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BIOASSAY OF
BUTYLATED HYDROXYTOLUENE (BHT)
FOR POSSIBLE CARCINOGENICITY

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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
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Bethesda, Maryland 20205

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FOREWORD: This report presents the results of the bioassay of butylated hydroxytoluene (BHT) conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. A negative result, in which the test animals do not have a greater incidence of cancer than control animals, does 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 carcinogenic for animals under the conditions of the test and indicates that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis.

CONTRIBUTORS: This bioassay of butylated hydroxytoluene (BHT) was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed the computer services. Histopathologic evaluations for rats were performed by Dr. J. F. Hardisty (3), and the histopathologic evaluations for mice were performed by Dr. L. J. Ackerman (3). The diagnoses included in this report represent the interpretations of Drs. Hardisty and Ackerman.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (4). Statistical analyses were

performed by Dr. J. R. Joiner (5) and Ms. P. L. Yong (5), using methods selected for the bioassay program by Dr. J. J. Gart (6). The chemicals used in this bioassay were analyzed at Frederick Cancer Research Center by Dr. W. Zielinsky (1). The chemical analyses and narrative were reviewed and approved by Dr. W. Lijinsky (1).

This report was prepared at Tracor Jitco (5) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. C. R. Angel, Acting Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. L. A. Owen, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The following scientists at NCI were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. A. R. Patel, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of butylated hydroxytoluene (BHT) for possible carcinogenicity was conducted by administering the test chemical in feed to F344 rats and B6C3F1 mice.

Groups of 50 rats and 50 mice of each sex were administered BHT at one of two doses, either 3,000 or 6,000 ppm; the rats for 105 weeks and the mice for 107 or 108 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of administration of the test chemical.

Mean body weights of the dosed rats and mice were lower than those of the corresponding controls and were dose related throughout most of the bioassay. Survival was not affected significantly in the dosed groups of rats or mice, and the survival was 60% or greater in all dosed or control groups of rats and mice of each sex at the end of the bioassay. Sufficient numbers of animals were at risk for the development of late-appearing tumors.

Alveolar/bronchiolar carcinomas or adenomas occurred in the female mice at a significant incidence in the low-dose group ($P = 0.009$) but not in the high-dose group, and the incidences were not significantly dose related (control 1/20, low-dose 16/46, high-dose 7/50). Thus, these lung tumors in the females cannot clearly be related to the administration of the BHT. No tumors occurred in either male or female rats at incidences that were significantly higher in dosed groups than in corresponding control groups. Nonneoplastic lesions that may have been related to the administration of the test chemical included focal alveolar histiocytosis at increased incidences in the dosed female rats and various lesions of the liver at increased incidences in the dosed male mice.

It is concluded that under the conditions of this bioassay, BHT was not carcinogenic for F344 rats or B6C3F1 mice.

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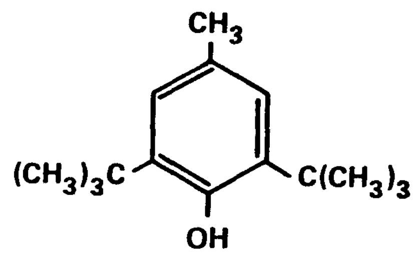
I. INTRODUCTION

The phenolic antioxidant 2,6-di-tert-butyl-p-cresol (CAS 128-37-0; NCI C03598), more commonly known as butylated hydroxytoluene, or BHT, was patented in 1947 (Stecher, 1968) and received approval for use as a food additive and preservative by the Food and Drug Administration (FDA) in 1954 (Federal Register, 1977). Since 1959, BHT has been generally recognized as safe (GRAS) for use in foods (Federal Register, 1977) and is one of the most commonly used antioxidants in foods containing fats (Stuckey, 1972). It is used alone or in combination with butylated hydroxyanisole or propyl gallate (Dugan, 1963; Stuckey, 1972). Acting on an evaluation of the toxicity of BHT by the Select Committee on GRAS Substances (1973), the Federal Register (1977) has recently proposed interim restrictions on use levels in foods until additional toxicity studies have been performed. The Select Committee had concluded that there was no evidence that BHT posed a hazard to public health when it was used at levels then current and in the manner then practiced, but that additional studies



BHT

would be necessary to resolve some uncertainties in the existing data. In particular, the Federal Register (1977) proposed that short-term metabolism studies be carried out to compare the metabolism of BHT in mice with that in man, and that if similar metabolisms were found, long-term feeding studies then be carried out to resolve conflicting reports (Clapp et al., 1976; Brooks et al., 1977) on the carcinogenicity of BHT for the lung in mice.

BHT prevents rancidity in foods containing fats by terminating chain reactions involving free radicals that are responsible for the oxidative degradation of the fats (Chapman and Kertesy, 1966; Noller, 1966). Oxidation not only produces undesirable flavor changes, but destroys both fat-soluble vitamins and the essential fatty acids, and may generate toxic products (Dugan, 1963).

BHT is approved for use in enriched rice, margarine, shortening, dehydrated potato products, dry breakfast cereals, chewing gum base, certain food-packaging materials (Federal Register, 1977; Code of Federal Regulations, 1977), and animal feed (Code of Federal Regulations, 1977a). It is cleared for use by the Meat Inspection Division of the U.S. Department of Agriculture in rendered animal fats, fresh and dried pork sausage, and freeze-dried meats (Furia, 1972). Among the nonfood items in which BHT acts as a stabilizer are pesticides (Code of Federal Regulations, 1976 and 1977); gasolines, lubricants, and rubber (Dugan, 1963); and oil-based lipsticks (Lauffer, 1972).

Although the level of BHT used in any food product has not been allowed to exceed 0.02% of the weight of fat present, the total amount of BHT used in foods in 1970 reached nearly 600,000 pounds, twice the figure reported in 1960 (Federal Register, 1977). By 1976, the annual production of BHT in the United States had increased to 19.81 million pounds, of which 8.86 million pounds were produced for use in foods and 10.95 million pounds for other uses (United States International Trade Commission, 1977).

Because humans are increasingly exposed to BHT through its wide use as a food additive, the chemical was selected for reevaluation of its potential carcinogenicity, using the protocols of the Carcinogenesis Testing Program.

II. MATERIALS AND METHODS

A. Chemical

Butylated hydroxytoluene (BHT), or 2,6-di-*tert*-butyl-p-cresol, was obtained from Koppers Co., Pittsburgh, Pennsylvania, as a fine, white, crystalline solid. Its purity was determined to be 99.9% by gas-liquid chromatography, with two to six contaminants comprising less than 0.1%. Mass spectral analysis showed a molecular ion at 220 m/e and a base peak at 205 m/e. The infrared spectrum was consistent with its chemical structure, and identical with that of a standard. The melting point was 69.6°C (Stecher, 1968: 70°C). Elemental analysis for carbon and hydrogen was in agreement with theoretical.

B. Dietary Preparation

Test diets containing BHT were prepared every 1 to 1-1/2 weeks in 6-to 12-kg batches at appropriate doses. A known weight of the chemical was first mixed with an equal weight of autoclaved Wayne® Sterilizable Lab Meal containing 4% fat (Allied Mills, Inc., Chicago, Ill.), using a mortar and pestle. The Wayne®

Sterilizable Lab Meal contained 4% fat but no added BHT (Drews, 1978). The mixing was continued with second and third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly® twin-shell blender with an intensifier bar.

The diets were stored at 7°C until used.

C. Animals

Male and female F344 (Fischer) rats and B6C3F1 mice were obtained as 4-week-old weanlings, all within 3 days of the same age, from the NCI Frederick Cancer Research Center (Frederick, Md.). The animals were housed within the test facility for 2 weeks and were then assigned four rats of the same sex to a cage and five mice of the same sex to a cage. The male rats used in the chronic study weighed 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N.J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N.Y.). The feed was presterilized Wayne® Sterilizable Lab Meal containing 4% fat, provided <u>ad libitum</u> in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied <u>ad libitum</u> from glass bottles with sipper tubes (Lab Products, Inc.) suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Corp., Mataway, N. J.), using the detergents, Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor (Oxford Chemicals, Atlanta, Ga.). The bottles and sipper tubes were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The animal rooms were maintained at 22 to 24°C, and the relative humidity was 45 to 55%. Incoming air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and expelled without recirculation through a "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). Room air was changed 15 times per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided automatically on a 12-hour-per-day cycle.

Rats administered BHT and their corresponding controls were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 88-96-0) phthalamide
(CAS 137-17-7) 2,4,5-trimethylaniline

Mice administered BHT and their corresponding controls were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 3165-93-3)	4-chloro-o-toluidine hydrochloride
(CAS 97-77-8)	tetraethylthiuram disulfide
(CAS 148-18-5)	sodium diethyldithiocarbamate
(CAS 636-21-5)	o-toluidine hydrochloride

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of BHT, on the basis of which two concentrations (referred to in this report as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats and five mice of each sex were fed diets containing BHT at one of several doses for 7 weeks, followed by 1 week of observation, and groups of five control animals of each species and sex were administered basal diet only. Each animal was weighed twice per week. Table 1 shows the doses fed, the survival of animals in each dosed group at the end of the study, and the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of the controls. At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. Histopathologic findings are shown as footnotes to the table.

Table 1. BHT Subchronic Feeding Studies in Rats and Mice

Dose (ppm)	Male Surviv-al (a)	Male Mean Weight at Week 7 as % of Control	Female Surviv-al (a)	Female Mean Weight at Week 7 as % of Control
Rats				
0	5/5	100	5/5	100
6,200	5/5	88	5/5	93
12,500(b)	4/5	74	5/5	84
25,000	5/5	38	5/5	44
50,000	0/5		0/5	
Mice				
0	5/5	100	5/5	100
3,100	5/5	89	5/5	88
6,200	5/5	94	5/5	83
12,500(c)	5/5	78	5/5	82
25,000(c)	5/5	79	4/5	74
50,000	4/5	73	1/5	97

(a) Number surviving/number in group.

(b) Slight increase in hematopoiesis in both sexes of rats.

(c) Histopathologic examination of male mice at 25,000 ppm and of female mice at 12,500 ppm showed a very small amount of centrilobular cytoplasmic vacuolation in the livers of the males.

Ten percent depression in body weight was a major criterion for the estimation of MTD's. The doses required to produce this response were determined by the following procedure: first, least squares regressions of mean body weights versus days on study were used to estimate mean body weights of each of the dosed groups at day 49. Next, probits of the percent weights of the dosed groups at day 49 relative to weights of corresponding control groups were plotted against the logarithms of the doses, and least squares regressions fitted to the data were used to estimate the doses required to induce 10% depression in weight.

The low and high doses for the rats and mice in the chronic study were set at 3,000 and 6,000 ppm, respectively.

F. Chronic Studies

The test groups, doses administered, and durations of the chronic studies are shown in tables 2 and 3.

G. Clinical and Pathologic Examinations

All animals were observed twice daily. Observations for sick,

Table 2. BHT Chronic Feeding Studies in Rats

Sex and Test Group	Initial No. of Animals(a)	BHT in Diet(b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	105
Low-Dose	50	3,000	105
High-Dose	50	6,000	105
Female			
Matched-Control	20	0	105
Low-Dose	50	3,000	105
High-Dose	50	6,000	105

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided *ad libitum* 7 days per week.

Table 3. BHT Chronic Feeding Studies in Mice

Sex and Test Group	Initial No. of Animals(a)	BHT in Diet(b) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	108
Low-Dose	50	3,000	108
High-Dose	50	6,000	107
Female			
Matched-Control	20	0	108
Low-Dose	50	3,000	108
High-Dose	50	6,000	107-108

(a) All animals were 6 weeks of age when placed on study.

(b) Test and control diets were provided *ad libitum* 7 days per week.

tumor-bearing, and moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month. Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 and necropsied.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestines, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, uterus, ovary, brain (cerebrum and cerebellum), and all tissue masses. Peripheral blood smears also were made for all animals, whenever possible.

Necropsies were also performed on all animals found dead, unless precluded in whole or in part by autolysis or cannibalization. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

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

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

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

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

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity (P less than 0.05, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared with its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a dosed group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the dosed group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (P 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) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male and female rats were lower than those of corresponding controls throughout the bioassay, and this depression was dose related (figure 1). Other clinical signs occurred at comparable incidences in dosed and control groups.

B. Survival (Rats)

Estimates of probabilities of survival for male and female rats administered BHT in the diet at the doses of this bioassay, together with those for the matched controls, are shown by the Kaplan and Meier curves in figure 2. The result of the Tarone test for dose-related trend in mortality is not significant in either sex.

In male rats, 36/50 (72%) of the high-dose group, 39/50 (78%) of the low-dose group, and 13/20 (65%) of the control group lived to the end of the bioassay. In females, 39/50 (78%) of the high-

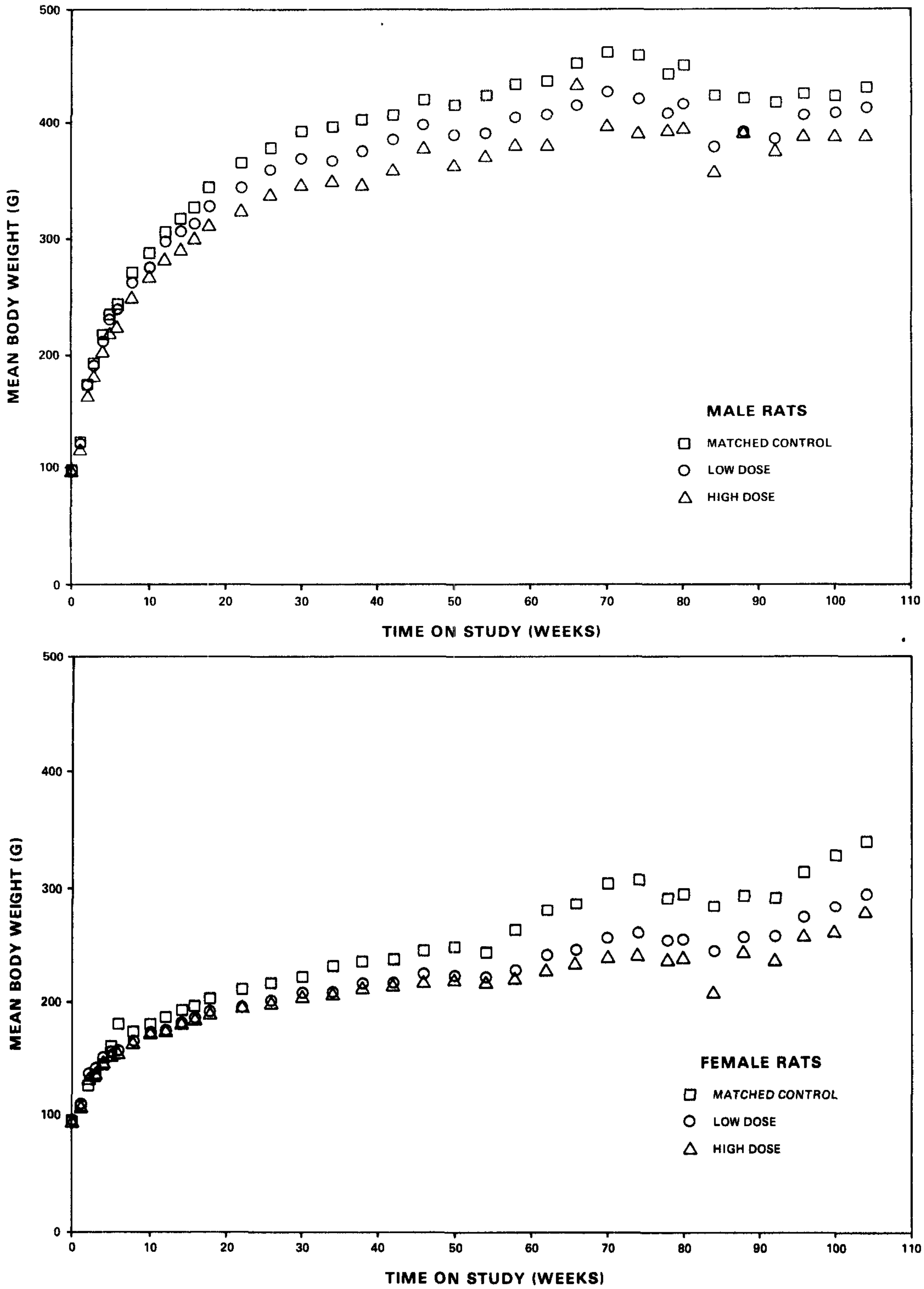
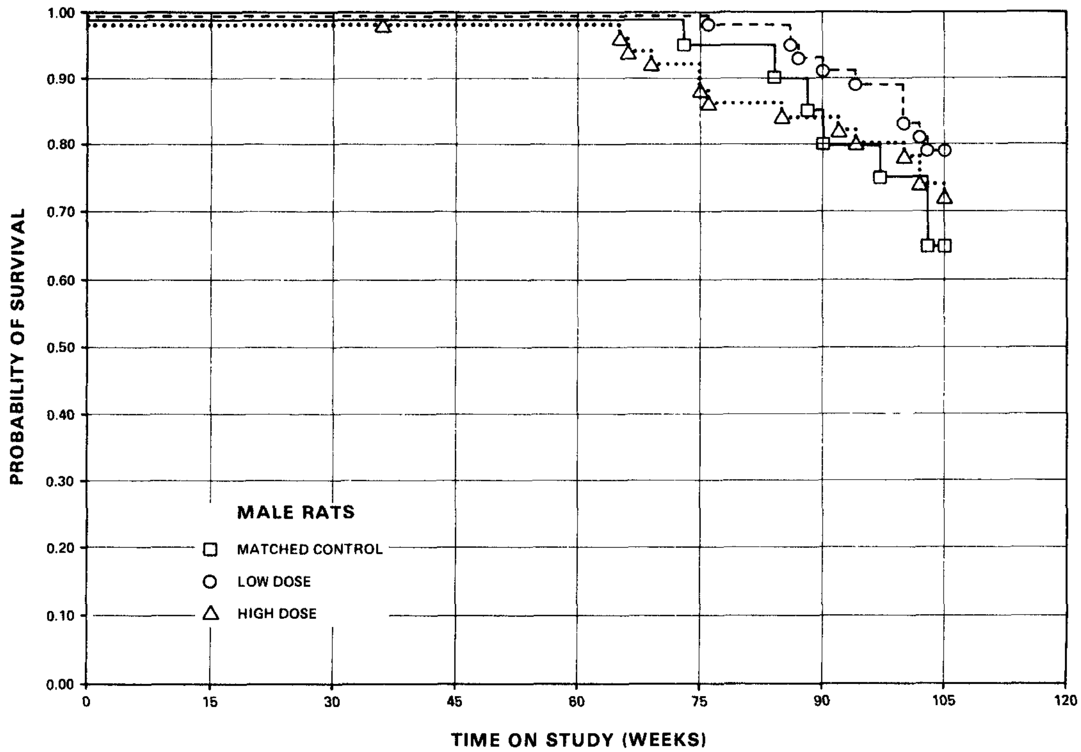


Figure 1. Growth Curves for Rats Administered BHT in the Diet



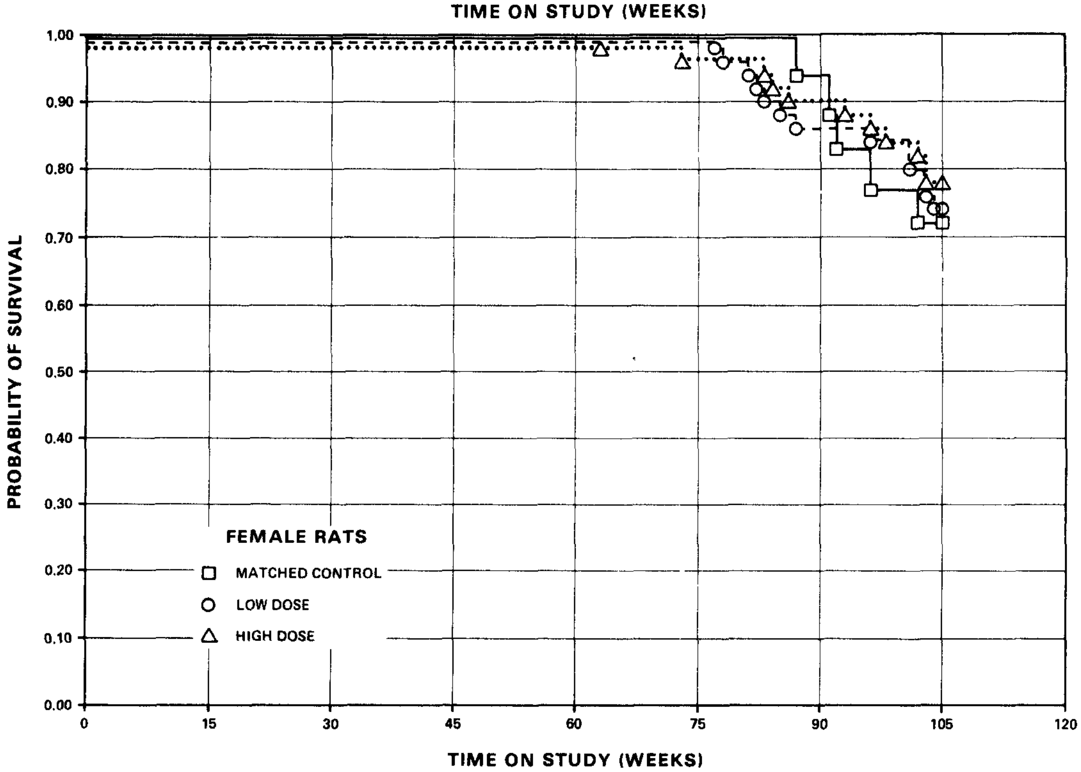


Figure 2. Survival Curves for Rats Administered BHT in the Diet

dose group, 37/50 (74%) of the low-dose group, and 13/20 (65%) of the control group lived to the end of the bioassay.

Sufficient numbers of rats of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms commonly seen in aged F344 rats occurred with approximately equal frequency in dosed and control rats. In the male rats, interstitial-cell tumors of the testes and pheochromocytomas of the adrenal were the most frequently observed neoplasms. In the female rats, fibroadenomas of the mammary gland and endometrial stromal polyps of the uterus were observed frequently.

Several inflammatory, degenerative, and proliferative lesions commonly seen in aged F344 rats occurred with approximately equal frequency in dosed and control animals. Focal alveolar histiocytosis in the lung was observed in both dosed and control animals, but this lesion was most often observed in the high-dose female rats. This lesion consisted of focal aggregates of large mononuclear cells within the alveolar lumen. These cells contained abundant foamy vacuolated cytoplasm. This lesion occurred in all dosed and control groups, as shown in the following table:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissues Examined	20	49	49	18	48	49
Focal Alveolar Histiocytosis	1(5%)	4(8%)	7(14%)	2(11%)	12(25%)	21(43%)

Based on the histopathologic examination, the administration of BHT at the doses used in this bioassay did not induce either neoplastic or nonneoplastic lesions in the F344 rat, with the possible exception of focal alveolar histiocytosis in the females.

D. Statistical Analyses of Results (Rats)

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

In each sex, the results of the Cochran-Armitage test for dose-related trend in the incidence of tumors and the results of the Fisher exact test comparing the incidence of tumors in each dosed group with that in the control group are not significant in the positive direction. However, significant results in the negative direction are observed in the incidence of adenomas of the pituitary in female rats.

In each of the 95% confidence intervals for relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals, except that for the incidence of adenomas of the pituitary in high-dose female rats, has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by BHT, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

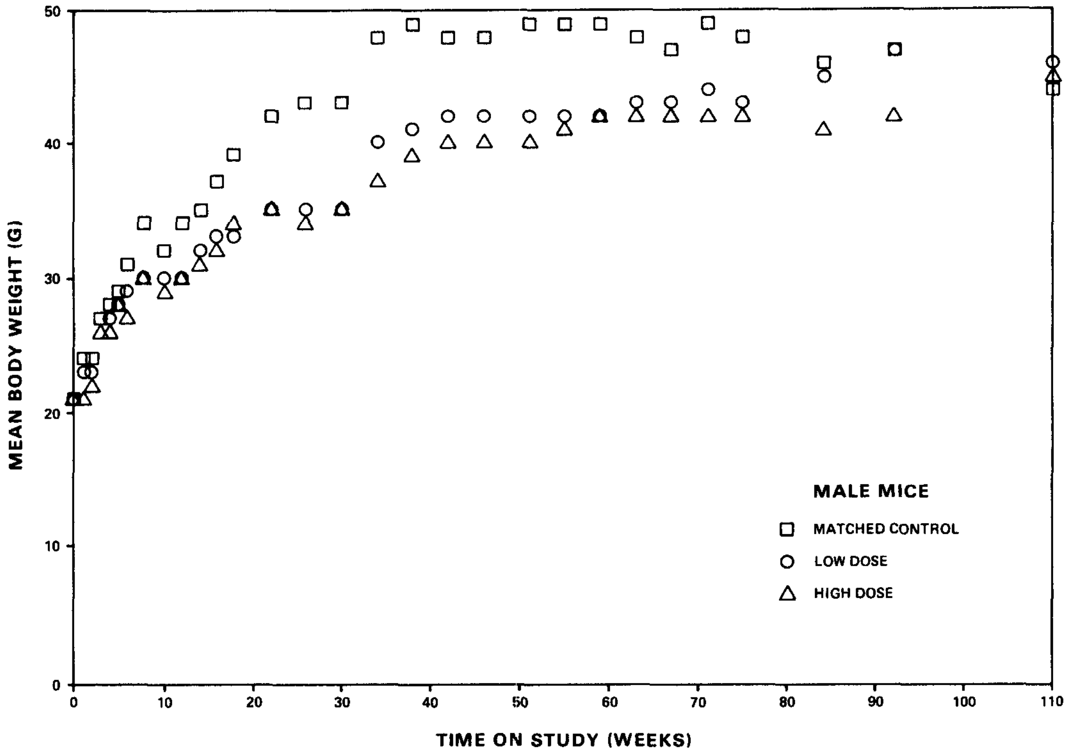
A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male and female mice were lower than those of corresponding controls throughout the bioassay, and were dose related (figure 3). Tissue masses occurred at comparable incidences in dosed and control groups.

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered BHT in the diet at the doses of this bioassay, together with those for the matched controls, are shown by the Kaplan and Meier curves in figure 4. In male mice, the result of the Tarone test for dose-related trend in mortality is significant ($P = 0.005$), but in the negative direction. In females, the result of the Tarone test is not significant.

In male mice, 46/50 (92%) of the high-dose group, 43/50 (86%) of the low-dose group, and 12/20 (60%) of the control group lived to the end of the bioassay. In female mice, 45/50 (90%) of the



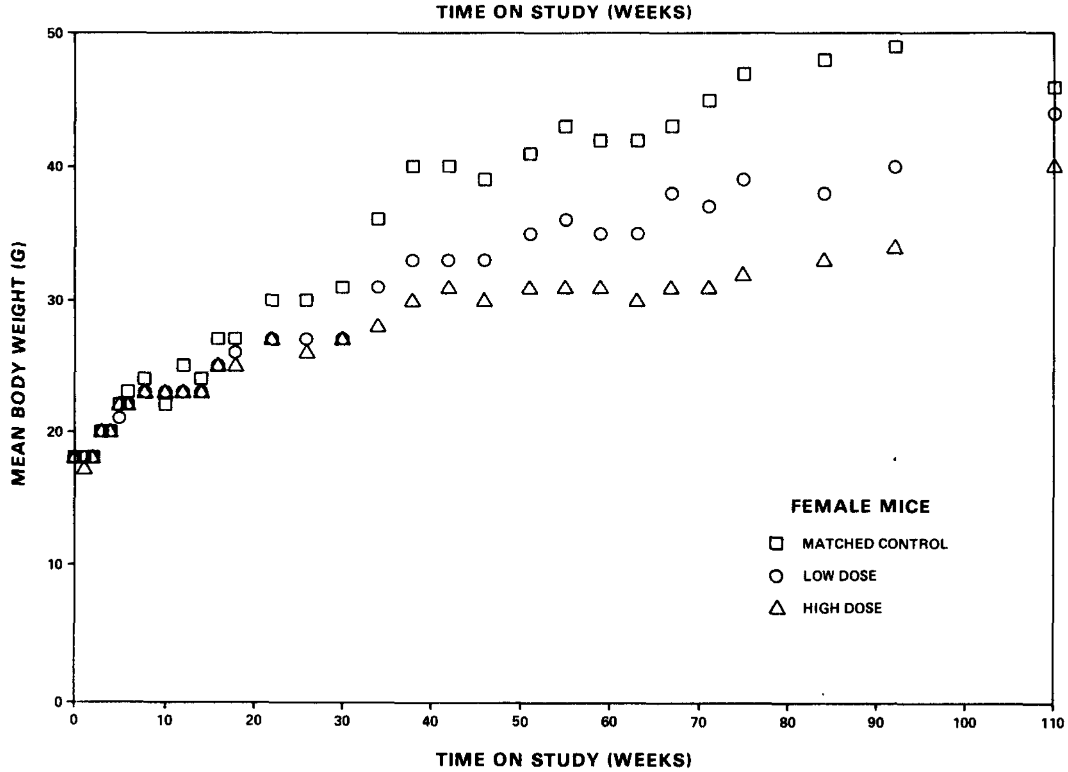


Figure 3. Growth Curves for Mice Administered BHT in the Diet

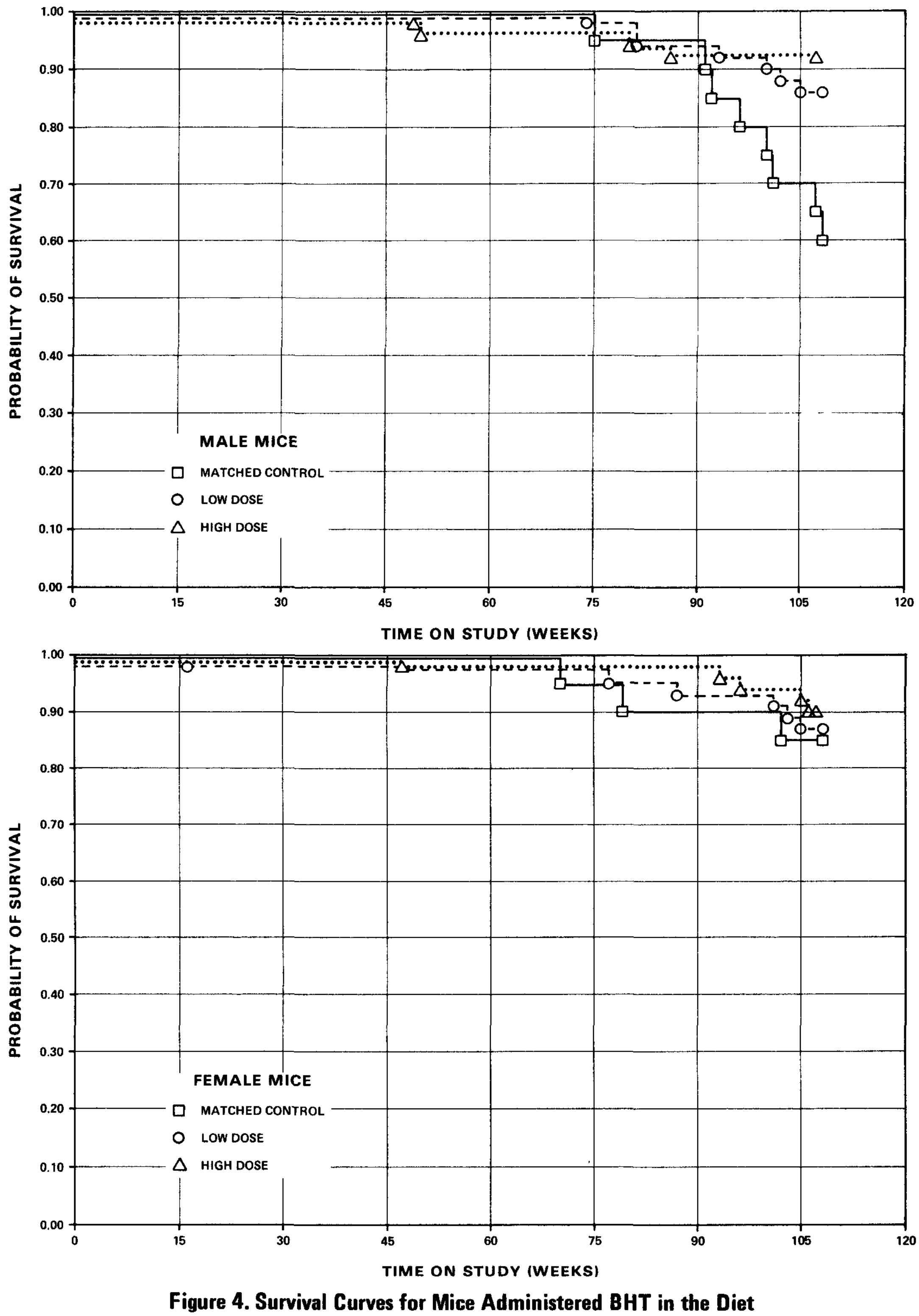


Figure 4. Survival Curves for Mice Administered BHT in the Diet

high-dose group, 41/50 (82%) of the low-dose group, and 17/20 (85%) of the control group lived to the end of the bioassay. Sufficient numbers of mice of each sex were at risk for the development of late-appearing tumors.

C. Pathology (Mice)

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

The liver was the most common organ to have proliferative lesions. The incidences of the lesions are summarized as follows:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissues Examined	20	48	49	20	46	49
LIVER						
Hepatocytomegaly	0(0%)	9(19%)	20(41%)	0(0%)	1(2%)	1(2%)
Hepatocellular Adenoma	2(10%)	11(23%)	7(14%)	0(0%)	3(7%)	2(4%)
Hepatocellular Carcinoma	9(45%)	12(25%)	6(12%)	1(5%)	1(2%)	3(6%)
Angiosarcoma	1(5%)	0(0%)	1(2%)	1(5%)	1(2%)	1(2%)
Peliosis	0(0%)	34(71%)	43(88%)	0(0%)	0(0%)	0(0%)
Hepatocellular Degeneration and Necrosis	2(10%)	34(71%)	45(92%)	0(0%)	0(0%)	0(0%)
Cytoplasmic Vacuolation	3(15%)	20(42%)	22(45%)	0(0%)	0(0%)	0(0%)

Focal hepatocytomegaly was characterized by well-demarcated areas of slightly enlarged hepatocytes. Typically, the cytoplasm of the hepatocytes was more eosinophilic and mildly to severely vacuolated. The edges of these foci were continuous with the surrounding hepatocytes, and there was little or no compression of the adjacent hepatic parenchyma. Multifocal hepatocytomegaly was used to describe less well-demarcated areas of hepatocytic enlargement and cellular change. The hepatocytes within these areas usually were vacuolated or had a slightly more eosinophilic staining quality than the surrounding liver parenchyma. The term "hepatocellular adenoma" was used to describe focal areas of hepatocellular proliferation which compressed the adjacent hepatic parenchyma. Within these foci, there was increased cellular pleomorphism, and mitotic figures were sometimes present. Typically, the cytoplasm of the cells was vacuolated, and it stained slightly more basophilic than the surrounding hepatocytes. Hepatocellular carcinomas were characterized by poorly circumscribed areas of proliferating hepatocytes. As a rule, the cells were basophilic and extremely variable in size, and the cytoplasm varied from being finely vacuolated to containing large, clear vacuoles or large eosinophilic-staining bodies. Nuclear atypia and mitotic figures were common. These growths compressed the adjacent liver parenchyma, but usually had areas of invasion into the adjacent liver lobules. Metastatic nodules of cells having similar morphologic characteristics were found in the lungs of three control and three low-dose male mice. Angiosarcomas were characterized by large, cavernous blood-filled spaces lined by proliferating spindle cells that invaded the adjacent liver parenchyma.

In addition to proliferative lesions of the liver, there was a high incidence of other liver lesions in most of the dosed male mice. These were peliosis, hepatocellular degeneration and necrosis, and varying degrees of hepatocellular vacuolation. Peliosis was characterized by areas of sinusoidal dilatation and spaces containing erythrocytes. These blood-filled spaces were surrounded by cellular màterial resembling hepatocytic cytoplasm and contained free hepatocytic nuclei. Many of these areas resembled foci of intrahepatocytic hemorrhage. These areas were scattered throughout the sections of liver and were primarily located in the midzonal portion of the lobules. Surrounding these areas of peliosis, there were areas of hepatocellular degeneration and necrosis. These hepatocytes showed varying degrees of swelling, hyalinization, and fine to coarse cytoplasmic vacuolation. Admixed with these areas of degenerating hepatocytes were single or multiple enlarged hepatocytes.

Other common neoplasms in mice of this study were pulmonary alveolar/bronchiolar adenomas and carcinomas. The incidence of these lung neoplasms is summarized as follows:

	MALES			FEMALES		
	Control	Low Dose	High Dose	Control	Low Dose	High Dose
Number of Animals with Tissues Examined	20	50	49	20	46	50
Alveolar/Bronchiolar Carcinoma	5(25%)	12(24%)	7(14%)	1(5%)	4(9%)	4(8%)
Adenoma	2(10%)	9(18%)	10(20%)	0(0%)	12(26%)	3(6%)

The alveolar/bronchiolar adenomas were characterized by circumscribed masses of well-differentiated cuboidal epithelial cells resting on a thin, fibrovascular stroma. These masses often compressed the surrounding pulmonary parenchyma, and on occasion protruded into the lumen of a bronchiole or elevated the pleura. The alveolar/bronchiolar carcinomas were usually large in size and less circumscribed than the adenomas; they usually invaded the surrounding lung parenchyma. The cells stained more basophilic, were piled up on one another, and showed cellular pleomorphism. In several of the mice with alveolar/bronchiolar adenocarcinomas, the pulmonary parenchyma adjacent to the tumor contained intra-alveolar mononuclear or multinucleated cells containing richly eosinophilic-staining cytoplasmic material.

Adenomas of the eye/lacrimal gland occurred in four high-dose male mice and in two low-dose females but not in corresponding controls. The significance of these findings is difficult to evaluate, however, since only animals with grossly apparent lesions at necropsy were examined microscopically.

Several inflammatory and neoplastic and nonneoplastic proliferative lesions commonly seen in aged B6C3F1 mice were observed, and the incidences were about the same in the control and dosed groups of mice.

Based on the histopathologic examination, under the conditions of this bioassay, the administration of BHT was associated with a high incidence of nonneoplastic hepatocellular changes in dosed male B6C3F1 mice compared with controls. Also, there was an increased incidence of lung tumors in the female mice.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, four adenomas of the eye/lacrimal gland are observed in the high-dose group, but none in the other two groups. The result of the Cochran-Armitage test for positive dose-related trend is significant (P = 0.039), but the results of the Fisher exact test are not significant. The historical records of this laboratory show an incidence of 5/422 (1.2%) as compared with 0/20 in the control group, 0/50 in the low-dose group, and 4/50 (8%) in the high-dose group of this study.

The incidence of alveolar/bronchiolar carcinomas or adenomas in low-dose female mice is significantly higher (P = 0.009) than that in the control group, but the incidence in the high-dose group is not significant. Historical records at this laboratory indicate that female control mice had an incidence of alveolar/bronchiolar carcinomas or adenomas of 21/440 (4.7%), compared with 1/20 (5%) in the female controls in this study, 16/46 (35%) in the low-dose group, and 7/50 (14%) in the high-dose group. The result of the Cochran-Armitage test also is not significant.

Significant results in the negative direction are observed in the incidence of tumors of the liver in male mice and in the incidence of sarcomas of multiple organs in female mice.

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

V. DISCUSSION

Mean body weights of the dosed rats and mice were lower than those of the corresponding controls and were dose related throughout most of the bioassay. Survival was not affected adversely in any of the dosed groups of rats or mice and was 60% or greater in all dosed or control groups of rats and mice of each sex at the end of the bioassay. Sufficient numbers of animals were at risk for the development of late-appearing tumors.

No neoplastic lesions occurred in the rats or mice at incidences that could clearly be related to administration of the BHT. Nonneoplastic lesions that may have been related to the test chemical consisted of focal alveolar histiocytosis at increased incidences in the lungs of dosed female rats and various lesions of the liver, including peliosis, hepatocellular degeneration and necrosis, cytoplasmic vacuolation, and hepatocytomegaly at increased incidences in the dosed male mice. Four high-dose male mice were observed to have adenomas of the lacrimal gland; however, these tumors cannot clearly be related to administration of the test compound, since all glands were not examined in the same manner. Alveolar/bronchiolar carcinomas or adenomas occurred at a significant incidence ($P = 0.009$) in the low-dose female mice; however, the incidence of the tumor in the high-dose group was not significant, and the overall incidences were not significantly dose related (control 1/20, low-dose 16/46, high-dose 7/50). Historical records at this laboratory indicate that female control mice had an incidence of alveolar/bronchiolar carcinomas or adenomas of 21/440 (4.7%), compared with 1/20 (5%) in the female controls in this study, 16/46 (35%) in the low-dose group, and 7/50 (14%) in the high-dose group. Thus, the occurrence of lung tumors in the low-dose female mice cannot clearly be related to administration of the test chemical.

In previous studies by others, the effects of BHT in tumor initiation, promotion, and protection have been investigated, and the results indicate that the temporal sequence between BHT administration and exposure to a known carcinogen may be important. Administration of BHT in feed at doses of 2,000, 5,000, 8,000, or 10,000 ppm for 2 years to male and female rats of unspecified strain induced no pathologic lesions; however, weight gain in the animals administered 10,000 ppm was subnormal indicating that a maximum tolerated dose may have been exceeded (Deichmann et al., 1955). Administration of BHT in a single oral dose of 200 mg in olive oil to female Sprague-Dawley rats prior to oral administration of 12 mg of dimethylbenz(a)anthracene (DMBA) in olive oil resulted in a decrease in the incidence of mammary tumors when comparisons were made with incidences of the tumors induced by DMBA alone (Wattenberg, 1972). Also, administration of BHT at 6,600 ppm for 24 weeks to male and for 32 weeks to female CD SPF rats that were simultaneously administered 2-acetylaminofluorene (AAF) at 223 ppm or N-hydroxy AAF at 239 ppm decreased the incidences of hepatomas in the males administered AAF or N-hydroxy AAF and the incidences of mammary carcinomas in the females administered N-hydroxy AAF when these organs were examined 12 to 13 weeks later and comparisons were made with incidences of the tumors induced by AAF or N-hydroxy AAF alone (Ulland et al., 1973). Administration of BHT alone in feed under the same conditions induced no tumors of the liver or mammary gland. In contrast, administration of BHT in feed at 5,000 ppm for 407 days to male Sprague-Dawley rats following previous administration of AAF in feed at 200 ppm for 18 days caused an increase in the incidences of liver tumors, compared with the incidences of the tumors induced by AAF alone (Peraino et al., 1977).

In a study using mice, administration of BHT alone in feed at 7,500 ppm to male BALB/c mice for 16 months increased the incidences of tumors of the lung and of the stomach, compared with incidences of the respective tumors in untreated controls, but decreased the incidence of reticulum-cell sarcomas (Clapp et al., 1974). Also, in another study using mice, administration of BHT alone in feed to CF1 mice at 1,000 ppm for the first 1 or 2 months, then at 1,000, 2,500, or 5,000 ppm for 22 to 23 months, led to dose-related increases in the incidences of lung tumors; in addition, the incidence of tumors of the ovary was reported to be increased in the female CF1 mice administered the BHT (Brooks et al., 1977). When, however, BHT was administered in tricaprylin by intraperitoneal injection at doses of 250 mg/kg three times daily for 8 weeks to male and female A/He mice and the animals held for an additional 16 weeks, it had no significant effect on the incidence of lung tumors (Stoner et al., 1973).

Administration of BHT in feed at 5,000 ppm for 2 weeks to female A/HeJ mice simultaneously administered benzo(a)pyrene (BP) at 1,000 ppm decreased the incidence of the tumors induced by BP alone (Wattenberg, 1972). Similarly, administration of BHT in feed at 7,500 ppm for 7 weeks to male and female BALB/c mice simultaneously administered diethylnitrosamine (DEN) in the drinking water at 350 mg/kg body weight decreased the incidence of carcinomas of the stomach in the females, but not in the males, when comparisons were made with the incidences induced by the DEN alone (Clapp et al., 1976).

However, when BHT was administered as a promotor, i.e., by intraperitoneal injection in corn oil to male Swiss-Webster mice at doses of 250 mg/kg weekly for 13 weeks following intraperitoneal injection of single doses of urethane at 1 mg/g, the numbers of tumors per lung was increased when comparisons were made with the numbers of tumors per lung induced by urethane alone. The opposite effect was observed when 0.9% NaCl was injected instead of the urethane, administration of the BHT then resulting in the complete absence of lung tumors, compared with the occurrence of lung tumors in the untreated controls (Witschi et al., 1977).

Thus, in previous studies, BHT administered alone did not increase the incidence of tumors in rats, but the incidences of tumors in mice were increased. In the present study, again using BHT alone, lung tumors were observed at an increased but equivocal incidence in female mice. In other previous studies, BHT protected against carcinogenesis in rats and mice when it was administered prior to or simultanously with exposure to a carcinogen. In contrast, however, when BHT was administered to rats and mice as a promoter, e.g., following a carcinogen, the incidence of tumors was increased.

It is concluded that under the conditions of this bioassay, increased incidences of focal alveolar histiocytosis in dosed female rats and various nonneoplastic lesions of the liver in dosed male mice may have been related to the administration of BHT. BHT was not, however, carcinogenic for F344 rats or B6C3F1 mice of either sex.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

RATS ADMINISTERED BHT IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(49)	(50)
SQUAMOUS CELL CARCINOMA		2 (4%)	
BASAL-CELL CARCINOMA			1 (2%)
*SUBCUT TISSUE	(20)	(49)	(50)
FIBROMA		2 (4%)	
AMELOBLASTIC ODONTOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA			2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(20)	(49)	(49)
MALIGNANT RETICULOSIS		1 (2%)	
*MULTIPLE ORGANS	(20)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE	4 (20%)	9 (18%)	10 (20%)
#SPLEEN	(20)	(48)	(47)
HEMANGIOSARCOMA	1 (5%)		
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(48)
SQUAMOUS CELL CARCINOMA, METASTA		1 (2%)	
#SALIVARY GLAND	(20)	(49)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(48)
BILE DUCT CARCINOMA			1 (2%)
NEOPLASTIC NODULE		1 (2%)	1 (2%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#SMALL INTESTINE	(18)	(48)	(48)
LIPOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
NEPHROBLASTOMA		1 (2%)	
#URINARY BLADDER	(20)	(47)	(46)
TRANSITIONAL-CELL CARCINOMA		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(47)
CARCINOMA,NOS	1 (5%)		
ADENOMA, NOS	6 (32%)	9 (19%)	9 (19%)
#ADRENAL	(19)	(49)	(48)
CORTICAL CARCINOMA			2 (4%)
PHEOCHROMOCYTOMA	2 (11%)	8 (16%)	10 (21%)
#ADRENAL/CAPSULE	(19)	(49)	(48)
PARAGANGLIOMA, NOS		1 (2%)	
#THYROID	(20)	(49)	(48)
FOLLICULAR-CELL ADENOMA		2 (4%)	
FOLLICULAR-CELL CARCINOMA	1 (5%)	2 (4%)	1 (2%)
C-CELL ADENOMA	1 (5%)	5 (10%)	1 (2%)
C-CELL CARCINOMA		1 (2%)	1 (2%)
#PARATHYROID	(18)	(45)	(43)
ADENOMA, NOS		1 (2%)	

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(19)	(48)	(48)
ISLET-CELL ADENOMA		2 (4%)	1 (2%)
ISLET-CELL CARCINOMA		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(49)	(50)
CARCINOMA,NOS		3 (6%)	
#TESTIS	(20)	(49)	(49)
INTERSTITIAL-CELL TUMOR	15 (75%)	42 (86%)	32 (65%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(49)	(49)
MENINGIOMA	1 (5%)		
#BRAIN	(20)	(49)	(49)
GLIOMA, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(20)	(49)	(50)
CARCINOMA,NOS			1 (2%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(49)	(50)
LIPOMA		1 (2%)	
*TUNICA VAGINALIS	(20)	(49)	(50)
MESOTHELIOMA, NOS	1 (5%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(49)	(50)
FIBROSARCOMA	1 (5%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	5	11
MORIBUND SACRIFICE	3	5	3
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	39	36
ANIMAL MISSING		1	

@ INCLUDES AUTOLYZED ANIMALS

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	19	46	44
TOTAL PRIMARY TUMORS	36	100	80
TOTAL ANIMALS WITH BENIGN TUMORS	18	45	41
TOTAL BENIGN TUMORS	25	72	57
TOTAL ANIMALS WITH MALIGNANT TUMORS	9	19	20
TOTAL MALIGNANT TUMORS	10	26	22
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	
TOTAL SECONDARY TUMORS		2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	1
TOTAL UNCERTAIN TUMORS	1	2	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	18	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(18)	(50)	(50)
CARCINOMA,NOS			1 (2%)
*SUBCUT TISSUE	(18)	(50)	(50)
FIBROMA		1 (2%)	
OSTEOSARCOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(49)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (6%)	2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#BRAIN	(18)	(49)	(50)
MALIGNANT RETICULOSIS		1 (2%)	
*MULTIPLE ORGANS	(18)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (6%)	2 (4%)	1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE	1 (6%)	8 (16%)	4 (8%)
#THYMUS	(17)	(43)	(45)
THYMOMA			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(49)
ADENOMA, NOS	8 (44%)	9 (19%)	5 (10%)
#ADRENAL	(17)	(47)	(49)
PHEOCHROMOCYTOMA		2 (4%)	1 (2%)
#THYROID	(18)	(48)	(49)
FOLLICULAR-CELL ADENOMA		2 (4%)	
FOLLICULAR-CELL CARCINOMA		1 (2%)	
C-CELL ADENOMA	2 (11%)	4 (8%)	4 (8%)
#PANCREATIC ISLETS	(17)	(46)	(47)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(18)	(50)	(50)
ADENOCARCINOMA, NOS		2 (4%)	
FIBROADENOMA	5 (28%)	7 (14%)	5 (10%)
*CLITORAL GLAND	(18)	(50)	(50)
CARCINOMA,NOS		1 (2%)	
#UTERUS	(17)	(49)	(49)
CARCINOMA,NOS			1 (2%)
ENDOMETRIAL STROMAL POLYP	2 (12%)	8 (16%)	6 (12%)
#OVARY	(17)	(49)	(49)
THECOMA	1 (6%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(18)	(50)	(50)
CARCINOMA,NOS			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	11	9
MORIBUND SACRIFICE	1	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	13	37	39
ANIMAL MISSING	2		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	36	26
TOTAL PRIMARY TUMORS	21	53	31
TOTAL ANIMALS WITH BENIGN TUMORS	11	27	18
TOTAL BENIGN TUMORS	19	36	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	16	9
TOTAL MALIGNANT TUMORS	2	17	9
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS

SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE ADMINISTERED BHT IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(49)
HEPATOCELLULAR CARCINOMA, METAST	3 (15%)	3 (6%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	9 (18%)	10 (20%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	5 (25%)	12 (24%)	7 (14%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	5 (10%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		4 (8%)	1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (10%)		
#SPLEEN	(19)	(50)	(48)
ANGIOSARCOMA	1 (5%)	1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#BRONCHIAL LYMPH NODE	(20)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
#MESENTERIC L. NODE	(20)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		2 (4%)	2 (4%)
#SMALL INTESTINE	(19)	(48)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYMUS	(10)	(39)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (10%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(49)
HEPATOCELLULAR ADENOMA	2 (10%)	11 (23%)	7 (14%)
HEPATOCELLULAR CARCINOMA	9 (45%)	12 (25%)	6 (12%)
ANGIOSARCOMA	1 (5%)		1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
ENDOCRINE SYSTEM			
#ADRENAL	(20)	(49)	(49)
CORTICAL ADENOMA	1 (5%)		
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(18)	(48)	(49)
FOLLICULAR-CELL ADENOMA		2 (4%)	2 (4%)
FOLLICULAR-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(49)
EPENDYMOMA		1 (2%)	

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(50)	(50)
ADENOMA, NOS			4 (8%)
*EAR	(20)	(50)	(50)
FIBROMA			2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(50)
SARCOMA, NOS, METASTATIC		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS	1 (5%)		1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	8	6	4
MORIBUND SACRIFICE		1	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	12	43	46
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	17	39	32
TOTAL PRIMARY TUMORS	28	65	48
TOTAL ANIMALS WITH BENIGN TUMORS	4	20	19
TOTAL BENIGN TUMORS	5	23	25
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	32	19
TOTAL MALIGNANT TUMORS	23	42	23
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	4	
TOTAL SECONDARY TUMORS	4	5	
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 ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	
ANIMALS NECROPSIED	20	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	46	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		12 (26%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	4 (9%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(46)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	2 (4%)	6 (12%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	2 (10%)	5 (11%)	
MALIGNANT LYMPHOMA, MIXED TYPE	1 (5%)		
#SPLEEN	(20)	(45)	(50)
ANGIOSARCOMA	2 (10%)		1 (2%)
MALIGNANT LYMPHOMA, NOS	2 (10%)		
#MESENTERIC L. NODE	(20)	(44)	(49)
ANGIOSARCOMA, METASTATIC	1 (5%)		
MALIGNANT LYMPHOMA, NOS			1 (2%)
#SMALL INTESTINE	(20)	(45)	(48)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
#THYMUS	(17)	(37)	(33)
MALIGNANT LYMPHOMA, NOS			1 (3%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(46)	(49)
HEPATOCELLULAR ADENOMA		3 (7%)	2 (4%)
HEPATOCELLULAR CARCINOMA	1 (5%)	1 (2%)	3 (6%)
SARCOMA, NOS		1 (2%)	
ANGIOSARCOMA	1 (5%)	1 (2%)	1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(45)	(47)
ADENOMA, NOS		4 (9%)	1 (2%)
#ADRENAL	(20)	(46)	(48)
CORTICAL ADENOMA	1 (5%)		1 (2%)
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(20)	(46)	(49)
FOLLICULAR-CELL ADENOMA			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(46)	(50)
ADENOCARCINOMA, NOS			2 (4%)
#UTERUS	(20)	(45)	(49)
PAPILLARY CYSTADENOCARCINOMA,NOS			1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (5%)	1 (2%)	
ANGIOMA			1 (2%)
#OVARY/OVIDUCT	(20)	(45)	(49)
PAPILLARY ADENOMA		1 (2%)	1 (2%)
#OVARY	(19)	(45)	(47)
PAPILLARY ADENOMA		1 (2%)	
PAPILLARY CYSTADENOMA, NOS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(46)	(50)
ADENOMA, NOS		2 (4%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(46)	(50)
SARCOMA, NOS	3 (15%)	1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	3	6	5
MORIBUND SACRIFICE			
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	41	45
ANIMAL MISSING		3	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	14	32	23
TOTAL PRIMARY TUMORS	17	42	31
TOTAL ANIMALS WITH BENIGN TUMORS	2	22	10
TOTAL BENIGN TUMORS	2	26	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	13	16	17
TOTAL MALIGNANT TUMORS	15	16	20
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC

LESIONS IN RATS ADMINISTERED BHT IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	20	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	49	49
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(49)
HEMORRHAGE		1 (2%)	
BRONCHOPNEUMONIA SUPPURATIVE	1 (5%)		
BRONCHOPNEUMONIA, ACUTE	1 (5%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM		3 (6%)	3 (6%)
#LUNG/ALVEOLI	(20)	(49)	(49)
HISTIOCYTOSIS	1 (5%)	4 (8%)	7 (14%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(48)	(48)
MYELOFIBROSIS			1 (2%)
#SPLEEN	(20)	(48)	(47)
HEMOSIDEROSIS			1 (2%)
HEMATOPOIESIS		9 (19%)	1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(48)
LYMPHANGIECTASIS	2 (10%)	5 (10%)	3 (6%)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(20)	(49)	(48)
LYMPHANGIECTASIS		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(49)	(49)
PERIARTERITIS		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#HEART/ATRIUM	(20)	(49)	(49)
THROMBOSIS, NOS	2 (10%)	1 (2%)	1 (2%)
#MYOCARDIUM	(20)	(49)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
FIBROSIS	1 (5%)	10 (20%)	8 (16%)
*CORONARY ARTERY	(20)	(49)	(50)
ARTERIOSCLEROSIS, NOS	1 (5%)		
MEDIAL CALCIFICATION		1 (2%)	
*PULMONARY ARTERY	(20)	(49)	(50)
MEDIAL CALCIFICATION		6 (12%)	
*MESENTERIC ARTERY	(20)	(49)	(50)
ARTERIOSCLEROSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(48)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL		2 (4%)	1 (2%)
METAMORPHOSIS FATTY	2 (10%)		1 (2%)
CYTOPLASMIC VACUOLIZATION		13 (27%)	9 (19%)
HEPATOCYTOMEGALY	3 (15%)	11 (23%)	2 (4%)
HYPERPLASIA, FOCAL	1 (5%)	3 (6%)	
#LIVER/CENTRILOBULAR	(20)	(48)	(48)
DEGENERATION, NOS	1 (5%)	1 (2%)	
NECROSIS, NOS			2 (4%)
NECROSIS, DIFFUSE			1 (2%)
#LIVER/PERIPORTAL	(20)	(48)	(48)
FIBROSIS		1 (2%)	
#BILE DUCT	(20)	(48)	(48)
HYPERPLASIA, NOS	16 (80%)	8 (17%)	5 (10%)
#PANCREAS	(19)	(48)	(48)
CYSTIC DUCTS			1 (2%)
PERIARTERITIS		4 (8%)	2 (4%)
#PANCREATIC ACINUS	(19)	(48)	(48)
ATROPHY, NOS		3 (6%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, FOCAL	2 (11%)	6 (13%)	6 (13%)
#STOMACH	(20)	(49)	(48)
ULCER, FOCAL			2 (4%)
#SMALL INTESTINE	(18)	(48)	(48)
HYPERPLASIA, LYMPHOID		3 (6%)	
#LARGE INTESTINE	(19)	(48)	(47)
NEMATODIASIS	2 (11%)	1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(48)
PYELONEPHRITIS, ACUTE			1 (2%)
INFLAMMATION, CHRONIC	19 (95%)	48 (98%)	46 (96%)
#KIDNEY/CORTEX	(20)	(49)	(48)
CYST, NOS			2 (4%)
#PROXIMAL CONVOLUTED	(20)	(49)	(48)
PIGMENTATION, NOS		1 (2%)	
#URINARY BLADDER	(20)	(47)	(46)
INFLAMMATION, ACUTE HEMORRHAGIC			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(47)	(47)
CYST, NOS	1 (5%)	1 (2%)	2 (4%)
HEMORRHAGE		1 (2%)	
INFARCT, NOS		1 (2%)	
ANGIECTASIS	1 (5%)		1 (2%)
#ADRENAL CORTEX	(19)	(49)	(48)
LIPOIDOSIS	2 (11%)	2 (4%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	2 (11%)	3 (6%)	
#ADRENAL MEDULLA	(19)	(49)	(48)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	1 (5%)	1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#THYROID	(20)	(49)	(48)
CYSTIC FOLLICLES		4 (8%)	1 (2%)
FOLLICULAR CYST, NOS			2 (4%)
HYPERPLASIA, C-CELL	4 (20%)	15 (31%)	15 (31%)
#PANCREATIC ISLETS	(19)	(48)	(48)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(49)	(50)
DILATATION/DUCTS		2 (4%)	1 (2%)
#PROSTATE	(20)	(49)	(48)
INFLAMMATION, SUPPURATIVE	2 (10%)	5 (10%)	11 (23%)
INFLAMMATION, ACUTE		4 (8%)	2 (4%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (5%)		3 (6%)
INFLAMMATION, ACUTE HEMORRHAGIC			1 (2%)
INFLAMMATION, CHRONIC		1 (2%)	
#TESTIS	(20)	(49)	(49)
ATROPHY, NOS	1 (5%)	1 (2%)	
HYPERPLASIA, INTERSTITIAL CELL		2 (4%)	4 (8%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
MINERALIZATION		1 (2%)	
HEMORRHAGE	2 (10%)		4 (8%)
SPECIAL SENSE ORGANS			
*EYE	(20)	(49)	(50)
CATARACT		4 (8%)	3 (6%)
*EYE/CORNEA	(20)	(49)	(50)
ULCER, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(20)	(49)	(50)
HEMORRHAGE			1 (2%)
PERIARTERITIS		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
ANIMAL MISSING/NO NECROPSY		1	
AUTO/NECROPSY/NO HISTO			1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	18	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	18	49	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(18)	(48)	(49)
BRONCHOPNEUMONIA, ACUTE		1 (2%)	1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	3 (17%)	2 (4%)	4 (8%)
#LUNG/ALVEOLI	(18)	(48)	(49)
HISTIOCYTOSIS	2 (11%)	12 (25%)	21 (43%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(17)	(48)	(49)
HEMOSIDEROSIS	1 (6%)	2 (4%)	
LYMPHOID DEPLETION		1 (2%)	
HEMATOPOIESIS	2 (12%)	5 (10%)	4 (8%)
#MANDIBULAR L. NODE	(18)	(48)	(49)
LYMPHANGIECTASIS	1 (6%)		
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(18)	(48)	(49)
LYMPHANGIECTASIS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(49)	(50)
PERIARTERITIS	1 (6%)		1 (2%)
#MYOCARDIUM	(18)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
*PULMONARY ARTERY	(18)	(50)	(50)
MEDIAL CALCIFICATION	1 (6%)	3 (6%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(17)	(48)	(49)
INFLAMMATION, NECROTIZING		2 (4%)	
GRANULOMA, NOS			1 (2%)
CHOLANGIOFIBROSIS	1 (6%)		2 (4%)
METAMORPHOSIS FATTY	1 (6%)		
LIPOIDOSIS	1 (6%)		
CYTOPLASMIC VACUOLIZATION	1 (6%)	3 (6%)	
HEPATOCYTOMEGALY		4 (8%)	
HYPERPLASIA, FOCAL	11 (65%)	16 (33%)	5 (10%)
ANGIECTASIS		1 (2%)	
#BILE DUCT	(17)	(48)	(49)
HYPERPLASIA, NOS	2 (12%)	15 (31%)	9 (18%)
#PANCREAS	(17)	(46)	(47)
PERIARTERITIS			1 (2%)
#PANCREATIC ACINUS	(17)	(46)	(47)
ATROPHY, FOCAL		5 (11%)	2 (4%)
#GASTRIC MUCOSA	(17)	(48)	(49)
MINERALIZATION	1 (6%)		
#SMALL INTESTINE	(17)	(46)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#SMALL INTEST./SEROSA	(17)	(46)	(49)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
#LARGE INTESTINE	(17)	(46)	(49)
NEMATODIASIS		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(17)	(48)	(49)
HEMORRHAGIC CYST			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
GLOMERULONEPHRITIS, ACUTE		1 (2%)	
PYELONEPHRITIS, ACUTE			1 (2%)
INFLAMMATION, CHRONIC	8 (47%)	23 (48%)	28 (57%)
NEPHROSIS, NOS		1 (2%)	
GLOMERULOSCLEROSIS, NOS	1 (6%)		
#PERIRENAL TISSUE	(17)	(48)	(49)
HEMORRHAGE			1 (2%)
#URINARY BLADDER	(16)	(47)	(48)
INFLAMMATION, ACUTE HEMORRHAGIC			1 (2%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(49)
CYST, NOS	2 (11%)	1 (2%)	4 (8%)
HEMORRHAGIC CYST		1 (2%)	
ANGIECTASIS	4 (22%)	3 (6%)	4 (8%)
#ADRENAL	(17)	(47)	(49)
NECROSIS, FOCAL			1 (2%)
#ADRENAL CORTEX	(17)	(47)	(49)
LIPOIDOSIS	3 (18%)	2 (4%)	
HYPERPLASIA, NOS			2 (4%)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
#ADRENAL MEDULLA	(17)	(47)	(49)
HYPERPLASIA, FOCAL	1 (6%)		
ANGIECTASIS	1 (6%)		
#THYROID	(18)	(48)	(49)
CYSTIC FOLLICLES		1 (2%)	3 (6%)
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, C-CELL	4 (22%)	7 (15%)	12 (24%)
HYPERPLASIA, FOLLICULAR-CELL		1 (2%)	
#PARATHYROID	(16)	(41)	(38)
HYPERPLASIA, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(18)	(50)	(50)
DILATATION/DUCTS	1 (6%)	4 (8%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#CERVIX UTERI	(17)	(49)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	
POLYP			1 (2%)
#UTERUS/ENDOMETRIUM	(17)	(49)	(49)
INFLAMMATION, ACUTE		1 (2%)	
HYPERPLASIA, CYSTIC		3 (6%)	
#OVARY	(17)	(49)	(49)
CYSTIC FOLLICLES	2 (12%)	2 (4%)	3 (6%)
NERVOUS SYSTEM			
#BRAIN	(18)	(49)	(50)
HEMORRHAGE		1 (2%)	4 (8%)
NECROSIS, FOCAL		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(18)	(50)	(50)
FIBROSIS, FOCAL	1 (6%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			3

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMAL MISSING/NO NECROPSY	2		
AUTO/NECROPSY/NO HISTO		1	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED BHT IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NOS			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
HEMORRHAGIC CYST			1 (2%)
RESPIRATORY SYSTEM			
#TRACHEA	(19)	(49)	(49)
HEMORRHAGE		4 (8%)	
#TRACHEAL GLAND	(19)	(49)	(49)
DILATATION, NOS	1 (5%)		
#LUNG	(20)	(50)	(49)
HEMORRHAGE		1 (2%)	3 (6%)
INFLAMMATION, NOS	4 (20%)	3 (6%)	5 (10%)
PROTEINOSIS, ALVEOLAR	2 (10%)	6 (12%)	3 (6%)
HYPERPLASIA, LYMPHOID		1 (2%)	
HEMATOPOIETIC SYSTEM			
*BLOOD	(20)	(50)	(50)
LEUKOCYTOSIS, NOS		1 (2%)	
RETICULOCYTOSIS		1 (2%)	
#SPLEEN	(19)	(50)	(48)
CONGESTION, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL	1 (5%)	4 (8%)	
HEMATOPOIESIS	5 (26%)	12 (24%)	7 (15%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(20)	(49)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(20)	(49)	(49)
MINERALIZATION			1 (2%)
HEMOSIDEROSIS	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)	2 (4%)	3 (6%)
#MESENTERIC L. NODE	(20)	(49)	(49)
CONGESTION, NOS		1 (2%)	2 (4%)
LIPOIDOSIS			1 (2%)
HYPERPLASIA, RETICULUM CELL	1 (5%)	2 (4%)	1 (2%)
HYPERPLASIA, LYMPHOID			4 (8%)
HEMATOPOIESIS	1 (5%)		
#THYMUS	(10)	(39)	(46)
HYPERPLASIA, LYMPHOID		1 (3%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(49)
MINERALIZATION	1 (5%)		
#MYOCARDIUM	(20)	(50)	(49)
INFLAMMATION, NOS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(48)	(49)
HEMORRHAGE			1 (2%)
INFLAMMATION, NOS		2 (4%)	
INFLAMMATION, FOCAL	11 (55%)	21 (44%)	27 (55%)
GRANULOMA, NOS		1 (2%)	
PELIOSIS HEPATIS		34 (71%)	43 (88%)
NECROSIS, FOCAL	2 (10%)	1 (2%)	2 (4%)
NECROSIS, CYTODEGENERATIVE		33 (69%)	43 (88%)
CYTOPLASMIC VACUOLIZATION	3 (15%)	20 (42%)	22 (45%)
BASOPHILIC CYTO CHANGE		2 (4%)	
EOSINOPHILIC CYTO CHANGE			1 (2%)
HEPATOCYTOMEGALY		9 (19%)	20 (41%)
HEMATOPOIESIS		1 (2%)	
*GALLBLADDER	(20)	(50)	(50)
CAST, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, PAPILLARY		1 (2%)	
#BILE DUCT	(20)	(48)	(49)
INFLAMMATION, NOS			2 (4%)
HYPERPLASIA, NOS			1 (2%)
#PANCREAS	(17)	(47)	(46)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL		1 (2%)	3 (7%)
NECROSIS, FAT	1 (6%)		1 (2%)
ATROPHY, NOS	2 (12%)	1 (2%)	1 (2%)
#PANCREATIC ACINUS	(17)	(47)	(46)
DEGENERATION, NOS	1 (6%)		
#ESOPHAGUS	(19)	(46)	(47)
HEMORRHAGE		1 (2%)	
#STOMACH	(18)	(49)	(48)
CYST, NOS			1 (2%)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, FOCAL			1 (2%)
#SMALL INTESTINE	(19)	(48)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
#LARGE INTESTINE	(18)	(48)	(46)
HYPERPLASIA, LYMPHOID	1 (6%)		2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(49)
HYDRONEPHROSIS		1 (2%)	
PYELONEPHRITIS, NOS	1 (5%)		
INFLAMMATION, INTERSTITIAL	2 (10%)	3 (6%)	
INFARCT, NOS		2 (4%)	
INFARCT, HEALED	2 (10%)		
CALCINOSIS, NOS			1 (2%)
HYPERPLASIA, TUBULAR CELL	14 (70%)	36 (72%)	40 (82%)
#KIDNEY/TUBULE	(20)	(50)	(49)
DILATATION, NOS		3 (6%)	2 (4%)
#URINARY BLADDER	(18)	(50)	(49)
CAST, NOS		7 (14%)	4 (8%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(14)	(46)	(45)
CYST, NOS			1 (2%)
#ADRENAL CORTEX	(20)	(49)	(49)
FIBROSIS			1 (2%)
HYPERPLASIA, NODULAR		4 (8%)	2 (4%)
HYPERPLASIA, NOS	16 (80%)	43 (88%)	48 (98%)
#ADRENAL MEDULLA	(20)	(49)	(49)
CYST, NOS			1 (2%)
DEGENERATION, NOS			1 (2%)
#THYROID	(18)	(48)	(49)
HYPERPLASIA, FOCAL			2 (4%)
HYPERPLASIA, C-CELL		1 (2%)	
#PANCREATIC ISLETS	(17)	(47)	(46)
HYPERPLASIA, NOS	4 (24%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(20)	(50)	(50)
CYST, NOS		4 (8%)	3 (6%)
INFLAMMATION, NOS			1 (2%)
#PROSTATE	(18)	(48)	(41)
CAST, NOS	1 (6%)	8 (17%)	7 (17%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
*SEMINAL VESICLE	(20)	(50)	(50)
CAST, NOS	1 (5%)		
#TESTIS	(20)	(50)	(49)
GRANULOMA, SPERMATIC		1 (2%)	
ATROPHY, NOS			1 (2%)
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)
#TESTIS/TUBULE	(20)	(50)	(49)
DEGENERATION, NOS			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
*EPIDIDYMIS	(20)	(50)	(50)
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(50)	(49)
INFLAMMATION, FOCAL		1 (2%)	
#BRAIN	(20)	(50)	(49)
MINERALIZATION	5 (25%)	19 (38%)	15 (31%)
HYDROCEPHALUS, INTERNAL		4 (8%)	3 (6%)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(50)
LIPOGRANULOMA	1 (5%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)		2 (4%)
MASTOCYTOSIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/NO HISTO			1

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED BHT IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		3	
ANIMALS NECROPSIED	20	46	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	46	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(46)	(50)
NECROSIS, FAT			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(46)	(50)
INFLAMMATION, NOS	1 (5%)		4 (8%)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	1 (2%)
PROTEINOSIS, ALVEOLAR	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(46)	(50)
MYELOFIBROSIS	15 (75%)	34 (74%)	28 (56%)
#SPLEEN	(20)	(45)	(50)
HEMATOPOIESIS	6 (30%)	20 (44%)	13 (26%)
#MANDIBULAR L. NODE	(20)	(44)	(49)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(20)	(44)	(49)
INFLAMMATION, GRANULOMATOUS	1 (5%)		1 (2%)
HYPERPLASIA, RETICULUM CELL		2 (5%)	
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	1 (5%)		
#THYMUS	(17)	(37)	(33)
HYPERPLASIA, LYMPHOID	1 (6%)		
CIRCULATORY SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(44)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#LIVER	(20)	(46)	(49)
CYST, NOS			1 (2%)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL	12 (60%)	27 (59%)	36 (73%)
NECROSIS, FOCAL	1 (5%)	3 (7%)	2 (4%)
EOSINOPHILIC CYTO CHANGE			1 (2%)
HEPATOCYTOMEGALY		1 (2%)	1 (2%)
LEUKEMOID REACTION			1 (2%)
HEMATOPOIESIS	2 (10%)		2 (4%)
#BILE DUCT	(20)	(46)	(49)
INFLAMMATION, NOS			1 (2%)
#PANCREAS	(18)	(45)	(48)
DILATATION/DUCTS		1 (2%)	
INFLAMMATION, FOCAL	1 (6%)	1 (2%)	
ATROPHY, NOS	1 (6%)	3 (7%)	
ATROPHY, DIFFUSE	1 (6%)		
#PEYERS PATCH	(20)	(45)	(48)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, LYMPHOID			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(46)	(49)
HYDRONEPHROSIS	1 (5%)		
INFLAMMATION, NOS		1 (2%)	
INFARCT, NOS	1 (5%)		
HYPERPLASIA, TUBULAR CELL	2 (10%)	6 (13%)	8 (16%)
HYPERPLASIA, LYMPHOID			4 (8%)
#URINARY BLADDER	(19)	(45)	(47)
INFLAMMATION, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(45)	(47)
HYPERPLASIA, FOCAL		1 (2%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#ADRENAL	(20)	(46)	(48)
HYPERPLASIA, NODULAR		1 (2%)	
LEUKEMOID REACTION			1 (2%)
#ADRENAL CORTEX	(20)	(46)	(48)
HYPERPLASIA, NODULAR		2 (4%)	1 (2%)
HYPERPLASIA, NOS	19 (95%)	39 (85%)	44 (92%)
#THYROID	(20)	(46)	(49)
HYPERPLASIA, FOLLICULAR-CELL		3 (7%)	3 (6%)
#PANCREATIC ISLETS	(18)	(45)	(48)
HYPERPLASIA, NOS			1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(20)	(45)	(49)
HEMORRHAGE		1 (2%)	
PYOMETRA			1 (2%)
#UTERUS/ENDOMETRIUM	(20)	(45)	(49)
HYPERPLASIA, CYSTIC	6 (30%)	24 (53%)	16 (33%)
#OVARY	(19)	(45)	(47)
CYST, NOS	1 (5%)	12 (27%)	4 (9%)
NERVOUS SYSTEM			
#BRAIN	(20)	(46)	(49)
MINERALIZATION	7 (35%)	15 (33%)	8 (16%)
HYDROCEPHALUS, INTERNAL	2 (10%)	4 (9%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*MESENTERY	(20)	(46)	(50)
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(46)	(50)
HYPERPLASIA, LYMPHOID			2 (4%)
HEMATOPOIESIS			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	1
ANIMAL MISSING/NO NECROPSY		3	
AUTO/NECROPSY/HISTO PERF			1
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED BHT IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered BHT in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20(5)	1/49(2)	3/49(6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.408	1.224
Lower Limit		0.005	0.108
Upper Limit		31.413	62.958
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Lymphoma (b)	5/20(25)	9/49(18)	12/50(24)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.735	0.960
Lower Limit		0.262	0.376
Upper Limit		2.517	3.124
Weeks to First Observed Tumor	88	100	76

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Carcinoma, NOS, or Adenoma, NOS (b)	7/19(37)	9/47(19)	9/47(19)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.520	0.520
Lower Limit		0.212	0.212
Upper Limit		1.440	1.440
Weeks to First Observed Tumor	90	76	102
Adrenal: Pheochromocytoma (b)	2/19(11)	8/49(16)	10/48(21)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.551	1.979
Lower Limit		0.355	0.486
Upper Limit		14.223	17.573
Weeks to First Observed Tumor	91	105	94

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	1/20(5)	4/49(8)	1/48(2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.633	0.417
Lower Limit		0.179	0.006
Upper Limit		78.704	32.058
Weeks to First Observed Tumor	105	100	94
Thyroid: C-cell Carcinoma or Adenoma (b)	1/20(5)	6/49(12)	2/48(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		2.449	0.833
Lower Limit		0.332	0.047
Upper Limit		110.166	48.155
Weeks to First Observed Tumor	105	103	94

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Carcinoma or Adenoma (b)	0/19(0)	4/48(8)	2/48(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.383	0.122
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	105	105
Preputial Gland: Carcinoma, NOS (b)	0/20(0)	3/49(6)	0/50(0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.044		
Relative Risk (f)		Infinite	--
Lower Limit		0.255	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	90	--

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Testis: Interstitial-cell Tumor (b)	15/20(75)	42/49(86)	32/49(65)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.143	0.871
Lower Limit		0.883	0.653
Upper Limit		1.577	1.333
Weeks to First Observed Tumor	73	90	75

(a) Dosed groups received 3,000 or 6,000 ppm.

(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 matched-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 the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered BHT in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/18(6)	3/48(6)	1/49(2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.125	0.367
Lower Limit		0.100	0.005
Upper Limit		57.811	28.279
Weeks to First Observed Tumor	105	105	105
Hematopoietic System: Lymphoma (b)	2/18(11)	10/50(20)	5/50(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.800	0.900
Lower Limit		0.445	0.168
Upper Limit		15.993	8.989
Weeks to First Observed Tumor	92	87	73

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	8/18(44)	9/48(19)	5/49(10)
P Values (c,d)	P = 0.003(N)	P = 0.038(N)	P = 0.004(N)
Relative Risk (f)		0.422	0.230
Lower Limit		0.184	0.074
Upper Limit		1.086	0.697
Weeks to First Observed Tumor	87	78	84
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	0/18(0)	3/48(6)	0/49(0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.049		
Relative Risk (f)		Infinite	--
Lower Limit		0.236	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	105	--

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Adenoma (b)	2/18(11)	4/48(8)	4/49(8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.750	0.735
Lower Limit		0.122	0.119
Upper Limit		7.883	7.727
Weeks to First Observed Tumor	105	105	105
Mammary Gland: Fibroadenoma (b)	5/18(28)	7/50(14)	5/50(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.504	0.360
Lower Limit		0.165	0.098
Upper Limit		1.814	1.416
Weeks to First Observed Tumor	87	101	98

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Uterus: Endometrial Stromal Polyp (b)	2/17(12)	8/49(16)	6/49(12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.388	1.041
Lower Limit		0.322	0.215
Upper Limit		12.696	10.000
Weeks to First Observed Tumor	105	105	93

(a) Dosed groups received 3,000 or 6,000 ppm.

(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 matched-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 the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE ADMINISTERED BHT IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered BHT in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	5/20(25)	12/50(24)	7/49(14)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.960	0.571
Lower Limit		0.376	0.184
Upper Limit		3.124	2.068
Weeks to First Observed Tumor	75	81	107
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	7/20(35)	21/50(42)	17/49(35)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.991
Lower Limit		0.609	0.482
Upper Limit		2.876	2.452
Weeks to First Observed Tumor	75	81	107

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	5/20(25)	14/50(28)	8/50(16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.120	0.640
Lower Limit		0.457	0.218
Upper Limit		3.556	2.250
Weeks to First Observed Tumor	108	74	107
Liver: Hepatocellular Carcinoma (b)	9/20(45)	12/48(25)	6/49(12)
P Values (c,d)	P = 0.003(N)	N.S.	P = 0.005(N)
Relative Risk (f)		0.556	0.272
Lower Limit		0.271	0.098
Upper Limit		1.283	0.749
Weeks to First Observed Tumor	91	81	107

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma (b)	11/20(55)	23/48(48)	13/49(27)
P Values (c,d)	P = 0.009(N)	N.S.	P = 0.025(N)
Relative Risk (f)		0.871	0.482
Lower Limit		0.537	0.262
Upper Limit		1.624	1.002
Weeks to First Observed Tumor	91	81	107
Thyroid: Follicular-cell Carcinoma or Adenoma (b)	0/18(0)	3/48(6)	2/49(4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.236	0.113
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	108	107

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Eye/Lacrimal Gland:			
Adenoma, NOS (b)	0/20(0)	0/50(0)	4/50(8)
P Values (c,d)	P = 0.039	--	N.S.
Relative Risk (f)		--	Infinite
Lower Limit		--	0.386
Upper LImit		--	Infinite
Weeks to First Observed Tumor	--	--	107

(a) Dosed groups received 3,000 or 6,000 ppm.

(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 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 matched-control group when P is less 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 the control group.

(e) The probability level for departure from linear trend is given when P is less less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered BHT in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	1/20(5)	4/46(9)	4/50(8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.739	1.600
Lower Limit		0.191	0.175
Upper Limit		83.697	77.169
Weeks to First Observed Tumor	108	108	107
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20(5)	16/46(35)	7/50(14)
P Values (c,d)	N. S.	P = 0.009	N.S.
Departure from Linear Trend (e)	P = 0.002		
Relative Risk (f)		6.957	2.800
Lower Limit		1.231	0.403
Upper Limit		282.404	123.407
Weeks to First Observed Tumor	108	101	107

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma (b)	7/20(35)	8/46(17)	8/50(16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.497	0.457
Lower Limit		0.191	0.175
Upper Limit		1.419	1.312
Weeks to First Observed Tumor	70	108	105
Liver: Hepatocellular Carcinoma (b)	1/20(5)	1/46(2)	3/49(6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.435	1.224
Lower Limit		0.006	0.108
Upper Limit		33.420	62.958
Weeks to First Observed Tumor	108	108	107

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma or Adenoma (b)	1/20(5)	4/46(9)	5/49(10)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.739	2.041
Lower Limit		0.191	0.254
Upper Limit		83.697	94.440
Weeks to First Observed Tumor	108	108	107
Pituitary: Adenoma, NOS (b)	0/20(0)	4/45(9)	1/47(2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.429	0.023
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	108	107

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered BHT in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Multiple Organs: Sarcoma, NOS (b)	3/20(15)	1/46(2)	0/50(0)
P Values (c,d)	P = 0.007(N)	N.S.	P = 0.021(N)
Relative Risk (f)		0.145	0.000
Lower Limit		0.003	0.000
Upper Limit		1.700	0.659
Weeks to First Observed Tumor	79	103	--

(a) Dosed groups received 3,000 or 6,000 ppm.

(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 matched-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 the control group.

(e) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

(f) The 95% confidence interval of the relative risk between each dosed group and the control group.

Review of the Bioassay of Butylated Hydroxytoluene (BHT)* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

December 13, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute on the Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of Butylated Hydroxytoluene (BHT).

The reviewer for the report on the bioassay of BHT raised a question regarding the possible significance of the increased incidence of lung tumors observed in low-dose treated female mice. He wondered if the lung tumors in the high-dose treated females might become statistically significant when compared with historic controls. He pointed out other studies, referenced in the report, indicating that BHT may induce lung tumors. Given the data from this bioassay and other studies, the reviewer expressed concern that the conclusionary statement in the report (". . . BHT was not carcinogenic . . ." in rats and mice) was worded too strongly. Finally, he noted that almost 9 million pounds of BHT were produced in 1976 for use in foods. Because of the large exposure to BHT, he emphasized the need to gain the best understanding of the significance of the bioassay data.

A Program staff pathologist said that the mean Program-wide incidence of lung tumors in male historic controls was about 11.7 percent and in females about 4.4 percent. He added that there is considerable variation around the mean for lung tumors. In regard to the significance of the response, the staff member said that greater credence could have been given to the findings if the high-dose treated female mice also had had a statistically significant increase in lung tumors. Without it, however, the possibility of a false positive in the low-dose treated females was increased. It was pointed out that BHT appears to be a promoting agent in the experimental induction of liver and lung tumors.

In view of the widespread human exposure to BHT in foods, evidence of its hepatotoxicity, and a suggestion of its tumorigenic effect in the lung, it was moved that the compound be considered for retest by the NCI Chemical Selection Working Group. It was further moved that the report on the bioassay of the compound be accepted as written. The motion was seconded and approved without objection.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Verald K. Rowe, Dow Chemical USA
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
Kenneth Wilcox, Michigan State Health Department

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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