NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 293

TOXICOLOGY AND CARCINOGENESIS STUDIES OF HC BLUE NO. 2

2,2'-((4-((2-HYDROXYETHYL)AMINO)-3-NITROPHENYL)IMINO)BIS(ETHANOL)

(CAS NO. 33229-34-4)

IN F344/N RATS AND B6C3F$_1$ MICE

(FEED STUDIES)

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

NATIONAL TOXICOLOGY PROGRAM

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

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

NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC 27709

August 1985

NTP TR 293

NIH Publication No. 85-2549

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
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NOTE TO THE READER

These studies are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected chemicals in laboratory animals (usually two species, rats and mice). Chemicals selected for testing in the NTP Carcinogenesis Program are chosen primarily on the bases of human exposure, level of production, and chemical structure. Selection per se is not an indicator of a chemical's carcinogenic potential. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that a test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical has the potential for hazard to humans. The determination of the risk to humans from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

Five categories of interpretative conclusions were adopted for use in June 1983 in the Technical Reports series to specifically emphasize consistency and the concept of actual evidence of carcinogenicity. For each definitive study result (male rats, female rats, male mice, female mice), one of the following quintet will be selected to describe the findings. These categories refer to the strength of the experimental evidence and not to either potency or mechanism.

- **Clear Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of malignant neoplasms, studies that exhibit a substantially increased incidence of benign neoplasms, or studies that exhibit an increased incidence of a combination of malignant and benign neoplasms where each increases with dose.
- **Some Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related increased incidence of benign neoplasms, studies that exhibit marginal increases in neoplasms of several organs/tissues, or studies that exhibit a slight increase in uncommon malignant or benign neoplasms.
- **Equivocal Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing a chemically related marginal increase of neoplasms.
- **No Evidence of Carcinogenicity** is demonstrated by studies that are interpreted as showing no chemically related increases in malignant or benign neoplasms.
- **Inadequate Study of Carcinogenicity** demonstrates that because of major qualitative or quantitative limitations, the studies cannot be interpreted as valid for showing either the presence or absence of a carcinogenic effect.

Additionally, the following concepts (as patterned from the International Agency for Research on Cancer Monographs) have been adopted by the NTP to give further clarification of these issues:

> The term *chemical carcinogenesis* generally means the induction by chemicals of neoplasms not usually observed, the earlier induction by chemicals of neoplasms that are commonly observed, or the induction by chemicals of more neoplasms than are generally found. Different mechanisms may be involved in these situations. Etymologically, the term *carcinogenesis* means induction of cancer, that is, of malignant neoplasms; however, the commonly accepted meaning is the induction of various types of neoplasms or of a combination of malignant and benign neoplasms. In the Technical Reports, the words *tumor* and *neoplasm* are used interchangeably.

This study was initiated by the National Cancer Institute's Carcinogenesis Bioassay Program, now part of the National Institute of Environmental Health Sciences, National Toxicology Program. The studies described in this Technical Report have been conducted in compliance with NTP chemical health and safety requirements and must meet or exceed all applicable Federal, state, and local health and safety regulations. All NTP toxicology and carcinogenesis studies are subjected to a data audit before being presented for peer review.

Although every effort is made to prepare the Technical Reports as accurately as possible, mistakes may occur. Readers are requested to identify any mistakes so that corrective action may be taken. Further, anyone who is aware of related ongoing or published studies not mentioned in this report is encouraged to make this information known to the NTP. Comments and questions about the National Toxicology Program Technical Reports on Toxicology and Carcinogenesis Studies should be directed to Dr. J.E. Huff, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709 (919-541-3780).

These NTP Technical Reports are available for sale from the National Technical Information Service, U.S. Department of Commerce, 5285 Port Royal Road, Springfield, VA 22161 (703-487-4650). Single copies of this Technical Report are available without charge (and while supplies last) from the NTP Public Information Office, National Toxicology Program, P.O. Box 12233, Research Triangle Park, NC 27709.

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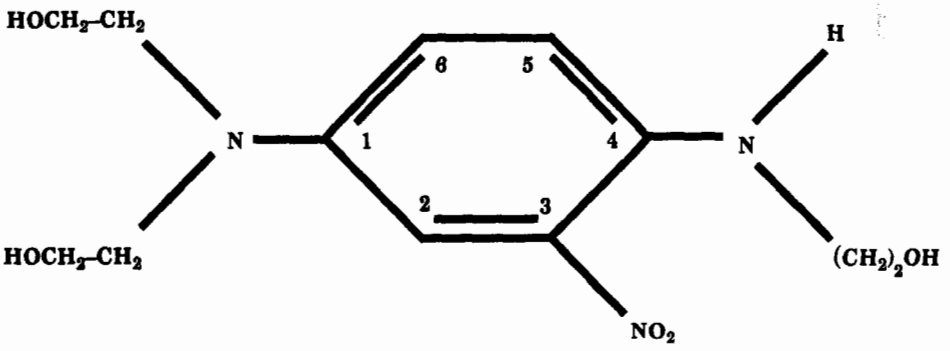

HC BLUE NO. 2
2,2'-((4-((2-HYDROXYETHYL)AMINO)-3-NITROPHENYL)IMINO)BIS(ETHANOL)
CAS No. 33229-34-4
Molecular Formula: $C_{12}H_{19}N_3O_5$ Molecular Weight: 285

ABSTRACT

Toxicology and carcinogenesis studies of HC Blue No. 2 (approximately 98% pure), a semipermanent hair dye, were conducted by administering the test chemical in feed for 103 weeks to groups of 50 F344/N rats of each sex and for 104 weeks to groups of 50 B6C3F$_1$ mice of each sex. The dietary concentrations used were 0, 5,000, or 10,000 ppm for male rats and male mice and 0, 10,000, or 20,000 ppm for female rats and female mice. These concentrations were selected on the basis of results from single-administration gavage and 14-day and 13-week feed studies. For the 2-year studies, the average daily doses were approximately 195 and 390 mg/kg in male rats, 465 and 1,000 mg/kg in female rats, 1,320 and 2,240 mg/kg in male mice, and 2,330 and 5,600 mg/kg in female mice.

The survival of high dose male rats and male mice was better than that for controls, and the survival of dosed female rats was comparable to that of the controls. The survival of high dose female mice was reduced ($P < 0.05$) relative to that of the controls (control, 35/50; low dose, 27/50; high dose 19/50); this reduced survival was attributed to a reproductive tract infection. Final mean body weights relative to those of the controls were depressed less than 10% in dosed male rats, whereas depressions of 13% and 22% were observed in the low dose and high dose groups of female rats. Final mean body weights for dosed male mice were within 5% of control values, but final mean body weights for dosed females were 15% (low dose) and 22% (high dose) lower than that of controls.

A dose-related increase in the incidence of hyperostosis of the skull was detected in rats (male, 5/50, 8/50, 25/49; female, 2/50, 19/50, 49/50) and in 1/49 high dose male and 4/50 high dose female mice. Mixed mesenchymal neoplasms of the kidney were detected in 2/50 high dose female rats; none was observed in any other group of female or male rats. This tumor is considered uncommon and has not been found in 1,863 historical control female F344/N rats. A negative trend in fibroadenomas of the mammary gland was seen in female rats (20/50, 10/50, 4/50).

A marginal ($P = 0.05$) positive trend occurred in the incidence of lymphomas in male mice (1/50; 5/48; 8/49); the incidences in the dosed groups were not significantly greater than that in the controls when survival differences were taken into account.

HC Blue No. 2 was mutagenic for strains TA97 and TA98 but not for strains TA100 or TA1535 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9. HC Blue No. 2 was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay in the presence of Aroclor 1254-induced male F344 rat liver S9.

An audit of the experimental data was conducted for these carcinogenesis studies on HC Blue No. 2 . No data discrepancies were found that influenced the final interpretations.

Under the conditions of these studies, there was *no evidence of carcinogenicity** in male and female F344/N rats or in male and female B6C3F$_1$ mice receiving HC Blue No. 2 in the diet at concentrations of 0.5% and 1.0% for males and 1.0% and 2.0% for females for 2 years. HC Blue No. 2 administration caused a dose-related increase in the incidence of hyperostosis of the skull in male and female rats.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

CONTRIBUTORS

The NTP Technical Report on the Toxicology and Carcinogenesis Studies of HC Blue No. 2 is based on the 13-week studies that began in June 1978 and ended in September 1978 and on the 2-year studies that began in February 1980 and ended in February 1982 at Southern Research Institute.

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The members of the Peer Review Panel who evaluated the draft Technical Report on HC Blue No. 2 on July 27, 1984, are listed below. Panel members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, Panel members have five major responsibilities: (a) to ascertain that all relevant literature data have been adequately cited and interpreted, (b) to determine if the design and conditions of the NTP studies were appropriate, (c) to ensure that the Technical Report presents the experimental results and conclusions fully and clearly, (d) to judge the significance of the experimental results by scientific criteria, and (e) to assess the evaluation of the evidence of carcinogenicity and other observed toxic responses.

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SUMMARY OF PEER REVIEW COMMENTS ON THE TOXICOLOGY AND CARCINOGENESIS STUDIES OF HC BLUE NO. 2

On July 27, 1984, the draft Technical Report on the toxicology and carcinogenesis studies of HC Blue No. 2 received peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9:00 a.m. in the Conference Center, Building 101, South Campus, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina.

Dr. S. Tannenbaum, a principal reviewer, agreed with the conclusions. He noted that two lots of test chemical were used, one 98 percent pure and one 75 percent pure, and requested clarification on which lots were used for which studies. Dr. J. Mennear, NTP, replied that the lot used for one 14-day study, the 2-year studies, and the mutagenicity experiments was 98% pure, whereas the lot used for the 13-week studies was 75% pure and that this would be emphasized in the report [see pp. 22-23]. Dr. Mennear reported that following Dr. Tannenbaum's suggestion at the review of HC Blue No. 1 (NTP TR 271, peer reviewed by the Panel in March 1984), nitrosamine contents had been measured for both dyes; based on dietary concentrations of these dyes, animals in the HC Blue No. 2 study received nearly twice as much nitrosamine (220 ppb for high dose rats and male mice; 440 ppb for female mice) in the feed as those in the HC Blue No. 1 study; HC Blue No. 1 was positive for carcinogenicity. [Results of these chemical analyses are presented in the final Technical Reports for both dye studies.]

As a second principal reviewer, Mr. L. Beliczky critiqued several issues, especially the comparisons of the toxicities of HC Blue No. 1 and 2 and that the presence of nitrosamines did not influence the findings. Dr. J. Huff, NTP, suggested that because the dietary concentrations of HC Blue No. 2 were about three times as great as those for HC Blue No. 1, some might consider HC Blue No. 2 to be at least three times less toxic; such extrapolations could be misleading and would not be presented in the Technical Report. Mr. Beliczky asked that more explanation be added as to why a second 14-day study was conducted and that an expanded discussion be included on the significance of mixed mesenchymal kidney tumors in female rats, C-cell carcinomas of the thyroid gland in male rats, and lymphomas in mice. Dr. G. Boorman, NTP, said that although the incidence in the high dose female rats was not significantly increased, the kidney tumors were uncommon and emphasizing the effect in the abstract seemed appropriate.

As a third principal reviewer, Dr. Turnbull agreed with the conclusions. He asked for inclusion of information as to why the dermal route was not chosen for the study, since that would be the likely route of human exposure [see page 54]. Dr. Mennear said the only use of HC Blue No. 2 was in hair dyes.

There was some discussion about the dose-related increases in the incidence of hyperostosis in male and female rats, and the Panel agreed that this was unusual and should be mentioned in the conclusion. Further discussion centered on whether the various nitrosamines present as impurities should be identified and whether significant in vivo nitrosation was likely.

Dr. Tannenbaum moved that the Technical Report on the toxicology and carcinogenesis studies of HC Blue No. 2 be accepted with the modifications discussed. Dr. Turnbull seconded the motion, and the Technical Report was approved by seven affirmative votes; there were two negative votes (Mr. Beliczky and Dr. Jones).

I. INTRODUCTION

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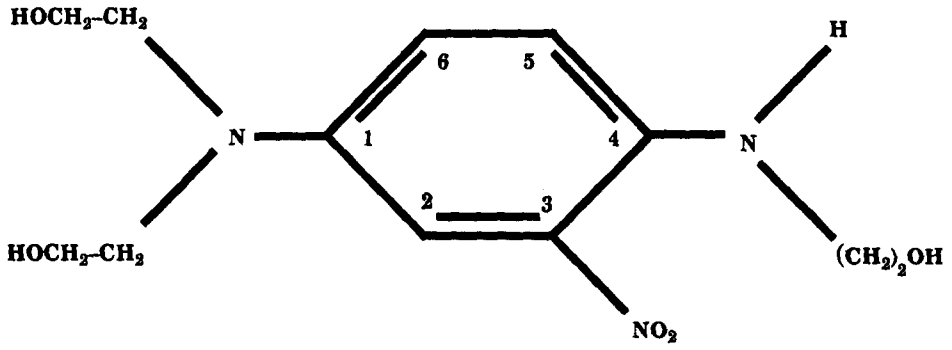

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CAS No. 33229-34-4
Molecular Formula: $C_{12}H_{19}N_3O_5$ Molecular Weight: 285

HC Blue No. 2 is a nitrophenylenediamine derivative used as a semipermanent hair dye. Semipermanent hair color products are generally "shampoo-in" preparations that are applied to the hair, lathered, and then allowed to remain in contact with the hair and scalp for 30-45 minutes. The concentration of HC Blue No. 2 used in these preparations ranges from 1.6% to 2%. Approximately 30,000 pounds of HC Blue No. 2 was used in the United States in 1983 (Burnett, C., personal communication to NTP, 1984).

Studies in which HC Blue No. 2 has been administered to laboratory animals have been conducted on complex mixtures of dyes, dye intermediates, and product base chemicals (solvents and detergents). Wernick et al. (1975) administered a composite of 15 semipermanent hair dyes, formulated in product base materials, to dogs, rats, and rabbits. The composite contained 6.95% dye chemicals, including 1.63% HC Blue No. 2. The mixture was tested for systemic effects in beagle dogs (dietary administration for 2 years), for teratologic effects in Sprague-Dawley rats (dietary administration on days 6 through 15 of gestation) and New Zealand white rabbits (gavage administration on days 6 through 18 of gestation), and for reproductive effects in Sprague-Dawley rats (dietary administration). The largest doses of HC Blue No. 2 delivered in the mixture were 1.59 mg/kg per day to dogs and rabbits and 12.7 mg/kg per day to rats. No compound-related effects were reported.

Burnett et al. (1976) studied a formulation containing 13 dyes and dye intermediates and 8 base chemicals. This mixture, which contained 1.7% HC Blue No. 2, was applied to the shaved skin of New Zealand white rabbits (1.0 ml/kg twice weekly for 13 weeks) and to pregnant Charles River rats (2.9 ml/kg on days 1, 4, 7, 13, 16, and 19 of gestation). Neither systemic nor teratologic effects were produced.

No published studies have been found concerning the absorption of HC Blue No. 2 through skin. Under conditions of use, the absorption of HC Blue No. 2 has been estimated to be less than 0.1% of the amount of the dye applied to the hair and scalp (Burnett, C., personal communication to NTP, 1984).

There are no literature citations on the mutagenicity of HC Blue No. 2; however, the NTP (Appendix N) found that HC Blue No. 2 (lot no. 9233, 98% pure) was mutagenic in strains TA97 and TA98 but not in strains TA100 or TA1535 of *Salmonella typhimurium* in the presence or absence of Aroclor 1254-induced Sprague-Dawley rat or Syrian hamster liver S9. HC Blue No. 2 also was mutagenic in the mouse lymphoma L5178Y/TK$^{+/-}$ assay.

HC Blue No. 2 is a structural analog of HC Blue No. 1, another semipermanent hair dye that has been studied for carcinogenic effects (NTP, 1985). The structures of these chemicals differ only in the nature of the substituent on the secondary amine in position number 4 of the molecule. In HC Blue No. 1, this substitution is a methyl group, whereas it is a hydroxyethyl group in HC Blue No. 2. In the earlier study, HC Blue No. 1 was found to be carcinogenic for both male and female mice, increasing the incidences of hepatocellular carcinomas in each sex (dietary administration of 0, 1,500, or 3,000 ppm to males and 0, 3,000, or 6,000 ppm to females). Dietary concentrations of 1,500 and 3,000 ppm also produced marginally significant increases in the incidences of neoplastic nodules/carcinomas of the liver in male rats. There was some evidence of carcinogenicity in female rats: dietary administration of 3,000 or 6,000 ppm was associated with increases in the incidence of alveolar/bronchiolar adenomas or carcinomas (NTP, 1985).

The International Agency for Research on Cancer (IARC, 1982) has published a monograph on aromatic amines, including hair dye preparations. The epidemiologic information concerning relationships between various human cancers and either employment as a hairdresser or the personal use of hair dyes was evaluated as inconclusive.

HC Blue No. 2 is one of five semipermanent hair dyes that were selected for toxicology and carcinogenicity assessment in a class study of hair color materials. The results of the evaluation of HC Blue No. 1 were reported earlier (NTP, 1985). The results for two other dyes (HC Red No. 3 and C.I. Disperse Blue 1) were peer reviewed in 1985, and a fifth dye, C.I. Acid Orange 3, is still under study.

The present report summarizes the results of 2-year toxicology and carcinogenesis studies of HC Blue No. 2 given in the diet of F344/N rats and B6C3F$_1$ mice. The oral (feed) route of administration was selected to maximize chances of detecting systemic effects associated with chemical administration.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HC BLUE NO. 2
PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS
ANALYSIS OF FORMULATED DIETS
SINGLE-ADMINISTRATION STUDIES
FOURTEEN-DAY REPEATED-EXPOSURE STUDIES
THIRTEEN-WEEK STUDIES
TWO-YEAR STUDIES
- Study Design
- Source and Specifications of Test Animals
- Animal Maintenance
- Clinical Examinations and Pathology
- Statistical Methods

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HC BLUE NO. 2

HC Blue No. 2 (2,2'-((4-((2-hydroxyethyl)amino)-3-nitrophenyl)imino)bis(ethanol)) was obtained in two lots. Lot no. 5130777 was obtained from Clairol Laboratories (Stamford, Connecticut), and lot no. 9233 was obtained from Southland Corp. (Dallas, Texas).

The identities of both lots of HC Blue No. 2 were confirmed by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy (Appendix G). All spectroscopic data were consistent with those expected for the structure of HC Blue No. 2 and the spectra of the purified dye provided by Clairol.

The purity of HC Blue No. 2 was determined to be approximately 75% for lot no. 5130777 and approximately 98% for lot no. 9233 by elemental analysis, water analysis, titration of the amine group, thin-layer chromatography, and high-performance liquid chromatography. Results of the elemental analyses agreed with the theoretical values. The water content was between 1% and 2%. By titration of the amine function, lot no. 5130777 was found to be 79% pure and lot no. 9233 to be 103% pure. When chromatographic analysis was performed on both lots in the same system, the chromatographic profiles of both lots were very similar; lot no. 513077 had more impurities than did lot no. 9233, and the impurities were present in greater concentrations. Chromatographic data indicated 10 impurities in lot no. 5130777; 3 impurities had areas of 1.4%, 17.3%, and 5.6% that of the major peak and the remaining 7 had a combined area of less than 1.5% that of the major peak. Five impurities were detected in lot no. 9233; one had an area of 1.5% that of the major peak, and the other four had a combined area that was less than 1% that of the major peak.

HC Blue No. 2 was found to be stable for 2 weeks at 60° C (Appendix G). HC Blue No. 2 was stored at 5° C at the testing laboratory. Periodic characterization of HC Blue No. 2 by infrared and ultraviolet/visible spectroscopy detected no deterioration over the course of the studies (Appendix G).

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

The formulated diets were prepared by adding a dry premix (approximately equal amounts of feed and HC Blue No. 2) to the appropriate amount of feed. The mixture was blended for 15 minutes. The homogeneity of diet mixtures formulated at the analytical chemistry and testing laboratories was evaluated (Appendix H). Further studies showed that HC Blue No. 2 was stable in feed when stored for 2 weeks at temperatures equal to or less than 25° C. Formulated diets were stored at 5° C for no longer than 14 days.

ANALYSIS OF FORMULATED DIETS

Periodic analyses for HC Blue No. 2 in feed mixtures were performed by the testing and analytical chemistry laboratories to determine if the formulated diets contained the correct concentrations of HC Blue No. 2 (Appendix I). The method of analysis involved a methanolic extraction followed by a spectrophotometric quantitation step. Occasionally, samples were not within 10% of the target concentration (Appendix J). Because 70 of 82 feed mixtures analyzed were within 10% of the target concentrations, the feed mixtures were estimated to have been within specifications 85% of the time throughout the entire study (Table 1). All mixtures were within 25% of the target concentrations.

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, Indiana) and held for approximately 13 days before the test began. The animals were approximately 8 weeks old when placed on study. Groups of five rats of each sex were administered a single dose of 31, 62, 125, 250, or 500 mg/kg HC Blue No. 2 (lot no. 5130777) in 1% carboxymethyl cellulose ether sodium salt in saline by gavage; and groups of five mice of each sex were administered 62, 125, 250, 500, or 1,000 mg/kg by the same route. Details of animal maintenance are presented in Table 2.

TABLE 1. RESULTS OF ANALYSES OF FORMULATED DIETS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

Date Mixed	Determined Concentration for Target Concentration of 5,000 ppm	10,000 ppm	20,000 ppm
Mean (ppm)	4,734	9,753	19,301
Standard Deviation	539.0	584.4	1,605.6
Coefficient of Variation (%)	11.4	6.0	8.3
Range (ppm)	3,380-6,160	8,380-11,500	15,660-22,900
Number of Samples	27	38	17

Animals were observed for mortality two times per day for 14 days. Body weights were taken on the day of dosing and on day 15. Necropsies were not performed.

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES

Fourteen-day repeated-exposure studies were conducted on both lots of HC Blue No. 2. The second study, conducted because of a substantial difference in the purity of the test material, began after the 13-week studies were completed. The lot used in the first study (lot. no. 5130777) was approximately 75% pure, whereas the purity of the lot used in the second study (lot no. 9233) was approximately 98% pure.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (first study) and from Charles River Laboratories (second study) and held for approximately 2 weeks before the study began.

Animals were approximately 8 weeks old when placed on study. Groups of five males and three or five females of each species were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 2 for 14 days. Rats and mice were observed two times per day for moribundity or mortality and were weighed on days 1 and 15. Necropsies were performed on all animals. Details of animal maintenance are presented in Table 2.

THIRTEEN-WEEK STUDIES

Thirteen-week studies were conducted to evaluate the cumulative toxic effects of repeated administration of HC Blue No. 2 and to determine the concentrations to be used in the 2-year studies. Lot no. 513077, 75% pure, was used in these studies.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries, observed for 15 days, and then assigned to cages according to a table of random numbers. Animals were approximately 8 weeks old when placed on test. The cages were assigned to dosed and control groups according to another table of random numbers.

Dosed animals received Wayne Lab Blox® mash and the required amount of HC Blue No. 2 (3,100, 6,200, 12,500, 25,000, or 50,000 ppm). Control diets consisted of Wayne Lab Blox® mash. Formulated diets, control diets, and water (via an automatic watering system) were available ad libitum. Further experimental details are summarized in Table 2.

Animals were checked two times per day for mortality and signs of moribundity. Feed consumption was measured weekly by cage. Individual animal weights were recorded weekly. At the end of the 13-week studies, survivors were killed. Necropsies were performed on all animals. Tissues and groups examined are listed in Table 2.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 5,000, or 10,000 ppm HC Blue No. 2 (lot no. 9233, 98% pure) were fed to groups of 50 male rats and 50 male mice. Groups of 50 female rats and 50 female mice were fed diets containing 0, 10,000, or 20,000 ppm. Rats received formulated diets for 103 weeks, and mice received formulated diets for 104 weeks.

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF HC BLUE NO. 2

	Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Rerun Fourteen-Day Repeated-Exposure Studies	Two-Year Studies
EXPERIMENTAL DESIGN					
Testing Laboratory	Southern Research Institute	Southern Research Institute	Southern Research Institute	Southern Research Institute	Southern Research Institute
Size of Test Groups	5 males and 5 females of each species	5 males and 3 or 5 females of each species	10 males and 10 females of each species	5 males and 5 females of each species	50 males and 50 females of each species
Doses	Rats--31, 62, 125, 250, or 500 mg/kg HC Blue No. 2 in 1% carboxymethyl cellulose in saline by gavage; mice--62, 125, 250, 500, or 1,000 mg/kg HC Blue No. 2	0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 2 in the diet	0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 2 in the diet	0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 2 in the diet	Males--0, 5,000, or 10,000 ppm HC Blue No. 2 in the diet; females--0, 10,000, or 20,000 ppm HC Blue No. 2 in the diet
Date of First Dose	1/24/78	2/15/78	6/07/78	10/25/79	2/06/80
Date of Last Dose	N/A	2/28/78	9/05/78	11/07/79	Rats--1/26/82; mice--2/02/82
Duration of Dosing	Single dose	14 d	91 d	14 d	Rats--103 wk; mice--104 wk
Type and Frequency of Observation	Observed 2 × d for mortality; weighed on d 0 and d 15	Observed 2 × d for moribundity and mortality; weighed on d 0 and d 15	Animals weighed weekly and observed 2 × d for moribundity and mortality. Clinical exams were made weekly; feed consumption measured 1 × wk	Animals weighed initially and weekly thereafter; observed 2 × d for moribundity and mortality	Animals weighed weekly for 13 wk; monthly thereafter; food consumption measured 1 × 4-6 wk. Observed 2 × d for moribundity and mortality
Necropsy and Histologic Examination	Not performed	Necropies were performed on all animals. No histopathologic examination was performed	All animals necropsied; all controls and all animals in the 50,000-ppm dose group were examined histopathologically. Tissues examined: skin, mandibular and mesenteric lymph nodes, mammary gland, salivary gland, thigh muscle, femur including marrow, thymus, trachea, lungs and mainstem bronchi, heart, thyroid gland, parathyroids, esophagus, small intestine, colon, liver, stomach,	Same as 14-d repeated-exposure studies	Necropsies were performed on all animals; tissues examined microscopically include: tissue masses, mandibular lymph nodes, salivary gland, femur including marrow, gallbladder (mice), thyroid gland, parathyroids, small intestine, colon, liver, prostate/ testes or ovaries/ uterus, lungs and mainstem bronchi,skin, heart, esophagus, stomach, urinary bladder, brain, thymus, trachea, pancreas, spleen, kidneys, adrenal glands,

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF HC BLUE NO. 2 (Continued)

	Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Rerun Fourteen-Day Repeated-Exposure Studies	Two-Year Studies
Necropsy and Histologic Examination (Continued)			pancreas, spleen, gallbladder (mice), kidneys, adrenal glands, urinary bladder, brain, vesicular gland, prostate/ testes or ovaries/ uterus, and pituitary gland		pituitary gland, mammary gland, eyes, regional lymph nodes, and spinal cord if abnormal
ANIMALS AND ANIMAL MAINTENANCE					
Strain and Species	F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice	F344/N rats; $B6C3F_1$ mice
Animal Source	Harlan Industries (Indianapolis, IN)	Same as single-administration studies	Same as single-administration studies	Charles River Breeding Labs (Portage, MI)	Charles River Breeding Labs (Portage, MI)
Method of Animal Identification	Earmarked with poultry punch	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Time Held Before Test	13 d	13 d	15 d	16 d	14 d
Age When Placed on Study	8 wk	8 wk	8 wk	8 wk	Rats--6-7 wk; mice--7 wk
Age When Killed	10 wk	10 wk	21 wk	10 wk	Rats--110-112 wk; mice--112-113 wk
Necropsy Dates	2/8/78	3/02/78-3/06/78	9/06/78-9/09/78	11/9/79-11/10/79	Rats--2/03/82-2/09/82; mice--2/10/82-2/15/82
Method of Animal Distribution	Randomized to cages and then to groups using two tables of random numbers	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Feed	Wayne Lab-Blox® pellets (Allied Mills, Inc., Chicago, IL); available ad libitum	Wayne Lab-Blox® Mash (Allied Mills, Inc., Chicago, IL); available ad libitum	Same as 14-d repeated-exposure studies	NIH 07 open formula (Ziegler Bros., Gardners, PA); available ad libitum	Same as rerun 14-d repeated-exposure studies
Bedding	Heat-treated hardwood chips (PWI, Inc, Lowville, NY)	Same as single-administration studies	Same as single-administration studies	Heat-treated hardwood chips (Northeastern Products Corp., Warrensburg, NY)	Same as rerun 14-d repeated-exposure studies
Water	Automatic watering system (Edstrom Industries, Waterford, WI); available ad libitum	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF HC BLUE NO. 2 (Continued)

	Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Rerun Fourteen-Day Repeated-Exposure Studies	Two-Year Studies
Cages	Polycarbonate (Lab Products, Inc., Garfield, NJ)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Cage Rotation	None	None	None	None	None
Cage Filters	Reemay spun-bonded polyester filters (Snow Filtration, Cincinnati, OH)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Animals per Cage	5	5	5	5	5
Animal Room Environment	Temp--21°-23° C; hum--40%-60%; fluorescent light 12 h/d; 15 room air changes/h	Same as single-administration studies	Temp--21°-23° C; hum--30%-50%; fluorescent light 12 h/d; 15 room air changes/h	Same as 13-wk studies	Temp--23° ± 1° C; hum--40%-60%; fluorescent light 12 h/d; 15 room air changes/h
CHEMISTRY					
Lot Numbers Used	5130777	5130777	5130777	9233	9233
Date of Initial Use of Subsequent Lot	N/A	N/A	N/A	N/A	N/A
Supplier	Clairol Research Laboratories (Stamford, CT)	Same as single-administration studies	Same as single-administration studies	Southland Corporation (Dallas, TX)	Same as rerun 14-d repeated-exposure studies
CHEMICAL/VEHICLE					
Preparation	Mixed on a w/v basis with 1% carboxymethyl cellulose ether sodium salt in saline by a sonifier with a probe for 10 min	Mixed on a w/w basis with feed. Premix was added to remaining feed in a 16-qt P-K® twin-shell blender and mixed for 15 min	Premix prepared in a Waring blender by mixing for 2 min; then sandwiched between three equal portions of the plain feed in a 16-qt P-K® twin-shell blender and mixed for 15 min	Premix sandwiched between two portions of feed in 16-qt blender with an intensifier bar and mixed for 15 min	Premix originally prepared by shaking manually in a specimen cup; premix later prepared with a mortar and pestle. Premix was sandwiched between two layers of plain feed in a 16-qt P-K® blender and mixed for 15 min (with an intensifier bar turned off for 5 min)
Maximum Storage Time	3 h	7 d	7 d	2 wk	2 wk
Storage Conditions	Stored in plastic bags in sealed plastic containers in the animal rooms	Same as single-administration studies	Stored in double plastic bags in sealed plastic containers at 5° C	Same as 13-wk studies	Same as 13-wk studies

Source and Specifications of Test Animals

The male and female F344/N rats and $B6C3F_1$ (C57BL/6N, female, × C3H/HeN MTV$^-$, male) mice used in this study were produced under strict barrier conditions at the Charles River Breeding Laboratories (Portage, Michigan) under a contract to the Carcinogenesis Program. Breeding starts for the foundation colony at the production facility originated at the National Institutes of Health Repository. Animals shipped for testing were progeny of defined microflora-associated parents that were transferred from isolators to barrier-maintained rooms. Animals were shipped to the testing laboratory at 4-5 weeks of age. The animals were quarantined at the testing facility for 2 weeks. Thereafter, a complete necropsy was performed on five animals of each sex and species to assess their health status. The rodents were placed on study at 6-7 weeks of age. The health of the animals was monitored during the course of the study according to the protocols of the NTP Sentinel Animal Program (Appendix K).

A quality control skin grafting program has been in effect since early 1978 to monitor the genetic integrity of the inbred mice used to produce the hybrid $B6C3F_1$ test animal. In mid-1981, data were obtained that showed incompatibility between the NIH C3H reference colony and the C3H colony from a Program supplier. In August 1981, inbred parental lines of mice were further tested for genetic integrity via isozyme and protein electrophoretograms that demonstrate phenotype expressions of known genetic loci.

The C57BL/6 mice were homogeneous at all loci tested. Eighty-five percent of the C3H mice monitored were variant at one to three loci, indicating some heterogeneity in the C3H line from this supplier. Nevertheless, the genome of this line is more homogeneous than that of randomly bred stocks.

Male mice from the C3H colony and female mice from the C57BL/6 colony were used as parents for the hybrid $B6C3F_1$ mice used in these studies. The influence of the potential genetic nonuniformity in the hybrid mice on these results is not known, but results of the studies are not affected because matched concurrent controls were included in each study.

Animal Maintenance

Rats and mice were housed five per cage. Formulated diets, control diets, and water (via an automatic watering system) were available ad libitum. Details of animal maintenance are presented in Table 2.

Clinical Examinations and Pathology

All animals were observed two times per day for signs of moribundity or mortality. Clinical signs were recorded once per week. Body weights by cage were recorded once per week for the first 13 weeks of the study and once per month thereafter. Feed consumption was recorded every 4-5 weeks. Mean body weights were calculated for each group. Moribund animals were killed, as were animals that survived to the end of the study. Necropsies were performed on all animals, including those found dead unless they were excessively autolyzed or cannibalized. Thus, the number of animals from which particular organs or tissues were examined microscopically varies and is not necessarily equal to the number of animals that were placed on study in each group.

Examinations for grossly visible lesions were performed on major tissues or organs. Tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues examined microscopically are listed in Table 2.

When the pathology examination was completed, the slides, individual animal data records, and summary tables were sent to an independent quality assurance laboratory. Individual animal records and tables were compared for accuracy, slides and tissue counts were verified, and histotechnique was evaluated. All tumor diagnoses, all target tissues, and all tissues from a randomly selected 10% of the animals were evaluated by a quality assurance pathologist. Slides of all target tissues and those about which the original and quality assurance pathologists disagreed were submitted to the Chairperson of the Pathology Working Group (PWG)

for evaluation. Representative coded slides selected by the Chairperson were reviewed by PWG pathologists, who reached a consensus and compared their findings with the original and quality assurance diagnoses. When diagnostic differences were found, the PWG sent the appropriate slides and comments to the original pathologist for review. This procedure has been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). The final diagnoses represent a consensus of contractor pathologists and the NTP Pathology Working Group.

Nonneoplastic lesions are not examined routinely by the quality assurance pathologist or the PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and the PWG if they are considered part of the toxic response to a chemical or if they are deemed of special interest.

Statistical Methods

Data Recording: Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969).

Survival Analyses: The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals were censored from the survival analyses at the time they were found dead of other than natural causes or were found to be missing; animals dying from natural causes were not 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 method for testing for a dose-related trend. All reported P values for the survival analysis are two-sided.

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

Analysis of Tumor Incidence: Three statistical methods are used to analyze tumor incidence data. The two that adjust for intercurrent mortality employ the classical method for combining contingency tables developed by Mantel and Haenszel (1959). Tests of significance included pairwise comparisons of high dose and low dose groups with vehicle controls and tests for overall dose-response trends.

For studies in which compound administration has little effect on survival, the results of the three alternative analyses will generally be similar. When differing results are obtained by the three methods, the final interpretation of the data will depend on the extent to which the tumor under consideration is regarded as being the cause of death. All reported P values for tumor analyses are one-sided.

Life Table Analyses--The first method of analysis assumed that all tumors of a given type observed in animals dying before the end of the study were "fatal"; i.e., they either directly or indirectly caused the death of the animal. According to this approach, the proportions of tumor-bearing animals in the dosed and control groups were compared at each point in time at which an animal died with a tumor of interest. The denominators of these proportions were the total number of animals at risk in each group. These results, including the data from animals killed at the end of the study, were then combined by the Mantel-Haenszel method to obtain an overall P value. This method of adjusting for intercurrent mortality is the life table method of Cox (1972) and of Tarone (1975).

Incidental Tumor Analyses--The second method of analysis assumed that all tumors of a given type observed in animals that died before the end of the study were "incidental"; i.e., they

were merely observed at necropsy in animals dying of an unrelated cause. According to this approach, the proportions of tumor-bearing animals in dosed and control groups were compared in each of five time intervals: 0-52 weeks, 53-78 weeks, 79-92 weeks, week 93 to the week before the terminal kill period, and the terminal kill period. The denominators of these proportions were the number of animals on which necropsies were actually performed during the time intervals. The individual time interval comparisons were then combined by the previously described method to obtain a single overall result. (See Haseman, 1984, for the computational details of both methods.)

Unadjusted Analyses--Primarily, survival-adjusted methods are used to evaluate tumor incidence. In addition, the results of the Fisher exact test for pairwise comparisons and the Cochran-Armitage linear trend test (Armitage, 1971; Gart et al., 1979) are given in the appendix containing the analyses of primary tumor incidence. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

Historical Control Data: Although the concurrent control group is always the first and most appropriate control group used for decision-making, there are certain instances in which historical control data can be helpful in the overall evaluation of tumor incidence. Consequently, control tumor incidences from the NTP historical control data base (Haseman et al., 1984) are included for those tumors in these studies appearing to show compound-related effects.

III. RESULTS

RATS

SINGLE-ADMINISTRATION STUDIES
FOURTEEN-DAY REPEATED-EXPOSURE STUDIES
RERUN FOURTEEN-DAY REPEATED-EXPOSURE STUDIES
THIRTEEN-WEEK STUDIES
TWO-YEAR STUDIES
Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

MICE

SINGLE-ADMINISTRATION STUDIES
FOURTEEN-DAY REPEATED-EXPOSURE STUDIES
RERUN FOURTEEN-DAY REPEATED-EXPOSURE STUDIES
THIRTEEN-WEEK STUDIES
TWO-YEAR STUDIES
Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

SINGLE-ADMINISTRATION STUDIES

All the rats survived to the end of the studies, and there were no dose-related effects on body weight gains (Table 3). The urine of dosed rats was blue on day 1 but not on day 2 or later.

TABLE 3. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF HC BLUE NO. 2

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams)		
		Initial	Final	Change (b)
MALE				
31	5/5	111 ± 4	178 ± 8	+67 ± 5
62	5/5	108 ± 2	171 ± 5	+63 ± 4
125	5/5	110 ± 5	173 ± 6	+63 ± 3
250	5/5	111 ± 2	187 ± 2	+76 ± 3
500	5/5	110 ± 3	178 ± 7	+68 ± 4
FEMALE				
31	5/5	94 ± 1	126 ± 3	+32 ± 2
62	5/5	103 ± 2	136 ± 2	+33 ± 2
125	5/5	97 ± 4	129 ± 5	+32 ± 1
250	5/5	97 ± 3	131 ± 3	+34 ± 2
500	5/5	96 ± 5	125 ± 8	+29 ± 4

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES (Lot No. 5130777)

All the rats survived to the end of the studies (Table 4). Rats that received 25,000 or 50,000 ppm HC Blue No. 2 gained notably less weight than did the controls. Dosed rats had dark violet urine. Bluish discoloration of various tissues was noted at necropsy in the rats that received 50,000 ppm.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	139 ± 6	190 ± 3	+ 51 ± 6	--
3,100	5/5	126 ± 9	170 ± 10	+ 44 ± 2	89
6,200	5/5	113 ± 9	155 ± 9	+ 42 ± 2	82
12,500	5/5	120 ± 9	165 ± 12	+ 45 ± 4	87
25,000	5/5	114 ± 5	146 ± 6	+ 32 ± 3	77
50,000	5/5	133 ± 9	146 ± 8	+ 13 ± 4	77
FEMALE					
0	3/3	111 ± 4	130 ± 0	+ 19 ± 5	--
3,100	5/5	97 ± 5	120 ± 5	+ 23 ± 2	92
6,200	5/5	117 ± 6	135 ± 2	+ 18 ± 5	104
12,500	5/5	104 ± 4	119 ± 3	+ 15 ± 3	92
25,000	5/5	93 ± 10	104 ± 11	+ 11 ± 4	80
50,000	5/5	108 ± 3	117 ± 5	+ 9 ± 2	90

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

RERUN FOURTEEN-DAY REPEATED-EXPOSURE STUDIES (Lot No. 9233)

These studies were conducted after the 13-week studies. All the rats survived to the end of the studies (Table 5). The final mean body weight of male rats that received 50,000 ppm HC Blue No. 2 was depressed 23% relative to that of the controls. Dosed rats had violet urine. The thymus gland was red in 2/5 males and 3/5 females that received 50,000 ppm HC Blue No. 2 and in 2/5 males that received 25,000 ppm. Since there were no marked differences in the results of the two 14-day repeated-exposure studies, the results of the 13-week studies with lot no. 5130777 were used to select doses for the 2-year studies with lot no. 9233.

TABLE 5. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE RERUN FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	131 ± 3	203 ± 4	+72 ± 1	--
3,100	5/5	124 ± 3	190 ± 3	+66 ± 2	94
6,200	5/5	128 ± 4	194 ± 4	+66 ± 2	96
12,500	5/5	129 ± 3	194 ± 4	+65 ± 3	96
25,000	5/5	129 ± 3	185 ± 6	+56 ± 3	91
50,000	5/5	134 ± 1	157 ± 1	+23 ± 2	77
FEMALE					
0	5/5	118 ± 2	146 ± 3	+28 ± 1	--
3,100	5/5	111 ± 1	139 ± 1	+28 ± 2	95
6,200	5/5	114 ± 5	144 ± 6	+30 ± 1	99
12,500	5/5	116 ± 3	142 ± 4	+26 ± 1	97
25,000	5/5	113 ± 4	138 ± 5	+25 ± 1	95
50,000	5/5	122 ± 1	141 ± 2	+19 ± 2	97

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

THIRTEEN-WEEK STUDIES

All the rats survived to the end of the studies (Table 6). Purple urine and dark feces were observed after day 9. Final mean body weights relative to those of the controls were depressed 12%-21% in males fed diets containing 6,200-50,000 ppm. Feed consumption by dosed and control rats was not dose related. At necropsy, the thyroid glands were dark in 40%-80% of the rats in each dose group; the incidences were dose related (8/10 males and 8/10 females that received 50,000 ppm and 7/10 males and 4/10 females that received 3,100 ppm). No compound-related histopathologic effects were observed.

Concentrations of 5,000 and 10,000 ppm were selected for male rats and 10,000 and 20,000 ppm were selected for female rats in the 2-year studies. These concentrations were selected on the basis of body weight gain data from the 13-week studies.

TABLE 6. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)	Feed Consumption (c) (grams) Day 30	Day 86
MALE							
0	10/10	112 ± 4	318 ± 8	+ 206 ± 8	--	18.2	15.6
3,100	10/10	102 ± 3	295 ± 7	+ 193 ± 6	93	16.0	15.3
6,200	10/10	102 ± 4	279 ± 10	+ 177 ± 9	88	16.4	14.2
12,500	10/10	101 ± 2	273 ± 7	+ 172 ± 7	86	19.4	15.0
25,000	10/10	110 ± 4	269 ± 11	+ 159 ± 8	85	18.9	14.6
50,000	10/10	98 ± 4	252 ± 10	+ 154 ± 8	79	26.2	16.4
FEMALE							
0	10/10	83 ± 2	170 ± 5	+ 87 ± 4	--	12.3	9.8
3,100	10/10	97 ± 1	187 ± 4	+ 90 ± 4	110	13.4	10.0
6,200	10/10	91 ± 3	177 ± 6	+ 86 ± 6	104	12.2	9.7
12,500	10/10	87 ± 3	171 ± 5	+ 84 ± 4	101	11.9	9.8
25,000	10/10	95 ± 3	175 ± 3	+ 80 ± 2	103	11.2	9.1
50,000	10/10	92 ± 3	169 ± 4	+ 77 ± 3	99	16.7	8.6

(a) Number surviving/number in group
(b) Mean weight change ± standard error of the mean
(c) Grams of feed per animal

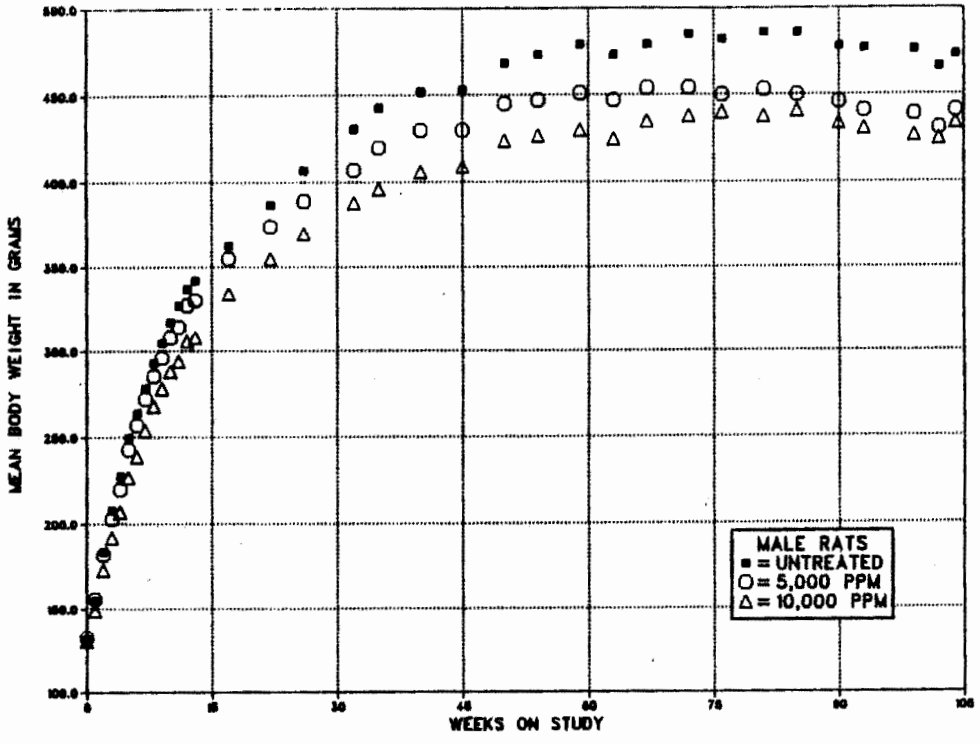
TWO-YEAR STUDIES

Body Weights and Clinical Signs

Throughout most of the studies, mean body weights of dosed rats of each sex were less than those of the controls (Table 7 and Figure 1). The mean body weights were dose related. Mean body weights of low dose and high dose female rats were less than 90% those of the controls after week 32, and the mean body weights of the low dose and high dose females were 87% and 78% that of the controls at the end of the studies. The mean body weight of high dose males was 92% that of the controls. The average daily feed consumption per rat by low dose and high dose rats was 99% and 96%, respectively, that of the controls for males and 91% and 92% for females (Appendix L, Tables L1 and L2). No compound-related clinical signs were observed. The average dose per day was approximately 195 and 390 mg/kg for low dose and high dose male rats and 465 and 1,000 mg/kg for low dose and high dose female rats.

TABLE 7. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

Weeks on Study	Control		Low Dose			High Dose		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE				5,000 ppm			10,000 ppm	
0	132	50	133	100.8	50	131	99.2	50
1	155	50	156	100.6	50	149	96.1	50
2	184	50	182	98.9	50	173	94.0	50
3	208	50	203	97.6	50	192	92.3	50
4	228	50	220	96.5	50	207	90.8	50
5	250	50	243	97.2	50	227	90.8	50
6	264	50	257	97.3	50	239	90.5	50
7	278	50	272	97.8	50	254	91.4	50
8	293	50	285	97.3	50	268	91.5	50
9	305	50	296	97.0	50	278	91.1	50
10	317	50	308	97.2	50	288	90.9	50
11	327	50	314	96.0	50	294	89.9	50
12	337	50	327	97.0	50	306	90.8	50
13	342	50	330	96.5	50	308	90.1	50
17	363	50	355	97.8	50	334	92.0	50
22	387	50	374	96.6	50	355	91.7	50
26	407	50	389	95.6	50	370	90.9	50
32	431	50	407	94.4	50	388	90.0	50
35	443	50	420	94.8	50	396	89.4	50
40	452	50	430	95.1	50	406	89.8	50
45	453	50	430	94.9	50	409	90.3	50
50	468	50	445	95.1	50	424	90.6	50
54	473	50	447	94.5	50	427	90.3	50
59	479	50	451	94.2	50	430	89.8	50
63	473	50	447	94.5	50	425	89.9	50
67	479	50	454	94.8	50	435	90.8	50
72	485	50	454	93.6	49	438	90.3	49
76	482	50	450	93.4	49	440	91.3	48
81	486	48	453	93.2	48	438	90.1	48
85	486	47	450	92.6	48	441	90.7	46
90	478	45	446	93.3	46	434	90.8	46
93	477	42	441	92.5	46	431	90.4	46
99	476	37	439	92.2	41	427	89.7	44
102	466	35	431	92.5	41	425	91.2	44
104	473	32	441	93.2	38	434	91.8	42
FEMALE				10,000 ppm			20,000 ppm	
0	102	50	103	101.0	50	104	102.0	50
1	114	50	113	99.1	50	110	96.5	50
2	128	50	125	97.7	50	124	96.9	50
3	137	50	135	98.5	50	133	97.1	50
4	147	50	142	96.6	50	140	95.2	50
5	160	50	153	95.6	50	151	94.4	50
6	165	50	156	94.5	50	156	94.5	50
7	171	50	162	94.7	50	161	94.2	50
8	177	50	166	93.8	50	166	93.8	50
9	179	50	170	95.0	50	171	95.5	50
10	186	50	174	93.5	50	176	94.6	50
11	188	50	174	92.6	50	176	93.6	50
12	193	50	179	92.7	50	180	93.3	50
13	193	50	178	92.2	50	179	92.7	50
17	201	50	189	94.0	50	190	94.5	50
22	208	50	193	92.8	50	196	94.2	50
26	216	50	200	92.6	50	200	92.6	50
32	230	50	208	90.4	50	204	88.7	50
35	234	50	209	89.3	50	203	86.8	50
40	244	50	218	89.3	50	209	85.7	50
45	248	50	220	88.7	50	209	84.3	50
50	262	50	229	87.4	50	216	82.4	50
54	269	50	232	86.2	50	216	80.3	50
59	276	50	238	86.2	47	223	80.8	50
63	282	50	239	84.8	47	222	78.7	50
67	293	50	253	86.3	47	231	78.8	50
72	307	49	263	85.7	47	238	77.5	50
76	315	49	271	86.0	47	238	75.6	47
81	325	48	275	84.6	47	244	75.1	46
85	329	47	280	85.1	47	248	75.4	46
90	333	45	280	84.1	46	248	74.5	44
93	337	45	277	82.2	46	244	72.4	43
99	342	45	286	83.6	43	251	73.4	40
102	340	44	287	84.4	41	248	72.9	40
104	344	41	298	86.6	40	268	77.9	39



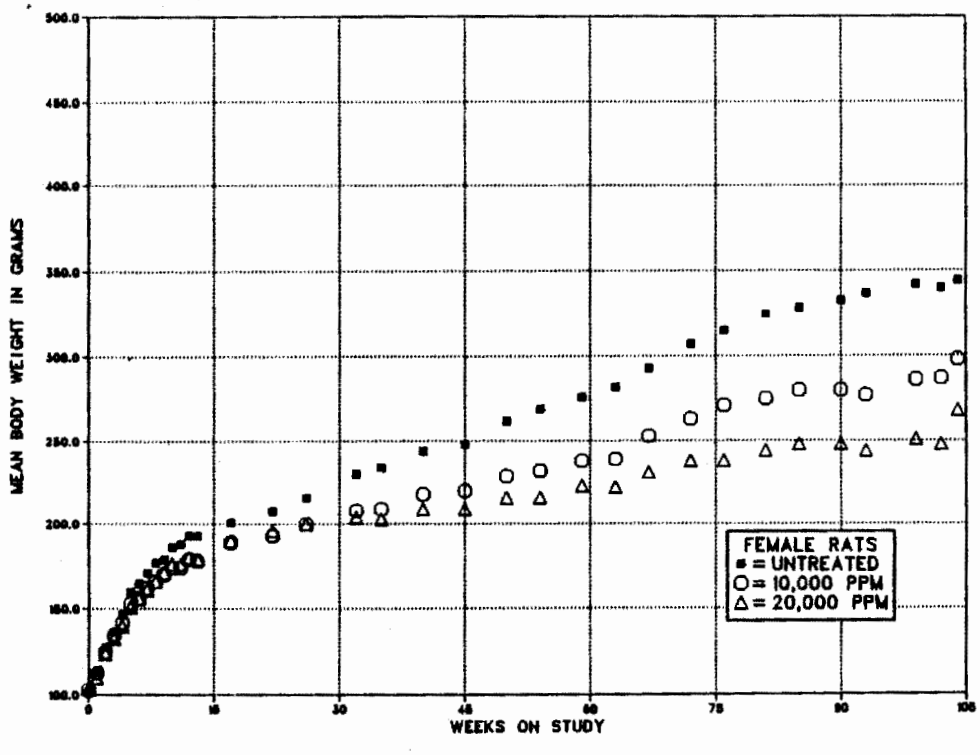


FIGURE 1. GROWTH CURVES FOR RATS FED DIETS CONTAINING HC BLUE NO. 2 FOR TWO YEARS

Survival

Estimates of the probabilities of the survival of male and female rats fed diets containing HC Blue No. 2 at the concentrations used in these studies and those of the controls are shown in the Kaplan and Meier curves in Figure 2. The survival of the control group of male rats was significantly less than that of the high dose group after week 102 (Table 8). HC Blue No. 2 had no significant effect on survival of female rats.

Pathology and Statistical Analyses of Results

This section describes significant or noteworthy changes in the incidences of rats with neoplastic or nonneoplastic lesions. Histopathologic findings on neoplasms in rats are summarized in Appendix A, Tables A1 and A2; Tables A3 and A4 give the survival and tumor status for individual male and female rats. Findings on nonneoplastic lesions are summarized in Appendix C, Tables C1 and C2. Appendix E, Tables E1 and E2, contains the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes). Historical incidences of tumors in control animals are listed in Appendix F.

Liver: Focal inflammation was observed at increased incidences in dosed male rats and in high dose female rats (male: control, 1/50; low dose, 17/50; high dose, 7/49; female: control, 19/50; low dose, 18/50; high dose, 28/50). The inflammation of the liver consisted of granulomas in the nonportal areas. These granulomas were small, consisting of six to eight cells, and were not numerous. Many livers had only one of these granulomas; some had three or four. When the livers were reevaluated in a blind fashion, the severity of inflammation did not appear to be dose related.

Cytoplasmic vacuolization (graded as minimal to mild) was observed at increased incidences in dosed male rats and low dose female rats (male: control, 6/50; low dose, 17/50; high dose, 22/49; female: control, 5/50; low dose, 15/50; high dose, 8/50).

Neoplastic nodules or carcinomas were observed in dosed female rats (control, 0/50; low dose, 2/50; high dose, 3/50) but not at statistically significant incidences.

TABLE 8. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

	Control	Low Dose	High Dose
MALE (a)		**5,000 ppm**	**10,000 ppm**
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	18	12	8
Killed at Termination	32	38	42
Survival P Values (c)	0.030	0.249	0.043
FEMALE (a)		**10,000 ppm**	**20,000 ppm**
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	9	10	11
Killed at Termination	41	40	39
Survival P Values (c)	0.660	0.952	0.740

(a) Terminal kill period: weeks 104-105
(b) Includes animals killed in a moribund condition
(c) The results of the life table trend test are in the control column, and the results of the life table exact pairwise comparisons with the controls are in the dosed columns.

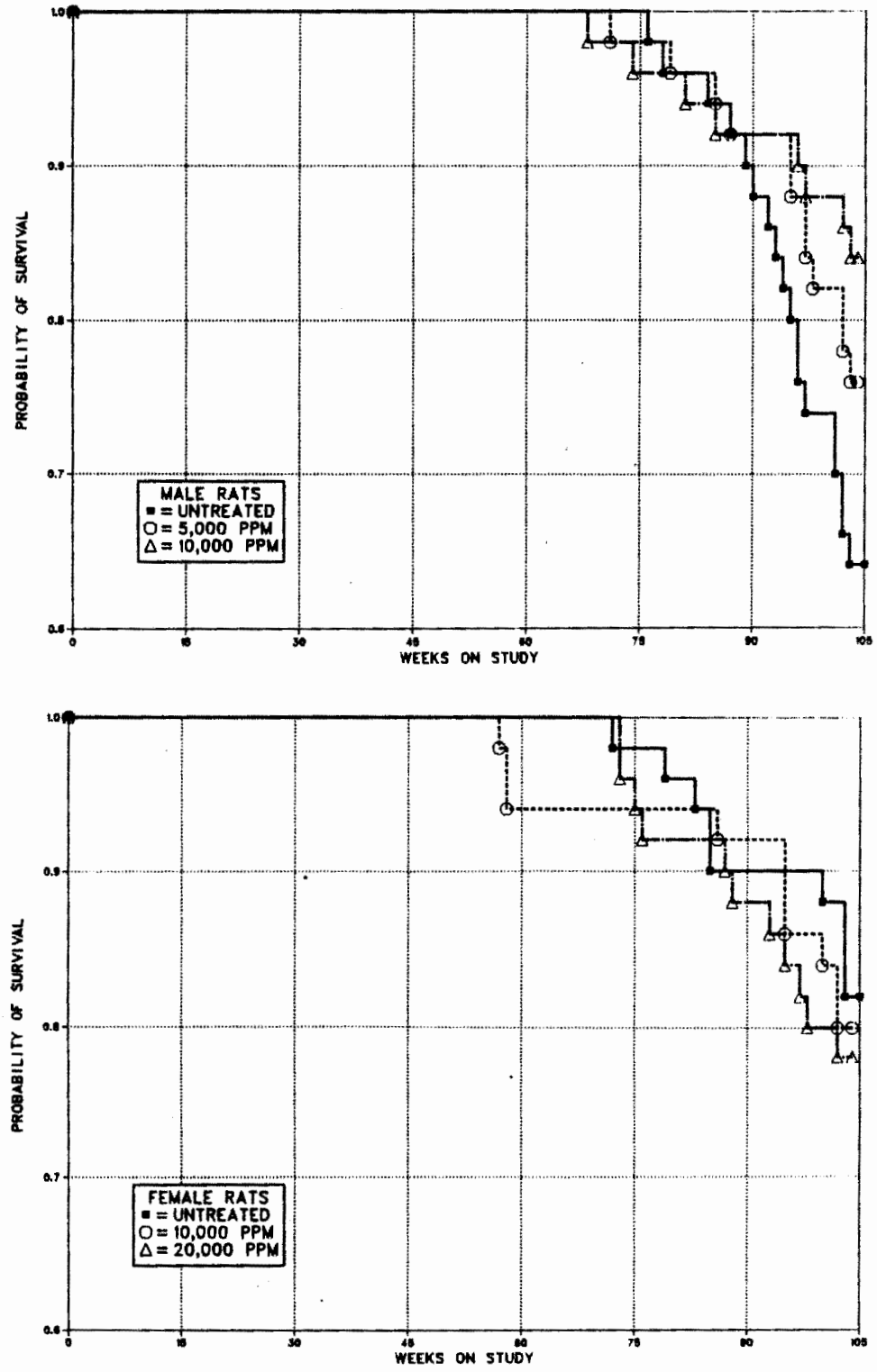


FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR RATS FED DIETS CONTAINING HC BLUE NO. 2 FOR TWO YEARS

Skull: Hyperostosis was observed at increased incidences in dosed rats of each sex (male: control, 5/50; low dose, 8/50; high dose, 25/49; female: control, 2/50; low dose, 19/50; high dose, 49/50). The hyperostosis consisted mainly of an increase in the number or thickness of the lamellae of the compact bone tissue of the calvaria, as compared with the calvaria of rats in the control groups. In the animals with hyperostosis, the new bone appeared similar to the old bone but was separated from it by a smooth basophilic cement line, suggesting that previous bone resorption did not precede the deposition of new bone. One high dose male and nine high dose females also had hyperostosis of the tympanic cavity.

Lung: Histiocytosis was observed at increased incidences in low dose male rats and in dosed female rats (male: control, 4/50; low dose, 14/50; high dose, 4/49; female: control, 6/50; low dose, 28/50; high dose, 19/50). The histiocytosis consisted of subpleural collections of macrophages in alveoli. These cells often contained fine cytoplasmic brown pigment.

Kidney: Malignant mixed mesenchymal tumors were observed in two high dose female rats. Both mesenchymal tumors were relatively large; one involved one-fourth and the other replaced three-fourths of the kidney. The tumors were characterized by spindle cells with round to irregular vesicular nuclei and moderate cytoplasm. Myxomatous and fibrosarcomatous areas were both present within the tumors. Neither tumor appeared to be encapsulated.

Thyroid Gland: C-cell carcinomas in male rats occurred with a significant positive trend, but the incidences of C-cell adenomas or carcinomas (combined) were not significant by any of the statistical tests (Table 9).

TABLE 9. ANALYSIS OF THYROID GLAND TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (a)

	Control	5,000 ppm (b)	10,000 ppm (b)
C-Cell Adenoma			
Overall Rates	7/50 (14%)	2/50 (4%)	5/49 (10%)
C-Cell Carcinoma			
Overall Rates	0/50 (0%)	3/50 (6%)	5/49 (10%)
Adjusted Rates	0.0%	7.6%	11.9%
Terminal Rates	0/32 (0%)	2/38 (5%)	5/42 (12%)
Life Table Tests	P=0.044	P=0.154	P=0.061
Incidental Tumor Tests	P=0.029	P=0.123	P=0.061
C-Cell Adenoma or Carcinoma			
Overall Rates	7/50 (14%)	5/50 (10%)	10/49 (20%)
Adjusted Rates	19.5%	12.0%	23.8%
Terminal Rates	5/32 (16%)	3/38 (8%)	10/42 (24%)
Life Table Tests	P=0.414	P=0.290N	P=0.510
Incidental Tumor Tests	P=0.273	P=0.395N	P=0.407

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) The equivalent dose in milligrams per kilogram per day is given in Chapter III (Body Weights and Clinical Signs) and Appendix L.

Eye: A number of rats were found to have cataracts and/or retinopathy (Table 10). These ocular changes are not believed to be related to the administration of HC Blue No. 2 but are thought to be due to the proximity of the animals' cages to the light source in the animal room. These studies were conducted before the initiation of routine animal cage rotation, a procedure done for the purpose of randomizing animals with respect to artificial light sources. High dose males and low dose females (the two groups most severely affected) were housed in the top rows of their respective cage racks. Control males and high dose females were housed in intermediate rows, and control females and low dose males (the least severely affected groups) were housed in the bottom rows. Ocular changes were not found in mice.

Negative Trends: Certain tumors occurred at decreased incidences in dosed groups relative to those of the controls. Significant negative trends for male rats included basal cell tumors or carcinomas of the skin (control, 4/50; low dose, 0/50; high dose, 0/49), integumentary system fibromas (control, 5/50; low dose, 2/50; high dose, 1/49), mononuclear cell leukemia (control, 12/50; low dose, 6/50; high dose, 5/49), adrenal pheochromocytoma (control, 13/50; low dose, 9/50; high dose, 7/49), and interstitial cell tumors of the testis (control, 45/50; low dose, 47/50; high dose, 37/49).

In female rats, a negative trend was observed for mammary gland fibroadenoma (control, 20/50; low dose, 10/50; high dose, 4/50).

TABLE 10. NUMBER OF RATS WITH RETINOPATHY OR CATARACTS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

	Control	Low Dose	High Dose
MALE		**5,000 ppm**	**10,000 ppm**
No. of animals examined	50	50	49
Retinopathy	6	2	16
Cataracts	3	2	14
FEMALE		**10,000 ppm**	**20,000 ppm**
No. of animals examined	50	50	50
Retinopathy	3	12	10
Cataracts	0	10	2

SINGLE-ADMINISTRATION STUDIES (Lot No. 5130777)

All the mice survived to the end of the studies, and there were no notable effects on body weight gain (Table 11). The urine of dosed mice that received 125, 250, 500, or 1,000 mg/kg was blue on day 1. The urine of one or two mice in these dosed groups was blue on day 2. The urine of one female mouse that received 1,000 mg was bluish red through day 4. Mice that received 1,000 mg/kg were slightly inactive for 4 days after dosing.

TABLE 11. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF HC BLUE NO. 2

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)
MALE				
62	5/5	19.4 ± 0.6	23.4 ± 0.4	+4.0 ± 0.6
125	5/5	23.0 ± 0.7	25.6 ± 0.9	+2.6 ± 0.7
250	5/5	20.8 ± 0.6	23.6 ± 0.5	+2.8 ± 0.7
500	5/5	20.6 ± 0.8	23.4 ± 0.5	+2.8 ± 0.7
1,000	5/5	20.0 ± 0.3	23.0 ± 0.5	+3.0 ± 0.3
FEMALE				
62	5/5	17.8 ± 0.9	19.4 ± 0.7	+1.6 ± 0.2
125	5/5	17.4 ± 0.4	19.8 ± 0.2	+2.4 ± 0.2
250	5/5	16.6 ± 0.7	17.4 ± 0.7	+0.8 ± 0.5
500	5/5	18.2 ± 0.8	20.0 ± 0.8	+1.8 ± 0.4
1,000	5/5	16.8 ± 0.8	18.4 ± 0.5	+1.6 ± 0.5

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES (Lot No. 5130777)

All the mice survived to the end of the studies (Table 12). Mice that received 50,000 ppm lost weight, and female mice receiving 25,000 ppm showed a 10% decrease in body weight relative to that of the controls. The final body weights of other dosed groups were similar to those of the controls. Dosed mice had violet urine throughout the studies. No compound-related effects were observed at necropsy.

TABLE 12. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	21.6 ± 0.7	22.0 ± 0.8	+0.4 ± 0.7	--
3,100	5/5	20.8 ± 0.4	21.6 ± 0.5	+0.8 ± 0.4	98.2
6,200	5/5	20.6 ± 0.6	21.0 ± 1.2	+0.4 ± 0.9	95.5
12,500	5/5	20.2 ± 0.7	21.8 ± 0.6	+1.6 ± 0.4	99.1
25,000	5/5	20.8 ± 0.7	21.0 ± 0.7	+0.2 ± 0.5	95.5
50,000	5/5	21.4 ± 0.7	20.6 ± 0.7	−0.8 ± 0.4	93.6
FEMALE					
0	5/5	18.4 ± 0.6	19.6 ± 0.5	+1.2 ± 0.4	--
3,100	5/5	17.4 ± 0.7	19.4 ± 1.0	+2.0 ± 0.3	99.0
6,200	5/5	17.6 ± 0.8	19.0 ± 0.4	+1.4 ± 0.4	96.9
12,500	5/5	18.6 ± 0.2	19.6 ± 0.4	+1.0 ± 0.3	100.0
25,000	5/5	17.2 ± 0.6	17.6 ± 0.5	+0.4 ± 0.2	89.8
50,000	5/5	18.4 ± 0.5	18.0 ± 0.3	−0.4 ± 0.5	91.8

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

RERUN FOURTEEN-DAY REPEATED-EXPOSURE STUDIES (Lot No. 9233)

These studies were conducted after the 13-week studies. All the mice survived to the end of the studies (Table 13). Dosed mice had violet urine. Control male mice gained no weight during the studies. Final mean body weights of mice were not dose related. No compound-related effects were observed at necropsy. It was concluded that the results of the 13-week studies with lot no. 5130777 could be used to select doses for the 2-year studies with lot no. 9223.

TABLE 13. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE RERUN FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)
MALE					
0	5/5	27.6 ± 0.7	27.6 ± 0.7	0.0 ± 0.5	--
3,100	5/5	28.0 ± 0.8	29.8 ± 0.8	+1.8 ± 0.7	108.0
6,200	5/5	28.0 ± 0.4	28.8 ± 0.6	+0.8 ± 0.4	104.3
12,500	5/5	28.4 ± 0.2	30.0 ± 0.5	+1.6 ± 0.5	108.7
25,000	5/5	28.4 ± 0.9	29.2 ± 1.2	+0.8 ± 0.4	105.8
50,000	5/5	26.6 ± 0.4	26.8 ± 0.6	+0.2 ± 0.6	97.1
FEMALE					
0	5/5	19.2 ± 0.2	20.8 ± 0.2	+1.6 ± 0.2	--
3,100	5/5	19.4 ± 0.6	20.8 ± 0.4	+1.4 ± 0.2	100.0
6,200	5/5	18.0 ± 0.4	19.2 ± 0.5	+1.2 ± 0.4	92.3
12,500	5/5	20.0 ± 0.5	20.2 ± 1.0	+0.2 ± 0.6	97.1
25,000	5/5	19.8 ± 0.6	20.4 ± 0.6	+0.6 ± 0.4	98.1
50,000	5/5	19.0 ± 0.5	20.0 ± 0.5	+1.0 ± 0.0	96.2

(a) Number surviving/number initially in the group
(b) Mean weight change ± standard error of the mean

THIRTEEN-WEEK STUDIES
(Lot No. 5130777)

All the mice survived to the end of the studies (Table 14). Final mean body weights of mice that received 50,000 ppm were depressed 11.5% for males and 9.9% for females, compared with their respective controls. Feed consumption was not dose related. The urine of dosed mice was purple. No compound-related histopathologic effects were observed.

Concentrations of 5,000 and 10,000 ppm HC Blue No. 2 were selected for male mice and 10,000 and 20,000 ppm were selected for female mice in the 2-year studies. The doses were selected primarily on the basis of body weight gain data.

TABLE 14. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF HC BLUE NO. 2

Concentration (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)	Feed Consumption (c) (grams per animal) Day 30	Day 86
MALE							
0	10/10	21.4 ± 0.6	31.2 ± 0.7	+ 9.8 ± 0.5	--	4.9	5.1
3,100	10/10	21.9 ± 0.4	31.1 ± 1.0	+ 9.2 ± 0.7	99.7	4.7	5.6
6,200	10/10	22.4 ± 0.6	29.8 ± 0.8	+ 7.4 ± 0.5	95.5	5.2	6.0
12,500	10/10	23.6 ± 0.5	30.8 ± 0.8	+ 7.2 ± 0.6	98.7	6.0	5.6
25,000	10/10	22.7 ± 0.5	29.2 ± 0.6	+ 6.5 ± 0.4	93.6	6.5	5.2
50,000	10/10	22.4 ± 0.5	27.6 ± 0.7	+ 5.2 ± 0.5	88.5	6.1	6.5
FEMALE							
0	10/10	18.7 ± 0.4	26.2 ± 0.6	+ 7.5 ± 0.5	--	6.0	7.2
3,100	10/10	19.5 ± 0.5	26.3 ± 0.6	+ 6.8 ± 0.5	100.4	3.9	4.3
6,200	10/10	18.0 ± 0.4	24.7 ± 0.4	+ 6.7 ± 0.4	94.3	3.6	3.2
12,500	10/10	18.4 ± 0.5	25.5 ± 0.4	+ 7.1 ± 0.3	97.3	5.7	5.1
25,000	10/10	19.0 ± 0.6	24.5 ± 0.6	+ 5.5 ± 0.6	93.5	5.6	3.8
50,000	10/10	18.1 ± 0.7	23.6 ± 0.6	+ 5.5 ± 0.2	90.1	5.5	4.5

(a) Number surviving/number in group
(b) Mean weight change ± standard error of the mean
(c) Grams of feed per animal

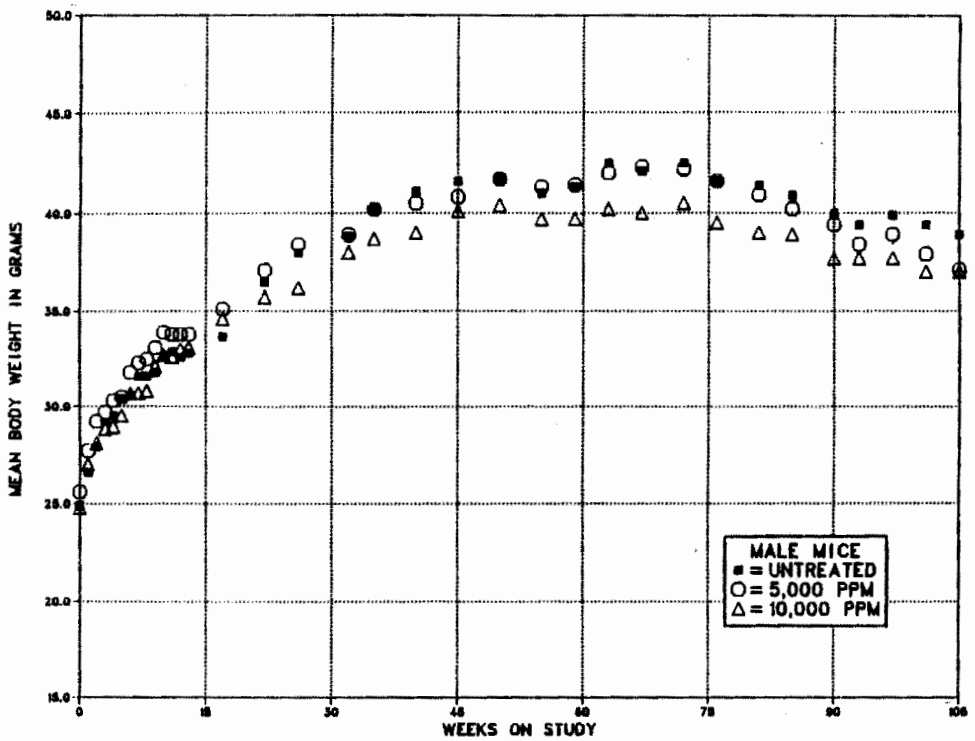
TWO-YEAR STUDIES

Body Weights and Clinical Signs

Mean body weights of high dose male mice were lower than those of the controls after week 22; mean body weights of dosed female mice were lower than those of the control group after week 12 (Table 15 and Figure 3). Low dose and high dose male mice consumed 104% and 85% as much feed as the controls per day; low dose and high dose female mice consumed 110% and 120% as much feed as the controls (Appendix L, Tables L3 and L4). The average dose per day was approximately 1,320 and 2,240 mg/kg for low dose and high dose male mice and 2,330 and 5,600 mg/kg for low dose and high dose female mice.

TABLE 15. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

Weeks on Study	Control		Low Dose			High Dose		
	Av. Wt. (grams)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors	Av. Wt. (grams)	Wt. (percent of controls)	No. of Survivors
MALE				5,000 ppm			10,000 ppm	
0	25	50	26	104.0	50	25	100.0	50
1	27	50	28	103.7	50	27	100.0	50
2	28	50	29	103.6	50	28	100.0	50
3	29	50	30	103.4	50	29	100.0	50
4	29	50	30	103.4	50	29	100.0	50
5	30	50	31	103.3	50	30	100.0	50
6	31	49	32	103.2	50	31	100.0	50
7	32	49	32	100.0	50	31	96.9	50
8	32	49	33	103.1	49	31	96.9	50
9	32	49	33	103.1	49	32	100.0	50
10	33	49	34	103.0	49	33	100.0	50
11	33	49	34	103.0	49	33	100.0	50
12	33	49	34	103.0	49	33	100.0	50
13	33	48	34	103.0	49	33	100.0	50
17	34	46	35	102.9	47	35	102.9	49
22	36	44	37	102.8	46	36	100.0	49
26	38	43	38	100.0	45	36	94.7	49
32	39	42	39	100.0	45	38	97.4	49
35	40	42	40	100.0	45	39	97.5	49
40	41	42	40	97.6	44	39	95.1	49
45	42	40	41	97.6	44	40	95.2	49
50	42	40	42	100.0	42	40	95.2	49
55	41	39	41	100.0	41	40	97.6	49
59	41	39	41	100.0	41	40	97.6	49
63	43	35	42	97.7	40	40	93.0	49
67	42	34	42	100.0	40	40	95.2	48
72	42	34	42	100.0	40	41	97.6	48
76	42	33	42	100.0	39	39	92.9	48
81	41	33	41	100.0	38	39	95.1	47
85	41	32	40	97.6	37	39	95.1	43
90	40	32	39	97.5	34	38	95.0	42
93	39	31	38	97.4	31	38	97.4	38
97	40	29	39	97.5	28	38	95.0	37
101	39	24	38	97.4	26	37	94.9	34
105	39	24	37	94.9	23	37	94.9	34
FEMALE				10,000 ppm			20,000 ppm	
0	19	50	20	105.3	50	19	100.0	50
1	21	50	20	95.2	50	20	95.2	50
2	21	50	22	104.8	50	21	100.0	50
3	22	50	22	100.0	50	21	95.5	50
4	23	50	23	100.0	50	22	95.7	50
5	23	50	23	100.0	50	22	95.7	49
6	23	50	23	100.0	50	23	100.0	49
7	24	50	24	100.0	50	23	95.8	49
8	25	50	25	100.0	50	24	96.0	49
9	25	50	23	92.0	50	24	96.0	49
10	25	50	25	100.0	50	25	100.0	49
11	25	50	26	104.0	50	25	100.0	49
12	24	50	25	104.2	50	25	104.2	49
13	25	50	24	96.0	50	24	96.0	49
17	26	50	26	100.0	50	26	100.0	49
22	30	50	29	96.7	50	28	93.3	49
26	32	50	30	93.8	50	28	87.5	49
32	35	50	32	91.4	50	30	85.7	49
35	35	50	32	91.4	50	29	82.9	49
40	37	50	34	91.9	50	31	83.8	49
45	40	48	34	85.0	50	31	77.5	49
50	43	48	36	83.7	50	32	74.4	49
55	44	48	37	84.1	50	32	72.7	49
59	44	48	36	81.8	49	31	70.5	49
63	45	48	37	82.2	49	32	71.1	49
67	45	48	37	82.2	49	33	73.3	49
72	46	48	37	80.4	49	33	71.7	48
76	45	46	36	80.0	47	32	71.1	47
81	46	45	38	82.6	43	32	69.6	41
85	45	44	37	82.2	42	32	71.1	40
90	45	39	36	80.0	40	31	68.9	35
93	43	39	35	81.4	36	31	72.1	31
97	43	38	36	83.7	34	32	74.4	27
101	42	38	36	85.7	30	32	76.2	21
105	41	35	35	85.4	27	32	78.0	19



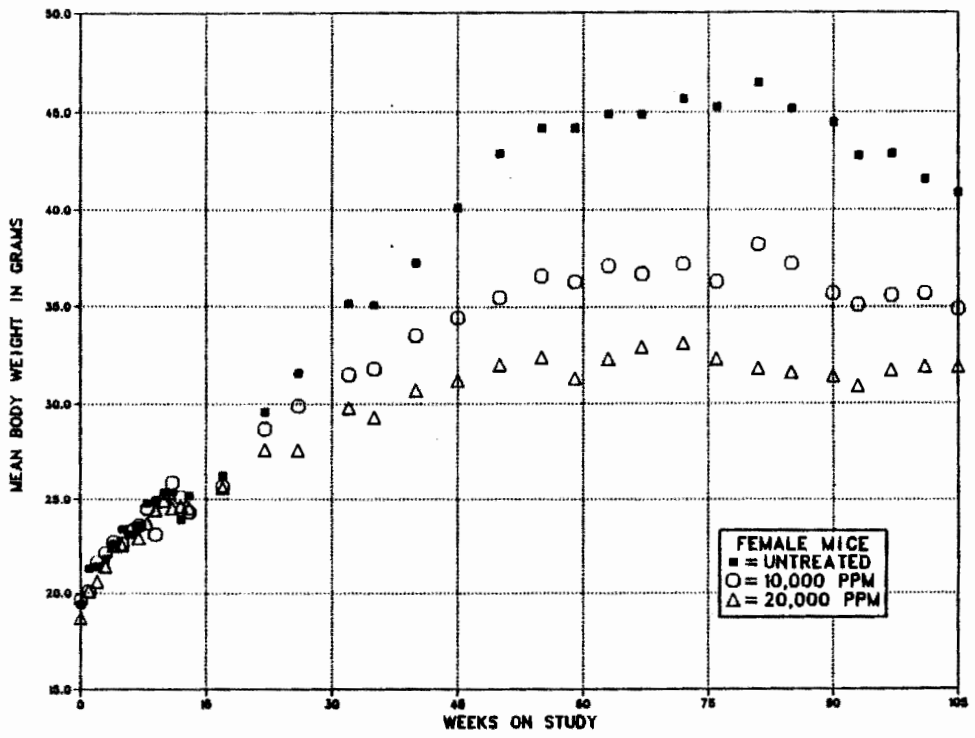


FIGURE 3. GROWTH CURVES FOR MICE FED DIETS CONTAINING HC BLUE NO. 2 FOR TWO YEARS

Survival

Estimates of the probabilities of survival of male and female mice fed diets containing HC Blue No. 2 at the concentrations used in these studies and those of the controls are shown in the Kaplan and Meier curves in Figure 4. The survival of the control group of male mice was significantly lower than that of the high dose group after week 100 and was somewhat lower than the historical survival of control male mice at this laboratory (Table 16). In the previous feeding studies at this laboratory, the mean survival in untreated control male $B6C3F_1$ mice was 76% ± 7% (range, 64%-84%). Survival of the high dose female group was significantly lower than that of the controls after week 97.

Pathology and Statistical Analyses of Results

This section describes significant or noteworthy changes in the incidences of mice with neoplastic or nonneoplastic lesions. Histopathologic findings on neoplasms in mice are summarized in Appendix B, Tables B1 and B2; Tables B3 and B4 give the survival and tumor status for individual male and female mice. Findings on nonneoplastic lesions are summarized in Appendix D, Tables D1 and D2. Appendix E, Tables E3 and E4, contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes). Historical incidences of tumors in control animals are listed in Appendix F.

TABLE 16. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

	Control	Low Dose	High Dose
MALE (a)		**5,000 ppm**	**10,000 ppm**
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	26	26	16
Animals Missing	0	1	0
Killed at Termination	24	23	34
Survival P Values (c)	0.027	0.965	0.030
FEMALE (a)		**10,000 ppm**	**20,000 ppm**
Animals Initially in Study	50	50	50
Nonaccidental Deaths Before Termination (b)	15	22	30
Killed at Termination	35	27	19
Died During Termination Period	0	1	1
Survival P Values (c)	0.005	0.256	0.008

(a) Terminal kill period: weeks 105-106
(b) Includes animals killed in a moribund condition
(c) The results of the life table trend test are in the control column, and the results of the life table exact pairwise comparisons with the controls are in the dosed columns.

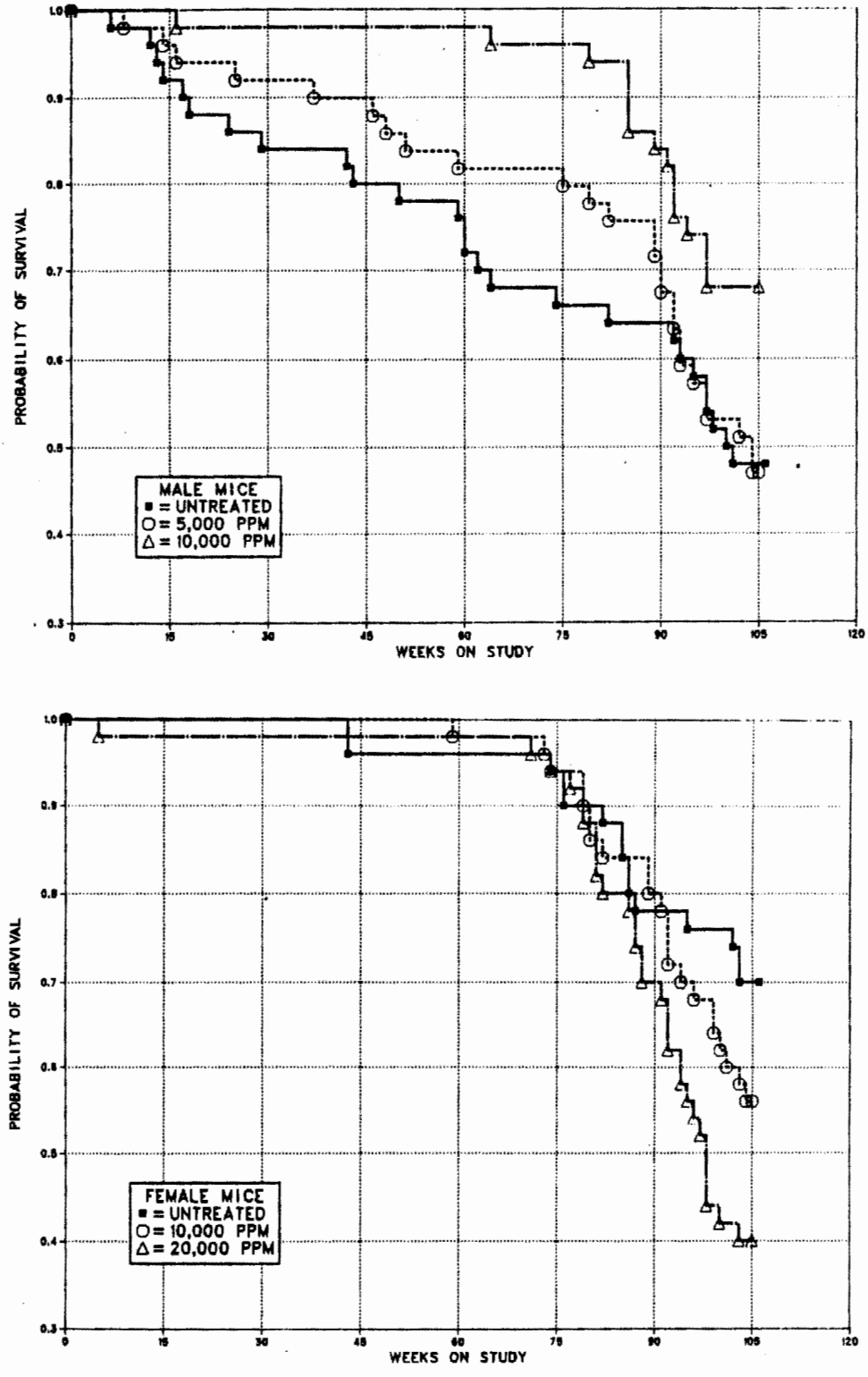


FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR MICE FED DIETS CONTAINING HC BLUE NO. 2 FOR TWO YEARS

Hematopoietic System: Lymphocytic malignant lymphomas and malignant lymphomas (all types) occurred in male mice with significant positive trends, but the incidences in the dosed groups were not significantly greater than those in the controls by pairwise comparisons (Table 17). The incidence of lymphomas in high dose female mice was lower than that in the controls. Leukemia was not observed in either male or female mice. Hematopoiesis was observed at an increased incidence in the liver and spleen of high dose female mice (Table 18).

TABLE 17. ANALYSIS OF HEMATOPOIETIC SYSTEM TUMORS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2 (a)

	Control	Low Dose	High Dose
MALE		**5,000 ppm (b)**	**10,000 ppm (b)**
Malignant Lymphoma, Lymphocytic Type			
Overall Rates	0/50 (0%)	2/48 (4%)	5/49 (10%)
Adjusted Rates	0.0%	8.7%	13.5%
Terminal Rates	0/24 (0%)	2/23 (9%)	3/34 (9%)
Life Table Tests	P=0.043	P=0.228	P=0.069
Incidental Tumor Tests	P=0.040	P=0.228	P=0.068
Lymphoma, All Malignant (c)			
Overall Rates	1/50 (2%)	5/48 (10%)	8/49 (16%)
Adjusted Rates	4.2%	17.6%	19.7%
Terminal Rates	1/24 (4%)	2/23 (9%)	4/34 (12%)
Life Table Tests	P=0.053	P=0.110	P=0.060
Incidental Tumor Tests	P=0.050	P=0.157	P=0.095
FEMALE		**10,000 ppm (b)**	**20,000 ppm (b)**
Lymphoma, All Malignant (d)			
Overall Rates	12/50 (24%)	11/50 (22%)	7/50 (14%)
Adjusted Rates	27.8%	32.1%	20.2%
Terminal Rates	5/35 (14%)	7/28 (25%)	1/20 (5%)
Life Table Tests	P=0.399N	P=0.523	P=0.397N
Incidental Tumor Tests	P=0.065N	P=0.479N	P=0.030N

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) The equivalent dose in milligrams per kilogram per day is given in Chapter III (Body Weights and Clinical Signs) and Appendix L.
(c) Historical incidence at testing laboratory (mean ± standard deviation): 8.9% ± 4.69%; historical incidence in NTP studies: 12.0% ± 6.7%
(d) Historical incidence at testing laboratory : 19.7% ± 7.7%; historical incidence in NTP studies: 25.1% ± 10.1%

TABLE 18. INCIDENCES OF MICE WITH HEMATOPOIESIS IN THE LIVER AND SPLEEN IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

	Control	Low Dose	High Dose
MALE		**5,000 ppm**	**10,000 ppm**
Liver	0/50	2/48	1/49
Spleen	10/50	6/47	4/49
FEMALE		**10,000 ppm**	**20,000 ppm**
Liver	2/50	3/50	11/49
Spleen	8/50	9/50	20/49

Pituitary: The incidence of pituitary adenomas in low dose female mice was significantly less than that in the controls (control, 9/49; low dose, 2/48; high dose, 5/49).

Forestomach: Squamous cell papillomas were observed in 2/49 high dose male mice and in 1/49 control female mice.

Bone: Fibrous osteodystrophy was observed at increased incidences in dosed female mice (control, 2/50; low dose, 5/50; high dose, 12/50) but not in any male mice. Hyperostosis of the skull was observed in 4/50 high dose female mice and 1/49 high dose male mice but not in any of the other groups of mice.

Multiple Organs: Suppurative inflammation of the prostate, preputial gland, seminal vesicle, urethra, bulbourethral gland, or multiple organs was observed in 7/50 control, 10/48 low dose, and 5/49 high dose male mice. Suppurative inflammation of the uterus or multiple organs or ovarian abscesses were observed in 8/15 (53%) control, 12/23 (52%) low dose, and 26/31 (84%) high dose female mice that died before the end of the study. The suppurative inflammation in the female mice usually caused death by spreading to multiple secondary sites and because of possible concurrent septicemia.

IV. DISCUSSION AND CONCLUSIONS

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The oral route of administration was selected to maximize chances of detecting systemic effects associated with administration of HC Blue No. 2. Absorption of HC Blue No. 2 from the gastrointestinal tract was evident by the observation of blue urine during single-administration gavage and 14-day repeated-exposure feed studies. Except for one mouse in the single-administration studies, blue urine was noted only on the day of dosing and on the following day. This observation is similar to those made during the HC Blue No. 1 studies (NTP, 1985) in which blue urine was observed for 1-2 days after dosing. HC Blue No. 2 is known to be absorbed through the skin, albeit less than 0.1% (Burnett, C., personal communication to NTP, 1984).

During the HC Blue No. 2 prechronic studies conducted on both species, the only endpoint that was noticeably influenced by dosing was body weight gain. All animals survived to the completion of the 13-week studies, which used diets with concentrations ranging from 0 to 50,000 ppm. All dosed rats and mice gained weight during this experiment, and weight gained by female rats was comparable to that gained by the control group. Male rats that received as little as 6,200 ppm HC Blue No. 2 exhibited a 12% depression in final mean body weight relative to the controls. This decrease in body weight gain could not be explained on the basis of decreased feed consumption. Male and female mice receiving diets with 50,000 ppm of HC Blue No. 2 had final mean body weights that were 88% (males) and 90% (females) those of the controls. Other dose groups also exhibited slight weight differences; these changes ranged from +0.4% in females receiving diets with the 3,100-ppm concentration to −6.5% in females receiving 25,000 ppm. The selection of dietary concentrations to be used in the 2-year studies (5,000 or 10,000 ppm for male rats and mice and 10,000 or 20,000 ppm for female rats and mice) was based largely on weight gains during the 13-week studies.

The survival of dosed male and female rats was not adversely affected by dietary administration of HC Blue No. 2 for 103-104 weeks. In fact, the survival of high dose males (42/50) was significantly greater than that of control males (32/50) at 104 weeks. The final mean body weights in dosed male rats were within 10% that of the controls, whereas the final mean body weights in low dose and high dose females were 13% and 22% lower than that of the controls.

Hyperostosis of the skull was produced by HC Blue No. 2 administration. This alteration was characterized by an increase in the number or thickness of lamellae of the compact bone of the calvaria. This unusual finding was present in a few control animals, whereas the incidence was increased considerably in the dosed groups with females being more affected than males (male: control, 5/50; low dose, 8/50; high dose, 25/49; female: control, 2/50; low dose, 19/50; high dose, 49/50). Hyperostosis of the skull was also observed in 4/50 high dose female mice and 1/49 high dose male mice. This skeletal alteration was not detected in control or low dose mice. This thickening of the bone was apparently not secondary to renal or parathyroid effects of HC Blue No. 2, since no parathyroid lesions were detected in the animals in these studies. Restriction of the hyperostosis to the skull may be more a reflection of the method of examination than the actual distribution of the lesion. The hyperostosis was visible grossly as white nodular raised areas on the calvaria when the brain was removed. The rest of the skeleton was not systematically examined grossly, but there was some bone deposition in other bones examined histologically. Bone changes are a dynamic process; thus, without studies using a time marker, it is not possible to determine the pathogenic mechanisms in the HC Blue No. 2 study.

A second skeletal change, fibrous osteodystrophy, was observed in all groups of female mice. Although the incidences were increased in a dose-related manner (control, 2/50; low dose, 5/50; high dose, 12/50), the change is not considered to be due to HC Blue No. 2 administration because fibrous osteodystrophy is a common finding in aged $B6C3F_1$ mice.

Malignant mixed mesenchymal neoplasms of the kidney were seen at the end of the study in two high dose female rats but not in any of the other groups of rats. Although those renal lesions are uncommon neoplasms in female F344/N rats at this laboratory and in NTP studies (0/1,863), the occurrence of these two neoplasms is not considered to be compound

related. The incidence of C-cell carcinomas of the thyroid gland increased in male rats (0/50, 3/50, 5/49), but this increase was accompanied by a decrease in C-cell adenomas, and the incidences of C-cell adenomas or carcinomas (combined) were not significantly different. Thus, the C-cell carcinomas were considered unrelated to HC Blue No. 2 administration.

The survival of male mice was not reduced by the administration of HC Blue No. 2, and, as in the rat studies, the survival of high dose male mice (34/50) was greater than that of the control males (24/50) at weeks 105-106. The survival of high dose female mice (19/50) was significantly lower than that of the controls (35/50), but it is difficult to attribute this decreased survival to the administration of HC Blue No. 2 since at week 90, 78% of the control females were alive as compared with 70% of the high dose females. During the final 15 weeks of the study, 4 control females died, whereas 16 high dose females died. Most of the early deaths among female mice in this study (control, 8/15, 53%; low dose, 12/23, 52%; high dose, 26/31, 84%) were attributable to a reproductive tract infection characterized by ovarian abscesses and suppurative inflammation of the uterus which spread to multiple organs. The dose-related increases in hematopoiesis of the liver and spleen in female mice are believed to be a part of a leukemoid reaction in response to this infection; similar infections have been found at this and other laboratories. Both *Klebsiella oxytoca* and *K. pneumoniae* have been isolated from ovarian abscesses; however, the exact source is not known. The early deaths among the high dose female mice are not considered to have been due to HC Blue No. 2. Despite the infection, over half of the animals in the high dose group (54%) survived for more than 97 weeks.

The final mean body weights of dosed male mice were within 5% of that of the control group, but final mean body weights for dosed females were 85% (10,000 ppm) or 78% (20,000 ppm) that of the control group.

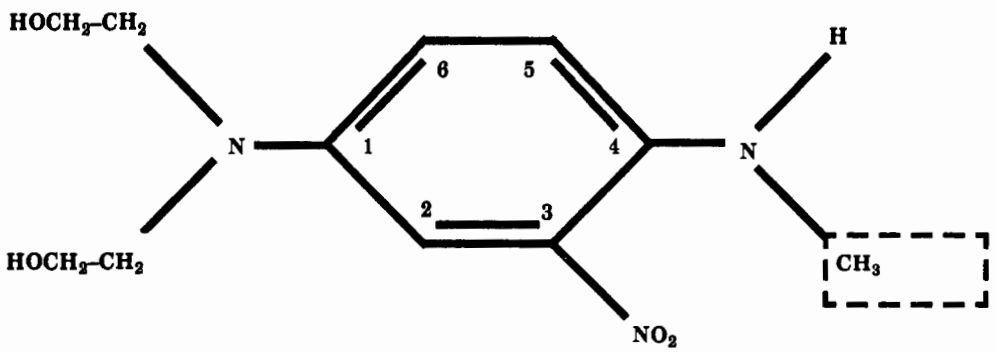
Negative trends occurred in the incidences of basal cell tumors or carcinomas of the skin, pheochromocytomas of the adrenal gland, mononuclear cell leukemia, skin or subcutaneous fibromas, and testicular interstitial cell tumors in male rats and of mammary gland fibroadenomas in female rats. Most of these negative trends could be related to greater than usual tumor incidences in the concurrent control groups. Reduced incidences of mammary gland fibroadenomas in female F344/N rats were found in previous NTP studies to be associated with decreased weight gain (Haseman, 1983); this association was observed in the current study as well.

A marginal positive trend ($P=0.05$) occurred in the incidences of lymphoma in male mice, but the incidences in the dosed groups were not significantly elevated relative to that in the controls. This trend may be due to a low incidence of these tumors in the control group. Lymphocytic-type malignant lymphoma was not observed in the controls; a single malignant lymphoma was diagnosed in the control group. The historical incidences of lymphomas (all types) are 9% for this laboratory and 12% for the Program. The marginal positive trend noted in this study is not considered to be associated with the administration of HC Blue No. 2.

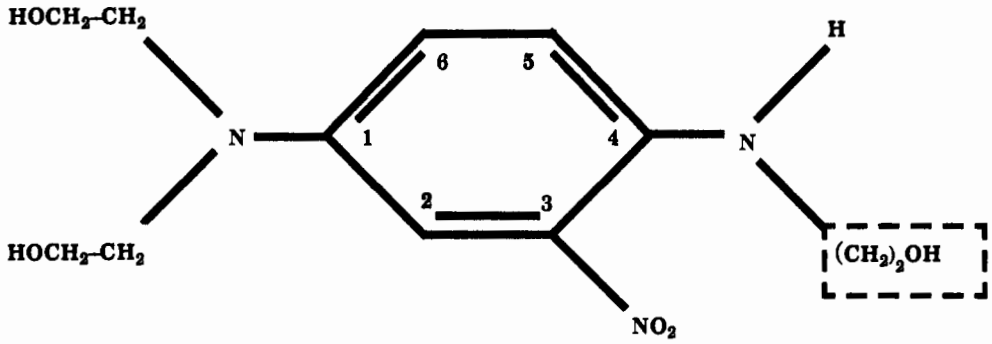
The results of these studies afford an opportunity to compare the toxicologic and carcinogenic properties of two closely related chemicals that are both used as hair dyes. Structurally, HC Blue No.1 and HC Blue No 2 differ only in the substituent on the secondary amine in position number 4 of the molecule. In HC Blue No. 1, this substituent is a methyl group, whereas in HC Blue No.2, it is a hydroxyethyl group (Figure 5).

Both studies were conducted at the same laboratory, in the same strains of animals, and at approximately the same time. The 2-year portion of the HC Blue No. 1 studies was conducted between May 1979 and April 1981, and the HC Blue No. 2 studies were conducted between February 1980 and February 1982. Also, the doses used for the single-administration and 14-day repeated-exposure studies were the same.

Because of the absence of any remarkable toxicologic effects during the 13-week phases of both studies, concentrations for the 2-year studies were based on chemical-induced reductions in body weight gains. Concentrations selected for the 13-week studies of HC Blue No. 1 ranged



HC BLUE NO. 1
2,2'-((4-METHYLAMINO)-3-NITROPHENYL)IMINO)BIS(ETHANOL)
CAS NO. 2784-94-3



HC BLUE NO. 2
2,2'-((4-((2-HYDROXYETHYL)AMINO)-3-NITROPHENYL)IMINO)BIS(ETHANOL)
CAS NO. 33229-34-4

FIGURE 5. CHEMICAL STRUCTURES OF HC BLUE NO. 1 AND HC BLUE NO. 2

from 750 to 12,500 ppm as compared with concentrations of 3,100 to 50,000 ppm in the 13-week HC Blue No. 2 studies. During the 13-week studies of HC Blue No. 1, a golden-brown pigment (not identified) was observed in the cytoplasm of thyroid epithelial cells in both rats and mice. No microscopic changes were detected in animals dosed for 13 weeks with HC Blue No. 2 (at dose concentrations four times greater than those used for HC Blue No. 1).

Hepatocellular neoplasia in mice and in male rats was the most striking effect of the 2-year administration of HC Blue No. 1 (NTP, 1985). Ninety-six percent of the high dose female mice in that study developed hepatocellular carcinomas. In addition, HC Blue No. 1 administration caused proliferative lesions of the thyroid gland in male mice and of the lung in female rats. In contrast, the long-term administration of HC Blue No. 2 was not considered to be associated with increases in the incidences of any primary tumors in either rats or mice. Also, proliferative changes of the thyroid gland and lung or pigmentation of the thyroid gland were not features of HC Blue No. 2 administration. The single effect produced by the administration of HC Blue No. 2 was hyperostosis of the skull, an effect seen primarily in rats, although it was found also in a small number of high dose mice.

The differences in the toxicologic potentials of these two closely related chemicals might be attributable to differing rates of absorption from the gastrointestinal tract, to excretion, or to different routes of biotransformation. Since colored urine is produced after single gavage administrations of the dyes to both rats and mice, both chemicals are absorbed to some extent, and both are excreted at least partly in the urine. In the earlier report on the HC Blue No. 1 studies (NTP, 1985), it was suggested that the compound might be subject to N-demethylation of the secondary amine, resulting in the formation of an aromatic amine. The presence of the hydroxyethyl substituent on the secondary amine moiety of HC Blue No. 2 may favor conjugation rather than dealkylation. Conjugation would favor more rapid urinary excretion, inhibit N-dealkylation, and therefore reduce the amount of free amine formed.

After this study ended, the dye sample used for the 2-year studies was found to contain approximately 22 ppm of nitrosamines. Five discrete nitrosamines were found in the sample, and only one (*N*-nitrosodiethanolamine, 2.7 ppm) was identified. Based on total nitrosamine content of the dye and concentrations of the dye in the diet, high dose male rats and mice received an estimated 220 ppb of total nitrosamines and high dose female rats and mice received 440 ppb. Since there was no evidence of carcinogenicity attributable to the administration of HC Blue No. 2, the presence of the nitrosamines is not considered to be a significant factor in this study.

Conclusions: Under the conditions of these studies, there was *no evidence of carcinogenicity** in male and female F344/N rats or in male and female B6C3F$_1$ mice receiving HC Blue No. 2 in the diet at concentrations of 0.5% and 1.0% for males and 1.0% and 2.0% for females for 2 years. HC Blue No. 2 administration caused a dose-related increase in the incidence of hyperostosis of the skull in male and female rats.

*Categories of evidence of carcinogenicity are defined in the Note to the Reader on page 2.

V. REFERENCES

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL TUMOR	3 (6%)		
BASAL-CELL CARCINOMA	1 (2%)		
ADNEXAL ADENOMA		2 (4%)	1 (2%)
KERATOACANTHOMA	3 (6%)	2 (4%)	1 (2%)
FIBROMA	2 (4%)		
*SUBCUT TISSUE	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
SARCOMA, NOS	1 (2%)		
FIBROMA	3 (6%)	2 (4%)	1 (2%)
FIBROSARCOMA			1 (2%)
LIPOMA	1 (2%)		
OSTEOSARCOMA, INVASIVE	1 (2%)		
NEUROFIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(48)	(43)	(47)
C-CELL CARCINOMA, INVASIVE		1 (2%)	
#LUNG	(50)	(50)	(49)
CARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
LEUKEMIA, MONONUCLEAR CELL	12 (24%)	6 (12%)	4 (8%)
#SPLEEN	(50)	(50)	(49)
LEIOMYOSARCOMA		1 (2%)	
#SPLENIC SEROSA	(50)	(50)	(49)
MESOTHELIOMA, NOS	1 (2%)		
#LIVER	(50)	(50)	(49)
LEUKEMIA, MONONUCLEAR CELL			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
NEOPLASTIC NODULE	1 (2%)		2 (4%)
#HEPATIC SEROSA	(50)	(50)	(49)
MESOTHELIOMA, NOS	1 (2%)		
#PANCREAS	(50)	(50)	(49)
ACINAR-CELL ADENOMA		1 (2%)	
#JEJUNUM	(50)	(50)	(49)
SARCOMA, NOS	1 (2%)		
LEIOMYOSARCOMA	1 (2%)		
#COLONIC MUCOSA	(49)	(50)	(49)
ADENOMATOUS POLYP, NOS		1 (2%)	

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(49)
LIPOMA	1 (2%)		
#KIDNEY/PELVIS	(50)	(50)	(49)
TRANSITIONAL-CELL CARCINOMA	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(49)	(48)
ADENOMA, NOS	9 (18%)	9 (18%)	10 (21%)
ACIDOPHIL ADENOMA	1 (2%)		
#ADRENAL	(50)	(50)	(49)
PHEOCHROMOCYTOMA	13 (26%)	9 (18%)	7 (14%)
#THYROID	(50)	(50)	(49)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	7 (14%)	2 (4%)	5 (10%)
C-CELL CARCINOMA		3 (6%)	5 (10%)
#PANCREATIC ISLETS	(50)	(50)	(49)
ISLET-CELL ADENOMA	1 (2%)	2 (4%)	1 (2%)
ISLET-CELL CARCINOMA	1 (2%)	1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
FIBROADENOMA	1 (2%)		1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(49)
CARCINOMA, NOS	1 (2%)	2 (4%)	
#TESTIS	(50)	(50)	(49)
INTERSTITIAL-CELL TUMOR	45 (90%)	47 (94%)	37 (76%)
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(49)
OSTEOSARCOMA, INVASIVE	1 (2%)		
SPECIAL SENSE ORGANS			
*EYELID	(50)	(50)	(49)
NEURILEMOMA			1 (2%)
*EAR	(50)	(50)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
*ZYMBAL GLAND	(50)	(50)	(49)
CARCINOMA, NOS		1 (2%)	
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOSQUAMOUS CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(49)
OSTEOSARCOMA	1 (2%)	1 (2%)	
BODY CAVITIES			
*PELVIC ORGANS	(50)	(50)	(49)
SARCOMA, NOS			1 (2%)
RHABDOMYOSARCOMA			1 (2%)
*TUNICA VAGINALIS	(50)	(50)	(49)
MESOTHELIOMA, NOS		3 (6%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(49)
C-CELL CARCINOMA, METASTATIC		1 (2%)	
SARCOMA, NOS	1 (2%)	1 (2%)	
LUMBAR REGION			
OSTEOSARCOMA	1		
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	4	6	3
MORIBUND SACRIFICE	14	6	5
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	32	38	42
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	50	49	47
TOTAL PRIMARY TUMORS	120	98	87
TOTAL ANIMALS WITH BENIGN TUMORS	49	48	45
TOTAL BENIGN TUMORS	92	78	67
TOTAL ANIMALS WITH MALIGNANT TUMORS	23	14	13
TOTAL MALIGNANT TUMORS	25	17	17
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	2	
TOTAL SECONDARY TUMORS	2	3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	3	3
TOTAL UNCERTAIN TUMORS	3	3	3
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 IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	1 (2%)	1 (2%)	
BASAL-CELL CARCINOMA		1 (2%)	
KERATOACANTHOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROMA			1 (2%)
RESPIRATORY SYSTEM			
*NOSE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
#LUNG	(50)	(50)	(50)
ADENOCARCINOMA, NOS, METASTATIC		1 (2%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
LEUKEMIA, MONONUCLEAR CELL	4 (8%)	6 (12%)	3 (6%)
#LIVER	(50)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	2 (4%)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#GASTRIC MUCOSA	(50)	(50)	(50)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
MIXED MESENCHYMAL TUMOR, MALIG			2 (4%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(50)	(49)
CARCINOMA, NOS	1 (2%)	1 (2%)	
ADENOMA, NOS	19 (39%)	18 (36%)	16 (33%)
#ADRENAL	(49)	(50)	(49)
PHEOCHROMOCYTOMA	3 (6%)	7 (14%)	5 (10%)
#THYROID	(49)	(50)	(49)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	6 (12%)	4 (8%)	5 (10%)
C-CELL CARCINOMA	1 (2%)	2 (4%)	2 (4%)
#PANCREATIC ISLETS	(49)	(50)	(50)
ISLET-CELL ADENOMA	1 (2%)		

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	
FIBROADENOMA	20 (40%)	10 (20%)	4 (8%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS		1 (2%)	
ADENOMA, NOS			1 (2%)
KERATOACANTHOMA			1 (2%)
*VAGINA	(50)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
#UTERUS	(50)	(50)	(50)
LEIOMYOSARCOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	13 (26%)	7 (14%)	15 (30%)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		
ADENOCARCINOMA, NOS	1 (2%)	1 (2%)	
#OVARY	(50)	(50)	(50)
GRANULOSA-CELL TUMOR	1 (2%)	1 (2%)	
GRANULOSA-CELL CARCINOMA	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
CARCINOMA, NOS, INVASIVE	1 (2%)		
ASTROCYTOMA		1 (2%)	
*CAUDA EQUINA	(50)	(50)	(50)
SARCOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EAR	(50)	(50)	(50)
NEURILEMOMA			1 (2%)
*ZYMBAL GLAND	(50)	(50)	(50)
ADENOSQUAMOUS CARCINOMA		1 (2%)	
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOSARCOMA	1 (2%)		
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIVE		1 (2%)	
*ABDOMINAL CAVITY	(50)	(50)	(50)
ADENOCARCINOMA, NOS, INVASIVE		1 (2%)	
ALL OTHER SYSTEMS			
NONE			

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

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	2	2	4
MORIBUND SACRIFICE	7	8	7
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	41	40	39
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	43	36	41
TOTAL PRIMARY TUMORS	78	68	65
TOTAL ANIMALS WITH BENIGN TUMORS	38	30	33
TOTAL BENIGN TUMORS	66	49	50
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	15	11
TOTAL MALIGNANT TUMORS	11	17	13
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	3	
TOTAL SECONDARY TUMORS	1	3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1	2	2
TOTAL UNCERTAIN TUMORS	1	2	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: UNTREATED CONTROL

ANIMAL NUMBER	0031	0032	0033	0034	0035	0036	0037	0038	0039	0040	0041	0042	0043	0044	0045	0046	0047	0048	0049	0050	0051	0052	0053	0054	0055
WEEKS ON STUDY	041	041	041	041	053	046	041	052	061	051	011	051	051	051	051	021	051	051	078	097	051	055	055	055	055
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA													X												
BASAL-CELL TUMOR																		X							
BASAL-CELL CARCINOMA																									
KERATOACANTHOMA																X									
FIBROMA						X																	X		
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA														X											
SARCOMA, NOS																									
FIBROMA																									
LIPOMA																				X					
OSTEOSARCOMA, INVASIVE													X												
NEUROFIBROSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA										X															
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MESOTHELIOMA, NOS											X														
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	-	+	-	+	+	-	+	-	+	+	+	+	+	-	+	-	+	-	+	-
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE																									
MESOTHELIOMA, NOS											X														
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS																									
LEIOMYOSARCOMA																									
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIPOMA						X																			
KIDNEY/PELVIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRANSITIONAL-CELL CARCINOMA						X																			
URINARY BLADDER	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS			X			X										X	X			X				X	X
ACIDOPHIL ADENOMA													X												
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA		X		X			X	X			X														
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA			X						X					X	X								X		
PARATHYROID	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA			X																						
ISLET-CELL CARCINOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROADENOMA																									
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA,NOS																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OSTEOSARCOMA, INVASIVE													X												
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N	N	N	N	N	N	N	N	N	N
OSTEOSARCOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SARCOMA, NOS		X																							
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									
LEUKEMIA,MONONUCLEAR CELL									X	X									X	X	X				
LUMBAR REGION																									
OSTEOSARCOMA																									

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
3: ANIMAL MIS-SEXED

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0225	0227	0228	0229	0231	0232	0233	0234	0235	0236	0237	0238	0239	0240	0241	0242	0243	0244	0245	0246	0247	0248	0249	0250	TOTAL
WEEKS ON STUDY	095	012	015	087	095	095	095	095	095	096	095	095	095	095	094	095	095	091	084	095	089	076	095	095	TISSUES TUMORS
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
SQUAMOUS CELL PAPILLOMA																									1
BASAL-CELL TUMOR												X										X		X	3
BASAL-CELL CARCINOMA																									1
KERATOACANTHOMA								X									X								3
FIBROMA																									2
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
SQUAMOUS CELL CARCINOMA																									1
SARCOMA, NOS																	X								1
FIBROMA			X															X				X			3
LIPOMA																									1
OSTEOSARCOMA, INVASIVE																									1
NEUROFIBROSARCOMA													X												1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA																									1
TRACHEA	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MESOTHELIOMA, NOS																									1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	-	+	+	+	+	+	-	+	-	+	-	-	-	-	+	+	+	+	+	-	+	+	34
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE						X																			1
MESOTHELIOMA, NOS																									1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SARCOMA, NOS															X										1
LEIOMYOSARCOMA									X																1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIPOMA																									1
KIDNEY/PELVIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRANSITIONAL-CELL CARCINOMA																									1
URINARY BLADDER	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS										X									X						9
ACIDOPHIL ADENOMA																									1
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA			X		X								X				X	X	X	X		X			13
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA																	X					X			7
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	48
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA																									1
ISLET-CELL CARCINOMA																								X	1
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	N	N	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
FIBROADENOMA																	X								1
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	45
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
CARCINOMA, NOS		X																							1
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
OSTEOSARCOMA, INVASIVE																									1
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
OSTEOSARCOMA																									1
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
SARCOMA, NOS		X																							1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									1
LEUKEMIA,MONONUCLEAR CELL	X		X	X					X							X						X	X		12
LUMBAR REGION																									
OSTEOSARCOMA																				X					1

+ ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADNEXAL ADENOMA								X												X					
KERATOACANTHOMA																			X						
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+
FIBROMA																	X								
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA, NOS, METASTATIC																									
TRACHEA	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL CARCINOMA, INVASIVE																									
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LEIOMYOSARCOMA																									
LYMPH NODES	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	-	+	-	+	+	-	-	+	-	+	+	+	-	+	+	+	-	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ACINAR-CELL ADENOMA																									
ESOPHAGUS	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMATOUS POLYP, NOS																									
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+
ADENOMA, NOS										X										X					
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA		X			X			X	X															X	
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA												+													+
C-CELL CARCINOMA									X			X													X
PARATHYROID	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
ISLET-CELL CARCINOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X		X	X	X	
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
CARCINOMA, NOS								X																	
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
EAR	N	N	N	+	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N
SQUAMOUS CELL PAPILLOMA																								X	
ZYMBAL GLAND	N	N	N	+	N	N	N	N	+	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N
CARCINOMA, NOS																									
SQUAMOUS CELL CARCINOMA																									
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	X	N	N	N	N	N	N	N	N	N	N	N
OSTEOSARCOMA																									
BODY CAVITIES																									
TUNICA VAGINALIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MESOTHELIOMA, NOS				X																					
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
C-CELL CARCINOMA, METASTATIC																									
SARCOMA, NOS												X													
LEUKEMIA, MONONUCLEAR CELL							X																		

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	0126	0127	0128	0129	0130	0131	0132	0133	0134	0135	0136	0137	0138	0139	0140	0141	0142	0143	0144	0145	0146	0147	0148	0149	0150	TOTAL
WEEKS ON STUDY	104	104	104	102	104	104	104	104	104	104	104	104	104	104	095	097	102	085	104	104	104	087	097	071	064	TISSUES / TUMORS
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	50M
ADNEXAL ADENOMA																								X		2
KERATOACANTHOMA																										2
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	50M
FIBROMA																	X									2
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA, NOS, METASTATIC																		X								1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	-	+	43
C-CELL CARCINOMA, INVASIVE				X																						1
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LEIOMYOSARCOMA						X																				1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	-	-	+	+	+	+	+	+	-	-	+	+	+	-	-	-	+	+	+	+	+	+	-	+	+	33
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	48
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ACINAR-CELL ADENOMA																					X					1
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMATOUS POLYP, NOS											X															1
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS	X	X								X	X				X						X			X		9
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA	X					X									X								X			9
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA																										2
C-CELL CARCINOMA				X		X																				1
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	47
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA						X				X																2
ISLET-CELL CARCINOMA				X																						1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
INTERSTITIAL-CELL TUMOR	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	47
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
CARCINOMA, NOS								X																		2
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
EAR	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	+	N	N	50M
SQUAMOUS CELL PAPILLOMA																										1
ZYMBAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	+	N	N	50M
CARCINOMA, NOS																		X								1
SQUAMOUS CELL CARCINOMA																							X			1
MUSCULOSKELETAL SYSTEM																										
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
OSTEOSARCOMA																										1
BODY CAVITIES																										
TUNICA VAGINALIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
MESOTHELIOMA, NOS							X											X								3
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
C-CELL CARCINOMA, METASTATIC				X																						1
SARCOMA, NOS																										1
LEUKEMIA, MONONUCLEAR CELL		X			X										X	X	X									6

+ ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: HIGH DOSE

ANIMAL NUMBER	0 0 1 1	0 0 1 2	0 0 1 3	0 0 1 4	0 0 1 5	0 0 1 6	0 0 1 7	0 0 1 8	0 0 1 9	0 1 1 0	0 1 1 1	0 1 1 2	0 1 1 3	0 1 1 4	0 1 1 5	0 1 1 6	0 1 1 7	0 1 1 8	0 1 1 9	0 2 2 0	0 2 2 1	0 2 2 2	0 2 2 3	0 2 2 4	0 2 2 5
WEEKS ON STUDY	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 4	1 0 2	1 0 4	1 0 4	1 0 4	1 0 7	1 0 4	1 0 4
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA												X													
SQUAMOUS CELL CARCINOMA																							X		
ADNEXAL ADENOMA																					X				
KERATOACANTHOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA		X																							
FIBROSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA									X																
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	-	-	+	-	+	-	+	+	-	+	+	+	+	-	-	-	-	+	+	+	+	+	+	-	-
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE							X													X					
LEUKEMIA,MONONUCLEAR CELL						X																			
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS		X			X				X		X	X			X						X			X	
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA																		X						X	
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA		X																							
C-CELL ADENOMA																				X					
C-CELL CARCINOMA		X			X																	X			
PARATHYROID	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA															X										
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROADENOMA		X																							
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
INTERSTITIAL-CELL TUMOR	X		X	X		X	X	X		X		X	X			X	X	X		X	X	X			X
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
EYE APPENDAGES	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
NEURILEMOMA						X																			
ZYMBAL GLAND	+	N	N	N	+	+	N	N	N	+	N	N	+	N	N	N	+	+	N	+	+	+	+	N	+
ADENOSQUAMOUS CARCINOMA																									
BODY CAVITIES																									
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SARCOMA, NOS																									
RHABDOMYOSARCOMA																									
TUNICA VAGINALIS	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+
MESOTHELIOMA, NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
LEUKEMIA,MONONUCLEAR CELL					X				X																

ANIMAL NUMBER	0 2 4	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0 4	6 8	0 0	0 7	0 5	0 4	0 6	0 4	0 4	0 5	0 4	0 4	0 4	0 4	0 8	0 4	0 4	0 5	0 6	0 4	0 4	0 3	0 4	0 4	0 4	
INTEGUMENTARY SYSTEM																										
SKIN	+	A	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
SQUAMOUS CELL PAPILLOMA																										1
SQUAMOUS CELL CARCINOMA																										1
ADNEXAL ADENOMA							X																			1
KERATOACANTHOMA																										1
SUBCUTANEOUS TISSUE	+	A	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
FIBROMA																					X					1
FIBROSARCOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA																										1
ALVEOLAR/BRONCHIOLAR CARCINOMA												X														1
TRACHEA	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LYMPH NODES	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	+	A	-	+	-	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	33
CIRCULATORY SYSTEM																										
HEART	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NEOPLASTIC NODULE																										2
LEUKEMIA,MONONUCLEAR CELL																										1
BILE DUCT	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	49M
PANCREAS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LARGE INTESTINE	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																										
KIDNEY	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY BLADDER	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	+	A	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ADENOMA, NOS									X						X											10
ADRENAL	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PHEOCHROMOCYTOMA	X		X					X													X				X	7
THYROID	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
FOLLICULAR-CELL CARCINOMA																										1
C-CELL ADENOMA												X	X					X					X			3
C-CELL CARCINOMA																X	X									5
PARATHYROID	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
PANCREATIC ISLETS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ISLET-CELL ADENOMA																										1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
FIBROADENOMA																										1
TESTIS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
INTERSTITIAL-CELL TUMOR	X		X	X	X	X	X	X		X	X		X		X	X	X	X	X	X	X	X	X	X	X	32
PROSTATE	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																										
BRAIN	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																										
EYE APPENDAGES	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	49M
NEURILEMOMA																										1
ZYMBAL GLAND	+	A	N	+	+	+	N	N	N	N	N	N	N	N	N	N	N	N	+	+	N	N	+	N	N	49M
ADENOSQUAMOUS CARCINOMA				X																						1
BODY CAVITIES																										
PERITONEUM	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	49M
SARCOMA, NOS												X														1
RHABDOMYOSARCOMA				X																						1
TUNICA VAGINALIS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
MESOTHELIOMA, NOS																X										1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	49M
LEUKEMIA,MONONUCLEAR CELL								X							X											4

M ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: UNTREATED CONTROL

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	079	105	105	105	105	105	105	105	105	105	100	105	105	105	105	105	105	105	105	072	105	103	105	103	105
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA												X													
KERATOACANTHOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	-	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPHOCYTIC LEUKEMIA																									
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																									
ADENOMA, NOS			X		X	X	X				X			X				X			X	X		X	
ADRENAL	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA			X																						
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA						X			X													X			
C-CELL CARCINOMA										X															
PARATHYROID	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
ISLET-CELL ADENOMA							X																		
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS																									
ADENOCARCINOMA, NOS																						X			
FIBROADENOMA		X				X		X		X	X	X	X		X		X	X						X	X
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS			X																						
ADENOCARCINOMA, NOS																									
ENDOMETRIAL STROMAL POLYP		X										X	X					X					X		
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GRANULOSA-CELL TUMOR																							X		
GRANULOSA-CELL CARCINOMA																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA, NOS, INVASIVE																									
MUSCULOSKELETAL SYSTEM																									
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
OSTEOSARCOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
LEUKEMIA,MONONUCLEAR CELL											X														

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
S: ANIMAL MIS-SEXED
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	026	027	028	029	030	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	105	105	105	105	085	105	105	105	105	105	105	105	105	085	105	105	105	104	105	105	105	085	105	105	105	
INTEGUMENTARY SYSTEM																										
SKIN	+	•	+	•	•	+	+	N	+	+	•	+	+	+	+	+	+	+	+	•	+	+	+	•	+	50M
SQUAMOUS CELL PAPILLOMA																										1
KERATOACANTHOMA							X																			1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRACHEA	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	-	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	-	+	+	+	+	-	+	43
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPHOCYTIC LEUKEMIA									X																	1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	+	48
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARCINOMA, NOS																								X		1
ADENOMA, NOS		X		X			X							X	X			X			X			X	X	19
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PHEOCHROMOCYTOMA										X												X				3
THYROID	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-CELL ADENOMA		X					X							X												6
C-CELL CARCINOMA																										1
PARATHYROID	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	43
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ISLET-CELL ADENOMA																										1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	•	+	+	•	50M
ADENOMA, NOS														X												1
ADENOCARCINOMA, NOS																										1
FIBROADENOMA	X	X					X							X	X			X		X	X					20
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS												X														1
ADENOCARCINOMA, NOS																										1
ENDOMETRIAL STROMAL POLYP								X	X	X	X		X			X	X								X	13
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GRANULOSA-CELL TUMOR																										1
GRANULOSA-CELL CARCINOMA			X																							1
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA, NOS, INVASIVE																								X		1
MUSCULOSKELETAL SYSTEM																										
BONE	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
OSTEOSARCOMA																		X								1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
LEUKEMIA, MONONUCLEAR CELL		X			X																			X		4

• ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: LOW DOSE

ANIMAL NUMBER	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021	0022	0023	0024	0025	0026	0027	0028	0029	0030	0031	0032	0033	0034	0035
WEEKS ON STUDY	058	104	105	057	104	095	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104	104
INTEGUMENTARY SYSTEM																									
SKIN	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA																									
BASAL-CELL CARCINOMA																X									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS, METASTATIC																									
ALVEOLAR/BRONCHIOLAR ADENOMA																X									
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	-	+	+	+	+	+	+	-	+	-	+	-	-	+	+	+	-	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE																X									
HEPATOCELLULAR CARCINOMA																								X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMATOUS POLYP, NOS		X																							
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																									
ADENOMA, NOS			X			X			X	X		X			X						X		X	X	
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA													X		X								X		X
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA																									
C-CELL CARCINOMA																									X
PARATHYROID	+	-	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS																									
FIBROADENOMA			X			X		X							X	X								X	
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
CARCINOMA,NOS																									
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS								X																	
ENDOMETRIAL STROMAL POLYP																					X	X			
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GRANULOSA-CELL TUMOR																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASTROCYTOMA																									
SPECIAL SENSE ORGANS																									
ZYMBAL GLAND	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	+	M	M	M	M	M	+
ADENOSQUAMOUS CARCINOMA												X													
BODY CAVITIES																									
MEDIASTINUM	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																									
PERITONEUM	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ADENOCARCINOMA, NOS, INVASIVE																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
LEUKEMIA,MONONUCLEAR CELL															X					X					

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: LOW DOSE (Continued)

ANIMAL NUMBER	021	021	021	021	031	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	104	041	041	001	041	041	041	041	042	041	041	041	095	041	041	041	041	041	041	095	055	041	042	041	041	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
SQUAMOUS CELL PAPILLOMA																	X									1
BASAL-CELL CARCINOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOCARCINOMA, NOS, METASTATIC																							X			1
ALVEOLAR/BRONCHIOLAR ADENOMA																										1
ALVEOLAR/BRONCHIOLAR CARCINOMA																					X					1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	48
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	-	-	+	-	-	-	+	-	+	+	+	-	+	-	+	+	-	+	-	+	-	+	+	-	+	32
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE																										1
HEPATOCELLULAR CARCINOMA																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMATOUS POLYP, NOS																										1
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA,NOS											X															1
ADENOMA, NOS				X					X		X					X	X	X	X					X		18
ADRENAL	+	•	•	+	+	+	+	+	•	•	+	+	+	+	+	+	+	+	+	+	•	+	+	+	+	50
PHEOCHROMOCYTOMA		X														X									X	7
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA										X	X		X											X		4
C-CELL CARCINOMA											X															2
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	46
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	50M
ADENOCARCINOMA, NOS							X				X						X						X			1
FIBROADENOMA																								X		10
PREPUTIAL/CLITORAL GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
CARCINOMA,NOS											X															1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOCARCINOMA, NOS												X														1
ENDOMETRIAL STROMAL POLYP				X			X				X	X														7
OVARY	+	+	+	+	+	+	+	+	+	+	+	•	•	+	+	+	+	•	•	+	+	+	+	•	+	50
GRANULOSA-CELL TUMOR																				X						1
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ASTROCYTOMA								X																		1
SPECIAL SENSE ORGANS																										
ZYMBAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
ADENOSQUAMOUS CARCINOMA																										1
BODY CAVITIES																										
MEDIASTINUM	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																					X					1
PERITONEUM	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
ADENOCARCINOMA, NOS, INVASIVE												X														1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50M
LEUKEMIA,MONONUCLEAR CELL		X											X									X			X	4

* ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: HIGH DOSE

ANIMAL NUMBER	0011	0021	0031	0041	0051	0061	0071	0081	0091	0101	0111	0121	0131	0141	0151	0161	0171	0181	0191	0201	0211	0221	0231	0241	0251
WEEKS ON STUDY	0 4	0 4	0 8 7	0 4	0 4	0 4	0 7 5	0 4	0 7 6	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 4	0 9 7	0 9 9	0 4	0 4	0 4
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	-	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NASAL CAVITY	M	M	+	M	M	+	M	M	M	M	+	M	M	+	M	+	M	M	+	+	M	M	M	M	+
SQUAMOUS CELL CARCINOMA			X																						
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEOPLASTIC NODULE														X											
HEPATOCELLULAR CARCINOMA																			X						
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MIXED MESENCHYMAL TUMOR, MALIGNAN																									
URINARY BLADDER	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS	X	X					X	X		X		X			X	X					X		X		
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA				X					X	X							X								
THYROID	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA																		X							
C-CELL ADENOMA											X														X
C-CELL CARCINOMA								X																	
PARATHYROID	+	-	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROADENOMA								X								X									
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ADENOMA, NOS						X																			
KERATOACANTHOMA																									
VAGINA	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
SQUAMOUS CELL PAPILLOMA				X																					
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LEIOMYOSARCOMA																								X	
ENDOMETRIAL STROMAL POLYP	X		X					X						X	X	X			X						X
ENDOMETRIAL STROMAL SARCOMA								X																	
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPINAL CORD	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
SARCOMA, NOS																									
SPECIAL SENSE ORGANS																									
EAR	+	+	M	+	+	+	M	M	M	M	+	+	M	+	M	+	+	M	+	+	M	M	+	+	+
NEURILEMOMA																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
LEUKEMIA,MONONUCLEAR CELL																									

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: HIGH DOSE (Continued)

ANIMAL NUMBER	0214	0227	0218	0229	0310	0311	0312	0313	0314	0315	0316	0317	0318	0319	0320	0411	0412	0413	0414	0415	0416	0417	0418	0419	0420	TOTAL
WEEKS ON STUDY	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	0 8 8	1 0 2	0 9 8	0 7 3	1 0 4	1 0 4	1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	0 7 3	0 9 3	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	1 1 0 4	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROMA																								X		1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	47
NASAL CAVITY	+	+	M	M	M	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	M	50
SQUAMOUS CELL CARCINOMA																										1
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	-	-	+	+	+	-	+	-	-	-	+	+	+	+	+	+	-	-	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE																	X									2
HEPATOCELLULAR CARCINOMA																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MIXED MESENCHYMAL TUMOR, MALIGNAN																								X	X	2
URINARY BLADDER	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	49
ADENOMA, NOS							X	X		X	X								X						X	16
ADRENAL	+	•	+	•	•	•	•	•	-	•	+	+	•	•	•	+	•	•	•	+	+	•	•	•	+	49
PHEOCHROMOCYTOMA					X																					5
THYROID	+	•	+	•	•	•	+	•	•	•	•	•	•	•	•	•	•	+	+	•	•	•	•	•	+	49
FOLLICULAR-CELL CARCINOMA																										1
C-CELL ADENOMA													X						X					X		5
C-CELL CARCINOMA																							X			2
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	44
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	•	+	+	+	+	•	•	•	+	•	•	+	•	+	•	+	+	•	•	•	•	+	+	+	+	50
FIBROADENOMA							X																X			4
PREPUTIAL/CLITORAL GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50
ADENOMA, NOS																										1
KERATOACANTHOMA				X																						1
VAGINA	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50
SQUAMOUS CELL PAPILLOMA																										1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LEIOMYOSARCOMA																										1
ENDOMETRIAL STROMAL POLYP						X		X		X			X	X				X							X	15
ENDOMETRIAL STROMAL SARCOMA																										1
OVARY	+	+	+	+	+	+	+	+	+	+	•	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	•	•	•	+	•	•	+	+	•	•	+	•	•	•	+	+	+	•	+	+	•	+	+	50
SPINAL CORD	M	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	+	M	M	M	M	M	M	M	M	50
SARCOMA, NOS									X																	1
SPECIAL SENSE ORGANS																										
EAR	+	+	+	+	+	M	M	M	+	M	+	+	M	+	M	M	M	M	M	+	+	M	+	+	+	50
NEURILEMOMA												X														1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	50
LEUKEMIA,MONONUCLEAR CELL			X														X	X								3

* ANIMALS NECROPSIED

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(48)	(49)
SQUAMOUS CELL CARCINOMA	1 (2%)		
FIBROMA		2 (4%)	
FIBROSARCOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(48)	(49)
CARCINOMA, NOS			1 (2%)
SARCOMA, NOS	4 (8%)		1 (2%)
FIBROMA	1 (2%)	3 (6%)	3 (6%)
FIBROSARCOMA	3 (6%)	8 (17%)	6 (12%)
RHABDOMYOSARCOMA			1 (2%)
OSTEOSARCOMA			2 (4%)
NEURILEMOMA	1 (2%)	1 (2%)	2 (4%)
NEURILEMOMA, MALIGNANT			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(48)	(49)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	8 (17%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)	1 (2%)	4 (8%)
SARCOMA, NOS, METASTATIC		1 (2%)	1 (2%)
FIBROSARCOMA, METASTATIC		3 (6%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		2 (4%)	4 (8%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	2 (4%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
#SPLEEN	(50)	(47)	(49)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
#LYMPH NODE	(50)	(47)	(49)
FIBROSARCOMA, METASTATIC			1 (2%)
#AXILLARY LYMPH NODE	(50)	(47)	(49)
SARCOMA, NOS, METASTATIC			1 (2%)
FIBROSARCOMA, METASTATIC			1 (2%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(48)	(49)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA, UNC PRIM OR MET	1 (2%)		
#SPLEEN	(50)	(47)	(49)
HEMANGIOSARCOMA	1 (2%)		
*PULMONARY VEIN	(50)	(48)	(49)
ALVEOLAR/BRONCHIOLAR CA, INVASIVE			1 (2%)
#LIVER	(50)	(48)	(49)
HEMANGIOSARCOMA	1 (2%)		1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(49)
HEPATOCELLULAR ADENOMA	6 (12%)	8 (17%)	8 (16%)
HEPATOCELLULAR CARCINOMA	5 (10%)	9 (19%)	12 (24%)
HEPATOBLASTOMA		1 (2%)	
#FORESTOMACH	(50)	(47)	(49)
SQUAMOUS CELL PAPILLOMA			2 (4%)
#COLON	(50)	(47)	(49)
ADENOMATOUS POLYP, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#ADRENAL	(50)	(47)	(49)
CORTICAL ADENOMA		1 (2%)	1 (2%)
PHEOCHROMOCYTOMA		1 (2%)	1 (2%)
#THYROID	(44)	(45)	(49)
FOLLICULAR-CELL ADENOMA	2 (5%)	2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(48)	(49)
ADENOMA, NOS			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*ABDOMINAL WALL	(50)	(48)	(49)
FIBROSARCOMA, INVASIVE			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(48)	(49)
SARCOMA, NOS		1 (2%)	
FIBROSARCOMA	1 (2%)		
FIBROSARCOMA, METASTATIC		1 (2%)	
MESOTHELIOMA, MALIGNANT		1 (2%)	
NEURILEMOMA, MALIGNANT		1 (2%)	

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

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	11	11	4
MORIBUND SACRIFICE	15	15	12
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	24	23	34
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING		1	
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	23	33	37
TOTAL PRIMARY TUMORS	33	56	58
TOTAL ANIMALS WITH BENIGN TUMORS	11	17	15
TOTAL BENIGN TUMORS	13	28	21
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	22	30
TOTAL MALIGNANT TUMORS	19	28	37
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	6	5
TOTAL SECONDARY TUMORS	1	6	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC	1		
TOTAL UNCERTAIN TUMORS	1		

** 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 IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
NEURILEMOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	3 (6%)	5 (10%)	2 (4%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	3 (6%)	2 (4%)
MALIGNANT LYMPHOMA, MIXED TYPE	7 (14%)	1 (2%)	2 (4%)
#SPLEEN	(50)	(50)	(49)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MAST-CELL TUMOR		1 (2%)	
#PEYER'S PATCH	(49)	(46)	(48)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
HEPATOCELLULAR ADENOMA	3 (6%)		3 (6%)
HEPATOCELLULAR CARCINOMA	4 (8%)	1 (2%)	7 (14%)
#FORESTOMACH	(49)	(48)	(49)
SQUAMOUS CELL PAPILLOMA	1 (2%)		
#DUODENUM	(49)	(46)	(48)
ADENOMATOUS POLYP, NOS	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(48)	(49)
ADENOMA, NOS	9 (18%)	2 (4%)	5 (10%)
#THYROID	(48)	(49)	(49)
FOLLICULAR-CELL ADENOMA	4 (8%)		1 (2%)
FOLLICULAR-CELL CARCINOMA	3 (6%)	1 (2%)	1 (2%)
#THYROID FOLLICLE	(48)	(49)	(49)
CYSTADENOMA, NOS	1 (2%)		

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOMA, NOS			1 (2%)
ADENOCARCINOMA, NOS			1 (2%)
MIXED TUMOR, MALIGNANT		1 (2%)	
#UTERUS	(50)	(50)	(50)
LEIOMYOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	3 (6%)		
#OVARY	(49)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		
GRANULOSA-CELL TUMOR	2 (4%)		
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
MYXOSARCOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
LEG			
OSTEOSARCOMA			1
FOOT			
SARCOMA, NOS	1		
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	10	15	21
MORIBUND SACRIFICE	5	8	10
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	35	27	19
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	31	19	22
TOTAL PRIMARY TUMORS	48	22	29
TOTAL ANIMALS WITH BENIGN TUMORS	17	6	11
TOTAL BENIGN TUMORS	22	6	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	15	15
TOTAL MALIGNANT TUMORS	24	15	18
TOTAL ANIMALS WITH SECONDARY TUMORS##	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	2	1	
TOTAL UNCERTAIN TUMORS	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 B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: UNTREATED CONTROL

ANIMAL NUMBER	01 02	01 03	01 04	01 05	01 06	01 07	01 08	01 09	01 10	01 11	01 12	01 13	01 14	01 15	01 16	01 17	01 18	01 19	01 20	01 21	01 22	01 23	01 24	01 25
WEEKS ON STUDY	012	051	051	051	051	051	051	051	096	030	054	037	051	051	011	061	053	091	041	021	031	010	042	046
INTEGUMENTARY SYSTEM																								
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA															X									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS															X			X						
FIBROMA			X																					
FIBROSARCOMA						X															X			
HEMANGIOSARCOMA, UNC PRIM OR META																							X	
NEURILEMOMA									X															
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA, METASTAT															X									
ALVEOLAR/BRONCHIOLAR ADENOMA		X																						
ALVEOLAR/BRONCHIOLAR CARCINOMA																							X	
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																						X		
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA											X							X						
HEPATOCELLULAR CARCINOMA			X					X			X													X
HEMANGIOSARCOMA											X													
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	N	+	+	+	+	N	+	+	+	N	+	N	N	+	+	N	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	+	+	+	+	+	+	•	+	•	•	•	•	•	+	-	+	•	-	+	+	+	•	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA									X															
PARATHYROID	+	-	+	+	+	+	+	+	-	-	+	+	-	+	+	-	-	+	+	-	+	+	+	+
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	N	N	N	N	N	N	N	N	+	N	N	N	N	N	+	+	N	N	N	N	+	N	+	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FIBROSARCOMA								X																
MALIG.LYMPHOMA, HISTIOCYTIC TYPE														X										

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
S: ANIMAL MIS-SEXED
: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	0 2 6	0 2 7	0 2 8	0 2 9	0 3 0	0 3 1	0 3 2	0 3 3	0 3 4	0 3 5	0 3 6	0 3 7	0 3 8	0 3 9	0 4 0	0 4 1	0 4 2	0 4 3	0 4 4	0 4 5	0 4 6	0 4 7	0 4 8	0 4 9	0 5 0	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 6	1 0 6	1 0 6	1 0 6	1 0 6	0 6 6	0 6 2	0 5 9	0 1 8	0 1 6	0 1 3	0 9 8	0 1 6	0 6 0	0 1 6	0 9 7	0 7 6	0 5 0	0 9 2	0 5 5	0 1 6	0 1 6	0 9 7	0 2 4	0 9 3	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
SQUAMOUS CELL CARCINOMA																										1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
SARCOMA, NOS												X					X									4
FIBROMA																										1
FIBROSARCOMA															X											3
HEMANGIOSARCOMA, UNC PRIM OR META																										1
NEURILEMOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SQUAMOUS CELL CARCINOMA, METASTAT																										1
ALVEOLAR/BRONCHIOLAR ADENOMA															X					X	X					3
ALVEOLAR/BRONCHIOLAR CARCINOMA																										2
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMANGIOSARCOMA																										1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA	X					X												X			X					6
HEPATOCELLULAR CARCINOMA													X													5
HEMANGIOSARCOMA																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	N	+	N	N	+	+	+	+	+	+	+	+	+	+	+	N	+	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	–	+	46
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYROID	+	+	+	+	+	–	+	+	–	+	+	+	–	+	+	+	+	+	+	+	+	–	+	+	+	44
FOLLICULAR-CELL ADENOMA																			X							2
PARATHYROID	+	+	+	+	+	–	–	+	–	+	–	+	–	–	+	–	–	+	–	+	+	+	+	–	+	34
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	+	N	N	+	N	N	+	50M
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
FIBROSARCOMA																										1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																										1

* ANIMALS NECROPSIED

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: LOW DOSE

ANIMAL NUMBER	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5
WEEKS ON STUDY	0 0 5	0 9 5	0 9 5	0 9 5	0 9 6	0 9 5	0 9 5	0 9 5	0 9 7	0 9 5	0 9 5	0 9 7	0 9 2	0 9 3	0 9 4	0 8 1	0 9 3	0 9 5	0 9 7	0 9 5	0 9 5	0 9 5	0 9 5	0 8 9	0 9 3
INTEGUMENTARY SYSTEM																								
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA						X																		
FIBROSARCOMA																								
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA		X	X				X					X		X						X				
FIBROSARCOMA				X																			X	
HEMANGIOMA																								
NEURILEMOMA																								
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA													X											
ALVEOLAR/BRONCHIOLAR ADENOMA				X	X			X	X							X	X							
ALVEOLAR/BRONCHIOLAR CARCINOMA																				X				
SARCOMA, NOS, METASTATIC																					X			
FIBROSARCOMA, METASTATIC																							X	
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA				X										X		X	X					X		
HEPATOCELLULAR CARCINOMA		X											X				X		X			X		
HEPATOBLASTOMA																	X							
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	M	+	+	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMATOUS POLYP, NOS																								X
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA																	X							
PHEOCHROMOCYTOMA							X																	
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
FOLLICULAR-CELL ADENOMA																	X					X		
PARATHYROID	+	-	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	N	N	N	N	N	N	N	N	+	N	N	+	+	N	N	N	N	N	+	N	N	N	N	+
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
SARCOMA, NOS																						X		
FIBROSARCOMA, METASTATIC		X																						
MESOTHELIOMA, MALIGNANT																								
NEURILEMOMA, MALIGNANT																		X						
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE							X																	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE												X												
MALIGNANT LYMPHOMA, MIXED TYPE									X															

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	02 06	02 07	02 08	02 09	03 01	03 11	03 12	03 31	03 41	03 51	03 61	03 71	03 81	03 91	04 01	04 11	04 21	04 31	04 41	04 51	04 61	04 71	04 81	04 91	05 01	TOTAL TISSUES TUMORS
WEEKS ON STUDY	10 05	10 14	09 31	10 08	09 02	08 09	10 05	02 05	00 05	00 05	03 07	08 51	11 41	01 07	09 14	09 01	09 20	10 20	10 05	08 21	10 05	10 16	09 22	10 05	10 05	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48M
FIBROMA																								X		2
FIBROSARCOMA					X																					1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48M
FIBROMA												X														3
FIBROSARCOMA	X																X			X					6
HEMANGIOMA																										1
NEURILEMOMA																							X			1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
HEPATOCELLULAR CARCINOMA, METASTA																										1
ALVEOLAR/BRONCHIOLAR ADENOMA												X												X		8
ALVEOLAR/BRONCHIOLAR CARCINOMA																										1
SARCOMA, NOS, METASTATIC																										1
FIBROSARCOMA, METASTATIC																	X			X						3
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
HEMATOPOIETIC SYSTEM																										
BONE MARROW	-	+	+	+	+	+	-	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	45
SPLEEN	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
LYMPH NODES	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
THYMUS	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
HEPATOCELLULAR ADENOMA												X							X				X			8
HEPATOCELLULAR CARCINOMA			X							X					X	X										9
HEPATOBLASTOMA																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
GALLBLADDER & COMMON BILE DUCT	+	N	N	N	+	+	+	N	+	+	N	+	A	+	N	+	+	M	+	+	+	+	+	+	N	48M
PANCREAS	+	-	+	+	+	+	+	+	+	+	+	+	A	+	-	+	+	M	+	+	+	+	+	+	+	46
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
STOMACH	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
SMALL INTESTINE	+	-	-	+	+	+	+	+	+	+	+	+	A	+	-	+	+	M	+	+	+	+	+	+	+	45
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	A	+	-	+	+	M	+	+	+	+	+	+	+	47
ADENOMATOUS POLYP, NOS																										1
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
URINARY BLADDER	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	A	+	-	+	+	M	+	+	+	+	+	+	+	47
ADRENAL	+	-	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	47
CORTICAL ADENOMA																										1
PHEOCHROMOCYTOMA																										1
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	-	+	M	+	+	+	-	+	+	+	45
FOLLICULAR-CELL ADENOMA																										2
PARATHYROID	+	+	+	-	+	+	-	-	+	-	-	-	A	+	+	-	+	M	-	+	+	-	+	+	+	35
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	N	N	+	N	+	+	N	N	N	N	N	N	A	N	N	N	N	M	N	N	N	N	+	N	N	48M
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	M	+	+	+	+	+	+	+	48
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	M	N	N	N	N	N	N	N	48M
SARCOMA, NOS																										1
FIBROSARCOMA, METASTATIC																										1
MESOTHELIOMA, MALIGNANT																X										1
NEURILEMOMA, MALIGNANT																										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE												X														2
MALIG.LYMPHOMA, HISTIOCYTIC TYPE						X																				2
MALIGNANT LYMPHOMA, MIXED TYPE																										1

* ANIMALS NECROPSIED

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: HIGH DOSE

ANIMAL NUMBER	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	1 0 5	1 0 5	1 0 7	1 0 7	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9	1 0 9
INTEGUMENTARY SYSTEM																								
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																							X	
SARCOMA, NOS																								
FIBROMA	X																X							
FIBROSARCOMA						X													X	X				
RHABDOMYOSARCOMA										X														
OSTEOSARCOMA																		X						
NEURILEMOMA																								
NEURILEMOMA, MALIGNANT										X														
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA					X																			
ALVEOLAR/BRONCHIOLAR ADENOMA																								
ALVEOLAR/BRONCHIOLAR CARCINOMA																					X			
SARCOMA, NOS, METASTATIC																								
FIBROSARCOMA, METASTATIC																								
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																								
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS, METASTATIC																								
FIBROSARCOMA, METASTATIC						X																		
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
BLOOD VESSELS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																					X			
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA												X			X			X			X			
HEPATOCELLULAR CARCINOMA		X				X		X									X			X				
HEMANGIOSARCOMA											X													
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA				X																				
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA																								
PHEOCHROMOCYTOMA																				X				
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																								
PARATHYROID	+	+	+	+	+	+	+	-	+	-	+	+	-	-	-	+	+	+	-	-	-	+	+	+
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	M	M	M	M	+	+	M	M	M	M	+	M	M	M	M	M	M	M	M	M	M	+	M	+
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																								
HARDERIAN GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
ADENOMA, NOS																								
BODY CAVITIES																								
PERITONEUM	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
FIBROSARCOMA, INVASIVE						X																		
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																								
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																								X
MALIGNANT LYMPHOMA, MIXED TYPE																		X						

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0212	0227	0228	0231	0310	0311	0312	0313	0314	0315	0316	0317	0318	0319	0320	0321	0401	0402	0403	0404	0405	0406	0407	0408	0409	0410	TOTAL TISSUES TUMORS
WEEKS ON STUDY	091	081	095	095	095	095	095	091	061	095	085	091	091	091	082	095	095	095	097	092	095	088	095	095	095	095	
INTEGUMENTARY SYSTEM																											
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
CARCINOMA,NOS																											1
SARCOMA, NOS	X																										1
FIBROMA			X																								3
FIBROSARCOMA						X		X																X			6
RHABDOMYOSARCOMA																											1
OSTEOSARCOMA																				X							2
NEURILEMOMA				X																							2
NEURILEMOMA, MALIGNANT																						X					1
RESPIRATORY SYSTEM																											
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR CARCINOMA, METASTA																											1
ALVEOLAR/BRONCHIOLAR ADENOMA			X	X																							2
ALVEOLAR/BRONCHIOLAR CARCINOMA						X																X			X		4
SARCOMA, NOS, METASTATIC	X																										1
FIBROSARCOMA, METASTATIC								X																			1
TRACHEA	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																											
BONE MARROW	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																	X										1
LYMPH NODES	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SARCOMA, NOS, METASTATIC	X																										1
FIBROSARCOMA, METASTATIC								X																			2
THYMUS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																											
HEART	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
BLOOD VESSELS	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49M
ALVEOLAR/BRONCHIOLAR CA, INVASIVE																											1
DIGESTIVE SYSTEM																											
SALIVARY GLAND	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA		X	X	X																					X		8
HEPATOCELLULAR CARCINOMA				X			X							X	X						X			X	X		12
HEMANGIOSARCOMA																											1
BILE DUCT	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	A	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48M
PANCREAS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SQUAMOUS CELL PAPILLOMA								X																			2
SMALL INTESTINE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LARGE INTESTINE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																											
KIDNEY	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY BLADDER	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																											
PITUITARY	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADRENAL	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CORTICAL ADENOMA																				X							1
PHEOCHROMOCYTOMA																											1
THYROID	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
FOLLICULAR-CELL ADENOMA			X																								1
PARATHYROID	+	-	+	+	+	+	+	+	A	+	+	+	-	-	+	+	+	+	+	-	-	+	+	+	+	+	37
REPRODUCTIVE SYSTEM																											
MAMMARY GLAND	+	M	M	M	M	M	M	M	A	M	M	+	M	M	+	M	M	+	+	M	M	M	M	M	M	M	49M
TESTIS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PROSTATE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																											
BRAIN	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																											
HARDERIAN GLAND	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49M
ADENOMA, NOS			X																								1
BODY CAVITIES																											
PERITONEUM	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49M
FIBROSARCOMA, INVASIVE																											1
ALL OTHER SYSTEMS																											
MULTIPLE ORGANS NOS	M	M	M	M	M	M	M	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	49M
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		X									X	X							X								4
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																											2
MALIGNANT LYMPHOMA, MIXED TYPE																											1

M ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: UNTREATED CONTROL

ANIMAL NUMBER	0 0 0 1	0 0 2 1	0 0 3 1	0 0 4 1	0 0 5 1	0 0 6 1	0 0 7 1	0 0 8 1	0 0 9 1	0 1 0 1	0 1 1 1	0 1 2 1	0 1 3 1	0 1 4 1	0 1 5 1	0 1 6 1	0 1 7 1	0 1 8 1	0 1 9 1	0 2 0 1	0 2 1 1	0 2 2 1	0 2 3 1	0 2 4 1	0 2 5 1
WEEKS ON STUDY	0 5	0 5	0 5	0 5	0 5	0 6	0 6	0 7	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 8	0 9
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA																							X		
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA							X					X									X				
HEPATOCELLULAR CARCINOMA																							X		
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA																									
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMATOUS POLYP, NOS																								X	
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS									X								X			X					
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																						X			X
FOLLICULAR-CELL CARCINOMA									X								X								
CYSTADENOMA, NOS																									
PARATHYROID	-	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOMETRIAL STROMAL POLYP																	X								
OVARY	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS		X																							
GRANULOSA-CELL TUMOR																									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MYXOSARCOMA																									X
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
FIBROUS HISTIOCYTOMA, MALIGNANT																									
MALIGNANT LYMPHOMA, NOS																									
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			X										X							X					
MALIG.LYMPHOMA, HISTIOCYTIC TYPE										X															
MALIGNANT LYMPHOMA, MIXED TYPE								X								X									
FOOT NOS																									
SARCOMA, NOS			X																						

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
S: ANIMAL MIS-SEXED

: NO TISSUE INFORMATION SUBMITTED
C: NECROPSY, NO HISTOLOGY DUE TO PROTOCOL
A: AUTOLYSIS
M: ANIMAL MISSING
B: NO NECROPSY PERFORMED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: UNTREATED CONTROL (Continued)

ANIMAL NUMBER	021	022	023	029	031	031	032	033	034	035	036	037	038	039	040	041	042	043	044	045	046	047	048	049	050	TOTAL TISSUES TUMORS
WEEKS ON STUDY	074	095	096	096	096	096	095	096	092	076	095	096	096	096	096	096	096	096	085	085	096	096	093	087	095	
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA																										1
ALVEOLAR/BRONCHIOLAR CARCINOMA					X																					1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA																										3
HEPATOCELLULAR CARCINOMA					X													X	X							4
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	50N
PANCREAS	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
STOMACH	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SQUAMOUS CELL PAPILLOMA												X														1
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMATOUS POLYP, NOS																										1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS			X			X							X	X								X			X	9
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	48
FOLLICULAR-CELL ADENOMA							X															X				4
FOLLICULAR-CELL CARCINOMA														X												3
CYSTADENOMA, NOS							X																			1
PARATHYROID	+	+	+	+	+	+	-	+	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	-	31
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOMETRIAL STROMAL POLYP													X	X												3
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOCARCINOMA, NOS									X																	1
GRANULOSA-CELL TUMOR																			X							2
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MYXOSARCOMA																										1
ALL OTHER SYSTEMS																										
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50N
FIBROUS HISTIOCYTOMA, MALIGNANT		X																								1
MALIGNANT LYMPHOMA, NOS										X																1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																										3
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																										1
MALIGNANT LYMPHOMA, MIXED TYPE							X					X								X		X	X			7
FOOT NOS																										
SARCOMA, NOS																										1

* ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: LOW DOSE

ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	105	105	104	105	105	105	079	105	105	091	105	105	091	105	105	094	091	105	105	105	073	105	105	082	089
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NEURILEMOMA																								X	
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA																	X								
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE											X														
MAST-CELL TUMOR																									
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA			X																						
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																									
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
ADENOMA, NOS						X																			
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA																									
PARATHYROID	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+
MIXED TUMOR, MALIGNANT	X																								
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
ADENOMA, NOS																									
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE					X										X		X						X		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE													X												
MALIGNANT LYMPHOMA, MIXED TYPE											X														

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: LOW DOSE (Continued)

ANIMAL NUMBER	0262	0271	0282	0293	0311	0312	0313	0314	0315	0316	0317	0318	0319	0320	0401	0402	0403	0404	0405	0406	0407	0408	0409	0410	0411	0412	TOTAL
WEEKS ON STUDY	1105	1101	1105	1105	0921	0907	0801	0905	0907	0905	1005	0905	0809	1003	1005	1005	1000	0808	0904	1005	0921	0905	0921	0905	0911	1005	TISSUES TUMORS
INTEGUMENTARY SYSTEM																											
SUBCUTANEOUS TISSUE NEURILEMOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M 1
RESPIRATORY SYSTEM																											
LUNGS AND BRONCHI ALVEOLAR/BRONCHIOLAR ADENOMA ALVEOLAR/BRONCHIOLAR CARCINOMA	+	+	+	+	+	+	+	+ X	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+ X	+	49 2 1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
HEMATOPOIETIC SYSTEM																											
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN MALIG.LYMPHOMA, LYMPHOCYTIC TYPE MAST-CELL TUMOR	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50 1 1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																											
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																											
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER HEPATOCELLULAR CARCINOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50 1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+ M	+	+	+	+	+ M	+	+ M	+	+	+	+	+	+ M	+	+	+	+	+	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	48
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SMALL INTESTINE MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+ X	+	+	+	+	+	+	+	46 1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																											
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																											
PITUITARY ADENOMA, NOS	+	+	+	+	+	+	+	+	+ -	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+	48 2
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYROID FOLLICULAR-CELL CARCINOMA	+	+	+	+	+	+	+	+	+	+	+	+	+ X	-	+	+	+	+	+	+	+	+	+	+	+	+	49 1
PARATHYROID	+	-	+	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	43
REPRODUCTIVE SYSTEM																											
MAMMARY GLAND MIXED TUMOR, MALIGNANT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M 1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																											
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																											
HARDERIAN GLAND ADENOMA, NOS	N	N	N	N	N	N	N	N	N	N	N X	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M 1
ALL OTHER SYSTEMS																											
MULTIPLE ORGANS NOS MALIG.LYMPHOMA, LYMPHOCYTIC TYPE MALIG.LYMPHOMA, HISTIOCYTIC TYPE MALIGNANT LYMPHOMA, MIXED TYPE	N	N	N	N	N	N	N	N X	N	N	N	N	N	N	N	N	N	N X	N	N	N	N	N	N X	N	N	50M 5 3 1

M ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2: HIGH DOSE

ANIMAL NUMBER	0 0 1	0 0 2	0 0 3	0 0 4	0 0 5	0 0 6	0 0 7	0 0 8	0 0 9	0 1 0	0 1 1	0 1 2	0 1 3	0 1 4	0 1 5	0 1 6	0 1 7	0 1 8	0 1 9	0 2 0	0 2 1	0 2 2	0 2 3	0 2 4	0 2 5
WEEKS ON STUDY	0 5 1	0 9 1	0 5 1	0 9 1	0 5 1	0 8 1	0 7 1	0 7 1	0 9 1	0 5 1	0 7 1	0 9 1	0 9 1	0 8 1	0 5 1	0 9 1	0 8 1	0 5 1	0 8 1	0 5 1	0 5 1	0 9 1	0 9 1	0 9 1	0 9 1
INTEGUMENTARY SYSTEM																								
SUBCUTANEOUS TISSUE HEMANGIOSARCOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+
LIVER HEPATOCELLULAR ADENOMA HEPATOCELLULAR CARCINOMA	+	+ X	+	+	+	+	+	+	+	+ X	+	+	+ X	+	+	+	+	-	+	+	+	+	+	+
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	N	+	N	+	+	+	+	N	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
SMALL INTESTINE MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY ADENOMA, NOS	+ X	+	+	+	+ X	+	+	+	+ X	+	+	+	+	+	+	+	+	-	+	+ X	+	+	+	+
ADRENAL	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+
THYROID FOLLICULAR-CELL ADENOMA FOLLICULAR-CELL CARCINOMA	+	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+	+	+	+	+	+	-	+	+
PARATHYROID	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND ADENOMA, NOS ADENOCARCINOMA, NOS	+ X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	N	+
UTERUS LEIOMYOMA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+ X	+	+	+
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS MALIG.LYMPHOMA, LYMPHOCYTIC TYPE MALIG.LYMPHOMA, HISTIOCYTIC TYPE MALIGNANT LYMPHOMA, MIXED TYPE	N	N	N	N	N	N	N	N X	N	N	N	N	N	N	N	N	N X	N	N	N	N	N	N	N
LEG NOS OSTEOSARCOMA																								

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: HIGH DOSE (Continued)

ANIMAL NUMBER	0 2 6	0 2 7	0 2 8	0 2 9	0 3 1	0 3 1	0 3 1	0 3 1	0 3 3	0 3 3	0 3 3	0 3 3	0 3 3	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 4	0 3 5	TOTAL TISSUES TUMORS
WEEKS ON STUDY	1 0 5	1 0 5	1 0 5	0 8 7	1 0 5	1 0 5	0 7 7	1 0 5	0 9 2	1 0 5	1 0 5	0 9 8	0 9 5	0 8 2	0 8 8	0 8 8	0 8 8	0 9 1	0 9 2	0 8 7	0 9 1	0 9 2	0 9 7	0 9 5	0 9 5	
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
HEMANGIOSARCOMA																									1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA									X														X		3
HEPATOCELLULAR CARCINOMA				X				X	X	X													X		7
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	50M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE									X																1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS																								X	5
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
FOLLICULAR-CELL ADENOMA																									1
FOLLICULAR-CELL CARCINOMA											X														1
PARATHYROID	+	M	+	+	M	M	-	+	M	+	M	M	M	+	M	M	+	M	M	+	M	+	M	M	45
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
ADENOMA, NOS																					X				1
ADENOCARCINOMA, NOS																									1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LEIOMYOMA																									1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALL OTHER SYSTEMS																									
MULTIPLE ORGANS NOS	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	50M
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																					X				2
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				X																					2
MALIGNANT LYMPHOMA, MIXED TYPE																		X	X						2
LEG NOS																									1
OSTEOSARCOMA																		X							1

+ ANIMALS NECROPSIED

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
INFLAMMATION, CHRONIC	1 (2%)		
ULCER, CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
*SUBCUT TISSUE	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)	3 (6%)	1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
NECROSIS, FAT		1 (2%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(49)
HEMORRHAGE		1 (2%)	
*NASAL MUCOSA	(50)	(50)	(49)
INFLAMMATION, FOCAL	1 (2%)		1 (2%)
#TRACHEA	(48)	(43)	(47)
INFLAMMATION, NOS	1 (2%)		
#PERITRACHEAL TISSUE	(48)	(43)	(47)
PIGMENTATION, NOS		1 (2%)	
#LUNG	(50)	(50)	(49)
CONGESTION, NOS	3 (6%)	2 (4%)	2 (4%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	2 (4%)	2 (4%)	1 (2%)
HISTIOCYTOSIS	4 (8%)	1 (2%)	
#LUNG/ALVEOLI	(50)	(50)	(49)
HYPERPLASIA, ADENOMATOUS			1 (2%)
HISTIOCYTOSIS		13 (26%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	1 (2%)
#BONE MARROW	(50)	(50)	(49)
ATROPHY, FATTY			1 (2%)
#SPLEEN	(50)	(50)	(49)
ACCESSORY STRUCTURE		1 (2%)	
CYST, NOS	1 (2%)		
HEMATOMA, NOS	1 (2%)		
FIBROSIS	1 (2%)		
INFARCT, NOS	3 (6%)		
HEMATOPOIESIS	2 (4%)	1 (2%)	1 (2%)
#LYMPH NODE	(50)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
#MANDIBULAR L. NODE	(50)	(49)	(49)
HYPERPLASIA, NOS	7 (14%)	1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	
#CERVICAL LYMPH NODE	(50)	(49)	(49)
HYPERPLASIA, NOS			1 (2%)
#MEDIASTINAL L. NODE	(50)	(49)	(49)
ANGIECTASIS	1 (2%)		
#PANCREATIC L. NODE	(50)	(49)	(49)
CONGESTION, NOS			1 (2%)
#MESENTERIC L. NODE	(50)	(49)	(49)
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, CYSTIC			1 (2%)
ANGIECTASIS	3 (6%)		
#LUNG	(50)	(50)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#LIVER	(50)	(50)	(49)
LEUKOCYTOSIS, NOS	1 (2%)	1 (2%)	
*MESENTERY	(50)	(50)	(49)
MASTOCYTOSIS		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(49)
INFLAMMATION, FOCAL	1 (2%)		
#MYOCARDIUM	(50)	(50)	(49)
INFLAMMATION, FOCAL	1 (2%)		
FIBROSIS, FOCAL	29 (58%)	23 (46%)	31 (63%)
*MESENTERIC ARTERY	(50)	(50)	(49)
PERIARTERITIS		1 (2%)	
*MESENTERY	(50)	(50)	(49)
PERIARTERITIS			1 (2%)
#TESTIS	(50)	(50)	(49)
PERIARTERITIS		1 (2%)	2 (4%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(48)	(49)
CYST, NOS	1 (2%)		
METAMORPHOSIS FATTY		1 (2%)	
ATROPHY, NOS		1 (2%)	
#LIVER	(50)	(50)	(49)
DEFORMITY, NOS	2 (4%)	1 (2%)	2 (4%)
CONGESTION, NOS	1 (2%)		
INFLAMMATION, FOCAL	1 (2%)	17 (34%)	7 (14%)
CHOLANGIOFIBROSIS	34 (68%)	40 (80%)	34 (69%)
DEGENERATION, CYSTIC	7 (14%)	10 (20%)	11 (22%)
NECROSIS, FOCAL	1 (2%)		
INFARCT, NOS	1 (2%)		
METAMORPHOSIS FATTY	3 (6%)	1 (2%)	
PIGMENTATION, NOS			1 (2%)
CYTOPLASMIC VACUOLIZATION	6 (12%)	17 (34%)	22 (45%)
BASOPHILIC CYTO CHANGE	1 (2%)	1 (2%)	5 (10%)
FOCAL CELLULAR CHANGE	1 (2%)	1 (2%)	2 (4%)
ANGIECTASIS		1 (2%)	1 (2%)
NODULAR REGENERATION	1 (2%)		1 (2%)
#LIVER/CENTRILOBULAR	(50)	(50)	(49)
NECROSIS, NOS	2 (4%)		
ATROPHY, NOS		1 (2%)	
#BILE DUCT	(50)	(50)	(49)
HYPERPLASIA, NOS	49 (98%)	50 (100%)	47 (96%)
HYPERPLASIA, FOCAL			1 (2%)
#PANCREAS	(50)	(50)	(49)
CYSTIC DUCTS	1 (2%)		1 (2%)
DEGENERATION, CYSTIC		1 (2%)	
ATROPHY, FOCAL	11 (22%)	10 (20%)	15 (31%)
HYPERPLASIA, FOCAL			1 (2%)
#STOMACH	(50)	(50)	(49)
EDEMA, NOS		1 (2%)	1 (2%)
ULCER, NOS	1 (2%)		
#GASTRIC MUCOSA	(50)	(50)	(49)
NECROSIS, FOCAL	1 (2%)		
#FORESTOMACH	(50)	(50)	(49)
ULCER, NOS	2 (4%)	1 (2%)	1 (2%)
INFLAMMATION, FOCAL	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(49)
CYST, NOS	1 (2%)	1 (2%)	
CONGESTION, NOS		1 (2%)	
NEPHROPATHY		1 (2%)	
NEPHROSIS, NOS	45 (90%)	44 (88%)	45 (92%)
NECROSIS, FOCAL	1 (2%)		
#KIDNEY/MEDULLA	(50)	(50)	(49)
CAST, NOS			1 (2%)
#PERIRENAL TISSUE	(50)	(50)	(49)
NECROSIS, FAT	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(49)	(48)
HEMATOMA, NOS		1 (2%)	
LIPOGRANULOMA			1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
FOCAL CELLULAR CHANGE	1 (2%)	1 (2%)	1 (2%)
HYPERPLASIA, FOCAL	7 (14%)	9 (18%)	2 (4%)
ANGIECTASIS	4 (8%)	5 (10%)	1 (2%)
#ADRENAL	(50)	(50)	(49)
CYTOPLASMIC CHANGE, NOS		1 (2%)	
FOCAL CELLULAR CHANGE	1 (2%)		1 (2%)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(50)	(50)	(49)
ACCESSORY STRUCTURE			1 (2%)
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	
CYTOPLASMIC VACUOLIZATION	2 (4%)	9 (18%)	9 (18%)
FOCAL CELLULAR CHANGE	5 (10%)	1 (2%)	
#ADRENAL MEDULLA	(50)	(50)	(49)
HYPERPLASIA, FOCAL	9 (18%)	5 (10%)	9 (18%)
ANGIECTASIS		1 (2%)	1 (2%)
#THYROID	(50)	(50)	(49)
ULTIMOBRANCHIAL CYST	3 (6%)		
CYSTIC FOLLICLES	5 (10%)	2 (4%)	3 (6%)
DEGENERATION, CYSTIC	5 (10%)		2 (4%)
PIGMENTATION, NOS	5 (10%)		1 (2%)
HYPERPLASIA, C-CELL	1 (2%)	4 (8%)	3 (6%)
#THYROID FOLLICLE	(50)	(50)	(49)
ACCESSORY STRUCTURE	1 (2%)		
INFLAMMATION, FOCAL	1 (2%)		
PIGMENTATION, NOS	2 (4%)	6 (12%)	5 (10%)
HYPERPLASIA, CYSTIC	1 (2%)		
#PARATHYROID	(48)	(47)	(48)
FOCAL CELLULAR CHANGE		2 (4%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
GALACTOCELE	1 (2%)		
CYSTIC DUCTS			2 (4%)
HEMORRHAGIC CYST		2 (4%)	
LIPOGRANULOMA			2 (4%)
CYSTIC DISEASE	23 (46%)	32 (64%)	28 (57%)
*MAMMARY DUCT	(50)	(50)	(49)
HEMORRHAGIC CYST			1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	3 (6%)	3 (6%)	
INFLAMMATION CHRONIC SUPPURATIVE			1 (2%)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, CYSTIC		1 (2%)	

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (*Continued*)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#PROSTATE	(49)	(49)	(49)
INFLAMMATION, SUPPURATIVE	23 (47%)	26 (53%)	33 (67%)
DEGENERATION, CYSTIC		2 (4%)	
HYPERPLASIA, CYSTIC	1 (2%)		
*SEMINAL VESICLE	(50)	(50)	(49)
COLLAPSE	1 (2%)	2 (4%)	
DEPLETION			1 (2%)
#TESTIS	(50)	(50)	(49)
EDEMA, NOS			1 (2%)
HEMORRHAGE			1 (2%)
DEGENERATION, CYSTIC		1 (2%)	
ATROPHY, NOS	14 (28%)	12 (24%)	20 (41%)
ATROPHY, FOCAL	1 (2%)		1 (2%)
HYPERPLASIA, INTERSTITIAL CELL	1 (2%)		2 (4%)
*EPIDIDYMIS	(50)	(50)	(49)
DILATATION, NOS	1 (2%)		
EDEMA, NOS		1 (2%)	
NECROSIS, FAT	1 (2%)		
*SPERMATIC CORD	(50)	(50)	(49)
NECROSIS, FAT	2 (4%)	5 (10%)	
NERVOUS SYSTEM			
#BRAIN	(49)	(50)	(49)
HEMORRHAGE	2 (4%)	2 (4%)	
NECROSIS, FOCAL	2 (4%)	1 (2%)	
#PALLIUM	(49)	(50)	(49)
DISPLACEMENT, NOS			1 (2%)
#CEREBRAL BASAL SURFA	(49)	(50)	(49)
DISPLACEMENT, NOS			1 (2%)
#HYPOTHALAMUS	(49)	(50)	(49)
DISPLACEMENT, NOS			2 (4%)
#PONS	(49)	(50)	(49)
DISPLACEMENT, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(49)
HEMORRHAGE	2 (4%)		
RETINOPATHY	6 (12%)	2 (4%)	16 (33%)
CATARACT	3 (6%)	2 (4%)	14 (29%)
PHTHISIS BULBI	1 (2%)		1 (2%)
*EAR CANAL	(50)	(50)	(49)
INFLAMMATION, NOS			2 (4%)
*ZYMBAL GLAND	(50)	(50)	(49)
HYPERPLASIA, FOCAL			1 (2%)
*TYMPANIC CAVITY	(50)	(50)	(49)
HYPEROSTOSIS			1 (2%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(49)
HYPEROSTOSIS	5 (10%)	8 (16%)	25 (51%)
BODY CAVITIES			
*MESENTERY	(50)	(50)	(49)
NECROSIS, FAT	1 (2%)	1 (2%)	2 (4%)
ANGIECTASIS			1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(49)
CONGESTION, NOS	3 (6%)		1 (2%)
SOLE OF FOOT			
CALLUS			1
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY			1

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

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
EPIDERMAL INCLUSION CYST	1 (2%)		
ABSCESS, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
RESPIRATORY SYSTEM			
*NASAL MUCOSA	(50)	(50)	(50)
EDEMA, NOS		1 (2%)	
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
*LARYNGEAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#LUNG	(50)	(50)	(50)
CONGESTION, NOS	1 (2%)	2 (4%)	3 (6%)
EDEMA, INTERSTITIAL			1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	
HISTIOCYTOSIS	1 (2%)	2 (4%)	
#LUNG/ALVEOLI	(50)	(50)	(50)
HYPERPLASIA, ADENOMATOUS	2 (4%)	1 (2%)	1 (2%)
HISTIOCYTOSIS	5 (10%)	26 (52%)	19 (38%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
LEUKOCYTOSIS, NOS			1 (2%)
#BONE MARROW	(50)	(50)	(50)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
#SPLEEN	(50)	(50)	(50)
PIGMENTATION, NOS		1 (2%)	
HEMOSIDEROSIS			1 (2%)
HEMATOPOIESIS	2 (4%)		3 (6%)
#MANDIBULAR L. NODE	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		9 (18%)
HYPERPLASIA, CYSTIC			1 (2%)
#MEDIASTINAL L. NODE	(50)	(50)	(50)
CONGESTION, NOS		1 (2%)	
ANGIECTASIS			2 (4%)
HYPERPLASIA, RETICULUM CELL	1 (2%)		
#CELIAC LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#PANCREATIC L. NODE	(50)	(50)	(50)
HYPERPLASIA, CYSTIC			1 (2%)
#MESENTERIC L. NODE	(50)	(50)	(50)
CONGESTION, NOS			1 (2%)
ANGIECTASIS	1 (2%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)
#RENAL LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#INGUINAL LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
#TRACHEA	(45)	(48)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
#LUNG	(50)	(50)	(50)
LEUKOCYTOSIS, NOS			1 (2%)
#LIVER	(50)	(50)	(50)
LEUKOCYTOSIS, NOS			1 (2%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#ADRENAL CORTEX	(49)	(50)	(49)
HEMATOPOIESIS	1 (2%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(50)	(50)
INFLAMMATION, FOCAL	1 (2%)		
FIBROSIS, FOCAL	3 (6%)	4 (8%)	2 (4%)
#CARDIAC VALVE	(50)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(50)	(49)
INFLAMMATION, NOS		1 (2%)	
#LIVER	(50)	(50)	(50)
DEFORMITY, NOS	2 (4%)	2 (4%)	3 (6%)
INFLAMMATION, FOCAL	19 (38%)	18 (36%)	28 (56%)
CHOLANGIOFIBROSIS	9 (18%)	14 (28%)	4 (8%)
DEGENERATION, CYSTIC		2 (4%)	1 (2%)
NECROSIS, FOCAL		1 (2%)	1 (2%)
METAMORPHOSIS FATTY	1 (2%)	1 (2%)	2 (4%)
PIGMENTATION, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	5 (10%)	15 (30%)	8 (16%)
BASOPHILIC CYTO CHANGE	13 (26%)	15 (30%)	2 (4%)
FOCAL CELLULAR CHANGE		1 (2%)	
CLEAR CELL CHANGE			1 (2%)
ANGIECTASIS	2 (4%)		
HISTIOCYTOSIS	1 (2%)		1 (2%)
#LIVER/PERIPORTAL	(50)	(50)	(50)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	35 (70%)	44 (88%)	43 (86%)
#PANCREAS	(49)	(50)	(50)
ATROPHY, NOS		1 (2%)	
ATROPHY, FOCAL	9 (18%)	9 (18%)	10 (20%)
#STOMACH	(50)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
#GASTRIC MUCOSA	(50)	(50)	(50)
EROSION			1 (2%)
#FORESTOMACH	(50)	(50)	(50)
DEFORMITY, NOS			1 (2%)
EDEMA, NOS		2 (4%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
#S. INTEST./MUSCULARIS	(50)	(50)	(50)
HYPERPLASIA, FOCAL			1 (2%)
#CECUM	(48)	(50)	(50)
PIGMENTATION, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
CONGESTION, NOS		3 (6%)	1 (2%)
INFLAMMATION, FOCAL	1 (2%)		
NEPHROSIS, NOS	25 (50%)	25 (50%)	16 (32%)
NECROSIS, MEDULLARY	1 (2%)		
PIGMENTATION, NOS	1 (2%)		
#KIDNEY/MEDULLA	(50)	(50)	(50)
CAST, NOS			1 (2%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM (Continued)			
#KIDNEY/TUBULE	(50)	(50)	(50)
CAST, NOS	1 (2%)		
DEGENERATION, HYALINE		1 (2%)	
#KIDNEY/PELVIS	(50)	(50)	(50)
DILATATION, NOS	1 (2%)		
CALCIFICATION, NOS			2 (4%)
#URINARY BLADDER/MUCOSA	(50)	(50)	(48)
POLYPOID HYPERPLASIA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(50)	(49)
CYST, NOS			3 (6%)
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, FOCAL	2 (4%)	7 (14%)	3 (6%)
ANGIECTASIS	3 (6%)	13 (26%)	8 (16%)
#ADRENAL	(49)	(50)	(49)
CONGESTION, NOS		1 (2%)	1 (2%)
ANGIECTASIS	1 (2%)	1 (2%)	
#ADRENAL CORTEX	(49)	(50)	(49)
ACCESSORY STRUCTURE	1 (2%)	2 (4%)	2 (4%)
CYTOPLASMIC VACUOLIZATION	3 (6%)	8 (16%)	3 (6%)
FOCAL CELLULAR CHANGE	3 (6%)	2 (4%)	
ANGIECTASIS	1 (2%)		
#ADRENAL MEDULLA	(49)	(50)	(49)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	2 (4%)	2 (4%)	4 (8%)
ANGIECTASIS	1 (2%)		
#THYROID	(49)	(50)	(49)
ULTIMOBRANCHIAL CYST	1 (2%)		2 (4%)
CYSTIC FOLLICLES	2 (4%)		
HYPERPLASIA, C-CELL	4 (8%)	3 (6%)	2 (4%)
#THYROID FOLLICLE	(49)	(50)	(49)
PIGMENTATION, NOS		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
GALACTOCELE	2 (4%)		
CYSTIC DUCTS	1 (2%)		
HYPERPLASIA, CYSTIC			1 (2%)
CYSTIC DISEASE	44 (88%)	44 (88%)	29 (58%)
*PREPUTIAL GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
*CLITORAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE		1 (2%)	1 (2%)
*VAGINA	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#UTERUS	(50)	(50)	(50)
HYDROMETRA		2 (4%)	2 (4%)
HEMORRHAGE			1 (2%)
HEMATOMETRA	1 (2%)		1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	3 (6%)
#UTERINE SEROSA	(50)	(50)	(50)
INFLAMMATION, CHRONIC			1 (2%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
CYST, NOS	2 (4%)	1 (2%)	1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, PAPILLARY	1 (2%)		
HYPERPLASIA, CYSTIC	4 (8%)	8 (16%)	14 (28%)
DECIDUAL ALTERATION, NOS		1 (2%)	
#ENDOMETRIAL GLAND	(50)	(50)	(50)
CYST, NOS			1 (2%)
#OVARY/PAROVARIAN	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
#OVARY	(50)	(50)	(50)
CYST, NOS	1 (2%)	1 (2%)	3 (6%)
FOLLICULAR CYST, NOS	3 (6%)	6 (12%)	3 (6%)
PAROVARIAN CYST	1 (2%)		3 (6%)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#MESOVARIUM	(50)	(50)	(50)
NECROSIS, FAT	1 (2%)		
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
HEMORRHAGE	1 (2%)	1 (2%)	
NECROSIS, FOCAL	1 (2%)		
#PALLIUM	(50)	(50)	(50)
DISPLACEMENT, NOS			6 (12%)
#CEREBRAL BASAL SURFACE	(50)	(50)	(50)
DISPLACEMENT, NOS	1 (2%)		4 (8%)
#HYPOTHALAMUS	(50)	(50)	(50)
DISPLACEMENT, NOS	4 (8%)	2 (4%)	
GLIOSIS		1 (2%)	
*SPINAL CORD	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
RETINOPATHY	3 (6%)	12 (24%)	10 (20%)
CATARACT		10 (20%)	2 (4%)
*EAR	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
*TYMPANIC CAVITY	(50)	(50)	(50)
HYPEROSTOSIS			9 (18%)
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
HYPEROSTOSIS	2 (4%)	19 (38%)	49 (98%)
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
*MESENTERY	(50)	(50)	(50)
NECROSIS, FAT	3 (6%)	3 (6%)	1 (2%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	
PIGMENTATION, NOS		1 (2%)	
SOLE OF FOOT			
ABSCESS, NOS	1		
INFLAMMATION, CHRONIC	1		
ULCER, CHRONIC	1		
HYPERPLASIA, EPITHELIAL	1		
CALLUS	12	6	
OMENTUM			
NECROSIS, FAT		1	
BROAD LIGAMENT			
NECROSIS, FAT	6	2	
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING		1	
ANIMALS NECROPSIED	50	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(48)	(49)
ULCER, NOS	10 (20%)	4 (8%)	6 (12%)
INFLAMMATION, FOCAL		1 (2%)	
ULCER, FOCAL		1 (2%)	
INFLAMMATION, SUPPURATIVE	2 (4%)		
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	17 (34%)	25 (52%)	5 (10%)
ULCER, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL		1 (2%)	1 (2%)
INFLAMMATION CHRONIC SUPPURATIVE	1 (2%)		
FIBROSIS	6 (12%)		13 (27%)
FIBROSIS, FOCAL	2 (4%)	1 (2%)	
INFECTION, FUNGAL		1 (2%)	
NECROSIS, NOS		1 (2%)	
PIGMENTATION, NOS		1 (2%)	
HYPERPLASIA, NOS		2 (4%)	1 (2%)
ACANTHOSIS		2 (4%)	
*SUBCUT TISSUE	(50)	(48)	(49)
EDEMA, NOS			1 (2%)
INFLAMMATION, NECROTIZING		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC	6 (12%)	1 (2%)	9 (18%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		1 (2%)
INFLAMMATION CHRONIC SUPPURATIVE	2 (4%)		
INFLAMMATION, GRANULOMATOUS		1 (2%)	
INFLAMMATION GRANULOMATOUS FOCAL		1 (2%)	
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
FIBROSIS	1 (2%)		
INFECTION, FUNGAL		1 (2%)	
METAPLASIA, OSSEOUS	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#TRACHEAL GLAND	(50)	(47)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
#LUNG/BRONCHUS	(50)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
#LUNG	(50)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		2 (4%)	2 (4%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, CHRONIC			1 (2%)
PNEUMONIA INTERSTITIAL CHRONIC		2 (4%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
CRYSTALS, NOS	1 (2%)		
ALVEOLAR MACROPHAGES	1 (2%)		1 (2%)
HYPERPLASIA, ALVEOLAR EPITHELIUM		1 (2%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
LEUKEMOID REACTION	1 (2%)		
HEMATOPOIESIS		1 (2%)	1 (2%)
#BONE MARROW	(50)	(45)	(49)
NECROSIS, NOS	1 (2%)		1 (2%)
ATROPHY, NOS	1 (2%)		
MYELOFIBROSIS	1 (2%)		
HYPERPLASIA, GRANULOCYTIC	2 (4%)	3 (7%)	2 (4%)
MYELOPOIESIS		1 (2%)	
#SPLEEN	(50)	(47)	(49)
ATROPHY, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	10 (20%)	6 (13%)	4 (8%)
#SPLENIC CAPSULE	(50)	(47)	(49)
ACCESSORY STRUCTURE			1 (2%)
#PANCREATIC L. NODE	(50)	(47)	(49)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
INFECTION, FUNGAL		1 (2%)	
#LUMBAR LYMPH NODE	(50)	(47)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#MESENTERIC L. NODE	(50)	(47)	(49)
ANGIECTASIS	8 (16%)	4 (9%)	7 (14%)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	2 (4%)
#AXILLARY LYMPH NODE	(50)	(47)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#INGUINAL LYMPH NODE	(50)	(47)	(49)
PIGMENTATION, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID	2 (4%)	2 (4%)	
#LIVER	(50)	(48)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
HEMATOPOIESIS		2 (4%)	1 (2%)
#PEYER'S PATCH	(48)	(45)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
THROMBUS, ORGANIZED			1 (2%)
*SKIN	(50)	(48)	(49)
LYMPHANGIECTASIS		1 (2%)	
*SUBCUT TISSUE	(50)	(48)	(49)
LYMPHANGIECTASIS			1 (2%)
#MYOCARDIUM	(50)	(48)	(49)
INFLAMMATION, CHRONIC FOCAL			1 (2%)
*SPERMATIC ARTERY	(50)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(49)
NECROSIS, COAGULATIVE	4 (8%)	3 (6%)	1 (2%)
NECROSIS, ISCHEMIC		1 (2%)	
CYTOPLASMIC VACUOLIZATION		1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE	1 (2%)	1 (2%)	3 (6%)
ANGIECTASIS			1 (2%)
#LIVER/CENTRILOBULAR	(50)	(48)	(49)
NECROSIS, FOCAL	1 (2%)		
#BILE DUCT	(50)	(48)	(49)
MULTIPLE CYSTS		1 (2%)	
#PANCREAS	(50)	(46)	(49)
HEMORRHAGIC CYST			1 (2%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#GASTRIC MUCOSA	(50)	(47)	(49)
CYST, NOS		1 (2%)	
HYPERPLASIA, ADENOMATOUS		1 (2%)	
#COLON	(50)	(47)	(49)
PARASITISM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(49)
HYDRONEPHROSIS	1 (2%)		
CYST, NOS			1 (2%)
MULTIPLE CYSTS	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	15 (30%)	16 (33%)	10 (20%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
GLOMERULONEPHRITIS, CHRONIC			1 (2%)
NEPHROSIS, NOS	1 (2%)		3 (6%)
#KIDNEY/CORTEX	(50)	(48)	(49)
CYST, NOS	1 (2%)		
#KIDNEY/TUBULE	(50)	(48)	(49)
DEGENERATION, HYALINE			1 (2%)
#URINARY BLADDER	(50)	(47)	(48)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
*URETHRA	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE	2 (4%)		
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(47)	(49)
CYST, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL CORTEX	(50)	(47)	(49)
CYTOPLASMIC VACUOLIZATION			1 (2%)
HYPERPLASIA, FOCAL			1 (2%)
#ADRENAL MEDULLA	(50)	(47)	(49)
HYPERPLASIA, FOCAL	1 (2%)	2 (4%)	1 (2%)
#THYROID	(44)	(45)	(49)
CYSTIC FOLLICLES		2 (4%)	2 (4%)
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
DEGENERATION, CYSTIC	2 (5%)	8 (18%)	6 (12%)
HYPERPLASIA, CYSTIC	1 (2%)		
HYPERPLASIA, FOLLICULAR-CELL	5 (11%)	2 (4%)	4 (8%)
#THYROID FOLLICLE	(44)	(45)	(49)
CRYSTALS, NOS			1 (2%)
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, CYSTIC	1 (2%)	1 (2%)	1 (2%)
#PANCREATIC ISLETS	(50)	(46)	(49)
HYPERPLASIA, NOS	1 (2%)		
REPRODUCTIVE SYSTEM			
*BULBOURETHRAL GLAND	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE			1 (2%)
*PREPUTIAL GLAND	(50)	(48)	(49)
CYST, NOS		1 (2%)	
CYSTIC DUCTS	6 (12%)	4 (8%)	3 (6%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		1 (2%)
INFLAMMATION, SUPPURATIVE	1 (2%)	2 (4%)	
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION CHRONIC SUPPURATIVE	3 (6%)	5 (10%)	4 (8%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#PROSTATE	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)	1 (2%)	
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION CHRONIC SUPPURATIVE	1 (2%)		
HYPERPLASIA, EPITHELIAL	1 (2%)		
*SEMINAL VESICLE	(50)	(48)	(49)
DILATATION, NOS		1 (2%)	
DISTENTION	2 (4%)		
RETENTION OF CONTENT	1 (2%)		
INFLAMMATION, SUPPURATIVE	2 (4%)	1 (2%)	
INFLAMMATION, ACUTE/CHRONIC		2 (4%)	
INFLAMMATION, CHRONIC	1 (2%)	3 (6%)	3 (6%)
HYPERPLASIA, EPITHELIAL		2 (4%)	
#TESTIS	(50)	(48)	(49)
ATROPHY, NOS		1 (2%)	
*EPIDIDYMIS	(50)	(48)	(49)
DILATATION, NOS		1 (2%)	
INFLAMMATION, CHRONIC FOCAL		2 (4%)	
*SPERMATIC CORD	(50)	(48)	(49)
STEATITIS		1 (2%)	1 (2%)
NERVOUS SYSTEM			
*CHOROID PLEXUS	(50)	(48)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
#BRAIN	(50)	(48)	(49)
HEMORRHAGE	1 (2%)		
CORPORA AMYLACEA	5 (10%)	1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(48)	(49)
HYPEROSTOSIS			1 (2%)
*ABDOMINAL MUSCLE	(50)	(48)	(49)
HEMORRHAGE	1 (2%)		
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
BODY CAVITIES			
*ABDOMINAL WALL	(50)	(48)	(49)
INFECTION, FUNGAL		1 (2%)	
*MESENTERY	(50)	(48)	(49)
STEATITIS		1 (2%)	2 (4%)
NECROSIS, FAT	2 (4%)		
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			2
ANIMAL MISSING/NO NECROPSY		1	
AUTOLYSIS/NO NECROPSY		1	1

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

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
ULCER, NOS	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
ALOPECIA		1 (2%)	
ACANTHOSIS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
CONGESTION, NOS	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		2 (4%)
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION GRANULOMATOUS FOCAL	2 (4%)		
PIGMENTATION, NOS		1 (2%)	
ALVEOLAR MACROPHAGES		1 (2%)	
HYPERPLASIA, ADENOMATOUS	1 (2%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MYELOPROLIFERATIVE DISORDER		1 (2%)	
HYPERPLASIA, LYMPHOID	2 (4%)		3 (6%)
HEMATOPOIESIS		3 (6%)	2 (4%)
*SUBCUT TISSUE	(50)	(50)	(50)
MASTOCYTOSIS	1 (2%)		
#BONE MARROW	(50)	(50)	(50)
DEGENERATION, NOS	1 (2%)		
MYELOFIBROSIS		1 (2%)	
HYPERPLASIA, GRANULOCYTIC		3 (6%)	4 (8%)
#SPLEEN	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	6 (12%)	1 (2%)	2 (4%)
HEMATOPOIESIS	8 (16%)	9 (18%)	20 (41%)
#SPLENIC CAPSULE	(50)	(50)	(49)
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS, DIFFUSE			1 (2%)
#MANDIBULAR L. NODE	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		
#MESENTERIC L. NODE	(50)	(50)	(48)
ANGIECTASIS		2 (4%)	
HYPERPLASIA, LYMPHOID	2 (4%)		1 (2%)
#RENAL LYMPH NODE	(50)	(50)	(48)
CONGESTION, NOS			1 (2%)
ANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID			4 (8%)
#LIVER	(50)	(50)	(49)
LEUKOCYTOSIS, NOS	2 (4%)	1 (2%)	4 (8%)
HEMATOPOIESIS	2 (4%)	3 (6%)	11 (22%)
#PEYER'S PATCH	(49)	(46)	(48)
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
#ADRENAL	(49)	(50)	(49)
HEMATOPOIESIS			2 (4%)

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#THYMUS	(49)	(49)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
CIRCULATORY SYSTEM			
#THYROID	(48)	(49)	(49)
PERIARTERITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(49)
CYST, NOS	1 (2%)		
MULTIPLE CYSTS	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR		3 (6%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFLAMMATION GRANULOMATOUS FOCAL		1 (2%)	
NECROSIS, FOCAL	1 (2%)		
NECROSIS, COAGULATIVE		4 (8%)	1 (2%)
NECROSIS, ISCHEMIC	1 (2%)		
PIGMENTATION, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	3 (6%)	1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE			1 (2%)
CYTOLOGIC ALTERATION, NOS	1 (2%)		
ANGIECTASIS	1 (2%)		
#PANCREAS	(49)	(48)	(49)
CYST, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE NECROTIZING		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		2 (4%)
#PANCREATIC ACINUS	(49)	(48)	(49)
ATROPHY, NOS	1 (2%)		1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
#STOMACH	(49)	(48)	(49)
CYST, NOS		1 (2%)	
#FORESTOMACH	(49)	(48)	(49)
ULCER, NOS		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERKERATOSIS	2 (4%)	1 (2%)	
#SMALL INTESTINE	(49)	(46)	(48)
AMYLOIDOSIS	1 (2%)		
#COLONIC MUSCULARIS PROPRIA	(50)	(49)	(49)
ABSCESS, CHRONIC		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
HYDRONEPHROSIS	1 (2%)		
GLOMERULONEPHRITIS, NOS			1 (2%)
PYELONEPHRITIS, NOS			2 (4%)
LYMPHOCYTIC INFLAMMATORY INFILTR	11 (22%)	25 (50%)	15 (30%)
INFLAMMATION, CHRONIC	1 (2%)		
GLOMERULONEPHRITIS, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#RENAL PAPILLA	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		
#KIDNEY/GLOMERULUS	(50)	(50)	(50)
AMYLOIDOSIS	1 (2%)		
#KIDNEY/PELVIS	(50)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)	1 (2%)	
#URINARY BLADDER	(49)	(50)	(49)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(48)	(49)
CYST, NOS	1 (2%)	1 (2%)	
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL	3 (6%)	3 (6%)	1 (2%)
ANGIECTASIS	6 (12%)	3 (6%)	6 (12%)
#ADRENAL	(49)	(50)	(49)
ANGIECTASIS			1 (2%)
#ADRENAL CORTEX	(49)	(50)	(49)
ECTOPIA		1 (2%)	
DEGENERATION, CYSTIC	1 (2%)		
CYTOPLASMIC VACUOLIZATION	1 (2%)		
HYPERPLASIA, NOS			1 (2%)
#ADRENAL MEDULLA	(49)	(50)	(49)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(48)	(49)	(49)
CYSTIC FOLLICLES	1 (2%)		1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
DEGENERATION, CYSTIC	3 (6%)	2 (4%)	1 (2%)
HYPERPLASIA, CYSTIC			2 (4%)
HYPERPLASIA, FOLLICULAR-CELL	3 (6%)	2 (4%)	1 (2%)
#THYROID FOLLICLE	(48)	(49)	(49)
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, CYSTIC			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS	2 (4%)		5 (10%)
*CLITORAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS	2 (4%)		2 (4%)
#UTERUS	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE SUPPURATIVE	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	4 (8%)	3 (6%)	5 (10%)
ABSCESS, CHRONIC		1 (2%)	1 (2%)
HYPERPLASIA, CYSTIC	43 (86%)	45 (90%)	38 (76%)
HYPERPLASIA, ADENOMATOUS	1 (2%)		
#UTERUS/MYOMETRIUM	(50)	(50)	(50)
ABSCESS, CHRONIC			1 (2%)
#OVARY	(49)	(50)	(50)
CYST, NOS	2 (4%)	1 (2%)	1 (2%)
CYSTIC FOLLICLES	2 (4%)	14 (28%)	3 (6%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	
ABSCESS, CHRONIC	5 (10%)	10 (20%)	23 (46%)
HYPERPLASIA, GRANULOSA-CELL	1 (2%)		
HYPERPLASIA, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(50)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
PIGMENTATION, NOS			1 (2%)
#CEREBRAL VENTRICLE	(50)	(50)	(50)
HYDROCEPHALUS, NOS	1 (2%)		
#BRAIN	(50)	(50)	(50)
CORPORA AMYLACEA	4 (8%)		
*SPINAL CORD	(50)	(50)	(50)
STATUS SPONGIOSUS		1 (2%)	

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE	(50)	(50)	(50)
FIBROUS OSTEODYSTROPHY	2 (4%)	5 (10%)	12 (24%)
*SKULL	(50)	(50)	(50)
HYPEROSTOSIS			4 (8%)
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
*PLEURA	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
*MESENTERY	(50)	(50)	(50)
STEATITIS		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION CHRONIC SUPPURATIVE			1 (2%)
ABSCESS, CHRONIC		1 (2%)	
NECROSIS, FAT	2 (4%)	1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
LYMPHOCYTIC INFLAMMATORY INFILTR		1 (2%)	
INFLAMMATION, SUPPURATIVE	3 (6%)	6 (12%)	14 (28%)
INFLAMMATION, ACUTE SUPPURATIVE	2 (4%)	2 (4%)	5 (10%)
INFLAMMATION CHRONIC SUPPURATIVE		4 (8%)	2 (4%)
NECROSIS, NOS			1 (2%)
OMENTUM			
STEATITIS	1		
NECROSIS, FAT	1		
BROAD LIGAMENT			
STEATITIS	4		
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1

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

APPENDIX E

ANALYSES OF PRIMARY TUMORS IN RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	Control	5,000 ppm	10,000 ppm
Skin: Basal Cell Tumor			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/49 (0%)
Adjusted Rates (b)	9.4%	0.0%	0.0%
Terminal Rates (c)	3/32 (9%)	0/38 (0%)	0/42 (0%)
Life Table Tests (d)	P=0.023N	P=0.092N	P=0.078N
Incidental Tumor Tests (d)	P=0.023N	P=0.092N	P=0.078N
Cochran-Armitage Trend Test (d)	P=0.038N		
Fisher Exact Test		P=0.121N	P=0.125N
Skin: Basal Cell Tumor or Carcinoma			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	0/49 (0%)
Adjusted Rates (b)	12.5%	0.0%	0.0%
Terminal Rates (c)	4/32 (13%)	0/38 (0%)	0/42 (0%)
Life Table Tests (d)	P=0.008N	P=0.043N	P=0.034N
Incidental Tumor Tests (d)	P=0.008N	P=0.043N	P=0.034N
Cochran-Armitage Trend Test (d)	P=0.015N		
Fisher Exact Test		P=0.059N	P=0.061N
Skin: Keratoacanthoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	8.9%	4.8%	2.4%
Terminal Rates (c)	2/32 (6%)	1/38 (3%)	1/42 (2%)
Life Table Tests (d)	P=0.155N	P=0.427N	P=0.219N
Incidental Tumor Tests (d)	P=0.256N	P=0.511N	P=0.312N
Cochran-Armitage Trend Test (d)	P=0.228N		
Fisher Exact Test		P=0.500N	P=0.316N
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	8.3%	5.0%	2.4%
Terminal Rates (c)	2/32 (6%)	1/38 (3%)	1/42 (2%)
Life Table Tests (d)	P=0.163N	P=0.440N	P=0.237N
Incidental Tumor Tests (d)	P=0.258N	P=0.527N	P=0.305N
Cochran-Armitage Trend Test (d)	P=0.228N		
Fisher Exact Test		P=0.500N	P=0.316N
Subcutaneous Tissue: Fibroma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	2/49 (4%)
Adjusted Rates (b)	11.3%	5.0%	4.8%
Terminal Rates (c)	3/32 (9%)	1/38 (3%)	2/42 (5%)
Life Table Tests (d)	P=0.174N	P=0.277N	P=0.240N
Incidental Tumor Tests (d)	P=0.259N	P=0.348N	P=0.297N
Cochran-Armitage Trend Test (d)	P=0.259N		
Fisher Exact Test		P=0.339N	P=0.349N
Integumentary System: Fibroma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	1/49 (2%)
Adjusted Rates (b)	14.4%	5.0%	2.4%
Terminal Rates (c)	4/32 (13%)	1/38 (3%)	1/42 (2%)
Life Table Tests (d)	P=0.034N	P=0.166N	P=0.061N
Incidental Tumor Tests (d)	P=0.061N	P=0.216N	P=0.082N
Cochran-Armitage Trend Test (d)	P=0.062N		
Fisher Exact Test		P=0.218N	P=0.107N
Integumentary System: Fibroma, Fibrosarcoma, or Neurofibrosarcoma			
Overall Rates (a)	6/50 (12%)	2/50 (4%)	2/49 (4%)
Adjusted Rates (b)	17.5%	5.0%	4.8%
Terminal Rates (c)	5/32 (16%)	1/38 (3%)	2/42 (5%)
Life Table Tests (d)	P=0.043N	P=0.095N	P=0.072N
Incidental Tumor Tests (d)	P=0.071N	P=0.127N	P=0.094N
Cochran-Armitage Trend Test (d)	P=0.084N		
Fisher Exact Test		P=0.134N	P=0.141N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	Control	5,000 ppm	10,000 ppm
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	12/50 (24%)	6/50 (12%)	5/49 (10%)
Adjusted Rates (b)	26.4%	13.6%	11.1%
Terminal Rates (c)	3/32 (9%)	2/38 (5%)	3/42 (7%)
Life Table Tests (d)	P=0.023N	P=0.078N	P=0.040N
Incidental Tumor Tests (d)	P=0.197N	P=0.217N	P=0.262N
Cochran-Armitage Trend Test (d)	P=0.039N		
Fisher Exact Test		P=0.097N	P=0.059N
Pituitary: Adenoma			
Overall Rates (a)	10/50 (20%)	9/49 (18%)	10/48 (21%)
Adjusted Rates (b)	28.8%	24.3%	22.5%
Terminal Rates (c)	8/32 (25%)	9/37 (24%)	8/42 (19%)
Life Table Tests (d)	P=0.316N	P=0.366N	P=0.369N
Incidental Tumor Tests (d)	P=0.456N	P=0.415N	P=0.585N
Cochran-Armitage Trend Test (d)	P=0.511		
Fisher Exact Test		P=0.520N	P=0.558
Adrenal: Pheochromocytoma			
Overall Rates (a)	13/50 (26%)	9/50 (18%)	7/49 (14%)
Adjusted Rates (b)	36.6%	22.7%	16.7%
Terminal Rates (c)	10/32 (31%)	8/38 (21%)	7/42 (17%)
Life Table Tests (d)	P=0.022N	P=0.131N	P=0.030N
Incidental Tumor Tests (d)	P=0.046N	P=0.182N	P=0.065N
Cochran-Armitage Trend Test (d)	P=0.089N		
Fisher Exact Test		P=0.235N	P=0.115N
Thyroid: C-Cell Adenoma			
Overall Rates (a)	7/50 (14%)	2/50 (4%)	5/49 (10%)
Adjusted Rates (b)	19.5%	4.6%	11.9%
Terminal Rates (c)	5/32 (16%)	1/38 (3%)	5/42 (12%)
Life Table Tests (d)	P=0.193N	P=0.058N	P=0.229N
Incidental Tumor Tests (d)	P=0.291N	P=0.088N	P=0.324N
Cochran-Armitage Trend Test (d)	P=0.314N		
Fisher Exact Test		P=0.080N	P=0.394N
Thyroid: C-Cell Carcinoma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	5/49 (10%)
Adjusted Rates (b)	0.0%	7.6%	11.9%
Terminal Rates (c)	0/32 (0%)	2/38 (5%)	5/42 (12%)
Life Table Tests (d)	P=0.044	P=0.154	P=0.061
Incidental Tumor Tests (d)	P=0.029	P=0.123	P=0.061
Cochran-Armitage Trend Test (d)	P=0.021		
Fisher Exact Test		P=0.121	P=0.027
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	5/50 (10%)	10/49 (20%)
Adjusted Rates (b)	19.5%	12.0%	23.8%
Terminal Rates (c)	5/32 (16%)	3/38 (8%)	10/42 (24%)
Life Table Tests (d)	P=0.414	P=0.290N	P=0.510
Incidental Tumor Tests (d)	P=0.273	P=0.395N	P=0.407
Cochran-Armitage Trend Test (d)	P=0.227		
Fisher Exact Test		P=0.380N	P=0.282
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	6.3%	7.6%	2.4%
Terminal Rates (c)	2/32 (6%)	2/38 (5%)	1/42 (2%)
Life Table Tests (d)	P=0.300N	P=0.576	P=0.405N
Incidental Tumor Tests (d)	P=0.369N	P=0.534	P=0.405N
Cochran-Armitage Trend Test (d)	P=0.407N		
Fisher Exact Test		P=0.500	P=0.508N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	Control	5,000 ppm	10,000 ppm
Testis: Interstitial Cell Tumor			
Overall Rates (a)	45/50 (90%)	47/50 (94%)	37/49 (76%)
Adjusted Rates (b)	91.8%	97.9%	84.1%
Terminal Rates (c)	28/32 (88%)	37/38 (97%)	35/42 (83%)
Life Table Tests (d)	P<0.001N	P=0.247N	P<0.001N
Incidental Tumor Tests (d)	P=0.008N	P=0.420	P=0.027N
Cochran-Armitage Trend Test (d)	P=0.025N		
Fisher Exact Test		P=0.357	P=0.050N
Tunica Vaginalis: Mesothelioma			
Overall Rates (a)	0/50 (0%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	0.0%	7.2%	2.4%
Terminal Rates (c)	0/32 (0%)	2/38 (5%)	1/42 (2%)
Life Table Tests (d)	P=0.443	P=0.145	P=0.554
Incidental Tumor Tests (d)	P=0.369	P=0.111	P=0.554
Cochran-Armitage Trend Test (d)	P=0.372		
Fisher Exact Test		P=0.121	P=0.495
All Sites: Mesothelioma			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	1/49 (2%)
Adjusted Rates (b)	3.1%	7.2%	2.4%
Terminal Rates (c)	1/32 (3%)	2/38 (5%)	1/42 (2%)
Life Table Tests (d)	P=0.532N	P=0.355	P=0.700N
Incidental Tumor Tests (d)	P=0.599N	P=0.306	P=0.700N
Cochran-Armitage Trend Test (d)	P=0.603		
Fisher Exact Test		P=0.309	P=0.748

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	Control	10,000 ppm	20,000 ppm
Hematopoietic System: Mononuclear Cell Leukemia			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	3/49 (6%)
Adjusted Rates (b)	8.6%	13.8%	6.7%
Terminal Rates (c)	1/41 (2%)	4/40 (10%)	1/39 (3%)
Life Table Tests (d)	P=0.455N	P=0.367	P=0.521N
Incidental Tumor Tests (d)	P=0.483N	P=0.183	P=0.482N
Cochran-Armitage Trend Test (d)	P=0.429N		
Fisher Exact Test		P=0.370	P=0.500N
Hematopoietic System: Leukemia			
Overall Rates (a)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	10.9%	13.8%	6.7%
Terminal Rates (c)	2/41 (5%)	4/40 (10%)	1/39 (3%)
Life Table Tests (d)	P=0.331N	P=0.491	P=0.384N
Incidental Tumor Tests (d)	P=0.348N	P=0.304	P=0.338N
Cochran-Armitage Trend Test (d)	P=0.303N		
Fisher Exact Test		P=0.500	P=0.358N
Liver: Neoplastic Nodule or Hepatocellular Carcinoma			
Overall Rates (a)	0/50 (0%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	0.0%	5.0%	7.0%
Terminal Rates (c)	0/41 (0%)	2/40 (5%)	2/39 (5%)
Life Table Tests (d)	P=0.079	P=0.233	P=0.118
Incidental Tumor Tests (d)	P=0.106	P=0.233	P=0.185
Cochran-Armitage Trend Test (d)	P=0.082		
Fisher Exact Test		P=0.247	P=0.121
Pituitary: Adenoma			
Overall Rates (a)	19/49 (39%)	18/50 (36%)	16/49 (33%)
Adjusted Rates (b)	42.0%	41.7%	37.1%
Terminal Rates (c)	14/40 (35%)	15/40 (38%)	12/39 (31%)
Life Table Tests (d)	P=0.361N	P=0.516N	P=0.402N
Incidental Tumor Tests (d)	P=0.271N	P=0.432N	P=0.284N
Cochran-Armitage Trend Test (d)	P=0.299N		
Fisher Exact Test		P=0.469N	P=0.337N
Pituitary: Adenoma or Carcinoma			
Overall Rates (a)	20/49 (41%)	18/50 (36%)	16/49 (33%)
Adjusted Rates (b)	43.2%	41.7%	37.1%
Terminal Rates (c)	14/40 (35%)	15/40 (38%)	12/39 (31%)
Life Table Tests (d)	P=0.294N	P=0.440N	P=0.333N
Incidental Tumor Tests (d)	P=0.225N	P=0.399N	P=0.240N
Cochran-Armitage Trend Test (d)	P=0.231N		
Fisher Exact Test		P=0.388N	P=0.265N
Adrenal: Pheochromocytoma			
Overall Rates (a)	3/49 (6%)	7/50 (14%)	5/49 (10%)
Adjusted Rates (b)	7.5%	17.5%	12.2%
Terminal Rates (c)	3/40 (7%)	7/40 (18%)	4/39 (10%)
Life Table Tests (d)	P=0.293	P=0.157	P=0.342
Incidental Tumor Tests (d)	P=0.326	P=0.157	P=0.413
Cochran-Armitage Trend Test (d)	P=0.308		
Fisher Exact Test		P=0.167	P=0.357
Thyroid: C-Cell Adenoma			
Overall Rates (a)	6/49 (12%)	4/50 (8%)	5/49 (10%)
Adjusted Rates (b)	14.2%	9.3%	12.8%
Terminal Rates (c)	5/41 (12%)	2/40 (5%)	5/39 (13%)
Life Table Tests (d)	P=0.471N	P=0.389N	P=0.538N
Incidental Tumor Tests (d)	P=0.402N	P=0.255N	P=0.500N
Cochran-Armitage Trend Test (d)	P=0.434N		
Fisher Exact Test		P=0.357N	P=0.500N

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	Control	10,000 ppm	20,000 ppm
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/49 (14%)	5/50 (10%)	7/49 (14%)
Adjusted Rates (b)	16.6%	11.7%	17.9%
Terminal Rates (c)	6/41 (15%)	3/40 (7%)	7/39 (18%)
Life Table Tests (d)	P=0.518	P=0.400N	P=0.572
Incidental Tumor Tests (d)	P=0.541N	P=0.276N	P=0.605
Cochran-Armitage Trend Test (d)	P=0.560		
Fisher Exact Test		P=0.365N	P=0.613
Mammary Gland: Fibroadenoma			
Overall Rates (a)	20/50 (40%)	10/50 (20%)	4/50 (8%)
Adjusted Rates (b)	45.3%	24.2%	10.0%
Terminal Rates (c)	17/41 (41%)	9/40 (23%)	3/39 (8%)
Life Table Tests (d)	P<0.001N	P=0.033N	P<0.001N
Incidental Tumor Tests (d)	P<0.001N	P=0.028N	P<0.001N
Cochran-Armitage Trend Test (d)	P<0.001N		
Fisher Exact Test		P=0.024N	P<0.001N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	13/50 (26%)	7/50 (14%)	15/50 (30%)
Adjusted Rates (b)	31.7%	17.0%	35.4%
Terminal Rates (c)	13/41 (32%)	6/40 (15%)	12/39 (31%)
Life Table Tests (d)	P=0.313	P=0.116N	P=0.357
Incidental Tumor Tests (d)	P=0.281	P=0.105N	P=0.312
Cochran-Armitage Trend Test (d)	P=0.361		
Fisher Exact Test		P=0.106N	P=0.412

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	Control	5,000 ppm	10,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	1/50 (2%)	3/48 (6%)	3/49 (6%)
Adjusted Rates (b)	4.2%	13.0%	8.8%
Terminal Rates (c)	1/24 (4%)	3/23 (13%)	3/34 (9%)
Life Table Tests (d)	P=0.383	P=0.287	P=0.436
Incidental Tumor Tests (d)	P=0.383	P=0.287	P=0.436
Cochran-Armitage Trend Test (d)	P=0.233		
Fisher Exact Test		P=0.293	P=0.301
Subcutaneous Tissue: Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	8/48 (17%)	6/49 (12%)
Adjusted Rates (b)	11.1%	25.5%	16.1%
Terminal Rates (c)	2/24 (8%)	3/23 (13%)	4/34 (12%)
Life Table Tests (d)	P=0.425	P=0.116	P=0.426
Incidental Tumor Tests (d)	P=0.518	P=0.290	P=0.514
Cochran-Armitage Trend Test (d)	P=0.206		
Fisher Exact Test		P=0.087	P=0.233
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	11/48 (23%)	9/49 (18%)
Adjusted Rates (b)	15.2%	36.7%	24.5%
Terminal Rates (c)	3/24 (13%)	6/23 (26%)	7/34 (21%)
Life Table Tests (d)	P=0.322	P=0.051	P=0.290
Incidental Tumor Tests (d)	P=0.388	P=0.135	P=0.351
Cochran-Armitage Trend Test (d)	P=0.102		
Fisher Exact Test		P=0.037	P=0.109
Skin: Fibroma or Fibrosarcoma			
Overall Rates (a)	0/50 (0%)	3/48 (6%)	0/49 (0%)
Adjusted Rates (b)	0.0%	11.5%	0.0%
Terminal Rates (c)	0/24 (0%)	2/23 (9%)	0/34 (0%)
Life Table Tests (d)	P=0.547N	P=0.119	(e)
Incidental Tumor Tests (d)	P=0.457N	P=0.185	(e)
Cochran-Armitage Trend Test (d)	P=0.633		
Fisher Exact Test		P=0.114	(e)
Integumentary System: Fibroma			
Overall Rates (a)	1/50 (2%)	5/48 (10%)	3/49 (6%)
Adjusted Rates (b)	4.2%	21.7%	8.8%
Terminal Rates (c)	1/24 (4%)	5/23 (22%)	3/34 (9%)
Life Table Tests (d)	P=0.435	P=0.088	P=0.436
Incidental Tumor Tests (d)	P=0.435	P=0.088	P=0.436
Cochran-Armitage Trend Test (d)	P=0.256		
Fisher Exact Test		P=0.093	P=0.301
Integumentary System: Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	9/48 (19%)	6/49 (12%)
Adjusted Rates (b)	11.1%	27.8%	16.1%
Terminal Rates (c)	2/24 (8%)	3/23 (13%)	4/34 (12%)
Life Table Tests (d)	P=0.437	P=0.076	P=0.426
Incidental Tumor Tests (d)	P=0.561N	P=0.245	P=0.514
Cochran-Armitage Trend Test (d)	P=0.211		
Fisher Exact Test		P=0.052	P=0.233
Integumentary System: Fibroma or Fibrosarcoma			
Overall Rates (a)	4/50 (8%)	14/48 (29%)	9/49 (18%)
Adjusted Rates (b)	15.2%	45.8%	24.5%
Terminal Rates (c)	3/24 (13%)	8/23 (35%)	7/34 (21%)
Life Table Tests (d)	P=0.360	P=0.011	P=0.290
Incidental Tumor Tests (d)	P=0.462	P=0.041	P=0.351
Cochran-Armitage Trend Test (d)	P=0.112		
Fisher Exact Test		P=0.007	P=0.109

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	Control	5,000 ppm	10,000 ppm
Subcutaneous Tissue: Sarcoma, NOS			
Overall Rates (a)	4/50 (8%)	0/48 (0%)	1/49 (2%)
Adjusted Rates (b)	12.4%	0.0%	2.7%
Terminal Rates (c)	0/24 (0%)	0/23 (0%)	0/34 (0%)
Life Table Tests (d)	P=0.056N	P=0.060N	P=0.118N
Incidental Tumor Tests (d)	P=0.280N	P=0.100N	P=0.627N
Cochran-Armitage Trend Test (d)	P=0.086N		
Fisher Exact Test		P=0.064N	P=0.187N
Subcutaneous Tissue: Neurilemoma or Neurilemoma, Malignant			
Overall Rates (a)	1/50 (2%)	1/48 (2%)	3/49 (6%)
Adjusted Rates (b)	3.3%	3.0%	7.9%
Terminal Rates (c)	0/24 (0%)	0/23 (0%)	2/34 (6%)
Life Table Tests (d)	P=0.301	P=0.760	P=0.427
Incidental Tumor Tests (d)	P=0.367	P=0.614N	P=0.444
Cochran-Armitage Trend Test (d)	P=0.198		
Fisher Exact Test		P=0.742	P=0.301
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/50 (6%)	8/48 (17%)	2/49 (4%)
Adjusted Rates (b)	12.5%	31.6%	5.9%
Terminal Rates (c)	3/24 (13%)	6/23 (26%)	2/34 (6%)
Life Table Tests (d)	P=0.235N	P=0.086	P=0.342N
Incidental Tumor Tests (d)	P=0.290N	P=0.091	P=0.342N
Cochran-Armitage Trend Test (d)	P=0.442N		
Fisher Exact Test		P=0.087	P=0.510N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	2/50 (4%)	1/48 (2%)	4/49 (8%)
Adjusted Rates (b)	7.9%	4.3%	10.1%
Terminal Rates (c)	1/24 (4%)	1/23 (4%)	2/34 (6%)
Life Table Tests (d)	P=0.374	P=0.510N	P=0.494
Incidental Tumor Tests (d)	P=0.429	P=0.494N	P=0.605
Cochran-Armitage Trend Test (d)	P=0.233		
Fisher Exact Test		P=0.515N	P=0.329
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/50 (10%)	9/48 (19%)	6/49 (12%)
Adjusted Rates (b)	19.9%	35.6%	15.7%
Terminal Rates (c)	4/24 (17%)	7/23 (30%)	4/34 (12%)
Life Table Tests (d)	P=0.402N	P=0.171	P=0.522N
Incidental Tumor Tests (d)	P=0.419N	P=0.182	P=0.441N
Cochran-Armitage Trend Test (d)	P=0.426		
Fisher Exact Test		P=0.172	P=0.486
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	0/50 (0%)	2/48 (4%)	5/49 (10%)
Adjusted Rates (b)	0.0%	8.7%	13.5%
Terminal Rates (c)	0/24 (0%)	2/23 (9%)	3/34 (9%)
Life Table Tests (d)	P=0.043	P=0.228	P=0.069
Incidental Tumor Tests (d)	P=0.040	P=0.228	P=0.068
Cochran-Armitage Trend Test (d)	P=0.016		
Fisher Exact Test		P=0.237	P=0.027
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	1/50 (2%)	5/48 (10%)	8/49 (16%)
Adjusted Rates (b)	4.2%	17.6%	19.7%
Terminal Rates (c)	1/24 (4%)	2/23 (9%)	4/34 (12%)
Life Table Tests (d)	P=0.053	P=0.110	P=0.060
Incidental Tumor Tests (d)	P=0.050	P=0.157	P=0.095
Cochran-Armitage Trend Test (d)	P=0.012		
Fisher Exact Test		P=0.093	P=0.014

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2 (Continued)

	Control	5,000 ppm	10,000 ppm
Liver: Hepatocellular Adenoma			
Overall Rates (a)	6/50 (12%)	8/48 (17%)	8/49 (16%)
Adjusted Rates (b)	21.1%	32.5%	22.5%
Terminal Rates (c)	4/24 (17%)	7/23 (30%)	7/34 (21%)
Life Table Tests (d)	P=0.492N	P=0.370	P=0.582N
Incidental Tumor Tests (d)	P=0.542	P=0.341	P=0.481
Cochran-Armitage Trend Test (d)	P=0.321		
Fisher Exact Test		P=0.355	P=0.371
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	5/50 (10%)	9/48 (19%)	12/49 (24%)
Adjusted Rates (b)	20.8%	27.3%	31.7%
Terminal Rates (c)	5/24 (21%)	2/23 (9%)	9/34 (26%)
Life Table Tests (d)	P=0.190	P=0.213	P=0.195
Incidental Tumor Tests (d)	P=0.120	P=0.256	P=0.237
Cochran-Armitage Trend Test (d)	P=0.039		
Fisher Exact Test		P=0.172	P=0.049
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	10/50 (20%)	(f) 16/48 (33%)	18/49 (37%)
Adjusted Rates (b)	36.9%	49.7%	46.7%
Terminal Rates (c)	8/24 (33%)	8/23 (35%)	14/34 (41%)
Life Table Tests (d)	P=0.306	P=0.138	P=0.294
Incidental Tumor Tests (d)	P=0.182	P=0.142	P=0.214
Cochran-Armitage Trend Test (d)	P=0.043		
Fisher Exact Test		P=0.103	P=0.052

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) No P value is presented because no tumors were observed in the 10,000-ppm and control groups.
(f) One animal also had an hepatoblastoma.

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	Control	10,000 ppm	20,000 ppm
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	3/49 (6%)	0/50 (0%)
Adjusted Rates (b)	2.9%	8.9%	0.0%
Terminal Rates (c)	1/35 (3%)	1/27 (4%)	0/20 (0%)
Life Table Tests (d)	P=0.518N	P=0.256	P=0.612N
Incidental Tumor Tests (d)	P=0.321N	P=0.372	P=0.612N
Cochran-Armitage Trend Test (d)	P=0.379N		
Fisher Exact Test		P=0.301	P=0.500N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	3/50 (6%)	7/50 (14%)	3/50 (6%)
Adjusted Rates (b)	8.0%	23.7%	11.3%
Terminal Rates (c)	2/35 (6%)	6/28 (21%)	1/20 (5%)
Life Table Tests (d)	P=0.297	P=0.094	P=0.483
Incidental Tumor Tests (d)	P=0.507	P=0.136	P=0.587N
Cochran-Armitage Trend Test (d)	P=0.571		
Fisher Exact Test		P=0.159	P=0.661N
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	1/50 (2%)	3/50 (6%)	2/50 (4%)
Adjusted Rates (b)	2.0%	6.7%	4.7%
Terminal Rates (c)	0/35 (0%)	0/28 (0%)	0/20 (0%)
Life Table Tests (d)	P=0.387	P=0.309	P=0.497
Incidental Tumor Tests (d)	P=0.565	P=0.206	P=0.631
Cochran-Armitage Trend Test (d)	P=0.399		
Fisher Exact Test		P=0.309	P=0.500
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	7/50 (14%)	1/50 (2%)	2/50 (4%)
Adjusted Rates (b)	17.3%	3.6%	5.4%
Terminal Rates (c)	3/35 (9%)	1/28 (4%)	0/20 (0%)
Life Table Tests (d)	P=0.092N	P=0.060N	P=0.195N
Incidental Tumor Tests (d)	P=0.015N	P=0.021N	P=0.023N
Cochran-Armitage Trend Test (d)	P=0.036N		
Fisher Exact Test		P=0.030N	P=0.080N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	12/50 (24%)	11/50 (22%)	7/50 (14%)
Adjusted Rates (b)	27.8%	32.1%	20.2%
Terminal Rates (c)	5/35 (14%)	7/28 (25%)	1/20 (5%)
Life Table Tests (d)	P=0.399N	P=0.523	P=0.397N
Incidental Tumor Tests (d)	P=0.065N	P=0.479N	P=0.030N
Cochran-Armitage Trend Test (d)	P=0.130N		
Fisher Exact Test		P=0.500N	P=0.154N
Liver: Hepatocellular Adenoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	3/49 (6%)
Adjusted Rates (b)	8.6%	0.0%	11.3%
Terminal Rates (c)	3/35 (9%)	0/28 (0%)	1/20 (5%)
Life Table Tests (d)	P=0.420	P=0.162N	P=0.441
Incidental Tumor Tests (d)	P=0.571	P=0.162N	P=0.634
Cochran-Armitage Trend Test (d)	P=0.593		
Fisher Exact Test		P=0.121N	P=0.651
Liver: Hepatocellular Carcinoma			
Overall Rates (a)	4/50 (8%)	1/50 (2%)	7/49 (14%)
Adjusted Rates (b)	11.4%	3.4%	29.1%
Terminal Rates (c)	4/35 (11%)	0/28 (0%)	5/20 (25%)
Life Table Tests (d)	P=0.050	P=0.251N	P=0.066
Incidental Tumor Tests (d)	P=0.102	P=0.190N	P=0.108
Cochran-Armitage Trend Test (d)	P=0.170		
Fisher Exact Test		P=0.181N	P=0.251

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF *HC BLUE NO. 2 (Continued)*

	Control	10,000 ppm	20,000 ppm
Liver: Hepatocellular Adenoma or Carcinoma			
Overall Rates (a)	7/50 (14%)	1/50 (2%)	8/49 (16%)
Adjusted Rates (b)	20.0%	3.4%	31.8%
Terminal Rates (c)	7/35 (20%)	0/28 (0%)	5/20 (25%)
Life Table Tests (d)	P=0.158	P=0.062N	P=0.151
Incidental Tumor Tests (d)	P=0.301	P=0.044N	P=0.275
Cochran-Armitage Trend Test (d)	P=0.422		
Fisher Exact Test		P=0.030N	P=0.483
Pituitary: Adenoma			
Overall Rates (a)	9/49 (18%)	2/48 (4%)	5/49 (10%)
Adjusted Rates (b)	26.5%	6.0%	25.0%
Terminal Rates (c)	9/34 (26%)	1/28 (4%)	5/20 (25%)
Life Table Tests (d)	P=0.379N	P=0.051N	P=0.579N
Incidental Tumor Tests (d)	P=0.339N	P=0.042N	P=0.579N
Cochran-Armitage Trend Test (d)	P=0.129N		
Fisher Exact Test		P=0.028N	P=0.194N
Thyroid: Follicular Cell Adenoma			
Overall Rates (a)	(e) 4/48 (8%)	0/49 (0%)	1/49 (2%)
Adjusted Rates (b)	10.4%	0.0%	5.0%
Terminal Rates (c)	3/35 (9%)	0/27 (0%)	1/20 (5%)
Life Table Tests (d)	P=0.158N	P=0.091N	P=0.334N
Incidental Tumor Tests (d)	P=0.312N	P=0.170N	P=0.519N
Cochran-Armitage Trend Test (d)	P=0.079N		
Fisher Exact Test		P=0.056N	P=0.175N
Thyroid: Follicular Cell Carcinoma			
Overall Rates (a)	3/48 (6%)	1/49 (2%)	1/49(2%)
Adjusted Rates (b)	8.6%	3.3%	5.0%
Terminal Rates (c)	3/35 (9%)	0/27 (0%)	1/20 (5%)
Life Table Tests (d)	P=0.367N	P=0.395N	P=0.519N
Incidental Tumor Tests (d)	P=0.255N	P=0.288N	P=0.519N
Cochran-Armitage Trend Test (d)	P=0.196N		
Fisher Exact Test		P=0.301N	P=0.301N
Thyroid: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	(e) 7/48 (15%)	1/49 (2%)	2/49 (4%)
Adjusted Rates (b)	18.8%	3.3%	10.0%
Terminal Rates (c)	6/35 (17%)	0/27 (0%)	2/20 (10%)
Life Table Tests (d)	P=0.120N	P=0.064N	P=0.253N
Incidental Tumor Tests (d)	P=0.145N	P=0.070N	P=0.374N
Cochran-Armitage Trend Test (d)	P=0.033N		
Fisher Exact Test		P=0.028N	P=0.075N
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	8.6%	0.0%	0.0%
Terminal Rates (c)	3/35 (9%)	0/28 (0%)	0/20 (0%)
Life Table Tests (d)	P=0.075N	P=0.162N	P=0.235N
Incidental Tumor Tests (d)	P=0.075N	P=0.162N	P=0.235N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Test		P=0.121N	P=0.121N

(a) Number of tumor-bearing animals/number of animals examined at the site
(b) Kaplan-Meier estimated tumor incidence at the end of the study after adjusting for intercurrent mortality
(c) Observed tumor incidence at terminal kill
(d) Beneath the control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between that dosed group and the controls. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The incidental tumor test regards these lesions as nonfatal. The Cochran-Armitage and Fisher exact test compare directly the overall incidence rates. A negative trend or lower incidence in a dosed group is indicated by (N).
(e) One animal also had cystadenoma, NOS, of the thyroid follicle.

APPENDIX F

HISTORICAL INCIDENCES OF TUMORS IN F344/N RATS AND B6C3F$_1$ MICE RECEIVING NO TREATMENT

TABLE F1. HISTORICAL INCIDENCE OF INTEGUMENTARY SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Basal Cell Tumor	Basal Cell Carcinoma	Basal Cell Tumor or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	0/49	0/49	0/49
Cytembena	0/50	1/50	1/50
Eugenol	0/40	1/40	1/40
Stannous chloride	0/50	0/50	0/50
Mannitol	0/50	0/50	0/50
Ziram	0/50	0/50	0/50
Propyl gallate	2/50	1/50	3/50
Zearalenone	1/50	0/50	1/50
HC Blue No. 1	0/50	0/50	0/50
TOTAL	3/439 (0.7%)	3/439 (0.7%)	6/439 (1.4%)
SD (b)	1.41%	1.09%	2.03%
Range (c)			
High	2/50	1/40	3/50
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	7/2,320 (0.3%)	14/2,320 (0.6%)	21/2,320 (0.9%)
SD (b)	0.85%	1.34%	1.57%
Range (c)			
High	2/50	3/50	3/50
Low	0/90	0/90	0/90

(a) Data as of March 16, 1983, for studies of at least 104 weeks. Four trichoepitheliomas, three sebaceous adenomas, and one sebaceous adenocarcinoma were also observed. The inclusion of these tumors does not affect the reported range.
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F2. HISTORICAL INCIDENCE OF ADRENAL TUMORS IN MALE F344/N RATS RECEIVING RECEIVING NO TREATMENT (a)

	Pheochromocytoma	Malignant Pheochromocytoma	Pheochromocytoma or Malignant Pheochromocytoma
Historical Incidence at Southern Research Institute			
Reserpine	3/48	1/48	3/48
Cytembena	11/48	0/48	11/48
Eugenol	9/40	0/40	9/40
Stannous chloride	4/50	1/50	5/50
Mannitol	14/50	0/50	14/50
Ziram	7/50	0/50	7/50
Propyl gallate	4/50	0/50	4/50
Zearalenone	5/50	2/50	7/50
HC Blue No. 1	20/49	1/49	21/49
TOTAL	77/435 (17.7%)	5/435 (1.1%)	81/435 (18.6%)
SD (b)	11.63%	1.46%	11.68%
Range (c)			
High	20/49	2/50	21/49
Low	3/48	0/50	3/48
Overall Historical Incidence			
TOTAL	(d) 388/2,280 (17.0%)	23/2,280 (1.0%)	409/2,280 (17.9%)
SD (b)	9.20%	1.44%	9.03%
Range (c)			
High	20/49	3/48	21/49
Low	2/50	0/50	3/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Includes eight diagnoses of pheochromocytoma of the adrenal medulla

TABLE F3. HISTORICAL INCIDENCE OF MAMMARY GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

	Fibroadenoma
Historical Incidence at Southern Research Institute	
Reserpine	14/50
Cytembena	17/49
Eugenol	14/40
Stannous chloride	16/50
Mannitol	10/50
Ziram	16/50
Propyl gallate	11/50
Zearalenone	9/50
HC Blue No. 1	13/50
TOTAL	120/439 (27.3%)
SD (b)	6.40%
Range (c)	
High	14/40
Low	9/50
Overall Historical Incidence	
TOTAL	(d) 543/2,370 (22.9%)
SD (b)	10.41%
Range (c)	
High	22/50
Low	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation.
(c) Range and SD are presented for groups of 35 or more animals.
(d) Includes four animals in one group diagnosed with cystfibroadenoma. Two fibroadenocarcinomas also have been observed.

TABLE F4. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

	Leukemia
Historical Incidence at Southern Research Institute	
Reserpine	18/49
Cytmebena	20/50
Eugenol	13/40
Stannous chloride	6/50
Mannitol	14/50
Ziram	10/50
Propyl gallate	16/50
Zearalenone	9/50
HC Blue No. 1	13/50
TOTAL	119/439 (27.1%)
SD (b)	9.19%
Range (c)	
High	20/50
Low	6/50
Overall Historical Incidence	
TOTAL	648/2,320 (27.9%)
SD (b)	10.18%
Range (c)	
High	23/50
Low	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F5. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

	Leukemia	Lymphoma	Leukemia or Lymphoma
Historical Incidence at Southern Research Institute			
Reserpine	2/50	6/50	8/50
Cytembena	0/49	1/49	1/49
Mannitol	1/50	7/50	8/50
Ziram	0/49	3/49	3/49
Eugenol	0/50	5/50	5/50
Propyl gallate	0/50	1/50	1/50
Zearalenone	0/50	6/50	6/50
HC Blue No. 1	0/50	4/50	4/50
Stannous chloride	0/50	7/50	7/50
TOTAL	3/448 (0.7%)	40/448 (8.9%)	43/448 (9.6%)
SD (b)	1.41%	4.69%	5.44%
Range (c)			
High	2/50	7/50	8/50
Low	0/50	1/50	1/50
Overall Historical Incidence			
TOTAL	17/2,343 (0.7%)	280/2,343 (12.0%)	297/2,343 (12.7%)
SD (b)	1.76%	6.74%	6.98%
Range (c)			
High	5/48	16/50	16/50
Low	0/50	1/50	1/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation.
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F6. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

	Leukemia	Lymphoma	Leukemia or Lymphoma
Historical Incidence at Southern Research Institute			
Reserpine	1/50	10/50	11/50
Cytembena	0/48	12/48	12/48
Mannitol	0/48	14/48	14/48
Ziram	5/50	6/50	11/50
Eugenol	1/50	12/50	13/50
Propyl gallate	1/50	8/50	9/50
Zearalenone	0/50	15/50	15/50
HC Blue No. 1	1/50	6/50	7/50
Stannous chloride	1/50	5/50	6/50
TOTAL	10/446 (2.2%)	88/446 (19.7%)	98/446 (22.0%)
SD (b)	3.07%	7.65%	6.33%
Range (c)			
High	5/50	15/50	15/50
Low	0/50	5/50	6/50
Overall Historical Incidence			
TOTAL	52/2,486 (2.1%)	625/2,486 (25.1%)	677/2,486 (27.2%)
SD (b)	4.71%	10.14%	9.95%
Range (c)			
High	13/46	31/50	31/50
Low	0/50	4/50	4/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

TABLE F7. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN FEMALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	0/48	0/48	0/48
Cytembena	0/45	1/45	1/45
Mannitol	0/45	0/45	0/45
Ziram	0/44	1/44	1/44
Eugenol	1/41	0/41	1/41
Propyl gallate	5/48	1/48	6/48
Zearalenone	3/46	0/46	3/46
HC Blue No. 1	4/44	0/44	4/44
Stannous chloride	0/43	0/43	0/43
TOTAL	13/404 (3.2%)	3/404 (0.7%)	16/404 (4.0%)
SD (b)	4.32%	1.10%	4.49%
Range (c)			
High	5/48	1/44	6/48
Low	0/48	0/48	0/48
Overall Historical Incidence			
TOTAL	163/2,051 (7.9%)	8/2,051 (0.4%)	171/2,051 (8.3%)
SD (b)	8.71%	0.99%	8.59%
Range (c)			
High	13/41	2/44	13/41
Low	0/48	0/49	0/48

(a) Data as of March 16, 1983, for studies of at least 104 weeks. Includes adenomas and carcinomas designated NOS or chromophobe.
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.

APPENDIX G

CHEMICAL CHARACTERIZATION

OF HC BLUE NO. 2

APPENDIX G. CHEMICAL CHARACTERIZATION

I. Identity and Purity Determinations Performed by the Analytical Chemistry Laboratory

A. Lot No. 5130777

1. Physical Properties

a. Appearance: Dark blue microcrystalline powder

b. Melting Point:

Determined	Literature Values
83.5°-90°C (visual melting point, capillary), small endotherm at 43.0°-51.0° C, larger endotherm at 90.5°-94.0° C (Dupont 900 DTA)	No literature reference found

2. Spectral Data

a. Infrared

	Determined	Literature Values
(1) Instrument:	Beckman IR-12	
(2) Phase:	1% in potassium bromide pellet	
(3) Results:	See Figure 6	Consistent with spectrum obtained from Clairol Research Laboratories and with structure

b. Ultraviolet/Visible

	Determined		Literature Values	
(1) Instrument:	Cary 118			
(2) Solvent:	Methanol		Water	
(3) Results:	λ_{max} (nm)	$\varepsilon \times 10^{-4}$	λ_{max} (nm)	$\varepsilon \times 10^{-4}$
	263	1.67 ± 0.09 (δ)		
	531	0.24 ± 0.01 (δ)	525	0.363 (Clairol)

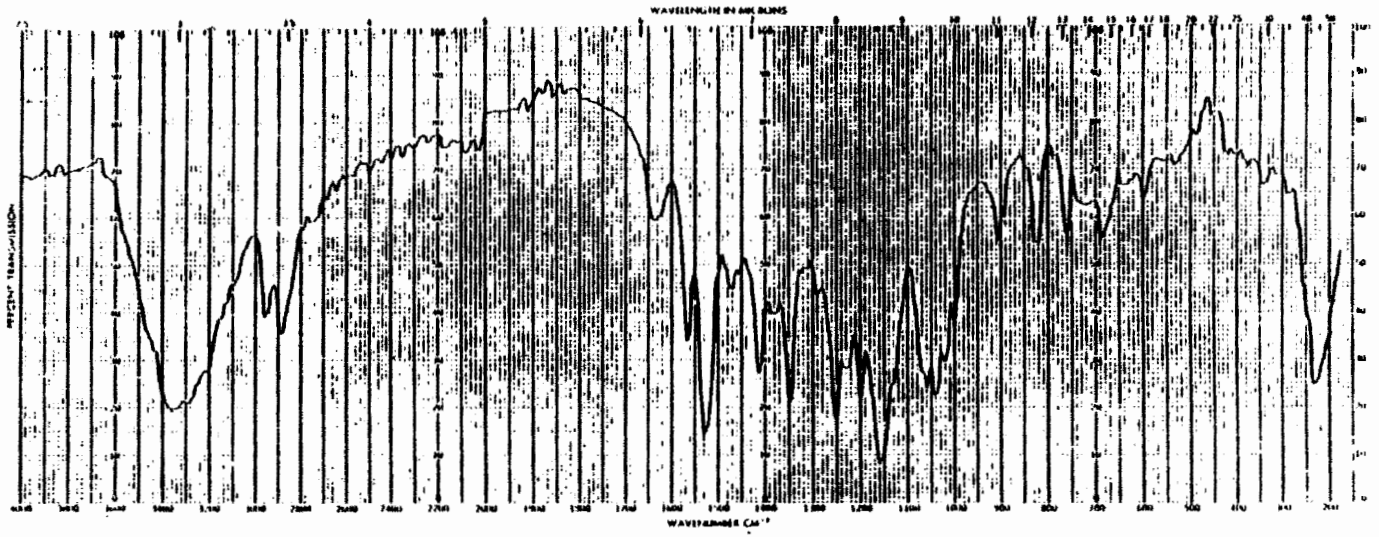

FIGURE 6. INFRARED ABSORPTION SPECTRUM OF HC BLUE NO. 2 (LOT NO. 5130777)

c. Nuclear Magnetic Resonance	Determined	Literature Values
(1) Instrument:	Varian EM-360 A	
(2) Solvent:	Deuterated methanol with internal tetramethylsilane	
(3) Assignments:	See Figure 7	No literature reference found. Spectrum is consistent with that expected for structure.
(4) Chemical Shift (δ):	a 2t, 3.00-3.94 ppm b d, 7.03 ppm c d of d, 7.35 ppm d d, 7.47 ppm e s, 4.82 ppm (H_2O and exchangeable protons) f s, 1.00 ppm (impurity)	
(5) Coupling Constant:	J_{b-c} = 10 Hz J_{c-d} = 4 Hz	
(6) Integration Ratios:	a 14.89 b 1.20 c, d 1.92 e H_2O and exchangeable protons f 0.12 (impurity)	

3. Titration: Titration of one amine function with perchloric acid, 78.5% ± 0.7(δ)%

4. Water Analysis (Karl Fischer): 1.61% ± 0.14(δ)%

5. Elemental Analysis:

Element	C	H	N
Theory (T)	50.52	6.71	14.73
Determined (D)	41.29 41.10	5.70 5.69	11.72 11.89
Percent D/T	81.5	84.9	80.4

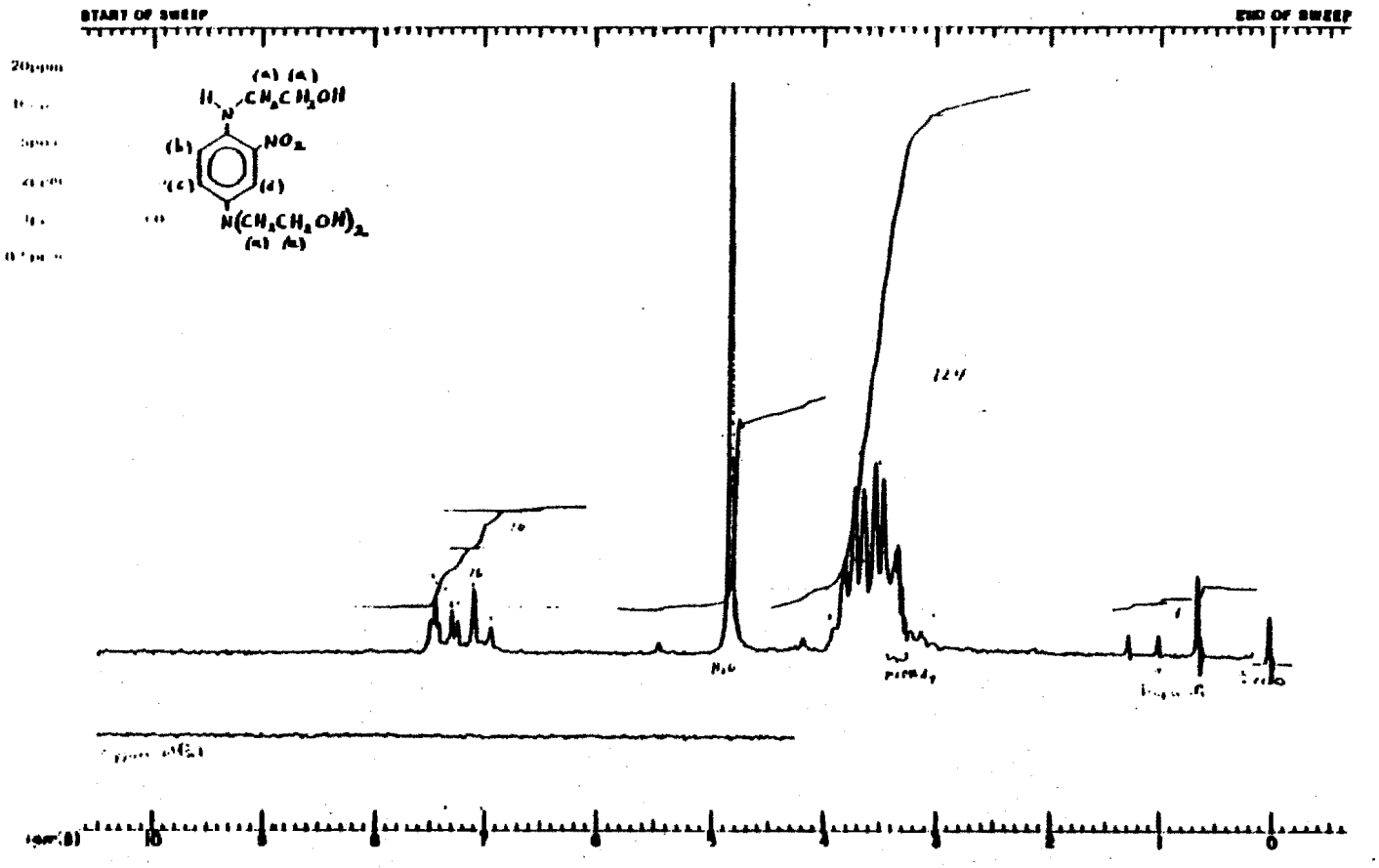

FIGURE 7. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HC BLUE NO. 2 (LOT NO. 5130777)

6. Chromatographic Analyses

a. Thin-Layer Chromatography

(1) Plates: Silica Gel-25; F-254

(2) Reference Standard: 2,6-diaminotoluene

(3) Amount Spotted: 100 and 300 µg, 10 µg/µl in methanol

(4) Visualization: Ultraviolet light (254 and 366 nm); furfural:glacial acetic acid (10 drops:10 ml) (Feigl, 1966)

System 1: Chloroform:methanol (75:25)

(a) R_f: 0.80 (trace), 0.72 (trace), 0.68 (trace), 0.65 (trace), 0.59 (major), 0.53 (minor), 0.42 (trace, 366 nm only), 0.19 (trace, 366 nm only), 0.14 (trace, 366 nm only) 0.06 (trace), 0.03 (trace, 366 nm only), origin (trace)

(b) R_{st}: 1.25, 1.13, 1.06, 1.02, 0.92, 0.83, 0.66, 0.30, 0.22, 0.09, 0.05, origin

System 2: Ethyl acetate:ethanol (88:12)

(a) R_f: 0.46 (trace), 0.41 (slight trace), 0.35 (minor), 0.32 (trace), 0.25 (major), 0.19 (minor), 0.16 (trace, 366 nm only), 0.11 (trace, 366 nm only), 0.03 (trace, 366 nm only), origin (minor)

(b) R_{st}: 1.00, 0.89, 0.76, 0.70, 0.54, 0.41, 0.35, 0.24, 0.07, origin

b. High-Performance Liquid Chromatography:

(1) Instrument: Waters Programmable Component System

(2) Column: µBondapak C_{18}, 300 × 4 mm, ID

(3) Detector: Ultraviolet, 254 nm

(4) Solvent Program: Water:acetonitrile (92:8), isocratic

(5) Solvent Flow: 1.0 ml/min

(6) Sample Injected: 10 µl of 1.0 mg/ml in methanol

(7) **Results:** Major peak and 10 impurities. Three impurities had areas of 1.4%, 17.3%, and 5.6% that of the major peak area. The other seven impurities had areas totaling less than 1.5% that of the major peak area. No other impurities were observed with higher acetonitrile:water ratios.

Peak	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	1.4	0.06	0.25
2	4.5 } unresolved	0.20 }	0.23
3	5.5 } unresolved	0.24 }	
4	9.5	0.42	0.09
5	11.3	0.51	1.4
6	13.2	0.59	0.09
7	16.8	0.75	17.3
8	22.5	1.00	100
9	27.0	1.20	0.41
10	33.2	1.48	0.27
11	41.0	1.82	5.6

7. Conclusions: Results of elemental analyses for carbon, hydrogen, and nitrogen were lower than the theoretical values. Titration of the amine function with perchloric acid indicated a purity of 78.5% ± 0.7(δ)%. Thin-layer chromatography by one system indicated 1 minor impurity and 10 trace impurities. A second thin-layer chromatography system indicated three minor impurities, five trace impurities, and one slight trace impurity. High-performance liquid chromatography indicated 10 impurities. Three impurities had areas of 1.4%, 17.3%, and 5.6% that of the major peak area. The other seven impurities totaled less than 1.5% that of the major peak area. The infrared, ultraviolet/visible, and the nuclear magnetic resonance spectra were consistent with the structure of HC Blue No. 2.

B. Lot No. 9233

1. Physical Properties

a. Appearance: Blackish-blue amorphous powder

b. Melting point: 93°-98°C (visual, capillary)

2. Spectral Data

a. Infrared

	Determined	Literature Values
(1) Instrument:	Beckman IR-12	
(2) Phase:	0.5% in potassium bromide pellet	
(3) Results:	See Figure 8	Consistent with spectrum from Clairol Research Labs and with that for lot no. 5130777

b. Ultraviolet/Visible

	Determined		Literature Values	
(1) Instrument:	Beckman model 25			
(2) Solvent:	Methanol		Water	
(3) Results:	λ_{max} (nm)	$\varepsilon \times 10^{-4}$	λ_{max} (nm)	$\varepsilon \times 10^{-4}$
	534	0.34 ± 0.04 (δ)	525	0.363
	264	2.16 ± 0.06 (δ)	(Clairol Research Labs)	

c. Nuclear Magnetic Resonance

	Determined	Literature Values
(1) Instrument:	Varian EM-360A	
(2) Solvent:	Deuterated methanol with internal tetramethylsilane	
(3) Assignments:	See Figure 9	No literature reference found. Spectrum consistent with structure.

(4) Chemical Shift (δ):

a 2t, 3.37-3.90 ppm
b d, 6.92 ppm
c d of d, 7.28 ppm
d d, 7.42 ppm
e s, 4.83 ppm (HDO and exchangeable protons)

(5) Coupling Constant: $J_{b\text{-}c} = 10$ Hz
$J_{c\text{-}d} = 4$ Hz

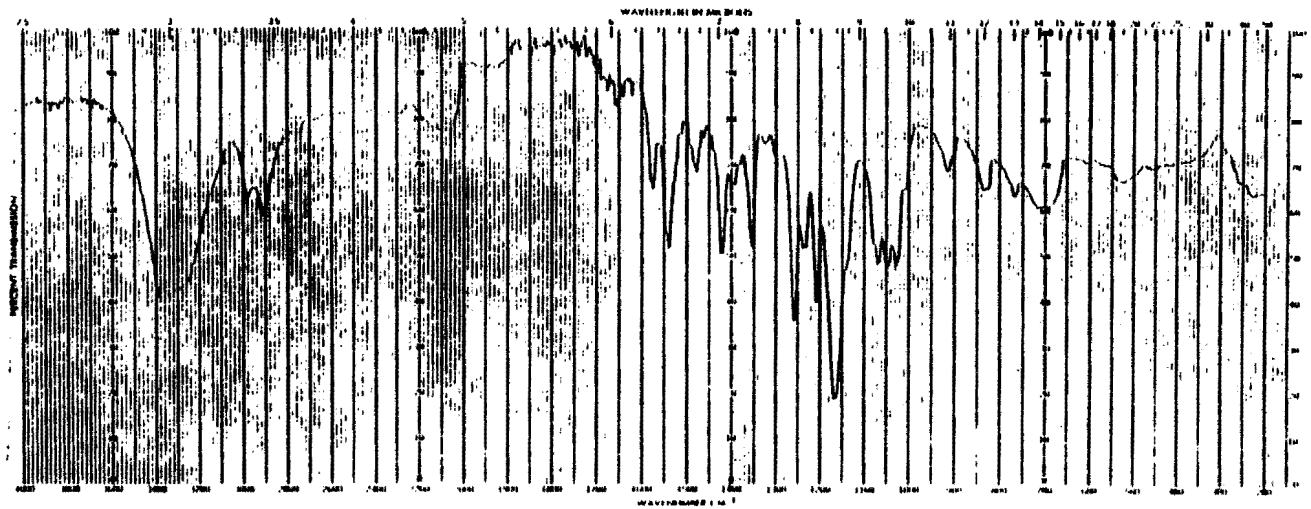

FIGURE 8. INFRARED ABSORPTION SPECTRUM OF HC BLUE NO. 2 (LOT NO. 9233)

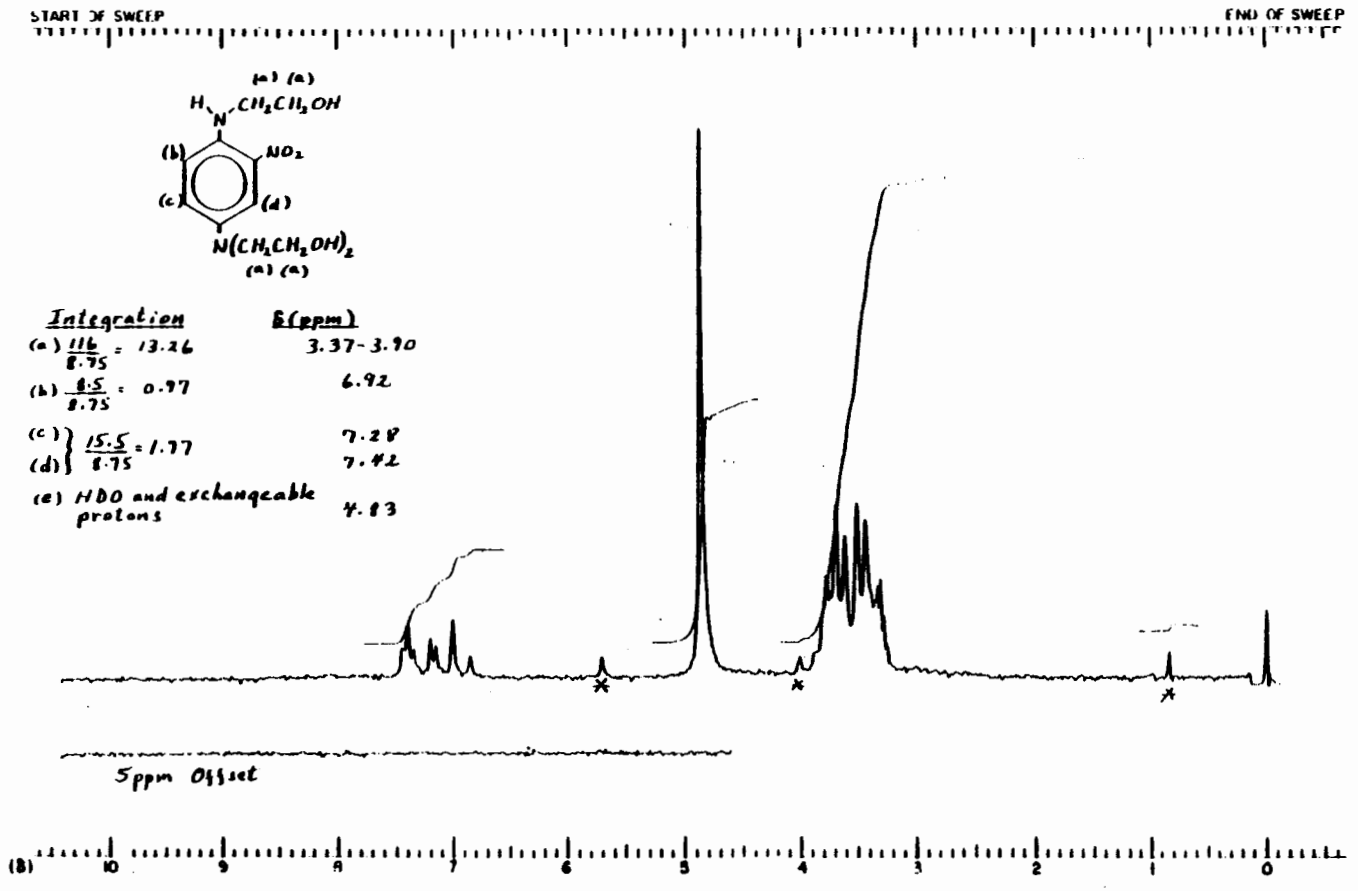

FIGURE 9. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HC BLUE NO. 2 (LOT NO. 9233)

(6) Integration Ratios:

a	13.26 (includes methanol)
b	0.97
c, d	1.77
e	HDO and exchangeable protons

3. Titration: Titration of one amine function with 0.1N perchloric acid, monitored potentiometrically with a combination electrode: 102.9% ± 0.4(δ)%

4. Water Analysis (Karl Fischer): 1.24% ± 0.08(δ)%

5. Elemental Analysis:

Element	C	H	N
Theory (T)	50.52	6.71	14.73
Determined (D)	48.11 48.35	6.90 6.84	14.41 14.37
Percent D/T	95.47	102.38	97.69

6. Chromatographic Analyses

a. Thin-Layer Chromatography

(1) Plates: Silica Gel 60 F-254

(2) Reference Standard: 1 µl of a solution (10 µg/µl) of 2,6-diaminotoluene in methanol

(3) Amount Spotted: 1, 10, and 30 µl of a solution (10 µg/µl) in methanol

(4) Visualization: Visible light, short (254 nm)and long (366 nm) wave ultraviolet; further visualization with furfural in acetic acid (1 drop/ml) (Feigl, 1966). Plates were warmed gently to intensify spots.

			Visualization			
Spot Intensity	R_f	R_{st}	Vis. Light	Spray	254 nm UV	366 nm UV
System 1: Chloroform:methanol (75:25)						
Slight trace	0.585	1.082	--	--	+	--
Trace	0.563	1.041	Brown	Brown	+/–	Gold
Major	0.474	0.877	Purple	Green	+	Purple
Slight trace	0.415	0.767	--	Purple	--	--
Trace	0.378	0.699	Gold	Gold	--	--
Trace	0.326	0.603	--	--	--	Gold
Slight trace	0.207	0.384	--	Purple	--	--
Trace	0.126	0.233	--	--	--	Blue
Trace	0.037	0.068	Brown	Brown	+/–	Gold
Minor	Origin		Brown	Brown	+	Brown
Reference	0.541		+/–	Orange	+	--

System 2: Ethylacetate:ethanol (88:12)

Trace	0.404	0.917	Purple	Purple	+	--
Trace	0.338	0.767	--	--	+	--
Major	0.250	0.567	Purple	Green	+	Purple
Trace	0.169	0.383	Yellow	Yellow	--	Yellow
Minor	0.125	0.283	Yellow	Pink	+	Yellow
Slight Trace	0.066	0.150	--	Blue	--	Blue
Trace	0.029	0.067	--	--	--	Blue
Minor	Origin		Brown	Brown	+	Gold
Reference	0.441		+/-	Orange	+	--

b. High-Peformance Liquid Chromatography:

(1) Instrument System:
Pump(s): Waters 6000A
Programmer: Waters 660
Detector: Waters 440
Injector: Waters U6K

(2) Column: Waters μBondapak C_{18}, 300 × 3.9 mm ID

(3) Detection: Ultraviolet, 254 nm

(4) Guard Column: Whatman CO:Pell ODS, 72 × 2.3 mm ID

(5) Solvent Program: 92% water:8% acetonitrile, isocratic

(6) Flow Rate: 2 ml/min

(7) Samples Injected: 20 μl of a solution (0.98 mg/ml) of HC Blue No. 2 in methanol, filtered

(8) Results: A major peak and one impurity before the major peak with an area of 1.5% that of the major peak area. Four other impurities were observed, but all were less than 0.1% that of the major peak area. Some residue was found on the Millipore filter after filtration of the sample solution. Sample injections at 100%, 80%, 60%, 40%, 20%, 15%, and 10% acetonitrile each indicated no additional impurities.

Peak	Retention Time (min)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	8.4	0.84	1.5
2	10.0	1.00	100

The previous lot (no. 5130777) was chromatographed by this same chromatographic system; more and larger impurities were observed for that lot. The chromatographic profile was very similar to that obtained in the original analysis. The major peak of lot no. 5130777 was 28% smaller than that for lot no. 9233 with the same sample concentration and injection volume.

7. Conclusions: Results of elemental analysis for carbon was low, for nitrogen very slightly low, and for hydrogen in agreement with the theoretical value. Water analysis by Karl Fischer titration indicated 1.24% ± 0.08(δ)%. Titration with perchloric acid of one amine group indicated a purity of 102.9% ± 0.4(δ)%. Thin-layer chromatography by one system indicated a major spot, a minor spot (at the origin), five trace, and three slight trace impurities. A second system indicated a major spot, two minor spots (one at the origin), four trace impurities, and a slight trace impurity. High-performance liquid chromatography indicated one impurity before the major peak with an area of 1.5% that of the major peak. Four other impurities were observed, all of which were less than 0.1% that of the major peak. The infrared, ultraviolet/visible and nuclear magnetic resonance spectra were consistent with the structure of HC Blue No. 2.

II. Test Chemical Stability Study Performed by the Analytical Chemistry Laboratory

A. Sample Preparation and Storage: HC Blue No. 2 samples were stored for 2 weeks at –20°, 5°, 25°, and 60° C.

B. Analytical Method: Titration of the amine function with perchloric acid

C. Results

Storage Temperature	Percent Purity
-20° C	100.5 ± 0.5 (δ)
5° C	100.4 ± 0.6 (δ)
25° C	100.1 ± 0.3 (δ)
60° C	99.6 ± 0.4 (δ)

D. Conclusion: HC Blue No. 2 is stable as the bulk chemical when stored for 2 weeks at temperatures of up to 60° C. The 60° C sample, however, was sticky and had condensed brown moisture.

III. Test Chemical Stability at the Testing Laboratory

A. Storage Conditions: The chemical was stored at 5° C.

B. Analytical Method:

1. Purity Determination: The absorbances of the bulk sample and reference aliquot were determined at 530 nm through the use of a Cary 17 spectrophotometer.

2. Identity Determination: The infrared absorption spectra of the sample was obtained as potassium bromide disks with a Perkin-Elmer 621.

C. Results:

1. Purity:

Date of Analysis	Lot No.	Absorptivity (a) Bulk	Absorptivity (a) Reference	Percent Purity (b)
05/24/78	5130777	0.26	0.27	96.3
08/25/78	5130777	0.26	0.27	96.3
12/19/78	5130777	0.231	0.237	97.5
04/26/79	5130777	0.244	0.229	106.6
08/10/79	5130777	0.276	0.257	107.4
12/09/79	9233	0.351	0.361	97.2
02/20/80	9233	0.333	0.338	98.5
06/10/80	9233	0.321	0.320	100.3
10/13/80	9233	0.339	0.338	100.3
02/11/81	9233	0.334	0.342	97.7
06/10/81	9233	0.349	0.357	97.8
10/21/81	9233	0.349	0.358	97.5
02/08/82	9233	0.351	0.355	98.9
		Mean Percent Purity		
		Lot no. 5130777		100.8
		Lot no. 9233		98.5

(a) (1/g-cm) $\times 10^{-3}$
(b) Purity of material stored at 5° C relative to reference standard stored at −20° C.

2. Identity: All spectra were consistent with the original spectra supplied by the analytical laboratory.

D. Conclusion: No notable degradation occurred during the studies.

APPENDIX H

PREPARATION AND CHARACTERIZATION OF FORMULATED DIETS

I. Studies Conducted at the Analytical Chemistry Laboratory

A. Preparation Procedure

1. Premix: HC Blue No. 2 (9.00 g) and NIH 07 feed (21.0 g) were ground together with a mortar and pestle until a uniform meal, free of lumps and agglomerates, was obtained. This weight of feed was approximately equal in volume to the volume of chemical used and was necessary to adequately disperse the chemical for the premix. The mixture in the mortar was transferred to a 2-liter stainless steel beaker and mixed by spatula with 170.0 g of feed for several minutes. About 40 g of the premix blend was placed in the mortar and stirred briefly to take up residual chemical and then returned to the beaker and mixed to complete preparation of 200-g premix.

2. Bulk Mixing: A 600-g portion of feed was layered evenly in the blender; then the 200 g of premix was added in roughly equal amounts to each blender shell. The fine material adhering to the beaker walls was taken up by stirring 100 g of feed in the beaker and pouring it onto the premix after adding an additional 600 g of feed in roughly equal amounts to both shells, and the blender ports were sealed. The feed and premix were blended with the intensifier bar turned on for the first 5 minutes and turned off for the last 10 minutes. The outside of the blender was periodically given a firm tap to dislodge any feed packed in the corners of the blender. The target concentration of HC Blue No. 2 in the blend was 6,000 ppm.

3. Extraction and Analysis: Each sample was placed in a 200-ml centrifuge bottle (quantitative transfer) and was extracted with 100 ml of methanol by shaking for 30 minutes on a wrist-action shaker set at maximum stroke. The extracts were clarified by centrifugation, and 5-ml aliquots were diluted to 100 ml with methanol. The absorbance of each sample and standard solution was measured versus methanol in 1-cm quartz cells at 538 nm with a Cary 219 spectrophotometer. Sample absorbances were corrected for the mean feed blank absorbance; then the concentration of HC Blue No. 2 in the sample solutions was calculated by the linear regression equation derived from the absorbance readings and concentrations of the standards.

4. Quality Control: Blank (undosed) feed samples and individual spiked mixtures (at the 6,000-ppm concentration) were extracted and prepared for analysis in the manner described above. Standard solutions of HC Blue No. 2 in methanol were used to determine the extinction coefficient for the compound at the analytical wavelength. Blank sample absorbance values were small and were subtracted from the absorbance values of samples containing HC Blue No. 2.

B. Homogeneity

1. Results:

Sample Location	Determined Concentration (ppm)	Determined Concentration / Target Concentration (a)
Right 1	5,990	99.8
Right 2	5,920	98.8
Right 3	5,790	96.5
		Avg = 98.3 ± 1.7(δ)
Left 1	5,960	99.4
Left 2	5,920	98.5
Left 3	5,840	97.3
		Avg = 98.4 ± 1.0(δ)
Bottom 1	5,690	94.8
Bottom 2	5,900	98.3
Bottom 3	5,680	94.7
		Avg = 95.9 ± 2.0(δ)

(a) Target concentration of chemical in feed, 6,000 ppm

2. Conclusion: A feed blend prepared by the recommended protocol at the 6,000 ppm concentration, and sampled at three blender locations, exhibited a maximum variation of 2.5% in dose concentration between any two sampling points.

C. Stability

1. Sample Mixing and Storage: A stock solution of HC Blue No. 2 in methanol (1.03 mg/ml) was prepared, and 5 ml of this solution was added to individual 5-g samples of Wayne Lab Blox® Rodent Feed. The methanol was removed from the samples on a rotary evaporator (20 minutes; water bath temperature, 35° C). The dried samples were thoroughly mixed with a vortex mixer and were stored in duplicate at −20°, 5°, 25°, or 45° C for 2 weeks.

2. Extraction and Analysis: Each stability sample was quantitatively transferred to a 200-ml centrifuge bottle and extracted according to the procedure described in Section I.A.3. A 10-ml aliquot of each extract solution was filtered through a 1.2-micron Millipore filter and then analyzed by high-performance liquid chromatography.

a. Instrument: Waters Programmable Component System
b. Column: μBondapak C_{18}, 300 × 4 mm, ID
c. Detector: Ultraviolet, 254 nm
d. Solvent: Water: acetonitrile (90:10), isocratic
e. Solvent flow rate: 10 ml/min
f. Retention time of compound: 15 min

3. Quality Control: Blank (undosed) feed samples and individual samples spiked at the 0.1% level were extracted and prepared for analysis in the manner described for test samples. The blank showed no feed interference.

4. Results

Storage Temperature	Average Percent Chemical Found in Chemical/Vehicle Mixture (a)
−20° C	0.103 ± 0.003
5° C	0.099 ± 0.003
25° C	0.095 ± 0.003
45° C	0.080 ± 0.003

(a) Mean ± standard deviation corrected for a spiked recovery yield of 94% ± 3%.
Target concentration of chemical in feed, 0.103% ± 0.001%

5. Conclusions: HC Blue No. 2 mixed with stock rodent feed at the 0.1% concentration was found to be stable to within experimental error over a 2-week storage period at 5° C or below. Samples stored at 25° C for 2 weeks showed a slight but significant loss of the major component when analyzed; samples stored at 45° C showed considerable loss of the major component.

II. Studies Conducted at the Testing Laboratory

A. Preparation: Formulated diets were prepared by adding a dry premix (approximately equal amounts of the feed and chemical) to the appropriate amount of feed and blending for 15 minutes. The mixtures were held at 5° C for no more than 2 weeks.

B. Homogeneity

1. Procedure: Five-gram feed samples were weighed, placed in a large test tube, and triturated with 20 ml of methanol for 2 minutes in a Polytron® high-speed blender. The mixture was filtered through a Millipore filtering apparatus. The feed residue was then twice mixed with an additional 20 ml of methanol and filtered through a Millipore filter. The residue was rinsed with additional aliquots of methanol until there was no trace of the dye in the feed residue. The combined filtrates were placed in a 100-ml volumetric flask and brought to volume with additional methanol. The absorbance of these solutions was measured at 532 nm to determine the HC Blue No. 2 content. These absorbances were compared with a standard absorption curve for HC Blue No. 2.

2. Results

Sample Location	Target Concentration (percent wt/wt)	Determined Concentration (percent wt/wt)	Percent of Target
Top left	0.31	0.29	93.5
Top right	0.31	0.31	100
Bottom	0.31	0.25	80.6
Top left	5.0	5.14	102.8
Top right	5.0	5.14	102.8
Bottom	5.0	5.47	109.4

C. Conclusion: The homogeneity of the low dose mixture was poor. The concentration at one sampling position was approximately 20% lower than the target concentration. All sampling locations of the high dose were within specifications.

APPENDIX I

ANALYSIS OF FORMULATED DIETS: METHODS

APPENDIX I. ANALYSIS: METHODS

The analytical procedures used by the testing and referee laboratories were similar. Both used a methanolic extraction procedure and a spectrophotometric quantitation step.

I. Testing Laboratory

A. Procedure

1. Samples of HC Blue No. 2 as a chemical/feed mixture were received for analysis.

2. Duplicate 5-g samples were weighed to the nearest 0.01 g into 50-ml test tubes.

3. Four 5-g samples of plain feed were weighed out, and two of these were spiked with 50 mg of HC Blue No. 2.

4. Twenty-five milliliters of reagent grade methanol was added to each sample, spiked plain feed, and plain feed.

5. The samples were triturated for 2 minutes on a Brinkman Polytron® Homogenizer.

6. Samples were filtered through a Millipore suction filter apparatus with a fiberglass filter.

7. Twenty-five milliliters of methanol was added to rinse the sample tube, and the rinse was added to the filtered feed residue with the suction off.

8. The feed mixture and methanolic rinse were stirred with a glass stirring rod.

9. The mixture was filtered by reattaching the suction.

10. Steps 7-9 were repeated.

11. The sample tube and feed residue were rinsed with 10-ml portions of methanol until no trace of HC Blue No. 2 was left in the feed residue.

12. The combined filtrates were transferred to a 100-ml volumetric flask and brought to volume with methanol.

13. The absorbance from 520 to 540 nm was measured against a methanol reference.

14. The concentration of HC Blue No. 2 in each sample and spike was calculated from the measured absorbance at 532 nm. The absorbance of the plain feed extracts was subtracted from the absorbances of the sample and spiked plain feed extracts.

B. Calculations

1. Concentrations of the sample may be read directly from a verified standard concentration-absorbance curve.

2. A 5-mg sample of HC Blue No. 2 was weighed on the Cahn G-2 electrobalance to the nearest microgram and transferred to a 50-ml volumetric flask.

3. Methanol was added to the 50-ml mark.

4. Five different dilutions were made of the solution.

5. The absorbance was measured from 520 to 540 nm on all of the dilutions and the original stock solution through the use of a Cary 17 Absorption Spectrometer.

6. With the absorbance as the independent variable and the concentration as the dependent variable, the slope and intercept of the calibration line were determined by the method of least squares (the zero-concentration, zero-absorbance point was included as a valid point in this treatment). The correlation coefficient and the standard deviation in the concentration were calculated as a measure of the goodness of fit of the data to a straight line.

II. Analytical Chemistry Laboratory

A. Procedure

1. Preparation of Spiked Feed Standards: Two standard solutions of HC Blue No. 2 were prepared independently in methanol. These solutions were diluted with methanol to span the range of the dosing concentration. Ten-milliliter aliquots of the six standard solutions were pipetted into individual 200-ml centrifuge bottles containing 5 g of undosed feed to make spiked feed samples bracketing the specified dose range. One 200-ml centrifuge bottle containing 5 g of undosed feed was treated with 10 ml of methanol for use as a blank. The spiked feed and the feed blank were sealed and allowed to stand overnight at room temperature before being used in the following analytical procedure.

2. Preparation of Dosed Feed Sample: Triplicate weights of the dosed feed sample (approximately 5 g weighed to the nearest 0.001 g) were transferred to individual 200-ml centrifuge bottles. Ten milliliters of methanol was pipetted into each sample; then the bottles were sealed and allowed to stand overnight at room temperature before being analyzed by the following procedure.

3. Analysis: Ninety milliliters of methanol was pipetted into each blank, standard, and sample bottle, and the bottles were shaken at maximum stroke for 30 minutes on a Burrell Model 75 Wrist Action® shaker. After the bottles were centrifuged for 10 minutes, a 5- to 7-ml aliquot from each extract was appropriately diluted with methanol (25-100 ml) and thoroughly mixed. The absorbance of each solution was measured at 532-538 nm versus methanol in 1-cm quartz cells on a Cary 118 spectrophotometer.

The total amount of HC Blue No. 2 in each feed sample was determined from the linear regression equation obtained from the standard data, relating the absorbance of each spiked feed and blank sample to the amount of chemical in the respective spiked feed.

APPENDIX J

ANALYSES OF FORMULATED DIETS: DATA

I. Thirteen-Week Studies: Formulated diets were analyzed twice during the 13-week studies: before administration and after 1 week of dosing. The results ranged from 90.3% to 112.9% of the target concentrations.

	Determined Concentration for Target Concentration of				
	3,100 ppm	6,200 ppm	12,500 ppm	25,000 ppm	50,000 ppm
Initial	2,800	7,000	13,500	26,000	52,500
Week One	3,000		13,900		52,000

II. Two-Year Studies: Samples of diet formulations were analyzed monthly. The results of the initial mixes ranged from 67.6% to 123.2% of the target concentrations (Table J1). It is assumed that the number of remixes required reflects the number of mixes out of specification (± 10%) of the target concentrations. The mixes were out of specification 14.6% of the time.

Split sample analyses were performed by the testing and analytical (referee) laboratories to verify analytical procedures. The analyses by both laboratories were within 10% of the target concentrations. The interlaboratory values were within 10% of each other (Table J2).

TABLE J1. CONCENTRATIONS OF HC BLUE NO. 2 IN FEED IN THE TWO-YEAR STUDIES (a)

Date Mixed	Determined Concentration for Target Concentration of 5,000 ppm	10,000 ppm	20,000 ppm
2/12/80	(b) 4,200	9,970	18,280
2/15/80	(c) 3,860		
3/11/80	4,790	10,740	
		10,880	
4/4/80	4,620	9,830	18,680
5/6/80	4,500	10,500	
		9,280	
6/3/80	(b) 6,160	9,740	20,860
6/6/80	(c) 4,240		
7/1/80	4,830	10,500	
		9,330	
7/29/80	5,210	9,980	20,000
8/26/80	5,090	9,510	
		10,200	
9/23/80	4,840	9,800	18,500
10/2/80	4,560	9,570	
		9,130	
11/18/80	4,100	9,340	20,200
12/16/80	4,610	9,390	
		9,460	
1/13/81	4,720	9,280	(b) 17,850
1/16/81			(c) 17,600
2/10/81	5,180	9,120	
		10,130	
3/10/81	(b) 4,450	10,140	20,920
3/13/81	(c) 4,730		
4/7/81	(b) 5,700	(b) 11,500	
		9,620	
4/9/81	(c) 4,630	(c) 9,360	
5/5/81	4,580	9,260	18,600
6/2/81	4,650	10,210	
		9,300	
6/30/81	(b) 4,110	9,680	(b) 15,660
7/2/81	(c) 4,850		(c) 17,300
7/21/81	(b) 3,380	9,240	
		9,570	
7/23/81	(c) 4,790		
8/25/81	4,950	9,980	18,400
9/22/81	4,930	(b) 8,380	18,430
		9,300	
9/24/81		(c) 9,260	
10/20/81	4,790	9,640	19,530
11/10/81	(b) 4,270	9,690	(b) 22,900
		9,390	
11/13/81	(c) 4,820		(c) 18,810
12/8/81	4,500	10,420	19,130
1/5/82	5,450	9,620	19,330
	4,660		20,840
Mean (ppm)	4,734	9,753	19,301
Standard deviation	539.0	584.4	1,605.6
Coefficient of variation (percent)	11.4	6.0	8.3
Range (ppm)	3,380-6,160	8,380-11,500	15,660-22,900
Number of samples	27	38	17

(a) The data presented are the results of duplicate analyses.
(b) Out of specifications. Not used in the study.
(c) Remix. Not included in the mean.

TABLE J2. REFEREE SAMPLE DATA IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

Date Mixed	Target Concentration (ppm)	Determined Concentration	
		Testing Laboratory	Analytical Laboratory
7/27/80	5,000	5,210	5,240
11/18/80	5,000	4,100	4,600
6/2/81	10,000	9,300	8,400
10/20/81	20,000	19,530	18,700
1/5/82	5,000	5,450	4,500

APPENDIX K

SENTINEL ANIMAL PROGRAM

APPENDIX K. SENTINEL ANIMAL PROGRAM

I. Methods

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect test results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via viral serology on sera from extra (sentinel) animals in the test rooms. These animals are untreated, and these animals and the test animals are both subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Fifteen $B6C3F_1$ mice and 15 F344/N rats of each sex are selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group are killed at 6, 12, and 18 months on study. Data from animals surviving 24 months are collected from 5/50 randomly selected control animals of each sex and species. The blood from each animal is collected and clotted and the serum is separated. The serum is cooled on ice and shipped to Microbiological Associates' Comprehensive Animal Diagnostic Service for determination of the viral antibody titers. The following tests are performed:

	Hemagglutination Inhibition	Complement Fixation	ELISA
Mice	PVM (pneumonia virus of mice) Reo 3 (reovirus type 3) GDVII (Theiler's encephalomyelitis virus) Poly (polyoma virus) MVM (minute virus of mice) Ectro (infectious ectromelia) Sendai	M.Ad. (mouse adenovirus) LCM (lymphocytic choriomeningitis virus	MHV (mouse hepatitis virus) (24 mo)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	

II. Results

Results are presented in Table K1.

TABLE K1. MURINE VIRUS ANTIBODY DETERMINATIONS FOR RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

	Interval (months)	No. of Animals	Positive Serologic Reaction for
RATS			
	6	1/10	KRV
	12	--	None positive
	18	4/10	KRV
	24	4/10	KRV
MICE			
	6	--	None positive
	12	(a) 1/9	Ectro
	18	(a) 1/8	MHV
	24	--	None positive

(a) Probably false positive

APPENDIX L

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 2

TABLE L1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

Week	Control		Low Dose				High Dose			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
2	15	184	16	182	1.1	440	14	173	0.9	809
6	14	264	15	257	1.1	292	14	239	1.0	586
12	15	337	14	327	0.9	214	15	306	1.0	490
17	15	363	15	355	1.0	211	15	334	1.0	449
22	15	387	15	374	1.0	201	14	355	0.9	394
26	15	407	14	389	0.9	180	14	370	0.9	378
32	16	431	15	407	0.9	184	15	388	0.9	387
35	17	443	15	420	0.9	179	15	396	0.9	379
40	17	452	17	430	1.0	198	16	406	0.9	394
45	16	453	15	430	0.9	174	14	409	0.9	342
50	16	468	15	445	0.9	169	14	424	0.9	330
54	16	473	15	447	0.9	168	14	427	0.9	328
59	15	479	15	451	1.0	166	15	430	1.0	349
63	16	473	15	447	0.9	168	15	525	0.9	286
57	16	479	15	454	0.9	165	15	435	0.9	345
72	16	485	15	454	0.9	165	15	438	0.9	342
76	16	482	15	450	0.9	167	16	440	1.0	364
81	16	486	17	453	1.1	188	16	438	1.0	365
85	16	486	16	450	1.0	178	15	441	0.9	340
90	15	478	15	446	1.0	168	15	434	1.0	346
93	14	477	16	441	1.1	181	12	431	0.9	278
99	14	476	15	439	1.1	171	15	427	1.1	351
102	15	466	14	431	0.9	162	15	425	1.0	353
104	14	473	15	441	1.1	170	16	434	1.1	369
Mean	15.4	433	15.2	409	1.0	194	14.8	397	1.0	390
SD (d)	0.9		0.8		0.1	59	0.9		0.1	110
CV (e)	5.8		5.3		10.0	30.4	6.1		10.0	28.2

(a) Grams of feed consumed per animal per day
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE L2. FEED AND COMPOUND CONSUMPTION BY FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

Week	Control		Low Dose				High Dose			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
2	12	128	11	125	0.9	880	11	124	0.9	1,774
6	11	165	10	156	0.9	641	10	156	0.9	1,282
12	11	193	9	179	0.8	503	9	180	0.8	1,000
17	10	201	10	189	1.0	529	9	190	0.9	947
22	10	208	9	193	0.9	466	9	196	0.9	918
26	10	216	10	200	1.0	500	11	200	1.1	1,100
32	11	230	10	208	0.9	481	11	204	1.0	1,078
35	11	234	10	209	0.9	478	11	203	1.0	1,084
40	11	244	10	218	0.9	459	9	209	0.8	861
45	11	248	9	220	0.8	409	9	209	0.8	861
50	11	262	10	229	0.9	437	10	216	0.9	926
54	12	269	10	232	0.8	431	10	216	0.8	926
59	11	276	10	238	0.9	420	8	223	0.7	717
63	8	282	11	239	1.4	460	11	222	1.4	991
57	12	293	11	253	0.9	435	10	231	0.8	866
72	12	307	11	263	0.9	418	10	238	0.8	840
76	13	315	12	271	0.9	443	11	238	0.8	924
81	14	325	12	275	0.9	436	12	244	0.9	984
85	13	329	11	280	0.8	393	12	248	0.9	968
90	12	333	11	280	0.9	393	11	248	0.9	887
93	12	337	11	277	0.9	397	13	244	1.1	1,066
99	13	342	11	286	0.8	385	11	251	0.8	876
102	13	340	11	287	0.8	383	13	248	1.0	1,048
104	11	344	11	298	1.0	369	14	268	1.3	1,045
Mean	11.5	268	10.5	234	0.9	464	10.6	217	0.9	999
SD (d)	1.3		0.8		0.1	106	1.5		0.1	201
CV (e)	11.3		7.6		11.1	22.8	14.2		11.1	20.1

(a) Grams of feed consumed per animal per day
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE L3. FEED AND COMPOUND CONSUMPTION BY MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

Week	Control		Low Dose				High Dose			
	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b) (grams)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b) (grams)	Dose/ Day (c)
2	6	27.9	7	29.2	1.2	1,199	7	28.1	1.2	2,491
6	8	30.6	8	31.8	1.0	1,258	9	30.7	1.1	2,932
12	9	32.6	10	33.8	1.1	1,479	9	33.0	1.0	2,727
17	8	33.7	8	35.1	1.0	1,140	7	34.6	0.9	2,023
22	8	36.5	9	37.1	1.1	1,213	8	35.7	1.0	2,241
26	9	38.0	10	38.4	1.1	1,302	8	36.2	0.9	2,210
32	8	38.8	8	38.9	1.0	1,028	8	38.0	1.0	2,105
35	9	40.3	10	40.2	1.1	1,244	8	38.7	0.9	2,067
40	9	41.1	9	40.5	1.0	1,111	8	39.0	0.9	2,051
45	9	41.6	11	40.8	1.2	1,348	9	40.1	1.0	2,244
50	10	41.7	9	41.7	0.9	1,079	8	40.4	0.8	1,980
55	9	41.0	9	41.3	1.0	1,090	8	39.7	0.9	2,015
59	10	41.3	10	41.4	1.0	1,208	9	39.7	0.9	2,267
63	9	42.5	10	42.0	1.1	1,190	7	40.2	0.8	1,741
67	11	42.1	11	42.3	1.0	1,300	8	40.0	0.7	2,000
72	10	42.5	11	42.2	1.1	1,303	9	40.5	0.9	2,222
76	11	41.6	9	41.6	0.8	1,082	8	39.5	0.7	2,025
81	13	41.4	11	40.9	0.8		9	39.0	0.7	2,308
85	11	40.9	10	40.2	0.9	1,244	8	38.9	0.7	2,057
90	11	40.0	10	39.4	0.9	1,269	7	37.7	0.6	1,857
93	13	39.4	13	38.4	1.0	1,693	9	37.7	0.7	2,387
97	12	39.9	14	38.9	1.2	1,799	9	37.7	0.8	2,387
101	11	39.4	14	37.9	1.3	1,847	9	37.0	0.8	2,432
105	12	38.9	14	37.1	1.2	1,887	11	37.0	0.9	2,973
Mean	9.8	38.9	10.2	38.8	1.0	1,319	8.3	37.5	0.9	2,239
SD (d)	1.7		1.9		0.1	247	0.9		0.1	308
CV (e)	17.3		18.6		10.0	18.7	10.8		11.1	13.8

(a) Grams of feed consumed per animal per day
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

TABLE L4. FEED AND COMPOUND CONSUMPTION BY FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 2

	Control		Low Dose				High Dose			
Week	Grams Feed/ Day (a)	Body Weight (grams)	Grams Feed/ Day (a)	Body Weight (grams)	Low/ Control (b)	Dose/ Day (c)	Grams Feed/ Day (a)	Body Weight (grams)	High/ Control (b)	Dose/ Day (c)
2	5	21.4	6	21.6	1.2	2,778	6	20.6	1.2	5,825
6	7	23.1	8	23.3	1.1	3,433	8	23.4	1.1	6,838
12	7	23.9	8	25.1	1.1	3,187	9	24.6	1.3	7,317
17	5	26.3	6	25.7	1.2	2,335	7	25.6	1.4	5,469
22	6	29.6	6	28.7	1.0	2,091	7	27.6	1.2	5,072
26	8	31.6	7	29.9	0.9	2,341	8	27.6	1.0	5,797
32	5	35.2	7	31.5	1.4	2,222	8	29.8	1.6	5,369
35	6	35.1	7	31.8	1.2	2,201	7	29.3	1.2	4,778
40	7	37.3	7	33.5	1.0	2,090	7	30.7	1.0	4,560
45	7	40.1	8	34.4	1.1	2,326	7	31.2	1.0	4,487
50	7	42.9	7	35.5	1.0	1,972	7	32.0	1.0	4,375
55	7	44.2	7	36.6	1.0	1,913	7	32.4	1.0	4,321
59	7	44.2	7	36.3	1.0	1,928	8	31.3	1.1	5,112
63	7	44.9	7	37.1	1.0	1,887	7	32.3	1.0	4,334
67	8	44.9	8	36.7	1.0	2,180	8	32.9	1.0	4,863
72	8	45.7	8	37.2	1.0	2,151	9	33.1	1.1	5,438
76	7	45.3	7	36.3	1.0	1,928	7	32.3	1.0	4,334
81	7	46.5	8	38.2	1.1	2,094	8	31.8	1.1	5,031
85	7	45.2	7	37.2	1.0	1,882	8	31.6	1.1	5,063
90	6	44.5	8	35.7	1.3	2,241	8	31.4	1.3	5,096
93	9	42.8	9	35.1	1.0	2,564	11	30.9	1.2	7,120
97	8	42.9	10	35.6	1.3	2,809	12	31.7	1.5	7,571
101	7	41.6	9	35.7	1.3	2,521	12	31.9	1.7	7,524
105	8	40.9	10	34.9	1.3	2,865	14	31.9	1.8	8,777
Mean	6.9	38.3	7.6	33.1	1.1	2,331	8.3	29.9	1.2	5,603
SD (d)	1.0		1.1		0.1	419	2.0		0.2	1,250
CV (e)	14.5		14.5		9.1	18.0	24.1		16.7	22.3

(a) Grams of feed consumed per animal per day
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of compound consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX M

INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS OF THE NIH 07 DIET

Meal Diet: December 1979 to January 1982

(Manufactured by Zeigler Bros., Inc., Gardners, PA)

TABLE M1. INGREDIENTS OF THE NIH 07 RAT AND MOUSE DIET (a)

Ingredients (b)	Percent by Weight
Ground #2 yellow shelled corn	24.50
Ground hard winter wheat	23.00
Soybean meal (49% protein)	12.00
Fish meal (60% protein)	10.00
Wheat middlings	10.00
Dried skim milk	5.00
Alfalfa meal (dehydrated, 17% protein)	4.00
Corn gluten meal (60% protein)	3.00
Soy oil	2.50
Brewer's dried yeast	2.00
Dry molasses	1.50
Dicalcium phosphate	1.25
Ground limestone	0.50
Salt	0.50
Premixes (vitamin and mineral)	0.25

(a) NIH, 1978; NCI, 1976
(b) Ingredients should be ground to pass through a U.S. Standard Screen No. 16 before being mixed.

TABLE M2. VITAMINS AND MINERALS IN THE NIH 07 DIET (a)

	Amount	Source
Vitamins		
A	5,500,000 IU	Stabilized vitamin A palmitate or acetate
D_3	4,600,000 IU	D activated animal sterol
K_3	2.8 g	Menadione activity
d-A-tocopheryl acetate	20,000 IU	
Choline	560.0 g	Choline chloride
Folic acid	2.2 g	
Niacin	30.0 g	
d-Pantothenic acid	18.0 g	d-Calcium pantothenate
Riboflavin	3.4 g	
Thiamine	10.0 g	Thiamine mononitrate
B_{12}	4,000 µg	
Pyridoxine	1.7 g	Pyridoxine hydrochloride
Biotin	140.0 mg	d-biotin
Minerals		
Iron	120.0 g	Iron sulfate
Manganese	60.0 g	Manganous oxide
Zinc	16.0 g	Zinc oxide
Copper	4.0 g	Copper sulfate
Iodine	1.4 g	Calcium iodate
Cobalt	0.4 g	Cobalt carbonate

(a) Per ton (2,000 lb) of finished product

TABLE M3. NUTRIENT COMPOSITION OF THE NIH 07 DIET(a)

	Mean	Range	Number of Samples
Nutrient (percent by weight)			
Crude protein	24.40 ± 0.01	22.6-26.3	24
Crude fat	4.92 ± 0.44	4.4-6.0	24
Crude fiber	3.37 ± 0.58	1.4-4.3	24
Ash	6.78 ± 0.42	5.97-7.42	24
Essential Amino Acids (percent of total diet)			
Arginine	1.260	1.21-1.31	2
Cystine	0.395	0.39-0.40	2
Glycine	1.175	1.15-1.20	2
Histidine	0.553	0.530-0.576	2
Isoleucine	0.908	0.881-0.934	2
Leucine	1.905	1.85-1.96	2
Lysine	1.250	1.20-1.30	2
Methionine	0.310	0.306-0.314	2
Phenylalanine	0.967	0.960-0.974	2
Threonine	0.834	0.840-0.827	2
Tryptophan	0.175	0.171-0.178	2
Tyrosine	0.587	0.566-0.607	2
Valine	1.085	1.05-1.12	2
Essential Fatty Acids (percent of total diet)			
Linoleic	2.37		1
Linolenic	0.308		1
Arachidonic	0.008		1
Vitamins			
Vitamin A (IU/kg)	10,275 ± 2,240	6,700-17,000	24
Vitamin D (IU/kg)	6,300		1
A-tocopherol (ppm)	37.6	31.1-44.0	2
Thiamine (ppm)	16.2 ± 0.428	7.8-23.0	24
Riboflavin (ppm)	6.9	6.1-7.4	2
Niacin (ppm)	75	65-85	2
Pantothenic acid (ppm)	30.2	29.8-30.5	2
Pyridoxine (ppm)	7.2	5.6-8.8	2
Folic acid (ppm)	2.1	1.8-2.4	2
Biotin (ppm)	0.24	0.21-0.27	2
Vitamin B_{12} (ppb)	12.8	10.6-15.0	2
Choline (ppm)	3,315	3,200-3,430	2
Minerals			
Calcium (percent)	1.32 ± 0.19	0.81-1.6	24
Phosphorous (percent)	1.01 ± 0.09	0.82-1.10	24
Potassium (percent)	0.809	0.772-0.846	2
Chloride (percent)	0.557	0.479-0.635	2
Sodium (percent)	0.304	0.258-0.349	2
Magnesium (percent)	0.172	0.166-0.177	2
Sulfur (percent)	0.278	0.270-0.285	2
Iron (ppm)	418	409-426	2
Manganese (ppm)	90.8	86.0-95.5	2
Zinc (ppm)	55.1	54.2-56.0	2
Copper (ppm)	12.68	9.65-15.70	2
Iodine (ppm)	2.58	1.52-3.64	2
Chromium (ppm)	1.86	1.79-1.93	2
Cobalt (ppm)	0.57	0.49-0.65	2

(a) One or two batches of feed analyzed for nutrients reported in this table were manufactured in January and/or April 1983.

TABLE M4. CONTAMINANT LEVELS OF THE NIH 07 DIET

Contaminant	Mean ± Standard Deviation	Range	Number of Samples
Arsenic (ppm)	0.37 ± 0.19	<0.05-0.93	24
Cadmium (ppm) (a)	0.11 ± 0.07	<0.05-0.40	24
Lead (ppm)	1.03 ± 0.61	0.33-2.62	24
Mercury (ppm) (b)	0.05		
Selenium (ppm)	0.27 ± 0.08	0.10-0.48	24
Aflatoxins (ppb) (b,c)	<10		24
Nitrate nitrogen (ppm) (d,e)	7.01 ± 4.08	<0.1-13.0	24
Nitrite nitrogen (ppm) (d,e)	1.46 ± 1.03	<0.1-3.7	24
BHA (ppm) (f,g)	3.08 ± 3.04	<0.2-11.0	24
BHT (ppm) (f)	2.84 ± 1.59	1.06-5.3	24
Aerobic plate count (CFU/g) (h)	61,000 ± 30,510	10,000-110,000	21
Aerobic plate count (CFU/g) (i)	87,312 ± 80,152	10,000-320,000	24
Coliform (MPN/g) (j)	66 ± 73	<3-240	15
Coliform (MPN/g) (k)	616 ± 823	<3-2,400	24
E. Coli (MPN/g) (l)	5.83 ± 6.08	<3-23	24
Total nitrosamines (ppb) (m,n)	7.42 ± 6.39	2.2-24.5	22
Total nitrosamines (ppb) (m,o)	15.22 ± 27.10	2.2-100.3	24
N-Nitrosodimethylamine (ppb) (m,n)	5.56 ± 5.87	0.7-20.0	22
N-Nitrosodimethylamine (ppb) (m,o)	13.30 ± 26.82	0.7-99.0	24
N-Nitrosopyrrolidine (ppb)	1.34 ± 0.82	0.5-3.5	24
Pesticides (ppm)			
Alpha BHC (b,p)	<0.01		24
Beta BHC (b)	<0.02		24
Gamma BHC-Lindane (b)	<0.01		24
Delta BHC (b)	<0.01		24
Heptachlor (b)	<0.01		24
Aldrin (b)	<0.01		24
Heptachlor epoxide (b)	<0.01		24
DDE (b,q)	<0.01	0.05 (7/14/81)	24
DDD (b)	<0.01		24
DDT (b)	<0.01		24
HCB (b)	<0.01		24
Mirex (b)	<0.01		24
Methoxychlor (b)	<0.05		24
Dieldrin (b)	<0.01		24
Endrin (b)	<0.01		24
Telodrin (b)	<0.01		24
Chlordane (b)	<0.05		24
Toxaphene (b)	<0.1		24
Estimated PCB's (b)	<0.2		24
Ronnel (b)	<0.01		24
Ethion (b)	<0.02		24
Trithion (b)	<0.05		24
Diazinon (b)	<0.1		24
Methyl parathion (b)	<0.02		24
Ethyl paration (b)	<0.02		24
Malathion (r)	0.10 ± 0.07	<0.05-0.25	24
Endosulfan I (b)	<0.01		24
Endosulfan II (b)	<0.01		24
Endosulfan sulfate (b)	<0.03		24

TABLE M4. CONTAMINANT LEVELS OF THE NIH 07 DIET (Continued)

(a) Three batches contained more than 0.1 ppm.
(b) All values were less than the detection limit, given in the table as the mean.
(c) Detection limit reduced from 10 ppb to 5 ppb after 7/81
(d) Source of contamination: Alfalfa, grains, and fish meal
(e) Two batches contained less than 0.2 ppm.
(f) Source of contamination: Soy oil and fish meal
(g) Six batches contained less than 0.5 ppm.
(h) Mean, standard deviation, and range exclude three extreme values (310,000, 310,000, and 320,000) obtained in batches produced on 12/21/79, 2/26/80, and 11/27/81. CFU = colony-forming units.
(i) Mean, standard deviation, and range include the two extreme values given in footnote h.
(j) Excludes nine very high values in the range 1,100-2,400 obtained in batches produced on 2/4/80, 2/26/80, 11/25/80, 12/16/80, 5/26/81, 7/14,81, 9/25/81, 10/23/81, and 11/27/81
(k) Includes the high values listed in footnote j
(l) Includes three values of 23. The remaining 21 values were less than 3 MPN/g. MPN = most probable number.
(m) All values were corrected for percent recovery.
(n) Mean, standard deviation, and range exclude two very high values of 101.6 and 100.3 ppb in batches produced on 1/26/81 and 4/27/81.
(o) Mean, standard deviation, and range includes the very high values given in footnote n.
(p) BHC = hexachlorocyclohexane or benzene hexachloride
(q) One observation was above the detection limit. The value and the date it was obtained is listed under the range.
(r) Eleven batches contained more than 0.05 ppm.

APPENDIX N

GENETIC TOXICOLOGY OF HC BLUE NO. 2

TABLE N1. MUTAGENICITY OF HC BLUE NO. 2 IN SALMONELLA

Strain	Dose (µg/plate)	Revertants/plate (a) −S9	+S9 (rat)	+S9 (hamster)
TA100	0	138 ± 13.2	152 ± 7.3	153 ± 6.1
	333	104 ± 7.8	151 ± 8.6	154 ± 6.7
	1,000	128 ± 8.2	152 ± 6.7	155 ± 3.5
	3,333	133 ± 6.5	149 ± 7.9	159 ± 9.6
	6,666	137 ± 6.8	165 ± 3.2	160 ± 17.6
	10,000	123 ± 12.2	158 ± 4.4	149 ± 17.0
TA1535	0	21 ± 3.8	10 ± 2.2	13 ± 1.8
	100	28 ± 5.4	--	--
	333	24 ± 4.3	7 ± 1.2	12 ± 0.3
	1,000	28 ± 1.5	7 ± 1.2	11 ± 2.5
	3,333	16 ± 5.2	9 ± 2.6	12 ± 2.2
	6,666	--	10 ± 1.2	12 ± 2.3
	10,000	25 ± 2.0	8 ± 3.2	10 ± 2.0
TA97	0	151 ± 4.6	165 ± 6.5	176 ± 16.8
	333	137 ± 5.5	167 ± 0.9	187 ± 6.4
	1,000	149 ± 4.4	160 ± 10.9	200 ± 7.4
	3,333	194 ± 4.9	156 ± 3.2	215 ± 4.8
	6,666	218 ± 8.5	179 ± 8.0	205 ± 9.3
	10,000	242 ± 15.8	194 ± 12.3	258 ± 6.9
TA98	0	15 ± 0.3	31 ± 4.6	35 ± 4.7
	333	14 ± 1.0	51 ± 2.2	38 ± 4.6
	1,000	25 ± 3.2	41 ± 3.2	52 ± 4.2
	3,333	43 ± 3.6	44 ± 3.8	61 ± 2.3
	6,666	62 ± 2.6	63 ± 3.4	82 ± 4.4
	10,000	66 ± 4.0	157 ± 11.0	93 ± 4.9

(a) The S9 fractions were prepared from the livers of Aroclor 1254-induced animals (male Sprague-Dawley rats and male Syrian hamsters). Cells and test compound or solvent (DMSO) were incubated for 20 minutes at 37° C in the presence of either S9 or buffer. After the addition of soft agar, the contents of each tube were poured onto minimal medium, and the plates were incubated at 37° C for 48 hours (Haworth et al., 1983). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

TABLE N2. MUTAGENICITY OF HC BLUE NO. 2 IN IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE PRESENCE OF S9 (a)

Compound	Dose (μg/ml)	Total Mutant Clones	Cloning Efficiency (percent)	Relative Total Growth (percent)	Mutation Frequency (mutants/10^6 clonable cells)
DMSO	(1%)	54	97	100	19
		63	99	100	21
		51	98	100	17
		56	107	100	17
Methyl methane-sulfonate	5	377	72	59	175
		383	80	70	160
		392	83	57	158
HC Blue No. 2	75	104	91	40	38
		89	81	33	36
		70	69	37	34
	150	75	66	32	38
		183	82	21	74
		145	63	16	77
	200	130	94	28	46
		187	88	30	71
		101	67	30	50
	300	180	83	15	72
		163	64	5	85
		155	48	4	107
	400	170	73	9	77
		232	59	5	132
		276	60	4	152
	600	145	27	2	177
		281	57	4	165
		230	37	3	204
	800	Toxic			

(a) Experiment was performed once, and all doses were tested in duplicate. The protocol was basically that of Clive et al. (1979): Cells (6×10^5/ml) were treated for 4 h at 37° C in medium, washed, resuspended in medium, and incubated for 48 h at 37° C. After expression, 3×10^6 cells were plated in medium supplemented with trifluorothymidine for selection of cells that were mutant at the thymidine kinase (TK) locus, and 600 cells were plated in nonselective medium to determine the percentage of viable cells. S9 was prepared from the livers of Aroclor 1254-induced male F344 rats.

APPENDIX O

DATA AUDIT SUMMARY

APPENDIX O. DATA AUDIT SUMMARY

The experimental data and tables of the draft NTP Technical Report on the Toxicology and Carcinogenesis Studies of HC Blue No. 2 in F344/N rats and B6C3F$_1$ mice were examined for completeness, consistency, and accuracy and for procedures consistent with Good Laboratory Practice requirements. The audit was conducted by Argus Research Laboratories (Contract No. 1-ES-38049) and NTP personnel. The following persons were involved in the audit: Jane E. Goeke, Ph.D., James H. Hills, Peter D. Ference, Richard E. Long, D.V.M., Ph.D., and Carol L. Veigle, H.T. The 2-year studies in rats and mice were conducted between February 1980 and February 1982 at Southern Research Institute, Birmingham, Alabama, under a subcontract with Tracor Jitco, Inc.

The full report of the audit is on file at the National Toxicology Program, NIEHS, and is available upon request. The audit included but was not limited to a review of the records of the in-life portion of the studies for 10% of the animals, 100% of the available chemistry data, and a random 10% sample of the chemical mix calculations. All Individual Animal Data Records were examined for correspondence between necropsy observations and histopathologic findings. All wet tissue bags were counted, and 10% were reviewed for animal identification and the presence of untrimmed lesions. A complete slide-block match for both sexes of both species in the high dose and control groups was performed.

The audit revealed no major problems with the conduct of the studies or with collection and documentation of the experimental data. The analytical chemistry data were adequate and support the stated conclusions of the Technical Report. On four occasions, chemical/diet mixtures 11%-16% below the target concentrations were administered to animals.

The wet tissue bag count revealed one missing bag in the high dose male rat group. Two bags were missing in the low dose male mouse group, and one was missing in the high dose male mouse group. Slide-block matchup of a total of 4,983 blocks and 4,986 slides revealed 2 questionable matches, 16 missing slides, and 1 missing block. Of the 81 bags opened for verification of animal identity, a total of 18 animals (2 rats and 16 mice) were not readily identified. For 14 of the 18 animals, the ear punch was not clearly interpretable but could be read as the correct animal number or an alternate number. A portion of the ear was missing from one mouse, and the ear punch was not evident. For the remaining two rats and one mouse, the ear punch did not appear to correspond to the bag number. In all three cases, however, the alternate wet tissue bag was opened and the animal identification was verified, indicating that the three animals in question were appropriately identified on the wet tissue bag and IADR. Wet tissue examination revealed one control male rat with an untrimmed lesion (raised focus on the liver) and and six high dose female rats with untrimmed white areas on the dorsal calvaria.

No discrepancies that significantly influenced the final interpretation of these studies in rats and mice were found. Additional minor problems, not necessarily pursued to final resolution but identified in the NTP audit report, were considered to be inconsequential. The data examined in this audit are considered adequate to support the conclusions of the Technical Report.

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