24 (major) (mannose) 0.17 (minor) (galactose)	R_f: 0.45 (major) 0.36 (minor)
R_{st}: 0.96, 0.68 relative to D-mannose 1.33, 0.94 relative to D-galactose	R_{st} 0.98, 0.78 relative to D-mannose 1.25, 1.00 relative to D-galactose

C. WATER ANALYSIS
(Karl Fisher) 5.7% ± 0.8(δ)%

D. TITRATION BY PERIODATE OXIDATION

Modification of U.S.P. Assay for Mannitol (USP, 1970)

Samples were dissolved in 25 ml concentrated sulfuric acid and 150 ml water in 250-ml volumetric flasks and left at room temperature for 65 hours. The solutions were then boiled for 55 minutes on a hot plate. The flasks were cooled and diluted to volume with water. Aliquots (5 ml) were transferred to 125-ml Erlenmeyer flasks and 50.0 ml potassium periodate/sulfuric acid solution was added. One sample and the blank were heated on a steam bath for 25 hours. Potassium iodide was added and the samples were titrated with sodium thiosulfate. A second sample was heated on a steam bath for 5 hours and left at room temperature for 16 hours before potassium iodide was added and the sample titrated. The assumption was made that each monomer unit reacted with 5 moles of periodate. A discussion of this procedure appears in Appendix F.

Purity: 77.2% $\pm$ 0.4(δ)% as compared to glucose

E. SPECTRAL DATA

Determined	Literature Values
1. Infrared Instrument: Beckman IR-12 Cell: 1% in potassium bromide Results: See Figure 5	Consistent with literature spectrum (McNaulty, 1960)
Determined	**Literature Values**
2. Ultraviolet/Visible Instrument: Cary 118 No absorbance between 220 and 350 nm (ultraviolet range) or between 350 and 800 nm (visible range) Concentration: 0.1 mg/ml Solvent: Water	No literature values found

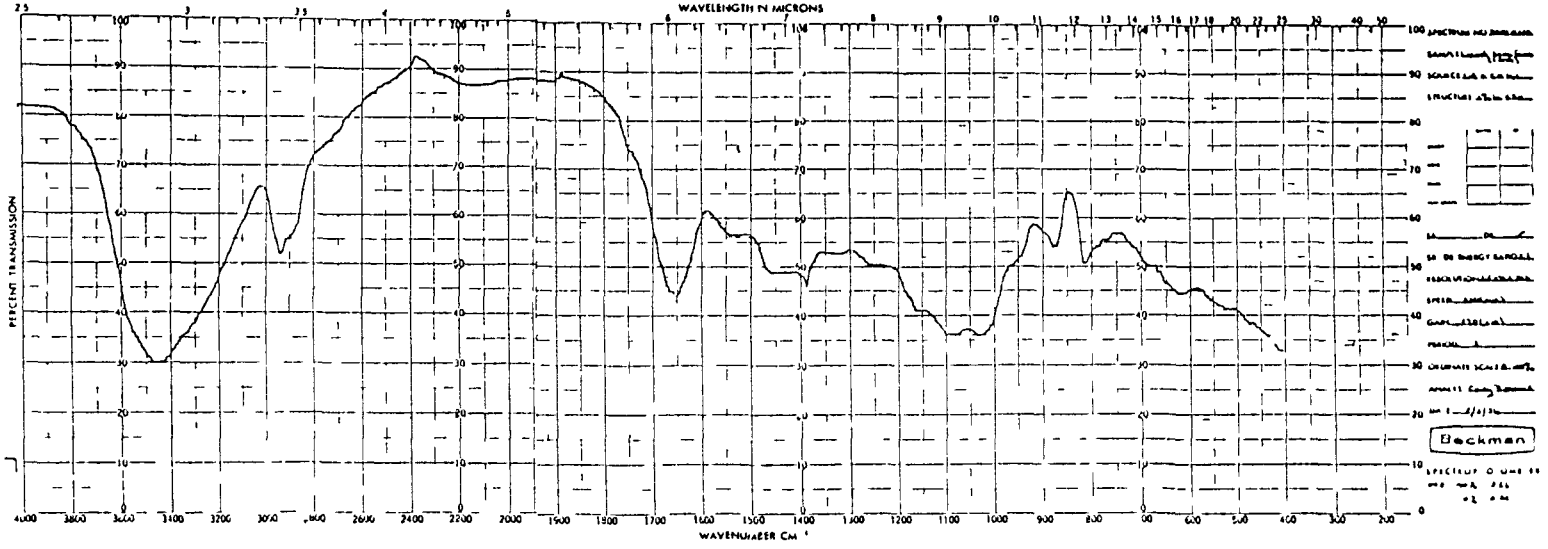

Figure 5. Infrared Absorption Spectrum (Lot No. CN-361) Locust Bean Gum

APPENDIX F

Analyses of Locust Bean Gum

(Lot No. 265-76)

Midwest Research Institute

APPENDIX F

Analyses of Locust Bean Gum Lot. (No. 265-76)
Midwest Research Institute

A. THIN-LAYER CHROMATOGRAPHY OF ACID HYDROLYSIS PRODUCTS

Plates Silica Gel 60 F-254

Ref. Standard: D-Galactose and D-Mannose (Varma et al., 1973)

Amount Spotted: 40 μg 2 μg/μl in methanol: H_2O (75:25)

Visualization: 0.5% Potassium permanganate in 1N sodium hydroxide

System 1:

n-Butanol:acetic acid:water (63:12:25)

R_f: 0.18 (minor)
0.28 (major)

R_{st}: 0.71, 1.01 relative to D-mannose
0.94, 1.33 relative to D-galactose

System 2:

n-Butanol:pyridine: water (46:31:23)

R_f: 0.49, 0.58

R_{st}: 0.88, 1.03 relative to D-mannose
1.03, 1.20 relative to D-galactose

B. WATER ANALYSIS

(Karl Fisher) 5.7% $\pm$ 0.4 (δ)%

C. TITRATION BY PERIODATE OXIDATION

Modification of U.S.P. Assay for Mannitol (USP, 1970)

Samples were dissolved in 25 ml concentrated sulfuric acid and 150 ml water in 250-ml volumetric flasks and left at room temperature for 18 hours. The solutions were then boiled on a hot plate until they started to discolor. All samples began to discolor before 15 minutes. The flasks were cooled and diluted to volume with water. Aliquots (5 ml) were transferred to 125-ml Erlenmeyer flasks and 50.0 ml potassium periodate/sulfuric acid solution added. Each sample and a blank were heated on a steam bath for 2.5 hours.

Potassium iodide was added and the samples titrated with sodium thiosulfate. The assumption was made that each monomer unit reacted with 5 moles of periodate.

Purity: 88.0% $\pm$ 2.5(δ)%

The use of this procedure for a comparative analysis of different lots at different points in time is tenuous because of the variability inherent in the procedure. Milder conditions were used for the hydrolysis of Lot No. 265-76 than for Lot No. CN-361, which may have led to further oxidation of the latter and to a lower calculated purity. Purities determined for both lots should be considered minimum values.

D. SPECTRAL DATA

1. Infrared

Instrument: Beckman IR-12	Consistent with
Cell: Thin Film	literature
Results: See Figure 6	spectrum (McNaulty, 1960)

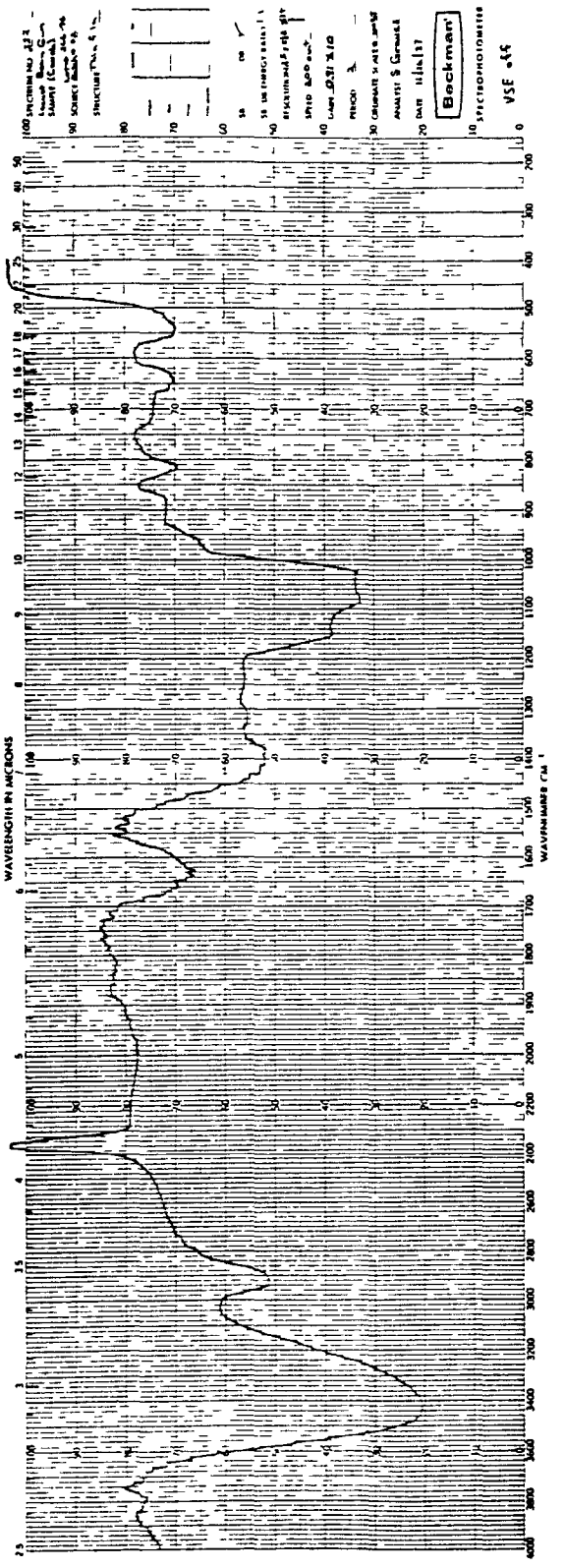

Figure 6. Infrared Absorption Spectrum (Lot No. 265-76) Locust Bean Gum

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