NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 230

CARCINOGENESIS BIOASSAY OF AGAR

(CAS NO. 9002-18-0)

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

CARCINOGENESIS BIOASSAY

of

AGAR

(CAS No. 9002-18-0)

in F344 RATS and $B6C3F_1$ MICE

(FEED STUDY)

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 circumstances. A positive result demonstrates that a test chemical is carçinogenic 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).

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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.

TABLE OF CONTENTS

TABLES

FIGURES

APPENDIXES

ABSTRACT

A carcinogenesis bioassay of agar isolated from *Pterocladia*, a gelling agent used in foods and pharmaceuticals, was conducted on groups of 50 F344 rats and 50 B6C3F1 mice of either sex which were fed diets containing 25,000 or 50,000 ppm of the test substance for 103 weeks. Groups of 50 untreated rats and mice of either sex served as controls.

Mean body weights of dosed and control male rats were comparable throughout the study. After week 80, mean body weights of dosed female rats were slightly lower than those of the controls. Mean body weights of dosed and control male mice were comparable throughout the study. The mean body weights of dosed female mice were lower than those of the controls at week 20 and remained lower throughout the study. No compound-related effects on survival, feed consumption, clinical signs of toxicity, or tumor incidence were observed. Although the rats of either sex and male mice might have been able to tolerate higher doses, 50,000 ppm was the administered high-dose level since that is the maximum concentration of a test substance in feed recommended in the guidelines of the Bioassay Program.

A statistically significant trend (P=0.015) was observed for the increased incidence of cortical adenomas of the adrenal gland (control, 0/50; low-dose, 0/50; high-dose, 4/50) in female rats; the difference between control and high-dose groups was not significant. In male mice the incidence of hepatocellular adenomas (control, 0/49; low-dose, 3/50; high-dose, 7/50) was significantly (P=0.007) increased in the high-dose group when compared with controls; likewise, the overall trend was significant (P=0.005). The incidence of total liver tumors (control, 9/49; low-dose, 8/50; high-dose, 13/50) did not differ significantly among the groups. Neither of these increases (in cortical adenomas or in liver tumors) was considered to be compound related.

Under the conditions of this bioassay, the agar isolated from *Pterocladia* was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

CONTRIBUTORS

The bioassay of agar was conducted at EG&G Mason Research Institute, Worcester, Massachusetts, under a subcontract to Tracor Jitco, Inc., the prime contractor for the NCI/NTP Bioassay Program. The prechronic study was started in October, 1976 and finished in April, 1977. The chronic study was begun in October, 1977 and completed in November, 1979.

The bioassay was conducted under the direction 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 R. Fogleman (3). 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. Dr. D. Wyand (1), pathologist, 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 (5). 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 (6). Chemical analyses were conducted at Midwest Research Institute (7).

This report was prepared at Tracor Jitco (3), and those responsible for the report were Dr. C. Cueto (8), Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. M. A. Stedham, pathologist; Dr. J. Tomaszewski, chemist; Dr. W. D. Theriault, reports manager; and Dr. A. C. Jacobs, bioscience writer.

The following scientists at NCI/NTP (9) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. J. Fielding Douglas, Dr. Charles K. Grieshaber, Dr. Joseph Haseman, Dr. James Huff, Dr. Ernest E. McConnell, Dr. Ronald Melnick (Chemical Manager), Dr. John A. Moore, Dr. Sherman F. Stinson, Dr. R. Tennant, and Dr. Jerrold M. Ward.

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SUMMARY OF PEER REVIEW COMMENTS

On February 18, 1981 this carcinogenesis bioassay report on Agar underwent peer review and was approved by the National Toxicology Program Board of Scientific Counselors' Technical Report Review Subcommittee and associated Panel of Experts at an open meeting held in Building 31C, National Institutes of Health, Bethesda, Maryland. Members of the Subcommittee are Drs. Margaret Hitchcock (Chairperson), Curtis Harper, and Alice Whittemore. Members of the Panel are Drs. Norman Breslow, Joseph Highland, Frank Mirer (Primary reviewer), Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore (Secondary reviewer), James Swenberg, and Gary Williams. Drs. Breslow and Whittemore were unable to attend this meeting.

Dr. Mirer, as the primary reviewer for the report on the bioassay of agar, agreed with the conclusion that under the conditions of this bioassay, there was no evidence that agar was carcinogenic for F344 rats or B6C3F1 mice of either sex. In female rats cortical adenomas of the adrenal glands were observed in increased incidence in the high-dose group, with a statistically significant increasing trend. However, the Fisher exact test was not significant, the incidence in the high-dose group was within the range for control animals in this laboratory, and no significantly increased incidence of this tumor was observed in male rats or in mice of either sex. In male mice, hepatocellular adenomas were observed in increased incidence which was statistically significant both in trend and incidence in the high-dose group. However, hepatocellular carcinomas were not increased significantly either in incidence or trend, and when carcinomas and adenomas were combined, the trend and incidence were not statistically significant and were approximately at the average rate for historical controls in this laboratory. There was no significantly increased incidence of this tumor in rats of either sex. Endometrial stromal polyps of the uterus in low-dose female rats and alveolar/bronchiolar adenomas or carcinomas in female mice were reduced; however, these observations were not consistently significant by all statistical tests.

Dr. Mirer said that the lower molecular weight constituents and impurities of the test material were not identified. Further, there are three algal sources of agar, with the test material being extracted from the alga species Pterocladia. A comparison of the analyses of agar from this source with the other varieties available would prove useful. For example, carageenan, at least the low molecular weight form, which is derived from agar of a different algal source, has toxic properties. His concern was that the commercial agar to which people are exposed in food might be from a different source, and thus, a lack of significant effects from this bioassay might not hold for agar from other sources.

As secondary reviewer, Dr. Shore agreed with the conclusion of the report. He too emphasized the marginal significance of the hepatocellular tumors in male mice. He said the experimental design was reasonable, and although there was little indication that the high dose was as high as could be tolerated, it was at the ceiling level used in the bioassay program, namely 5 percent of the diet.

Dr. Mirer moved that the report on the bioassay of agar be accepted; he indicated these results pertain to only the Pterocladia-derived algae. Dr. Shore seconded the motion, and the report was approved unamiously by the Peer Review Panel.

I. INTRODUCTION

Agar (CAS No. 9002-18-0) is an extract of red algae, principally Gracilaria, Gelidium, and Pterocladia (Kirk and Othmer, 1978).

Structurally, agar consists of alternating 1,3-linked β-D-galactopyranose and 1,4- linked 3,6-anhydro-α-L-galactopyranose units. Varying amounts of ester sulfates may be present, depending on the seaweed source. Agar can be separated into a neutral gelling fraction called agarose and a sulfated, nongelling fraction called agaropectin (Kirk and Othmer, 1968).

The Food Chemicals Codex (1972) specifies that agar must not contain more than 20% water, 6.5% ash, and 1% insoluble matter. Agar is approved for use as a food additive by the U. S. Food and Drug Administration and is on the list of substances "generally recognized as safe" (CFR, 1974; Fed. Register, 1979). It is widely used (200,000 pounds per year) as a gelling agent in bakery and confectionery goods, particularly icings, and may also be found in meat, fish, and dairy products at concentrations of 600 to 2,000 ppm. Agar is sometimes used as a clarifying agent for beer and wine, as a suspending agent for pharmaceuticals, and as a laxative. The daily intake of agar by an adult in the United States has been estimated to be 5-13 mg (LSRO, 1973).

Agar is also used for dental impressions, as a component of bacterial culture media, and as a sizing agent for silk and textiles (Merck, 1968; Kirk-Othmer, 1968; and Furia, 1972). Most of the one million pounds used annually in the United States originates in the Mediteranean or off the coast of South America, although some is harvested off the California coast (Furia, 1972; Colony Import and Export, 1979).

The oral LD_{50} values of agar in rats and mice are 11.4 and 15.7 g/kg, respectively (Bailey and Morgareidge, 1976).

Agar was tested by the carcinogenesis testing program because agar is widely used as a food additive and therefore, widespread exposure of the general population is likely, and because there are no carcinogenesis bioassay data available on this material.

II. MATERIALS AND METHODS

A. Chemical

USP food grade, low-gel strength, type 700 agar (CAS No. 9002-18-0) was obtained in two batches from Colony Import and Export Company (New York, NY). The agar was isolated from *Pterocladia* that had been harvested off the coast of the Azore Islands. Lot No. JO-6646 was used for the subchronic studies and for the first 20 weeks of the chronic studies. Lot No. JO-7785 was used for the remainder of the chronic studies.

Purity and identity analyses were performed at Midwest Research Institute (Appendix E). The results of carbon and hydrogen analyses were 87% of the calculated values for Lot No. JO-6646 and 94% for Lot No. JO-7785. Variable amounts of sulfur, presumably as sulfate (Kirk-Othmer, 1968), and nitrogen were also found by elemental analysis, as were small amounts of sodium, potassium, magnesium, and calcium salts. Lot No. JO-6646 contained 9.14% water, and Lot No. JO-7785 contained 5.83% water. The compound and the rest of the material, including water, sulfur, nitrogen, alkaline metals, and calculated oxygen account for more than 97% of the total for Lot No. JO-6646 and 100.3% of the total for Lot No. JO-7785. The infrared spectra of both lots were identical to spectra reported in the literature for agar.

Results of thin-layer chromatography of the hydrolysis products of Lot No. JO-7785 indicated that the major component was galactose. Two trace impurities were also detected but were not identified.

Agar was stored in the dark at 4°C.

B. Dietary Preparation

Test diets were prepared by first mixing the chemical with an aliquot of Wayne® Lab Blox animal meal (Table 1) in a mortar using a pestle and then

Table 1. Source and Description of Materials Used for Animals 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. (Rochelle, Park, NJ)
Filter Sheets	Diposable, nonwoven fiber	Lab Products, Inc. (Rochelle Park, NJ)
Bedding	Hardwood chips: Aspen bed	American Excelsior (Baltimore, MD)
	Beta Chips®	Agway Corp. (Syracuse, NY)
Watering System	Edstrom Automatic	Edstrom Industries (Waterford, WI)

adding this mixture to an appropriate additional amount of feed contained in a Patterson-Kelly twin-shell V-blender and mixing for 15 minutes. Test diets were sealed in labelled plastic bags and stored at $4^{o}C$ for no longer than 14 days.

Due to similarities between some of the components of agar and feed, quantitative methodology could not be developed to reproducibly measure chronic dose levels of agar in feed to within 10%. Historically, dose levels of similar feed mixtures from this laboratory have been within 10% of the prescribed concentrations. Therefore, it was assumed that the chronic dose levels of agar in the feed were also within 10%.

C. Animals

Subchronic Studies

Three-week-old F344 rats and 3- to 4-week-old B6C3F1 mice were obtained from Frederick Cancer Research Center (Frederick, MD) and observed for the presence of parasites and other disease conditions for 8 days (rats) or 13 days (mice). The animals were randomly assigned to individual cages, and the cages were randomly assigned to test group.

Chronic Studies

Four-week-old F344 rats and 4- to 5-week old B6C3F1 mice were obtained from the Charles River Breeding Laboratories (Wilmington, MA) and observed for the presence of parasites and other disease conditions for 8 days (rats) or 13 days (mice). The animals were randomly assigned to individual cages, and the cages were randomly assigned to test groups.

D. Animal Maintenance

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

The temperature in the animal rooms varied from 17.8° to 29.4°C (average 22.9°C) and relative humidity was uncontrolled and ranged from 5% - 83% (average 39%). Incoming air was filtered through Tri-Dek 15/40 denier Dacron filters to remove particulate matter. Room air was changed 10-12 times per hour. Fluorescent lighting was provided 12 hours per day.

E. Dose Selection for the Chronic Study

Acute Toxicity and 14-Day Repeated-Dose Study

Acute oral toxicity and 14-day repeated-dose feed studies were conducted with F344 rats and B6C3F1 mice obtained from Frederick Cancer Research Center. The studies were conducted to determine the toxicity of the test material and the concentrations of agar to be used in the subchronic studies.

In the acute toxicity test, groups of five males and five females of each species were administered a single dose of agar (0.63, 1.75, or 2.5 g/kg) in distilled water by gavage. One male rat died as a result of a gavage accident. All surviving animals were killed on day 15 and one animal from each group was necropsied. No chemical-related effects were observed at necropsy for either rats or mice.

In the repeated-dose study, groups of five males and five females of each species were fed diets containing 6,300, 12,500, 25,000, 50,000, or 100,000 ppm agar for 14 days. No animals died in this study. On day 15, all animals were killed and necropsied. No compound-related effects were observed for either rats or mice.

Subchronic Studies

In subchronic studies conducted to determine the concentrations of the test compound to be used in the chronic studies, groups of 10 males and 10 females of each species were given feed containing 0, 3,100, 6,300, 12,500, and 25,000, or 50,000 ppm agar for 13 weeks. Animals were observed twice daily and weighed weekly. One male control rat and one male control mouse died. At the end of the 91-day period, all surviving animals were killed and necropsied.

Mean body weights of dosed and control groups were comparable in both the rat and mouse studies. No compound-related gross or histopathologic effects were observed.

F. Chronic Studies

Doses selected for the rats and the mice for the chronic study were 25,000 and 50,000 ppm agar in the feed. The latter dose is the upper limit recommended for chronic feeding studies (NCI, 1976).

The initial number of animals in the test groups, the concentration of agar administered in the feed, and the number of weeks on study of rats and mice in the chronic studies are shown in Table 2. Dosed groups were given diets containing agar for 103 consecutive weeks followed by 2 weeks on basal feed before the animals were killed.

G. Clinical Examinations and Pathology

Mortality and morbidity checks were made twice daily, and individual animal weights and clinical signs of toxicity were recorded monthly. Animals that were moribund and those that survived to the end of the study were killed with carbon dioxide and immediately necropsied.

Table 2. Experimental Design of Chronic Feeding Studies with Agar in Rats and Mice

Test Group	Initial No. of Animals	Agar (ppm)	Weeks on Study Dosed(a)	Weeks on Study Undosed
Male Rats				
Control (b)	50	0	0	106
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2
Female Rats				
Control (b)	50	0	0	106
Low-Dose	50	25,000	103	2-3
High-Dose	50	50,000	103	2
Male Mice				
Control (b)	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2
Female Mice				
Control (b)	50	0	0	105
Low-Dose	50	25,000	103	2
High-Dose	50	50,000	103	2

(a) The start dates were October 4, 1977, for rats and October 24, 1977 for mice. The terminal kill was initiated on October 10, 1979 for rats and on October 30, 1979 for mice.

(b) Control and dosed groups were of the same strain, sex, and age range and from the same source and shipment. All animals of the same strain shared the same room, and all aspects of animal care and maintenance were similar. Animals were randomized to dosed and control groups as described in Section II.C.-Chronic.

The mean body weight of each dosed or control group was calculated as

$$\frac{\text{the total weight of all surviving animals in the group}}{\text{the number of surviving animals in the group}}$$

Feed consumption was measured per cage. The average feed consumption per animal was calculated as

$$\frac{\text{the total feed consumption measured for all cages in a group}}{\text{the number of surviving animals in the group}}$$

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from 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. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following 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.

H. 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).

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time 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 number 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, statistical tests were based on animals that survived at least 52 weeks, unless a tumor was found at an anatomic site of interest before that time. In such cases, comparisons were based exclusively on animals that survived at least until 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 test, etc.) were followed.

Life table methods were used to analyze the incidence of tumors, as described in Saffiotti et al. (1972). The week during which an animal died naturally or was killed was entered as the time point of examination for tumors. 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 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 0.050 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 possibility of the indication of tumors by the test chemicals, which is not detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed and control male rats were comparable throughout the study (Figure 1 and Table 3). After week 80, mean body weights of dosed female rats were slightly lower than those of the untreated controls. No compound-related clinical signs of toxicity or effects on feed consumption were observed (Appendix F). For male rats, feed consumption in the low- and high-dose groups averaged 97% and 99% of control values, respectively. For female rats, the corresponding figures were 92% and 95%.

B. Survival (Rats)

Estimates of the probabilities of survival of male and female rats fed diets containing agar 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 decreases in survival were observed between the dosed groups of rats compared with controls.

In male rats, 31/50 (62%) of the untreated controls, 40/50 (80%) of the low-dose, and 35/50 (70%) of the high-dose group lived to the end of the study. In female rats, 35/50 (70%) of the untreated controls, 41/50 (82%) of the low-dose, and 42/50 (84%) of the high-dose group lived to the end of the study.

A sufficient number of dosed rats were at risk for the development of late-appearing tumors.

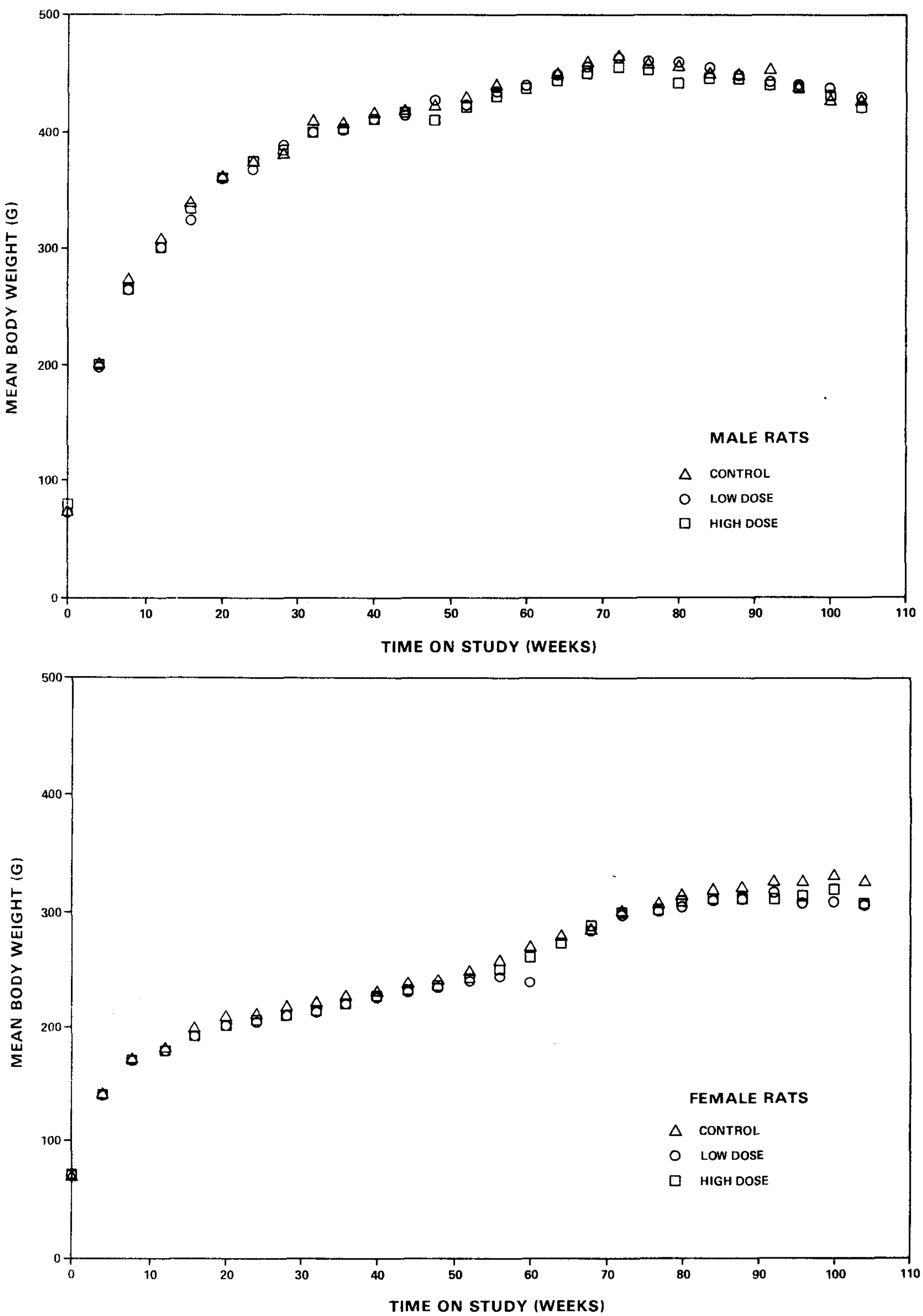

Figure 1. Growth Curves for Rats Fed Diets Containing Agar

Table 3. Mean Body Weight Change (Relative to Controls) of Rats Fed Diets Containing Agar

	Week No.	Cumulative Mean Body Weight Change (grams) Control	Low Dose	High Dose	Weight Change Relative to Controls (a) (%) Low Dose	High Dose
	0	74(b)	73(b)	78(b)		
Male	4	128	124	121	-3	-5
Rats	24	301	293	297	-3	-1
	44	345	342	339	-1	-2
	64	376	376	367	0	-2
	84	379	383	368	+1	-3
	104	353	356	345	+1	-2
	0	68(b)	67(b)	69(b)		
Female	4	73	71	71	-3	-3
Rats	24	145	138	138	-5	-5
	44	170	164	163	-4	-4
	64	213	205	205	-4	-4
	84	250	242	242	-3	-3
	104	258	239	238	-7	-8

(d) Weight Change Relative to Controls =

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

(b) Initial weight.

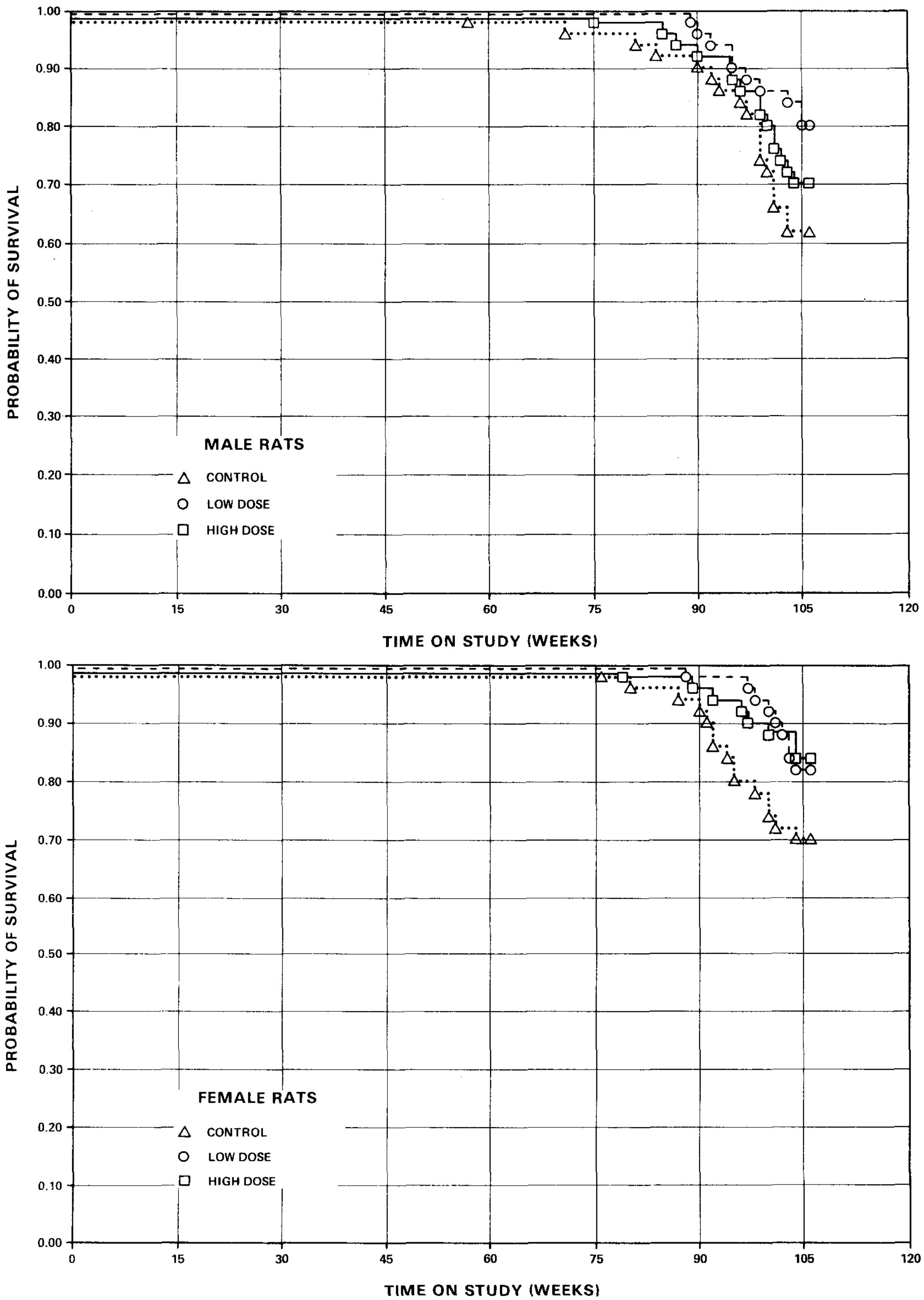

Figure 2. Survival Curves for Rats Fed Diets Containing Agar

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. There were no tumors judged to be due to adminstration of the test compound.

Nonneoplastic lesions were found in control and dosed rats; however, none were considered to be compound related.

D. Statistical Analyses of Results (Rats)

Tables 4 and 5 contain the statistical analyses of those primary tumors that satisfied both of the following criteria: (1) the tumor incidence was at least 5% in one of the three experimental groups and (2) the tumor occurred in at least two animals from one group.

Endometrial stromal polyps of the uterus 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, but there was a departure from linear trend due to decreased incidence in the low-dose group compared with the other two groups. The Fisher exact test between the low-dose group and the untreated control group was significant (P=0.036). This value of P=0.036 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 decreased incidence was observed in the high-dose group.

Cortical adenomas of the adrenal in female rats were observed in increased incidence in the high-dose group (0/50, 0%, in the controls; 0/50, 0%, in the low-dose; and 4/50, 8% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the positive direction (P=0.015), but the Fisher exact tests were not significant. Cortical adenomas of the adrenal have been observed in 19/958 (2%) of the untreated female F344 rats at this laboratory, with one untreated group having an incidence of 4/47, 9%. In male rats, this tumor was not observed in statistically significant proportions.

No rats died before 52 weeks on study, so a time-adjusted analysis was not performed. Life table analysis, using the death of an animal as the time point of examination for tumors, did not materially alter the significance of the results reported above.

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Agar (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue: Fibroma (b)	2/50(4)	1/50(2)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	2.000
Lower Limit		0.009	0.301
Upper Limit		9.290	21.316
Weeks to First Observed Tumor	106	92	99
Hematopoietic System: Myelomonocytic Leukemia (b)	9/50(18)	8/50(16)	11/50(22)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.889	1.222
Lower Limit		0.325	0.506
Upper Limit		2.382	3.041
Weeks to First Observed Tumor	96	90	85
Pituitary: Adenoma NOS (b)	12/44(27)	21/50(42)	11/42(26)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.540	0.960
Lower Limit		0.828	0.433
Upper Limit		3.005	2.105
Weeks to First Observed Tumor	81	92	100

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	3/44(7)	1/50(2)	2/42(5)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.293	0.698
Lower Limit		0.006	0.061
Upper Limit		3.500	5.788
Weeks to First Observed Tumor	93	105	105
Pituitary: Adenoma, NOS or Carcinoma, NOS (b)	15/44(34)	22/50(44)	13/42(31)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.291	0.908
Lower Limit		0.741	0.455
Upper Limit		2.313	1.784
Weeks to First Observed Tumor	81	92	100
Adrenal: Cortical Adenoma (b)	1/50(2)	3/50(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.000	2.000
Lower Limit		0.251	0.108
Upper Limit		154.270	115.621
Weeks to First Observed Tumor	103	105	105

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	5/50(10)	4/50(8)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.800	1.000
Lower Limit		0.168	0.245
Upper Limit		3.499	4.082
Weeks to First Observed Tumor	99	105	102
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	6/50(12)	4/50(8)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	0.833
Lower Limit		0.147	0.215
Upper Limit		2.635	3.604
Weeks to First Observed Tumor	99	105	102
Thyroid: C-Cell Adenoma (b)	0/49(0)	3/49(6)	1/44(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.602	0.060
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	---	105	105

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Carcinoma (b)	2/49(4)	3/49(6)	3/44(7)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.500	1.670
Lower Limit		0.180	0.200
Upper Limit		17.316	19.213
Weeks to First Observed Tumor	99	105	104
Thyroid: C-Cell Adenoma or Carcinoma (b)	2/49(4)	6/49(12)	4/44(9)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.000	2.227
Lower Limit		0.569	0.337
Upper Limit		29.224	23.629
Weeks to First Observed Tumor	99	105	102
Testis: Interstitial-Cell Tumor (b)	36/50(72)	41/50(82)	41/50(82)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.139	1.139
Lower Limit		0.898	0.898
Upper Limit		1.413	1.413
Weeks to First Observed Tumor	81	89	75

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Tunica Vaginalis:			
Mesothelioma, NOS (b)	4/50(8)	3/50(6)	2/50(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.750	0.500
Lower Limit		0.115	0.047
Upper Limit		4.206	3.318
Weeks to First Observed Tumor	99	105	105

(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 untreated 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.

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Agar (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Myelomonocytic Leukemia (b)	10/50(20)	2/50(10)	10/50(20)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.500	1.000
Lower Limit		0.144	0.410
Upper Limit		1.482	2.437
Weeks to First Observed Tumor	76	100	105
Adrenal: Cortical Adenoma (b)	0/50(0)	0/50(0)	4/50(8)
P Values (c),(d)	P=0.015	N.S.	N.S.
Relative Risk (Control) (e)		-	Infinite
Lower Limit		-	0.927
Upper Limit		-	Infinite
Weeks to First Observed Tumor	-	-	105
Pituitary: Adenoma, NOS (b)	19/48(40)	20/48(42)	17/49(35)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.053	0.876
Lower Limit		0.618	0.493
Upper Limit		1.796	1.552
Weeks to First Observed Tumor	80	101	89

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS (b)	5/48(10)	4/48(8)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.800	0.588
Lower Limit		0.168	0.096
Upper Limit		3.490	2.846
Weeks to First Observed Tumor	106	102	92
Pituitary: Adenoma, NOS or Carcinoma, NOS (b)	24/48(50)	24/48(50)	20/49(41)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.000	0.816
Lower Limit		0.645	0.504
Upper Limit		1.549	1.319
Weeks to First Observed Tumor	80	101	89
Thyroid: C-Cell Carcinoma (b)	4/49(8)	3/50(6)	2/49(4)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.735	0.500
Lower Limit		0.113	0.047
Upper Limit		4.120	3.315
Weeks to First Observed Tumor	106	102	105

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Thyroid: C-Cell Adenoma or Carcinoma	4/49(8)	3/50(6)	3/49(6)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.735	0.750
Lower Limit		0.113	0.115
Upper Limit		4.120	4.201
Weeks to First Observed Tumor	106	102	105
Mammary Gland Adenocarcinoma, NOS (b)	3/50(6)	1/50(2)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.333	0.333
Lower Limit		0.006	0.006
Upper Limit		3.983	3.983
Weeks to First Observed Tumor	80	101	100
Mammary Gland: Fibroadenoma (b)	14/50(28)	12/50(24)	17/50(34)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.857	1.214
Lower Limit		0.404	0.636
Upper Limit		1.790	2.354
Weeks to First Observed Tumor	92	88	92

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing Agar (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Uterus: Edometrial Stromal Polyp (b)	17/50(34)	8/49(16)	16/50(32)
P Values (c),(d)	N.S.	P=0.036(N)	N.S.
Departure from Linear Trend (f)	P=0.032		
Relative Risk (Control) (e)		0.480	0.941
Lower Limit		0.199	0.505
Upper Limit		1.056	1.746
Weeks to First Observed Tumor	76	105	97

(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 untreated 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.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of dosed and control male mice were comparable throughout the study. The mean body weights of dosed female mice were lower than those of the untreated controls after week 20 (Figure 3 and Table 6) and remained lower throughout the study. No compound-related clinical signs of toxicity were observed. Feed consumption by dosed and control mice was comparable (Appendix F). For male mice, feed consumption in the low- and high-dose groups averaged 103% and 111% of control values, respectively. For female mice the corresponding figures were 100% and 107%.

B. Survival (Mice)

Estimates of the probabilities of survival of male and female mice fed diets containing agar 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 decreases in survival were observed between any of the dosed groups of either sex of mice compared with the control groups.

In male mice, 32/50 (64%) of the untreated controls, 35/50 (70%) of the low-dose, and 32/50 (64%) of the high-dose group lived to the end of the study. In female mice, 29/50 (58%) of the untreated controls, 34/50 (68%) of the low-dose, and 39/50 (78%) of the high-dose group lived to the end of the study.

A sufficient number of dosed mice were at risk for the development of late-appearing tumors.

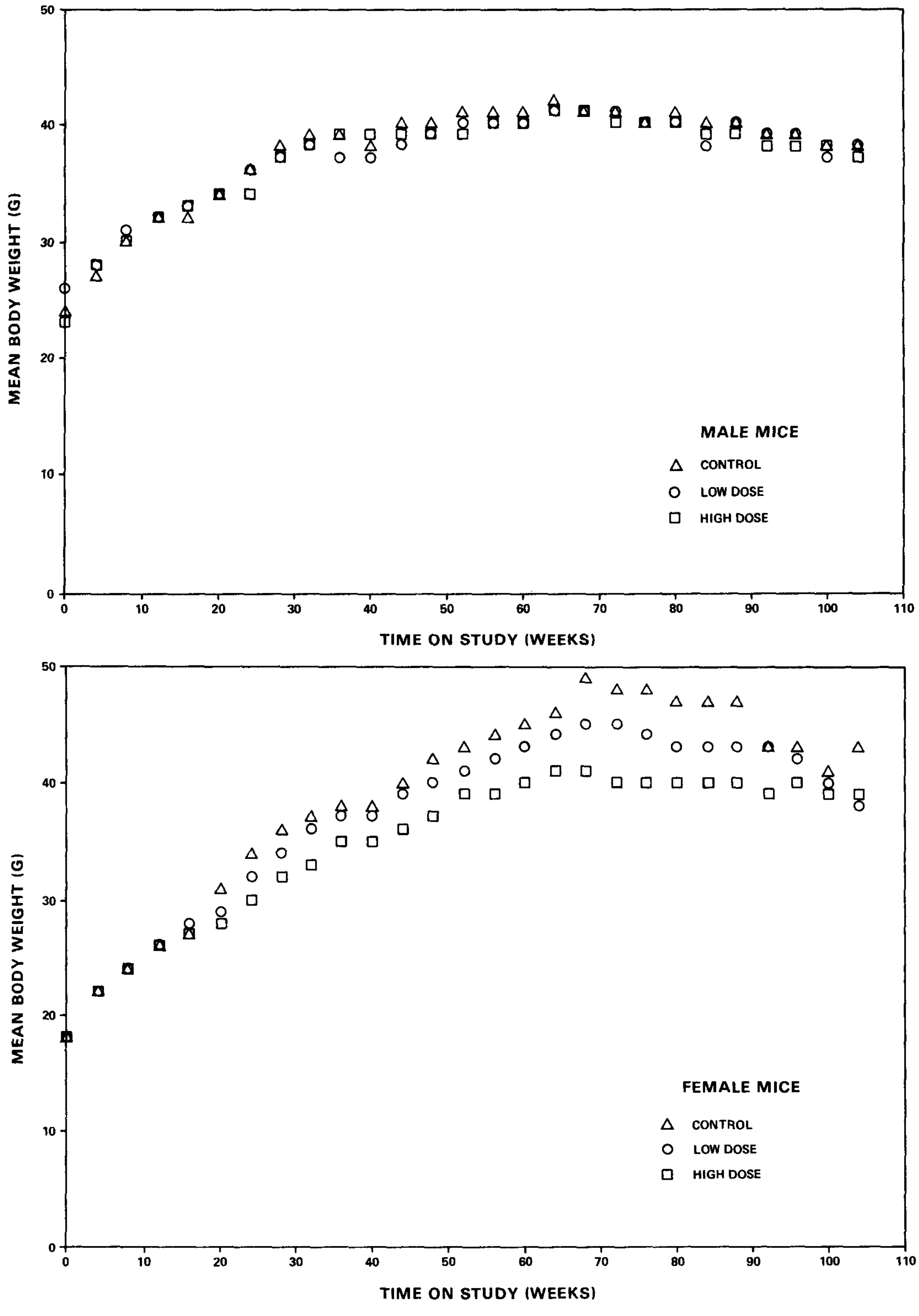

Figure 3. Growth Curves for Mice Fed Diets Containing Agar

Table 6. Mean Body Weight Change (Relative to Controls) of Mice Fed Diets Containing Agar

	Week No.	Cumulative Mean Body Weight Change (grams)			Weight Change Relative to Controls (a) (%)	
		Control	Low Dose	High Dose	Low Dose	High Dose
	0	24(b)	26(b)	23(b)		
Male	4	3	2	5	-33	+67
Mice	24	12	10	11	-17	-8
	44	16	12	16	-25	0
	64	18	15	18	-17	0
	84	16	12	16	-25	0
	104	14	12	14	-14	0
	0	18(b)	18(b)	18(b)		
Female	4	4	4	4	0	0
Mice	24	16	14	12	-13	-25
	44	22	21	18	-5	-18
	64	28	26	23	-7	-18
	84	29	25	22	-14	-24
	104	25	20	21	-20	-16

(a) Weight Change Relative to Controls =

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

(b) Initial weight

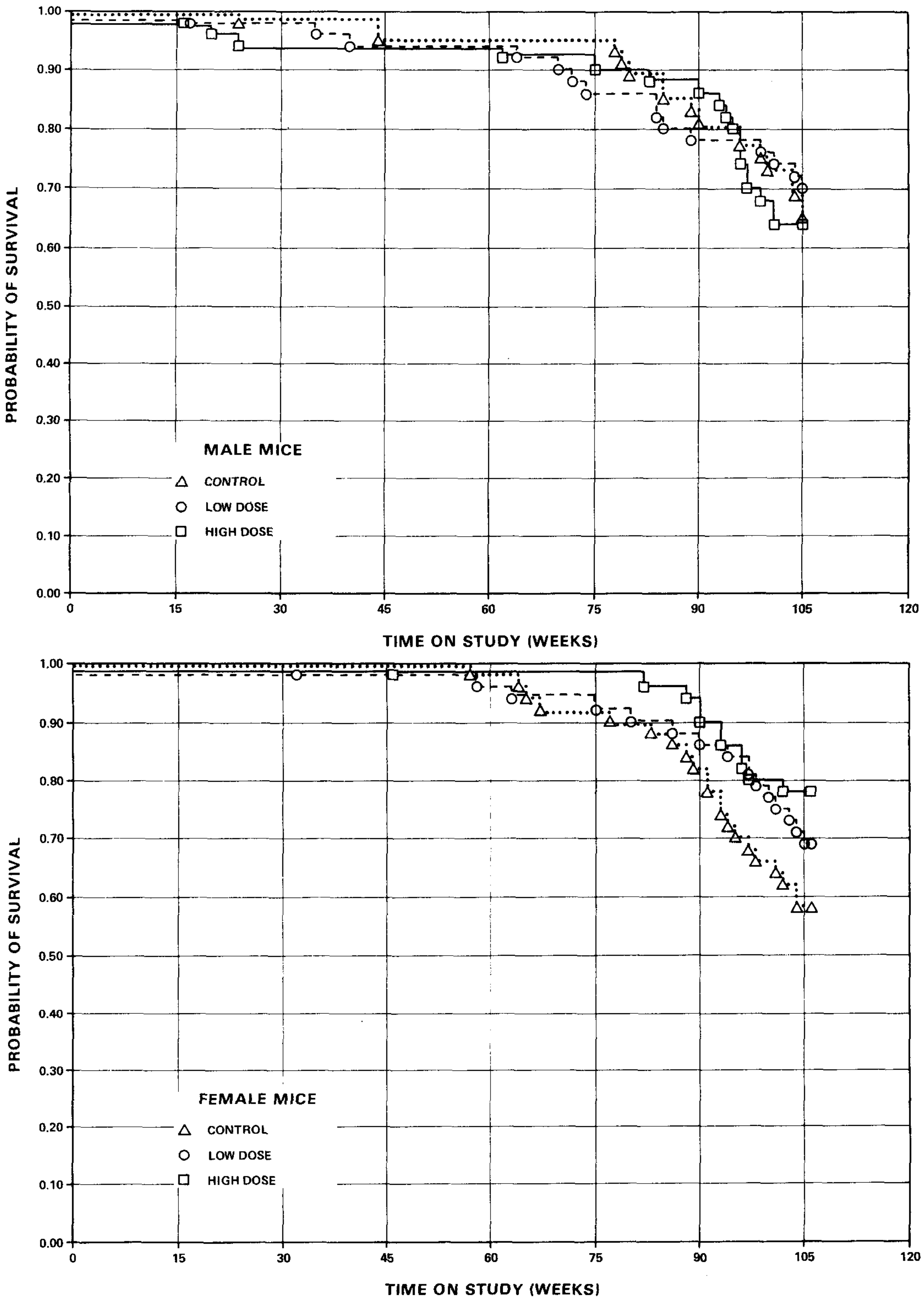

Figure 4. Survival Curves for Mice Fed Diets Containing Agar

C. Pathology (Mice)

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

A variety of neoplasms and nonneoplastic lesions occurred in both control and dosed mice. None of these appeared to be compound related.

D. Statistical Analyses of Results (Mice)

Tables 7 and 8 contain the statistical analyses of those primary tumors that satisfied both of the following criteria: (1) the tumor incidence was at least 5% in one of the three experimental groups and (2) the tumor occurred in at least two animals from one group.

Hepatocellular adenomas in male mice were observed in a statistically significant positive relation (0/49, 0% in the controls; 3/50, 6% in the low-dose; and 7/50, 14% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the postive direction (P=0.005), and the Fisher exact test between the high-dose group and the untreated control group was significant (P=0.007). Although this tumor occurred in increased incidence in the low-dose group compared with the untreated control group, the level of incidence was not significant. The total combined incidence of each group of male mice with either adenoma or carcinoma of the liver was not statistically significant. The incidence of male controls with hepatocellular carcinomas exceeded that in either of the dosed groups. The historical incidence of control B6C3F1 male mice in tests of 105 weeks duration with either adenoma or carcinoma of the liver observed in bioassays at this laboratory is 234/699 (33.5%). In female mice, these tumors were not observed at statistically significant levels.

The total number of female mice with alveolar/bronchiolar adenomas or carcinomas was less in each dosed group than in the untreated control group

(7/50, 14% in the controls; 3/49, 6% in the low-dose; and 1/50, 2% in the high-dose). The Cochran-Armitage test for linear trend was statistically significant in the negative direction (P=0.018). The Fisher exact tests between the high-dose group and the untreated control group indicated a value of P=0.030. This value 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. In male mice, this tumor was not observed at statistically significant levels.

Two male mice in each group and a total of two female mice died before the 52nd week of the study. Time adjusted analyses, eliminating those animals dying before 52 weeks, and life table analyses, using the week of the death of an animal as the time point of examination for tumors, did not materially alter the significance of the results reported above.

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

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	4/49(4)	5/50(10)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		1.225	1.470
Lower Limit		0.280	0.372
Upper Limit		5.833	6.681
Weeks to First Observed Tumor	105	89	96
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	6/49(12)	6/50(12)	7/50(14)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		0.980	1.143
Lower Limit		0.281	0.355
Upper Limit		3.418	3.831
Weeks to First Observed Tumor	105	89	96
Hematopoietic System: Lymphoma, Malignant, NOS (b)	2/49(4)	6/50(12)	4/50(8)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		2.940	1.960
Lower Limit		0.558	0.296
Upper Limit		28.662	20.886
Weeks to First Observed Tumor	104	99	96

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

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma, Malignant, NOS or Leukemia (b)	3/49(6)	8/50(16)	5/50(10)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (f)		2.613	1.633
Lower Limit		0.672	0.337
Upper Limit		14.517	10.018
Weeks to First Observed Tumor	104	70	96
Liver: Hepatocellular Adenoma (b)	0/49(0)	3/50(6)	7/50(14)
P Values (c),(d)	P=0.005	N.S.	P=0.007
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.590	1.903
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	---	105	105
Liver: Hepatocellular Carcinoma (b)	9/49(18)	5/50(10)	6/50(12)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.544	0.653
Lower Limit		0.154	0.207
Upper Limit		1.673	1.895
Weeks to First Observed Tumor	80	89	83

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

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma or Carcinoma (b)	9/49(18)	8/50(16)	13/50(26)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.871	1.416
Lower Limit		0.319	0.619
Upper Limit		2.333	3.402
Weeks to First Observed Tumor	80	89	83
Harderian Gland: Adenoma, NOS (b)	4/49(8)	3/50(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.735	0.245
Lower Limit		0.113	0.005
Upper Limit		4.120	2.362
Weeks to First Observed Tumor	105	89	105

(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 untreated 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.

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

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	5/50(10)	3/49(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.612	0.200
Lower Limit		0.100	0.004
Upper Limit		2.967	1.699
Weeks to First Observed Tumor	105	105	105
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	7/50(14)	3/49(6)	1/50(2)
P Values (c),(d)	P=0.018(N)	N.S.	P=0.030(N)
Relative Risk (Control) (e)		0.437	0.143
Lower Limit		0.077	0.003
Upper Limit		1.793	1.052
Weeks to First Observed Tumor	89	105	105
Hematopoietic System: Lymphoma, All Malignant (b)	9/50(18)	7/49(14)	9/50(18)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.794	1.000
Lower Limit		0.272	0.384
Upper Limit		2.201	2.603
Weeks to First Observed Tumor	93	90	102

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

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma (b)	1/50(2)	3/49(6)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.061	1.000
Lower Limit		0.256	0.013
Upper Limit		157.341	76.970
Weeks to First Observed Tumor	105	101	105
Liver: Hepatocellular Carcinoma (b)	3/50(6)	2/49(4)	0/50(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.680	0.000
Lower Limit		0.059	0.000
Upper Limit		5.680	1.663
Weeks to First Observed Tumor	104	105	--
Liver: Hepatocellular Adenoma or Carcinoma (b)	4/50(8)	5/49(10)	1/50(2)
P Values (c),(d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.276	0.250
Lower Limit		0.292	0.005
Upper Limit		6.070	2.411
Weeks to First Observed Tumor	104	101	105

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

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	6/45(13)	2/43(5)	4/38(11)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.349	0.789
Lower Limit		0.036	0.175
Upper Limit		1.824	3.066
Weeks to First Observed Tumor	105	97	105
Harderian Gland: Adenoma, NOS or Cystadenoma (b)	1/50(2)	5/49(10)	0/50(0)
P Values (c),(d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.007		
Relative Risk (Control) (e)		5.102	0.000
Lower Limit		0.601	0.000
Upper Limit		236.025	18.658
Weeks to First Observed Tumor	98	105	---

(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 untreated 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

Fifty F344 rats and 50 B6C3F1 mice of either sex were fed diets containing 25,000 or 50,000 ppm of agar isolated from Pterocladia for 103 weeks to test for the potential carcinogenicity of this compound. Mean body weights of dosed and control male rats were comparable throughout the study. After week 80, mean body weights of dosed female rats were slightly lower than those of the untreated controls. The mean body weights of dosed and control male mice were comparable throughout the study, while mean body weights of dosed female mice were lower than those of the untreated controls at week 20 and remained lower throughout the study. There were no compound-related effects on survival, feed consumption, or clinical signs of toxicity, nor were there any sites in rats or mice at which an increase in tumor incidence could be associated with the administration of agar.

Although the incidence of cortical adenoma of the adrenal in female rats in the high-dose group revealed a statistically significant linear trend by the Cochran-Armitage test, the increased incidence was not significant by the Fisher exact test. There was a significantly increased incidence of hepatocellular adenomas in male mice in the high-dose group. However, when the incidences of adenomas and carcinomas of the liver of male mice are combined, the net result was not statistically different from that of the control group. There was a significant, decreased incidence of endometrial stromal polyps of the uterus in female rats in the low-dose group compared with the control group. This change was not observed in the high-dose group. The trend of decreased incidences of alveolar/bronchiolar adenomas or carcinomas in female mice was statistically significant. The decrease in alveolar/bronchiolar adenomas or carcinomas in the high-dose group of female mice was significant compared with the control group.

Thirty-five to 42 dosed rats of either sex and 32-39 dosed mice of either sex received as much as 5% agar in their diets for 103 weeks. Although the rats and mice might have tolerated higher doses, this is the maximum concentration (5%) of a test substance in feed recommended in the Bioassay Program Guidelines.

Besides agar, 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 both sexes. Under these test conditions, all were considered not carcinogenic (gum arabic, NTP 1982a; guar gum, NTP 1982b; locust bean gum, NTP 1982c; and tara gum, NTP 1982d).

VI. CONCLUSION

Under the conditions of this bioassay, agar isolated from *Pterocladia* was not carcinogenic for F344 rats or B6C3F1 mice of either sex.

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APPENDIX A

Summary of the Incidence of Neoplasms in Rats Fed Diets Containing Agar

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING AGAR

	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%)	
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROMA	2 (4%)	1 (2%)	4 (8%)
FIBROSARCOMA	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA, METAST	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA	9 (18%)	8 (16%)	11 (22%)
CIRCULATORY SYSTEM			
*LYMPHATICS OF NECK	(50)	(50)	(50)
SEBACEOUS ADENOCARCINOMA, METAST	1 (2%)		
#HEART	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA, INVASIV	1 (2%)		
#LEFT VENTRICLE	(50)	(50)	(49)
SARCOMA, 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
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(49)	(50)	(50)
SEBACEOUS ADENOCARCIMONA, INVASI	1 (2%)		
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#FORESTOMACH	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#JEJUNUM	(49)	(50)	(50)
MUCINOUS CYSTADENOCARCINOMA	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(50)	(42)
CARCINOMA,NOS	3 (7%)	1 (2%)	2 (5%)
ADENOMA, NOS	12 (27%)	21 (42%)	11 (26%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	1 (2%)	3 (6%)	2 (4%)
PHEOCHROMOCYTOMA	5 (10%)	4 (8%)	5 (10%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)		
#THYROID	(49)	(49)	(44)
C-CELL ADENOMA		3 (6%)	1 (2%)
C-CELL CARCINOMA	2 (4%)	3 (6%)	3 (7%)
#PANCREATIC ISLETS	(49)	(48)	(49)
ISLET-CELL ADENOMA	1 (2%)		1 (2%)
ISLET-CELL CARCINOMA	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA		2 (4%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
#TESTIS	(50)	(50)	(50)
INTERSTITIAL-CELL TUMOR	36 (72%)	41 (82%)	41 (82%)
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
OLIGODENDROGLIOMA	1 (2%)		
#PONS	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
SEBACEOUS ADENOCARCINOMA	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*THORACIC CAVITY	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
*PERITONEUM	(50)	(50)	(50)
MESOTHELIOMA, NOS	1 (2%)		
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS	4 (8%)	3 (6%)	2 (4%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TAIL			
FIBROMA		1	
OMENTUM			
MESOTHELIOMA, NOS	1	1	

ANIMAL DISPOSITION SUMMARY

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	9	4	10
MORIBUND SACRIFICE	10	6	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	31	40	35
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

TUMOR SUMMARY

	CONTROL	LOW DOSE	HIGH DOSE
TOTAL ANIMALS WITH PRIMARY TUMORS*	47	50	49
TOTAL PRIMARY TUMORS	88	97	90
TOTAL ANIMALS WITH BENIGN TUMORS	43	49	45
TOTAL BENIGN TUMORS	59	77	67
TOTAL ANIMALS WITH MALIGNANT TUMORS	19	14	18
TOTAL MALIGNANT TUMORS	23	15	21
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	1	
TOTAL SECONDARY TUMORS	5	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	4	4	2
TOTAL UNCERTAIN TUMORS	6	5	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS FED DIETS CONTAINING AGAR

	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)
FIBROMA	2 (4%)	1 (2%)	1 (2%)
FIBROSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)		
C-CELL CARCINOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MYELOMONOCYTIC LEUKEMIA	10 (20%)	5 (10%)	10 (20%)
#SPLEEN	(49)	(50)	(50)
C-CELL CARCINOMA, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
#UTERUS	(50)	(49)	(50)
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
NONE			
URINARY SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(48)	(49)
CARCINOMA,NOS	5 (10%)	4 (8%)	3 (6%)
ADENOMA, NOS	19 (40%)	20 (42%)	17 (35%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA			4 (8%)
PHEOCHROMOCYTOMA	2 (4%)	1 (2%)	2 (4%)
GANGLIONEUROMA	1 (2%)		
#THYROID	(49)	(50)	(49)
FOLLICULAR-CELL CARCINOMA	1 (2%)	1 (2%)	
C-CELL ADENOMA			1 (2%)
C-CELL CARCINOMA	4 (8%)	3 (6%)	2 (4%)
#PANCREATIC ISLETS	(50)	(48)	(48)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	3 (6%)	1 (2%)	1 (2%)
FIBROADENOMA	14 (28%)	12 (24%)	17 (34%)
#UTERUS	(50)	(49)	(50)
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP	17 (34%)	8 (16%)	16 (32%)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	1 (2%)
#CERVIX UTERI	(50)	(49)	(50)
LEIOMYOSARCOMA		1 (2%)	
#OVARY	(50)	(50)	(49)
C-CELL CARCINOMA, METASTATIC			1 (2%)
GRANULOSA-CELL TUMOR	1 (2%)	1 (2%)	
SARCOMA, NOS	1 (2%)		
NERVOUS SYSTEM			
#CEREBRUM	(50)	(50)	(50)
ASTROCYTOMA		2 (4%)	

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#BRAIN	(50)	(50)	(50)
CARCINOMA, NOS, INVASIVE	2 (4%)	1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
*EAR CANAL	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*MANDIBLE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH[a]	11	3	4
MORIBUND SACRIFICE	4	6	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	35	41	42
ANIMAL MISSING			

[a] 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*	47	43	43
TOTAL PRIMARY TUMORS	83	63	78
TOTAL ANIMALS WITH BENIGN TUMORS	39	35	36
TOTAL BENIGN TUMORS	57	42	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	16	19
TOTAL MALIGNANT TUMORS	25	20	19
TOTAL ANIMALS WITH SECONDARY TUMORS#	2	1	2
TOTAL SECONDARY TUMORS	2	1	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT	1	1	
TOTAL UNCERTAIN TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

Summary of the Incidence of Neoplasms in Mice Fed Diets Containing Agar

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING AGAR

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
PAPILLOMA, NOS			1 (2%)
FIBROMA		1 (2%)	
FIBROSARCOMA		1 (2%)	1 (2%)
*SUBCUT TISSUE	(49)	(50)	(50)
FIBROMA	1 (2%)		
FIBROSARCOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(49)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	3 (6%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (8%)	5 (10%)	6 (12%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	1 (2%)	1 (2%)
FIBROSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)	5 (10%)	4 (8%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
MYELOMONOCYTIC LEUKEMIA		1 (2%)	
GRANULOCYTIC LEUKEMIA		1 (2%)	
MAST-CELL LEUKEMIA	1 (2%)		
#ILEUM	(48)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(49)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#SPLEEN	(48)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
#HEART	(49)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
#LIVER	(49)	(50)	(50)
HEMANGIOSARCOMA	1 (2%)		1 (2%)
#URINARY BLADDER	(49)	(49)	(50)
HEMANGIOMA		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
HEPATOCELLULAR ADENOMA		3 (6%)	7 (14%)
HEPATOCELLULAR CARCINOMA	9 (18%)	5 (10%)	6 (12%)
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
TUBULAR-CELL ADENOMA	1 (2%)		
#URINARY BLADDER	(49)	(49)	(50)
TRANSITIONAL-CELL PAPILLOMA	1 (2%)		
ENDOCRINE SYSTEM			
#ADRENAL	(46)	(47)	(47)
CORTICAL ADENOMA		1 (2%)	
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(49)	(48)	(50)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
#PROSTATE	(49)	(48)	(48)
CARCINOMA,NOS			1 (2%)
#TESTIS	(49)	(50)	(50)
INTERSTITIAL-CELL TUMOR		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(49)	(50)	(50)
ADENOMA, NOS	4 (8%)	3 (6%)	1 (2%)
*EAR	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*RIB	(49)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
BODY CAVITIES			
*ABDOMINAL CAVITY	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(49)	(50)	(50)
SARCOMA, NOS	1 (2%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	16	13	14
MORIBUND SACRIFICE	1	2	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	35	32
ANIMAL MISSING	1		

@ 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*	24	24	25
TOTAL PRIMARY TUMORS	29	32	34
TOTAL ANIMALS WITH BENIGN TUMORS	10	14	13
TOTAL BENIGN TUMORS	11	16	16
TOTAL ANIMALS WITH MALIGNANT TUMORS	17	15	16
TOTAL MALIGNANT TUMORS	18	16	18
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	1	
TOTAL SECONDARY TUMORS	5	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE FED DIETS CONTAINING AGAR

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(49)	(50)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA		1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	5 (10%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	6 (12%)	4 (8%)	8 (16%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
*SUBCUT TISSUE	(50)	(49)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#SPLEEN	(50)	(48)	(48)
MALIGNANT LYMPHOMA, NOS	1 (2%)	2 (4%)	1 (2%)
#LUMBAR LYMPH NODE	(45)	(43)	(45)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(48)	(48)
HEMANGIOSARCOMA	1 (2%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(50)
HEPATOCELLULAR ADENOMA	1 (2%)	3 (6%)	1 (2%)
HEPATOCELLULAR CARCINOMA	3 (6%)	2 (4%)	
#GASTRIC MUCOSA	(49)	(49)	(49)
ADENOCARCINOMA, NOS		1 (2%)	
#FORESTOMACH	(49)	(49)	(49)
SQUAMOUS CELL CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(38)
ADENOMA, NOS	6 (13%)	2 (5%)	4 (11%)
#THYROID	(47)	(46)	(46)
FOLLICULAR-CELL ADENOMA			1 (2%)
FOLLICULAR-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
#UTERUS	(48)	(48)	(48)
ENDOMETRIAL STROMAL POLYP	1 (2%)		
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#OVARY	(44)	(45)	(50)
GRANULOSA-CELL TUMOR			1 (2%)
TERATOMA, BENIGN	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(47)	(49)	(48)
GLIOMA, NOS		1 (2%)	

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(49)	(50)
ADENOMA, NOS	1 (2%)	4 (8%)	
CYSTADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
OSTEOSARCOMA			1 (2%)
SITE UNKNOWN			
LEIOMYOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH[a]	20	14	11
MORIBUND SACRIFICE	1	1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	29	34	39
ANIMAL MISSING		1	

[a] 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*	26	24	16
TOTAL PRIMARY TUMORS	32	26	21
TOTAL ANIMALS WITH BENIGN TUMORS	13	13	7
TOTAL BENIGN TUMORS	16	13	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	12	12
TOTAL MALIGNANT TUMORS	16	13	13
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Diets Containing Agar

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING AGAR

	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)
PUS	1 (2%)		
FIBROSIS	1 (2%)		
NECROSIS, NOS	1 (2%)		
HYPERKERATOSIS	1 (2%)	1 (2%)	1 (2%)
ACANTHOSIS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
ABSCESS, NOS		2 (4%)	2 (4%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(50)	(49)
HEMATOPOIESIS			1 (2%)
#MANDIBULAR L. NODE	(48)	(49)	(47)
HYPERPLASIA, PLASMA CELL		1 (2%)	1 (2%)
#PANCREATIC L.NODE	(48)	(49)	(47)
CONGESTION, NOS			1 (2%)
#MESENTERIC L. NODE	(48)	(49)	(47)
FIBROSIS			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
CIRCULATORY SYSTEM			
#HEART/VENTRICLE	(50)	(50)	(49)
FIBROSIS	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
#MYOCARDIUM	(50)	(50)	(49)
FIBROSIS	1 (2%)		
#ENDOCARDIUM	(50)	(50)	(49)
SCLEROSIS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
FIBROSIS, FOCAL	1 (2%)		
NECROSIS, FOCAL	2 (4%)		
METAMORPHOSIS FATTY	4 (8%)	6 (12%)	4 (8%)
BASOPHILIC CYTO CHANGE	5 (10%)	3 (6%)	4 (8%)
GROUND-GLASS CYTO CHANGE			1 (2%)
CLEAR-CELL CHANGE	3 (6%)	1 (2%)	5 (10%)
MEGALOCYTOSIS	1 (2%)		
ANGIECTASIS	3 (6%)	2 (4%)	1 (2%)
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
NECROSIS, NOS	2 (4%)	1 (2%)	
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	39 (78%)	35 (70%)	40 (80%)
#PANCREAS	(49)	(48)	(49)
INFLAMMATION, INTERSTITIAL	1 (2%)		2 (4%)
HYPERPLASTIC NODULE			1 (2%)
#PANCREATIC ACINUS	(49)	(48)	(49)
ATROPHY, NOS	1 (2%)		
#STOMACH	(50)	(50)	(50)
ACANTHOSIS	1 (2%)		
#FORESTOMACH	(50)	(50)	(50)
ULCER, NOS	1 (2%)		
ULCER, ACUTE	1 (2%)	1 (2%)	
#COLON	(50)	(50)	(49)
NEMATODIASIS	2 (4%)	3 (6%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
NEPHROSIS, NOS	45 (90%)	44 (88%)	45 (90%)

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	(44)	(50)	(42)
HEMOSIDEROSIS			1 (2%)
ANGIECTASIS	1 (2%)		
#ADRENAL CORTEX	(50)	(50)	(50)
METAMORPHOSIS FATTY	1 (2%)	2 (4%)	
ANGIECTASIS			1 (2%)
#ADRENAL MEDULLA	(50)	(50)	(50)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#THYROID	(49)	(49)	(44)
CYSTIC FOLLICLES	1 (2%)		
HYPERPLASIA, C-CELL	2 (4%)		
#PANCREATIC ISLETS	(49)	(48)	(49)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC		2 (4%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
PUS		1 (2%)	
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, NOS	1 (2%)	1 (2%)	
#PROSTATE	(49)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	4 (8%)
#TESTIS	(50)	(50)	(50)
CALCIFICATION, NOS	2 (4%)		
HYPERPLASIA, INTERSTITIAL CELL	2 (4%)	1 (2%)	2 (4%)
#TESTIS/TUBULE	(50)	(50)	(50)
DEGENERATION, NOS	4 (8%)	1 (2%)	3 (6%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERICARDIUM	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
*MESENTERY	(50)	(50)	(50)
STEATITIS			1 (2%)
ALL OTHER SYSTEMS			
OMENTUM			
INFLAMMATION, GRANULOMATOUS	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 AGAR

	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, CHRONIC		1 (2%)	
FIBROSIS		1 (2%)	
ACANTHOSIS			1 (2%)
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)
#LUNG	(50)	(50)	(49)
INFLAMMATION, INTERSTITIAL			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
HEMOSIDEROSIS			1 (2%)
HEMATOPOIESIS	1 (2%)	1 (2%)	
#MANDIBULAR L. NODE	(46)	(46)	(46)
HYPERPLASIA, PLASMA CELL			1 (2%)
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
EMBOLUS, SEPTIC			1 (2%)
#MYOCARDIUM	(50)	(49)	(48)
INFLAMMATION, CHRONIC		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
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
NECROSIS, FOCAL	2 (4%)		1 (2%)
INFARCT, NOS			1 (2%)
METAMORPHOSIS FATTY	5 (10%)	5 (10%)	2 (4%)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
BASOPHILIC CYTO CHANGE	24 (48%)	24 (48%)	27 (54%)
EOSINOPHILIC CYTO CHANGE	1 (2%)	1 (2%)	
CLEAR-CELL CHANGE	1 (2%)		
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	11 (22%)	6 (12%)	17 (34%)
HYPERPLASIA, CYSTIC		1 (2%)	
#GASTRIC MUCOSA	(49)	(50)	(50)
NECROSIS, NOS			1 (2%)
#GASTRIC SUBMUCOSA	(49)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
#FORESTOMACH	(49)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
HYPERKERATOSIS	1 (2%)		
#COLON	(47)	(49)	(49)
NEMATODIASIS	1 (2%)	6 (12%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
HYDRONEPHROSIS		1 (2%)	
NEPHROSIS, NOS	15 (30%)	16 (33%)	18 (36%)
TUBULONECROSIS		1 (2%)	
HEMOSIDEROSIS		2 (4%)	
ENDOCRINE SYSTEM			
#PITUITARY	(48)	(48)	(49)
CYST, NOS		3 (6%)	2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
MULTIPLE CYSTS	1 (2%)		
HEMORRHAGIC CYST	3 (6%)	1 (2%)	1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL	(50)	(50)	(50)
METAMORPHOSIS FATTY	1 (2%)		
#ADRENAL CORTEX	(50)	(50)	(50)
CYST, NOS		1 (2%)	
METAMORPHOSIS FATTY	2 (4%)		
#ADRENAL MEDULLA	(50)	(50)	(50)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#THYROID	(49)	(50)	(49)
HYPERPLASIA, C-CELL	1 (2%)	1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS	2 (4%)	2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC	5 (10%)	8 (16%)	5 (10%)
*CLITORAL GLAND	(50)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	2 (4%)
*VAGINA	(50)	(50)	(50)
POLYP		1 (2%)	
#UTERUS	(50)	(49)	(50)
INFARCT, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(50)	(49)	(50)
CYST, NOS		1 (2%)	
HYPERPLASIA, CYSTIC	2 (4%)	8 (16%)	7 (14%)
#OVARY	(50)	(50)	(49)
CYST, NOS		2 (4%)	
FOLLICULAR CYST, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT			1 (2%)
ALL OTHER SYSTEMS			
FACE			
INFLAMMATION, SUPPURATIVE	1		
ADIPOSE TISSUE			
NECROSIS, FAT	1		
CALCIFICATION, NOS	1		
CALCIFICATION, FOCAL	1		
OMENTUM			
NECROSIS, FAT		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

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 Agar

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING AGAR

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	49	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(50)	(50)
INFLAMMATION, ACUTE	2 (4%)		
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
ACANTHOSIS		1 (2%)	
*SUBCUT TISSUE	(49)	(50)	(50)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	
GRANULATION, TISSUE			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(47)	(49)	(49)
MYELOID METAPLASIA			1 (2%)
#SPLEEN	(48)	(49)	(49)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
HEMATOPOIESIS	1 (2%)	1 (2%)	4 (8%)
#AORTIC LYMPH NODE	(38)	(41)	(44)
HYPERPLASIA, LYMPHOID		1 (2%)	
#MESENTERIC L. NODE	(38)	(41)	(44)
CONGESTION, NOS	2 (5%)	2 (5%)	2 (5%)
INFLAMMATION, GRANULOMATOUS		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
HYPERPLASIA, LYMPHOID	4 (11%)		
#LUNG	(49)	(50)	(50)
LEUKOCYTOSIS, NOS			1 (2%)
#LIVER	(49)	(50)	(50)
HEMATOPOIESIS		1 (2%)	
#PEYER'S PATCH	(48)	(49)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)	2 (4%)	
CIRCULATORY SYSTEM			
#HEART	(49)	(50)	(50)
ENDOCARDITIS, BACTERIAL			1 (2%)
#MYOCARDIUM	(49)	(50)	(50)
INFLAMMATION, ACUTE	1 (2%)	1 (2%)	1 (2%)
#CARDIAC VALVE	(49)	(50)	(50)
ENDOCARDITIS, BACTERIAL	1 (2%)	2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, NOS	1 (2%)		
NECROSIS, FOCAL	2 (4%)		1 (2%)
NECROSIS, DIFFUSE	1 (2%)		
METAMORPHOSIS FATTY	2 (4%)		
CLEAR-CELL CHANGE		1 (2%)	
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
NECROSIS, NOS		2 (4%)	1 (2%)
ATROPHY, NOS	1 (2%)		
#FORESTOMACH	(49)	(50)	(49)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
#COLON	(45)	(46)	(43)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(50)
INFLAMMATION, FOCAL	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, INTERSTITIAL	2 (4%)		1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
GLOMERULOSCLEROSIS, NOS			1 (2%)
#KIDNEY/TUBULE	(49)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
#KIDNEY/PELVIS	(49)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
#URINARY BLADDER	(49)	(49)	(50)
INFLAMMATION, ACUTE	1 (2%)		1 (2%)
ULCER, ACUTE		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(40)	(39)
CYST, NOS			1 (3%)
#ADRENAL/CAPSULE	(46)	(47)	(47)
HYPERPLASIA, NOS			1 (2%)
#ADRENAL CORTEX	(46)	(47)	(47)
HYPERPLASIA, NODULAR		2 (4%)	
#THYROID	(49)	(48)	(50)
CYSTIC FOLLICLES	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL		2 (4%)	4 (8%)
#THYROID FOLLICLE	(49)	(48)	(50)
HYPERPLASIA, CYSTIC			1 (2%)
REPRODUCTIVE SYSTEM			
*PENIS	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
*PREPUCE	(49)	(50)	(50)
INFLAMMATION, SUPPURATIVE	2 (4%)		
INFLAMMATION, ACUTE	1 (2%)	2 (4%)	1 (2%)
*PREPUTIAL GLAND	(49)	(50)	(50)
DILATATION/DUCTS			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
PUS			1 (2%)
INFLAMMATION, SUPPURATIVE	2 (4%)		
INFLAMMATION, ACUTE	1 (2%)		
#PROSTATE	(49)	(48)	(48)
INFLAMMATION, ACUTE	1 (2%)		1 (2%)
#TESTIS	(49)	(50)	(50)
GRANULOMA, SPERMATIC			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL		1 (2%)	
NERVOUS SYSTEM			
#CEREBRAL CORTEX	(48)	(50)	(50)
HEMORRHAGE	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(49)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
LEG			
HEMORRHAGE	1		
INFLAMMATION, SUPPURATIVE	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	10	13	16

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY	1		

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING AGAR

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
ABSCESS, NOS		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(50)
INFLAMMATION, ACUTE	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
INFLAMMATION, INTERSTITIAL	1 (2%)	2 (4%)	1 (2%)
BRONCHOPNEUMONIA, ACUTE	1 (2%)		
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(48)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)	2 (4%)	
HEMATOPOIESIS	8 (16%)	10 (21%)	3 (6%)
#LYMPH NODE	(45)	(43)	(45)
CYST, NOS		3 (7%)	
CONGESTION, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(45)	(43)	(45)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
#BRONCHIAL LYMPH NODE	(45)	(43)	(45)
INFLAMMATION, ACUTE		1 (2%)	
#MEDIASTINAL L.NODE	(45)	(43)	(45)
INFLAMMATION, ACUTE		1 (2%)	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, PLASMA CELL	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
#LUMBAR LYMPH NODE	(45)	(43)	(45)
DILATATION, NOS	1 (2%)		
CYST, NOS		2 (5%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, PLASMA CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)	2 (5%)	
#MESENTERIC L. NODE	(45)	(43)	(45)
CYST, NOS	1 (2%)		
CONGESTION, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)	2 (5%)	
#RENAL LYMPH NODE	(45)	(43)	(45)
HYPERPLASIA, NOS	1 (2%)		
#LIVER	(50)	(49)	(50)
HEMATOPOIESIS	2 (4%)	6 (12%)	1 (2%)
#ADRENAL	(45)	(47)	(48)
HEMATOPOIESIS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(49)	(50)
CALCIFICATION, FOCAL		1 (2%)	
#MYOCARDIUM	(50)	(49)	(50)
INFLAMMATION, ACUTE		1 (2%)	
#CARDIAC VALVE	(50)	(49)	(50)
ENDOCARDITIS, BACTERIAL			1 (2%)
*CORONARY ARTERY	(50)	(49)	(50)
INFLAMMATION, ACUTE NECROTIZING	1 (2%)		
#UTERUS	(48)	(48)	(48)
THROMBOSIS, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(49)	(50)
INFLAMMATION, CHRONIC		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
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL	1 (2%)	2 (4%)	1 (2%)
NECROSIS, DIFFUSE	1 (2%)		
METAMORPHOSIS FATTY		1 (2%)	
HEPATOCYTOMEGALY		1 (2%)	
ANGIECTASIS	3 (6%)	1 (2%)	1 (2%)
#HEPATIC CAPSULE	(50)	(49)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
#LIVER/CENTRILOBULAR	(50)	(49)	(50)
METAMORPHOSIS FATTY	1 (2%)		
*GALLBLADDER/SEROSA	(50)	(49)	(50)
INFLAMMATION, ACUTE	3 (6%)	3 (6%)	3 (6%)
#PANCREAS	(46)	(46)	(48)
CYSTIC DUCTS		2 (4%)	
INFLAMMATION, INTERSTITIAL	1 (2%)		
METAMORPHOSIS FATTY		1 (2%)	
#GASTRIC MUCOSA	(49)	(49)	(49)
NECROSIS, FOCAL		1 (2%)	
#GASTRIC SEROSA	(49)	(49)	(49)
INFLAMMATION, ACUTE		1 (2%)	
#FORESTOMACH	(49)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	1 (2%)
ACANTHOSIS			1 (2%)
#PEYER'S PATCH	(48)	(48)	(46)
HYPERPLASIA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
INFLAMMATION, INTERSTITIAL			2 (4%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
PYELONEPHRITIS, ACUTE	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
GLOMERULOSCLEROSIS, NOS	2 (4%)		
AMYLOIDOSIS		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
#URINARY BLADDER	(50)	(47)	(46)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(38)
CYST, NOS		2 (5%)	
#ADRENAL	(45)	(47)	(48)
INFLAMMATION, ACUTE	2 (4%)		1 (2%)
METAMORPHOSIS FATTY	2 (4%)		
#ADRENAL/CAPSULE	(45)	(47)	(48)
HYPERPLASIA, NOS		1 (2%)	
#THYROID	(47)	(46)	(46)
CYSTIC FOLLICLES			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL	2 (4%)	1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(48)	(48)	(48)
HYDROMETRA	1 (2%)	1 (2%)	
INFLAMMATION, ACUTE	1 (2%)		
#CERVIX UTERI	(48)	(48)	(48)
CALCINOSIS, NOS		1 (2%)	
#UTERUS/ENDOMETRIUM	(48)	(48)	(48)
INFLAMMATION, SUPPURATIVE	7 (15%)	8 (17%)	4 (8%)
HYPERPLASIA, CYSTIC	27 (56%)	27 (56%)	34 (71%)
#OVARY/PAROVARIAN	(44)	(45)	(50)
NECROSIS, FAT	1 (2%)		
#OVARY	(44)	(45)	(50)
CYST, NOS	9 (20%)	13 (29%)	11 (22%)
CYSTIC FOLLICLES			1 (2%)
HEMORRHAGIC CYST			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
INFLAMMATION, SUPPURATIVE	2 (5%)	3 (7%)	
INFLAMMATION, ACUTE	1 (2%)		
ABSCESS, NOS	5 (11%)	5 (11%)	8 (16%)
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	
HYPERPLASIA, CYSTIC		1 (2%)	
HYPERPLASIA, ADENOMATOUS	1 (2%)	1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*EYE/CORNEA	(50)	(49)	(50)
INFLAMMATION, ACUTE		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(50)	(49)	(50)
INFLAMMATION, ACUTE		1 (2%)	
BODY CAVITIES			
*MEDIASTINUM	(50)	(49)	(50)
INFLAMMATION, ACUTE	1 (2%)		
*ABDOMINAL CAVITY	(50)	(49)	(50)
STEATITIS		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
ABSCESS, NOS	1 (2%)	1 (2%)	
NECROSIS, FAT	2 (4%)	1 (2%)	
*PERITONEUM	(50)	(49)	(50)
INFLAMMATION, ACUTE	1 (2%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(49)	(50)
INFLAMMATION, ACUTE		1 (2%)	
ADIPOSE TISSUE			
INFLAMMATION, CHRONIC		1	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
OMENTUM			
STEATITIS			1
INFLAMMATION, ACUTE		1	
INFLAMMATION, CHRONIC	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	1	3
ANIMAL MISSING/NO NECROPSY		1	

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

APPENDIX E

Analysis of Agar

APPENDIX E
Analysis of Agar
(Batch 01, Lot No. JO-6646)
and
(Batch 02, Lot No. JO-7785)

A. ELEMENTAL ANALYSIS

Batch 01	Element	C	H	N	S	Na	K	Mg	Ca
Theory	(a)	44.40	6.22	-	0.3-2.0%	-	-	-	-
	(b)	40.38	6.67		0.3-2.0%				
Determined		38.35	6.45	0.03	1.17	0.34	0.08	0.15	0.70
		38.09	6.28	0.02	1.18	0.32		0.14	0.70
						0.28		0.16	0.71
								0.19	0.71

Batch 02	Element	C	H	N	S	Na	K	Mg	Ca
Theory	(a)	44.40	6.22		0.3-2.0%				
	(c)	41.85	6.51	-	0.3-2.0%	-	-	-	-
Determined		38.89	6.47	1.60	0.70	0.28	0.064	0.090	0.46
		39.03	6.46	1.66	0.68	0.29	0.640	0.088	0.48

(a) Smith and Montgomery, 1959.
(b) C and H based on 90.86% $C_6H_{10}O_5$ + 9.14% H_2O
(c) C and H based on 94.17% $C_6H_{10}O_5$ and 5.83% H_2O

B. WATER ANALYSIS (Karl Fisher)

Batch 01 9.14 ± 1.2% (δ)%(Karl Fisher); 02 5.83 ± 0.29 (δ)%.

C. MELTING POINT

Determined (01)	Literature Values
220° to 250°C, decomposition (visual; capillary) Endotherm: 238° to 275°C (Dupont 900 DTA)	No literature reference found

D. ANALYSIS (Modification of USP Assay for Mannitol)

Batch 01: Samples were dissolved in 25 ml concentrated sulfuric acid and 150 ml water in 250-ml volumetric flasks and left at room temperature for 20 hours. The solutions were then boiled for 12 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 an 50.0 ml potassium periodate/ sulfuric acid solution added. Samples and blank were heated on a steam bath for 5 hr. Potassium iodide was added, and the samples were titrated with sodium thiosulfate.

Results: 68.1 + 1.6 (δ)% (It was assumed that each mole of monomer requires 5 moles of periodate) (USP, 1970)

The percentage purity obtained by this assay is probably not the actual purity because of decomposition during the hydrolysis step. Therefore, this analysis is useful only in checking similarities or differences between different lots.

Batch 02: 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 hr.

The solutions were then heated on a hot plate until they started to discolor. None of the samples reached boiling temperature before discoloration began. 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. One sample and the blank were heated on a steam bath for 2.5 hr. Potassium iodide was added and the samples were titrated with sodium thiosulfate. The assumption was made that each mole of monomer reacts with 5 moles of periodate.

Results 83.9 $\pm$ 2.4 (δ)%

E. THIN-LAYER CHROMATOGRAPHY OF ACID HYDROLYSIS PRODUCTS (Varma et al., 1973)

Batch 02

Plates: Silica Gel 60 F-254

Ref. Standard: D-Galactose

Amount Spotted: 41 μg 2.0 $\mu g/\mu l$ H_2O:methanol

Visualization: 0.5% Potassium permanganate in 1 N sodium hydroxide

System 1: n-Butanol:acetic acid:water (63:12:25)

System 2: n-Butanol:pyridine: water (46:31:23)

System 1 R_f: 0.04 (trace), 0.17 (major) 0.68 (slight trace)

System 2 R_f: 0.01 (slight trace), 0.49 (major), 0.55 (trace)

System 1 R_{st}: 0.23, 0.89, 3.52

System 2 R_{st}: 0.02, 1.02, 1.14

F. SPECTRAL DATA

1. Infrared

Instrument: Beckman, IR-12	Identical to literature spectrum (Tsuchiya and Hong, 1965)

Batch 01: a. Cell: 1% potassium bromide pellet
Results: See Figure 5

Batch 01: b. Cell: Neat film
Results: See Figure 6

Batch 02: c. Cell: Thin film
Results: See Figure 7

2. Ultraviolet/Visible
Batches 01 and 02

Instrument: Cary 118	No literature reference found

No UV or visible absorbance detectable at approximately 0.1 mg/ml

Solvent: H_2O

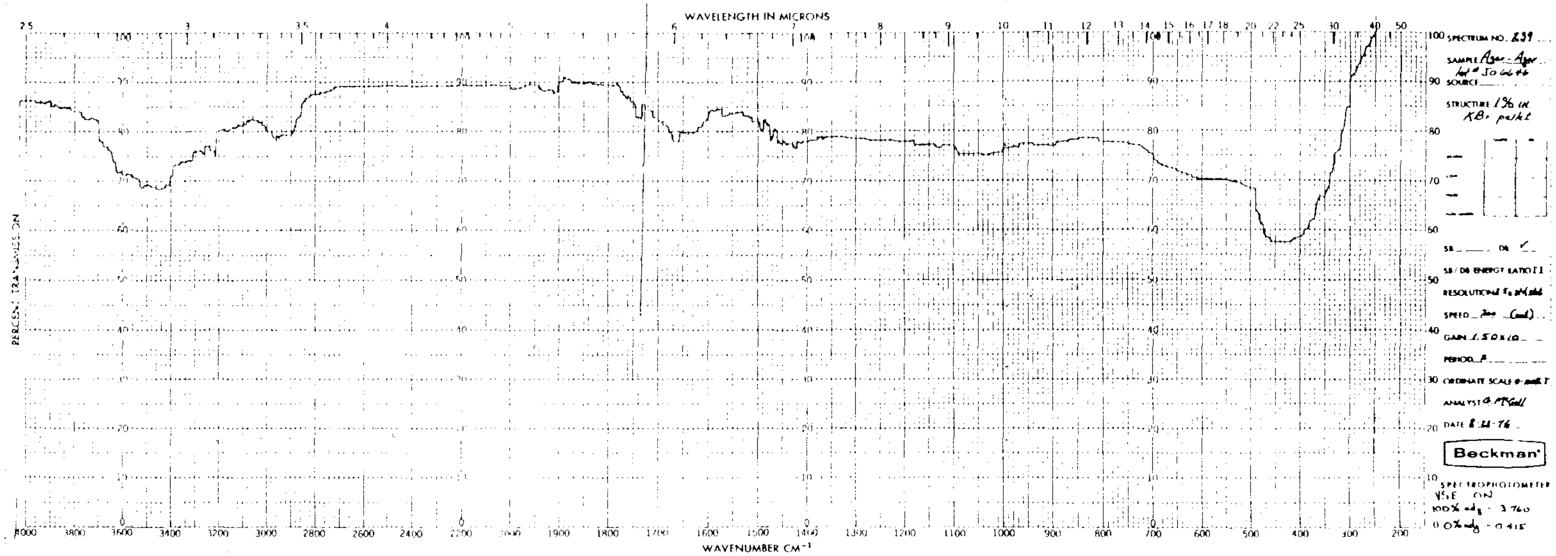

Figure 5. Infrared Absorption Spectrum of Agar (Lot No. JO-6646) KBr Pellet

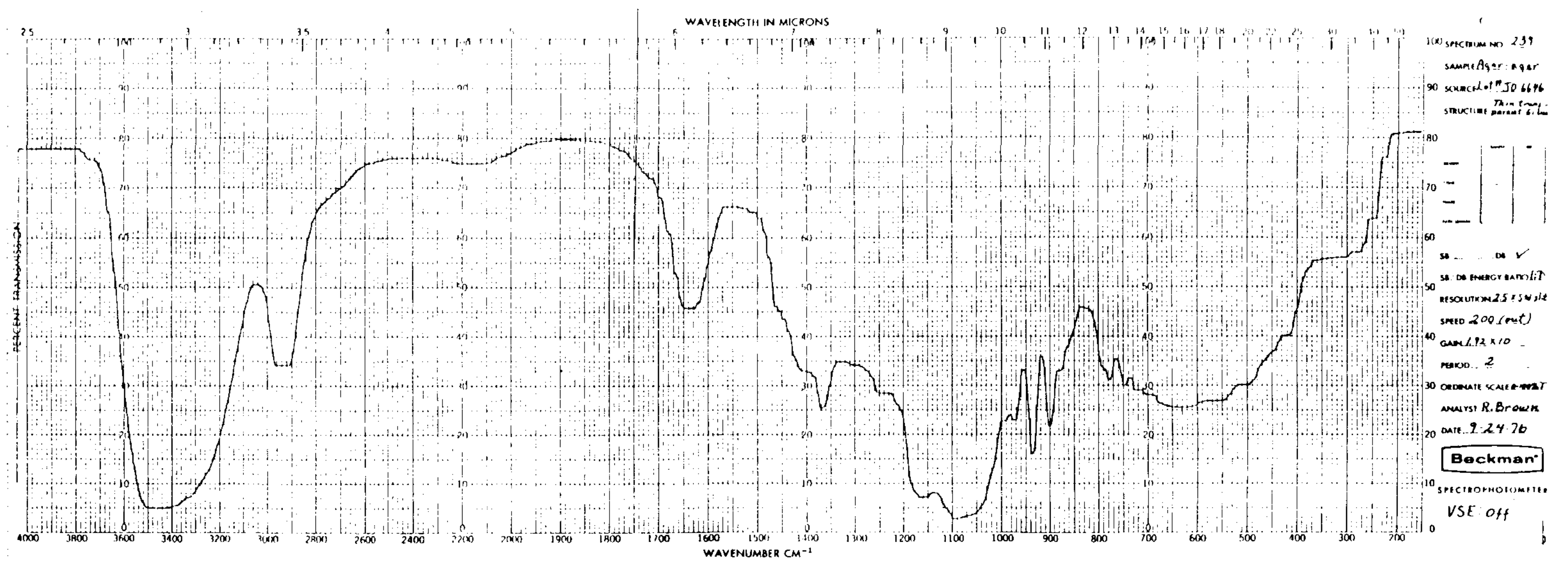

Figure 6. Infrared Absorption Spectrum of Agar (Lot No. JO-6646) - Neat

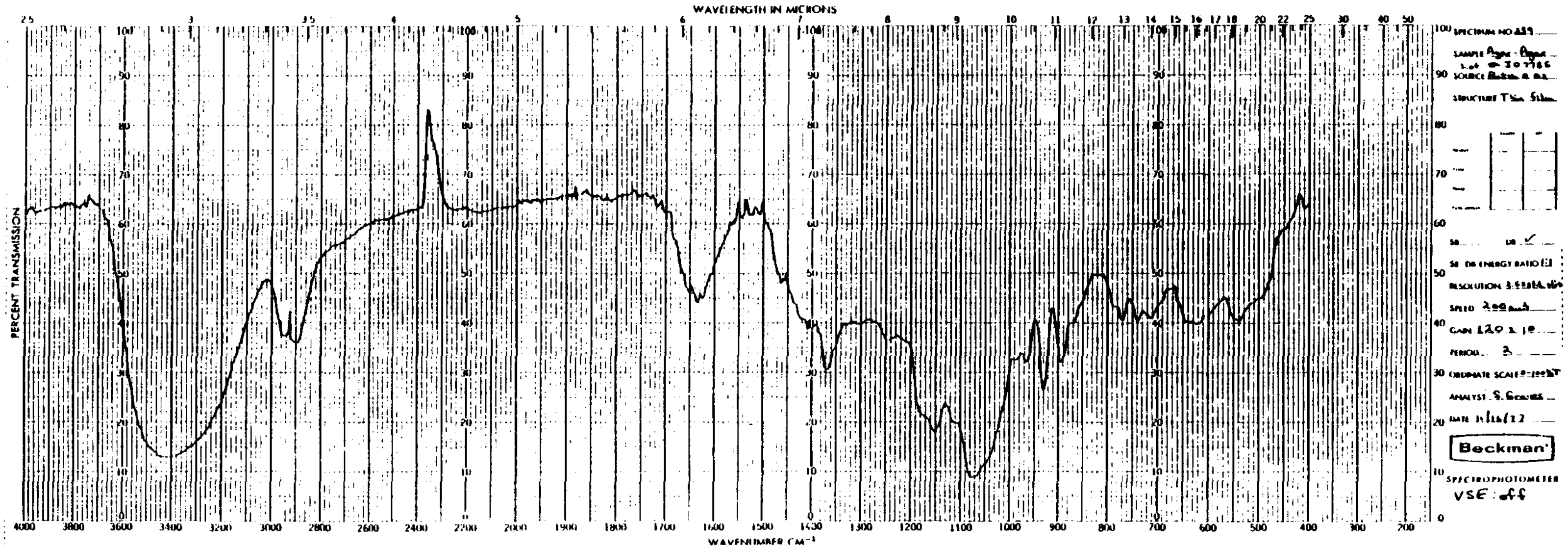

Figure 7. Infrared Absorption Spectrum of Agar (Lot No. JO-7785)

APPENDIX F
Feed Consumption by Rats and
Mice Receiving Agar

Table F1. Feed Consumption by Male Rats Receiving Agar

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low/ Control (b)	High Grams Feed/ Day(a)	High/ Control (b)
4	18.7	18.9	1.0	19.9	1.1
8	19.1	17.7	0.9	19.1	1.0
12	23.1	24.9	1.1	14.9	0.6
16	20.0	18.9	0.9	21.1	1.1
20	19.0	18.1	1.0	18.6	1.0
24	17.4	17.7	1.0	18.3	1.1
28	21.6	19.1	0.9	22.3	1.0
32	19.1	19.6	1.0	20.4	1.1
36	20.9	19.9	1.0	18.9	0.9
40	19.9	19.1	1.0	19.0	1.0
44	20.4	19.6	1.0	20.6	1.0
48	23.6	22.1	0.9	22.6	1.0
52	24.4	23.1	0.9	24.6	1.0
56	20.3	20.1	1.0	20.9	1.0
60	20.7	20.9	1.0	21.4	1.0
64	21.6	21.6	1.0	21.6	1.0
68	23.1	22.4	1.0	23.6	1.0
72	19.1	20.3	1.1	19.1	1.0
76	21.6	19.9	0.9	21.1	1.0
80	22.3	21.9	1.0	22.1	1.0
84	23.4	22.6	1.0	23.3	1.0
88	21.6	20.1	0.9	21.7	1.0
92	22.9	23.4	1.0	23.7	1.0
96	20.0	20.1	1.0	20.6	1.0
100	23.1	20.1	0.9	21.7	0.9
Mean	21.1	20.5	1.0	20.8	1.0
SD (c)	1.8	1.8	0.1	2.1	0.1
CV (d)	8.5	8.8	10.0	10.1	10.0

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/mean) x 100.

Table F2. Feed Consumption by Female Rats Receiving Agar

	Control	Low		High	
Week	Grams Feed/ Day(a)	Grams Feed/ Day(a)	Low/ Control (b)	Grams Feed/ Day(a)	High/ Control (b)
4	14.3	13.9	1.0	14.1	1.0
8	16.7	13.4	0.8	13.3	0.8
12	18.4	14.4	0.8	18.0	1.0
16	15.0	14.4	1.0	15.6	1.0
20	17.1	13.9	0.8	15.4	0.9
24	16.6	14.9	0.9	15.1	0.9
28	15.4	15.6	1.0	16.9	1.1
32	15.4	14.1	0.9	15.3	1.0
36	17.6	16.0	0.9	15.4	0.9
40	16.3	15.1	0.9	15.9	1.0
44	14.0	14.7	1.1	15.3	1.1
48	18.3	17.1	0.9	18.9	1.0
52	21.1	19.1	0.9	18.9	0.9
56	18.7	17.1	0.9	16.9	0.9
60	20.9	18.0	0.9	19.9	0.9
64	21.9	19.6	0.9	19.9	0.9
68	22.3	20.4	0.9	21.6	1.0
72	16.6	17.6	1.1	16.9	1.0
76	17.4	16.7	1.0	17.6	1.0
80	19.1	17.6	0.9	18.3	1.0
84	18.4	16.9	0.9	18.1	1.0
88	18.4	17.6	1.0	17.0	0.9
92	16.7	18.0	1.1	18.1	1.1
96	17.3	15.3	0.9	16.4	0.9
100	19.1	16.0	0.8	16.0	0.8
Mean	17.7	16.3	0.9	16.9	1.0
SD (c)	2.1	1.9	0.1	1.9	0.1
CV (d)	11.9	11.7	11.1	11.2	10.0

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/mean) x 100.

Table F3. Feed Consumption by Male Mice Receiving Agar

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low/ Control (b)	High Grams Feed/ Day(a)	High/ Control (b)
4	6.7	7.6	1.1	8.7	1.3
8	6.9	6.9	1.0	7.1	1.0
12	6.7	7.0	1.0	7.1	1.1
16	6.9	9.9	1.4	7.6	1.1
20	6.4	6.6	1.0	7.1	1.1
24	7.1	7.6	1.1	8.3	1.2
28	6.9	6.7	1.0	7.6	1.1
32	6.3	6.4	1.0	6.9	1.1
36	7.0	7.3	1.0	7.9	1.1
40	7.1	7.4	1.0	7.9	1.1
44	5.6	5.6	1.0	5.9	1.1
48	8.1	7.7	1.0	9.4	1.2
52	7.6	6.9	0.9	7.7	1.0
56	7.7	7.0	0.9	7.6	1.0
60	8.0	8.1	1.0	13.0	1.6
64	7.0	7.0	1.0	7.0	1.0
68	6.6	7.6	1.2	7.6	1.2
72	6.1	6.3	1.0	6.7	1.1
76	7.1	7.6	1.1	8.0	1.1
80	6.3	7.6	1.2	7.9	1.3
84	7.7	7.4	1.0	7.7	1.0
88	6.9	7.7	1.1	7.9	1.1
92	7.4	7.1	1.0	7.6	1.0
96	9.0	7.9	0.9	7.9	0.9
100	9.6	8.4	0.9	10.4	1.1
Mean	7.1	7.3	1.0	7.9	1.1
SD (c)	0.9	0.8	0.1	1.4	0.1
CV (d)	12.7	11.0	10.0	17.7	9.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/mean) x 100.

Table F4. Feed Consumption by Female Mice Receiving Agar

Week	Control Grams Feed/ Day(a)	Low Grams Feed/ Day(a)	Low/ Control (b)	High Grams Feed/ Day(a)	High/ Control (b)
4	6.6	7.9	1.2	8.0	1.2
8	6.9	8.7	1.3	9.1	1.3
12	6.6	8.4	1.3	8.7	1.3
16	9.3	8.9	1.0	10.3	1.1
20	8.1	8.6	1.1	5.9	0.7
24	8.3	8.6	1.0	9.6	1.2
28	7.1	7.1	1.0	8.4	1.2
32	6.6	7.1	1.1	7.6	1.2
36	7.9	7.9	1.0	8.3	1.1
40	7.6	7.6	1.0	8.6	1.1
44	6.0	6.3	1.1	6.9	1.2
48	9.3	9.6	1.0	11.3	1.2
52	9.6	9.1	0.9	9.7	1.0
56	9.0	8.4	0.9	8.9	1.0
60	10.6	9.6	0.9	11.1	1.0
64	9.0	8.1	0.9	9.0	1.0
68	7.3	9.3	1.3	8.9	1.2
72	7.9	7.6	1.0	8.4	1.1
76	9.0	8.9	1.0	10.3	1.1
80	9.1	8.9	1.0	8.9	1.0
84	9.7	9.1	0.9	9.4	1.0
88	9.6	9.1	0.9	9.1	0.9
92	9.9	9.0	0.9	9.4	0.9
96	10.1	9.4	0.9	10.6	1.0
100	12.1	9.6	0.8	10.0	0.8
Mean	8.5	8.5	1.0	9.1	1.1
SD (c)	1.5	0.9	0.1	1.2	0.1
CV (d)	17.6	10.6	10.0	13.2	9.1

(a) Grams of feed consumed per animal per day.
(b) Ratio of feed consumed per day for the dosed group to that for the controls.
(c) Standard deviation.
(d) (Standard deviation/mean) x 100.

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