NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 271

TOXICOLOGY AND CARCINOGENESIS STUDIES OF HC BLUE NO. 1

(CAS NO. 2784-94-3)

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.

HC Blue No. 1, NTP TR 271

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 271

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U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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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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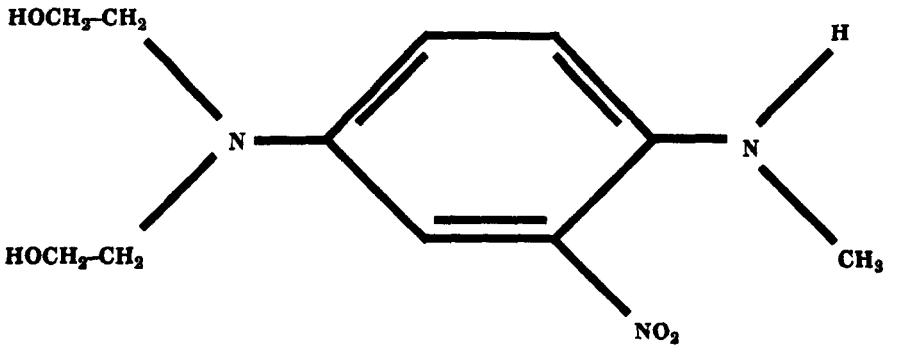

HC BLUE NO. 1

CAS No. 2784-94-3

2,2'((4-(METHYLAMINO)-3-NITROPHENYL)IMINO)BIS(ETHANOL)
$C_{11}H_{17}N_3O_4$ Molecular Weight: 255.3

ABSTRACT

Toxicology and carcinogenesis studies of HC Blue No. 1 (97% pure), a semipermanent hair dye, were conducted by administering the test chemical in feed for 103 weeks to groups of 50 F344/N rats and 50 B6C3F$_1$ mice of each sex. The dietary concentrations used were 0, 1,500, or 3,000 ppm for rats and male mice and 0, 3,000, or 6,000 ppm for female mice. These concentrations were selected on the basis of results from single-administration gavage studies and 14-day and 13-week feed studies.

The survival of male and female rats and male mice was not affected by administration of HC Blue No. 1. Survival of high dose female mice was reduced ($P<0.05$); the early deaths in this group are believed to have been caused by hepatocellular carcinomas. Body weights of high dose rats and dosed mice were lower than those of the respective control groups; female rats and mice were more affected than were males.

Administration of HC Blue No. 1 produced significant positive trends in the incidences of male rats with hepatocellular neoplastic nodules/carcinomas (neoplastic nodules: control, 0/49; low dose, 0/50; high dose, 3/50; neoplastic nodules/carcinomas: 1/49; 0/50; 6/50). In male and female mice, both doses of HC Blue No. 1 increased the incidences of hepatocellular carcinoma (male: 11/50; 20/50; 30/50; female: 1/50; 24/48; 47/49) and the low doses increased the incidences of hepatocellular adenomas (male: 4/50; 17/50; 10/50; female: 2/50; 11/48; 4/49).

HC Blue No. 1 produced dose-related increases in the incidences of proliferative lesions of the lungs (adenomatous hyperplasia and alveolar/bronchiolar adenomas or carcinomas) in female rats (hyperplasia: 2/50; 5/49; 8/50; adenoma/carcinoma: 1/50; 3/49; 7/50).

In male mice, HC Blue No. 1 at the 6,000-ppm dose increased the incidences of thyroid gland follicular cell hyperplasia and adenomas (hyperplasia: 3/47; 7/49; 14/50; adenoma: 0/47; 0/49; 5/50).

HC Blue No. 1 was mutagenic in strains TA97, TA98, and TA100 of *Salmonella typhimurium* in the presence or absence of Aroclor-induced male Sprague-Dawley rat or Syrian hamster liver S9; HC Blue No. 1 was negative in strain TA1535. HC Blue No. 1 was mutagenic in the absence of activation in the L5178Y/TK$^{+/-}$ mouse lymphoma assay and induced unscheduled DNA synthesis in rat hepatocytes in vitro.

An audit of the experimental data was conducted for these carcinogenesis studies on HC Blue No. 1. No data discrepancies were found that influenced the final interpretations.

Under the conditions of these feed studies, there was *equivocal evidence of carcinogenicity** in male F344/N rats, since HC Blue No. 1 caused a marginal increase in the incidence of hepatocellular neoplastic nodules/carcinomas. For female F344/N rats, there was *some evidence of carcinogenicity* in that HC Blue No. 1 induced increased incidences of alveolar/bronchiolar neoplasms. There was *clear evidence of carcinogenicity* of HC Blue No. 1 for male and female B6C3F$_1$ mice as shown by increased incidences of hepatocellular carcinomas. The incidences of follicular cell adenomas of the thyroid gland were also increased in male mice receiving HC Blue No. 1.

*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. 1 is based on the 13-week studies that began in May 1978 and ended in August 1978 and on the 2-year studies that began in May 1979 and ended in April 1981 at Southern Research Institute.

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The members of the Peer Review Panel who evaluated the draft Technical Report on March 23, 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. 1

On March 23, 1984, the draft Technical Report on HC Blue No. 1 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 Hubert Humphrey Building in Washington, D.C.

Dr. S. Friess, a principal reviewer, was unable to attend the meeting; his comments were read by Dr. J. Hook, Panel Chair. Dr. Freiss agreed with the conclusions as given. He noted that cytoplasmic pigmentation of the follicular epithelial cells of the thyroid gland occurred at increased incidences in dosed animals of both species and sexes and asked if the unidentified pigment may have had some causative role in enhanced incidences of follicular cell adenomas. Further, he inquired whether the positive Sendai virus titers in rats and mice could have had any impact on development of proliferative lesions in the lungs or in other target tissues. Dr. J. Mennear, NTP, responded that although a relationship between pigment deposition and proliferative changes in follicular cells could not be ruled out, no consistent relationship was observed in other NTP dye studies or in studies by other laboratories. With regard to the possible role of Sendai, the available data do not allow a definitive conclusion about any interaction between virus and chemical in tumor causation. NTP unpublished data show no difference in incidence of pulmonary tumors in controls that have had positive Sendai titers and those that did not.

As a second principal reviewer, Dr. C. Harper agreed with the conclusions. He thought the doses selected for the 2-year study in female mice might have been rather high, perhaps contributing to low survival, but he did not think the low survival invalidated the interpretation of the findings in female mice. Dr. Mennear observed that the early deaths were likely due to hepatocellular carcinomas. Dr. Harper asked for a statement in the Technical Report explaining why the feed route was used rather than dermal application. [See page 19.]

As a third principal reviewer, Dr. B. Turnbull said he agreed with the conclusions in mice but was not totally convinced about those for rats. He questioned the use of a one-sided P value of 0.05 (5%) as the nominal limit value for significance, and he expressed concern about a high probability of false positives. He proposed that a 1% criterion ($P<0.01$) would be preferable. As further reasons for a lower level of evidence for the rat studies, he mentioned the different primary tumors in male versus female rats and the marginally significant increase of lung tumors in female rats. Dr. Turnbull supported a designation of equivocal evidence of carcinogenicity for both sexes of rats. Dr. J. Haseman, NIEHS, said that the NTP does not employ a rigid decision rule (5% or 1%) in the final interpretation of carcinogenicity data. He stated that the designation of some evidence of carcinogenicity in female rats was supported by the seven lung tumors (14%) in the high dose group, which exceeded the highest control incidence seen in the Program, and the increase in neoplasms being paralleled by an increase in adenomatous hyperplasias.

Dr. S. Tannenbaum (and two of the principal reviewers) expressed concern about the incomplete chemical identification of the 3% impurities. He said that during the synthesis of nitroaromatic chemicals, nitrosamines are often formed as contaminants, in this case, probably derivatives of nitrosomethylaniline. Dr. C. Jameson, NTP, said a more definitive characterization would be made of the impurities. [See page 55.] Mr. L. Beliczky asked whether there were uses for HC Blue No. 1 other than as a hair dye and whether there was more than one manufacturer. [Dr. Mennear subsequently determined that there were no other uses or other manufacturers in the United States.]

In other discussion, Dr. Turnbull commented on how animal cage assignment randomly by rows rather than by individual cages might affect the power of statistical inference. Dr. R. Kociba asked that the doses that were expressed as parts per million also be given as milligrams per kilogram per day in the report and that the sections on clinical observations and nonneoplastic toxicology be expanded. Dr. Swenberg stated that the designation of clear evidence based on liver tumors in mice was strengthened by the lack of hepatotoxicity even in females, where the maximal tolerated dose likely was exceeded, and because the mouse liver is a target organ for aromatic amines. The interpretation of lung tumors in female rats as associated with the chemical was more difficult. Dr. G. Boorman, NTP, said that the NTP Pathology Working Group [see page 12] was in unanimous agreement that the lung diagnoses supported a designation of a carcinogenic response.

Dr. Harper moved that the Technical Report on the toxicology and carcinogenesis studies of HC Blue No. 1 be accepted with the conclusions as stated and with the modifications as discussed. Dr. D. Davis seconded the motion, and the Technical Report was approved by 10 affirmative votes with 1 abstention (Dr. Turnbull).

I. INTRODUCTION

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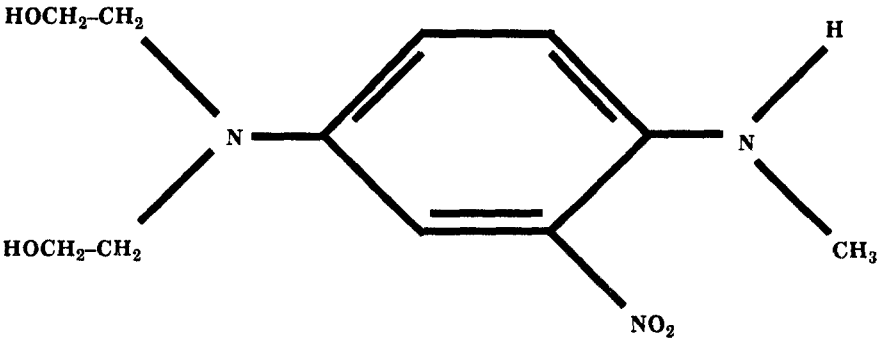

HC BLUE NO. 1

CAS No. 2784-94-3

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HC Blue No. 1 is a nitrophenylenediamine derivative used exclusively as a semipermanent hair dye. Semipermanent hair color products are generally "shampoo-in" preparations that are applied to the hair, lathered, and allowed to remain in contact with the hair and scalp for 30-45 minutes (Frenkel and Brody, 1973). The concentration of HC Blue No. 1 used in these preparations ranges from 1% to 2%. Approximately 20,000-30,000 pounds of HC Blue No. 1 are used annually (C. Burnett, personal communication to NTP, 1983).

Frenkel and Brody (1973) reported that dermally applied HC Blue No. 1, dissolved in saline, is absorbed through the skin of rabbits and rats and that the dye is excreted unchanged in the urine and bile. Intraperitoneally and subcutaneously administered HC Blue No. 1 is also excreted unchanged in rabbit and rat urine and bile.

Under conditions of use, HC Blue No. 1 is absorbed through the human scalp (Maibach and Wolfram, 1981). A commercial hair dye formulation, which had been enriched with ^{14}C-HC Blue No. 1, was applied to the hair of human volunteers and allowed to remain in contact with the hair and scalp for 30 minutes. Radioactivity, accounting for 0.09% of that in the dye applied to the hair, was detected in the urine over the 7-day period after the dye application. Since the urinary excretion products were not identified, the metabolic fate of HC Blue No. 1 in humans remains unknown.

HC Blue No. 1 has been administered to laboratory animals in studies of 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 1.54% HC Blue No. 1. 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. 1 delivered in the mixture were 1.5 mg/kg per day to dogs and rabbits and 12 mg/kg per day (estimated) 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 1.6% HC Blue No. 1, was applied to the skin of New Zealand white rabbits (1.0 ml/kg twice weekly for 13 weeks) and pregnant Charles

River rats (2.0 ml/kg on days 1, 4, 7, 13, 16, and 19 of gestation). Neither systemic nor teratologic effects were observed.

The National Toxicology Program found that HC Blue No. 1 was mutagenic in *Salmonella typhimurium* strains TA97, TA98, and TA100 in the presence or absence of Aroclor-induced Sprague-Dawley rat or Syrian hamster liver S9 (Appendix M). The compound was not mutagenic in strain TA1535. HC Blue No. 1 was mutagenic in the L5178Y/TK$^{+/-}$ mouse lymphoma forward mutation assay without activation. These combined results suggest that HC Blue No. 1 is a direct-acting frameshift mutagen whose mutagenic activity is either not changed or is slightly enhanced by the addition of S9. The sample used for the Salmonella study was taken from the same lot (no. 3670379) that was used for the last 13 months of the 2-year studies described in this report. In addition, the NTP found that this lot of HC Blue No. 1 induced unscheduled DNA synthesis in rat hepatocytes (Appendix M).

Apparently, a different sample of HC Blue No. 1 (not used in the NTP 2-year studies) was tested for genetic toxicity in a variety of short-term tests by Darroudi et al. (1983), Loprieno et al. (1983), and Shahin and Bugaut (1983). This sample was not mutagenic in strains TA98, TA100, TA1535, TA1537, or TA1538 of Salmonella in the presence or absence of Aroclor-induced rat liver S9 (Shahin and Bugaut, 1983); purity was confirmed by high-performance liquid chromatography and thin-layer chromatography.

Loprieno et al. (1983) studied the genetic toxicity of HC Blue No. 1 in four test systems using yeast and mammalian cells. Although HC Blue No. 1 was negative for forward mutation and mitotic gene conversion in yeast, the doses used never decreased cell survival below 66% or 80%, respectively. Thus, the negative results in yeast should be considered preliminary until higher doses are used. HC Blue No. 1 did not induce either unscheduled DNA synthesis in HeLa cells or 6-thioguanine-resistant mutants in Chinese hamster V79 cells. Survival data were not reported for either mammalian cell test, so it is unclear whether the doses tested (30 mM and 100 mM, respectively) were adequate. HC Blue No. 1 reportedly did not induce either sex-linked recessive lethal mutations in Drosophila or micronuclei in mouse bone marrow (Darroudi et al., 1983). Once again, LD_{50} values were not reported, so it is uncertain whether adequate doses were tested. HC Blue No. 1 also was tested for cytogenetic effects in vitro in Chinese hamster ovary cells. Although HC Blue No. 1 did not induce chromosomal aberrations, exposure of the cells to 200 µg/ml for 24 hours yielded a frequency of sister-chromatid exchanges twice that of the solvent control.

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. 1 is one of five semipermanent hair dyes selected for toxicology and carcinogenicity assessment in a class study of hair color materials. The other dye studies that have been peer reviewed and will be printed in 1985 are HC Blue No. 2 (NTP, 1985a), C.I. Disperse Blue 1 (NTP, 1985b), and HC Red No. 3 (NTP, 1985c). A fifth study, C.I. Acid Orange 3, is currently in histopathology analysis. HC Blue No. 1 is absorbed through the skin of rats (strain unspecified) and rabbits in small but detectable amounts (Frenkel and Brody, 1973). Because a larger proportion of a dose would be absorbed through the gastrointestinal tract than through the skin, the dosed feed route of administration was selected for these studies to provide more systemic exposure than would have been possible by dermal application.

II. MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION OF HC BLUE NO. 1
PREPARATION AND 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. 1

HC Blue No. 1 was obtained in two lots (lot no. 3270877 and lot no. 3670379) from Clairol Research Laboratories (Stamford, CT). Purity and identity determinations were conducted on both lots (Appendix G). Lot no. 3270877 was used for the single-administration, 14-day repeated-exposure, and 13-week studies and for the first 11 months of the 2-year studies. Lot no. 3670379 was used for the last 13 months of the 2-year studies.

Chemical identity was confirmed by infrared, ultraviolet, and nuclear magnetic resonance spectroscopy. All spectroscopic data were consistent with the structure of HC Blue No. 1 and with the spectra of the purified dye provided by Clairol.

Purity of the two lots was determined 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 quite low, the maximum concentration being 0.35%. Titration of the amine function indicated that the material was 99% pure. Chromatographic data indicated six impurities greater than or equal to 0.1% (lot no. 3270877) and three impurities greater than or equal to 0.1% (lot no. 3670379); these impurities were not identified. For both lots, the combined area of the impurities was less than 3% that of the major component. The overall data obtained in these studies indicate that both lots were approximately 97% pure.

A heat stability test performed by the analytical chemistry laboratory indicated that the compound should be stable in storage for at least 2 weeks at 60° C (Appendix G). After receipt of HC Blue No. 1 from the analytical chemistry laboratory, the testing laboratory stored the material at 5° C. Periodic characterization of HC Blue No. 1 by infrared and ultraviolet/visible spectroscopy detected no deterioration over the course of the studies (Appendix G).

PREPARATION AND ANALYSIS OF FORMULATED DIETS

Formulated diet mixtures were shown to be homogeneous (Appendix H). Further studies demonstrated that HC Blue No. 1 was stable in feed when stored for 2 weeks at temperatures equal to or less than 25° C. The testing laboratory prepared the formulated diets by adding a dry premix (approximately equal amounts of feed and HC Blue No. 1) to the appropriate amount of feed. The mixture then was blended for 15 minutes. Formulated diets were stored at 5° C for no longer than 14 days.

Analyses for HC Blue No. 1 in feed mixtures were performed to confirm that the animals were administered the dye in feed at the correct concentrations. The method of analysis involved methanolic extraction as a purification step and a spectrophotometric reading as a quantitation step (Appendix I). Occasionally, the testing laboratory's periodic analysis indicated that a sample was not within 10% of the target concentration (Table 1). Because 6 of 80 samples examined were not within 10% of the target concentrations, the feed mixtures were estimated to have been within specifications 92% of the time.

TABLE 1. ANALYSES OF FORMULATED DIETS IN THE THIRTEEN-WEEK AND TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

Target Concentration (ppm)	Measured Concentration (ppm)	Number of Samples	Coefficient of Variation (percent)	Range (ppm)
Thirteen-Week				
750	780	1	--	--
1,500	1,700	1	--	--
3,000	2,920	1	--	--
6,250	6,560	1	--	--
12,500	12,300	1	--	--
Two-Year				
1,500	1,465	26	6.7	1,250-1,660
3,000	2,926	26	6.9	2,470-3,300
6,000	5,865	13	3.4	5,600-6,200

SINGLE-ADMINISTRATION STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries (Indianapolis, IN) and held for approximately 13 days before the test began. The animals were 8-9 weeks old when placed on study.

Groups of five F344/N rats of each sex were administered a single dose of 31, 62, 125, 250, or 500 mg/kg HC Blue No. 1 in 1% carboxymethylcellulose 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.

Animals were observed for mortality twice daily for 14 days. Weights were taken on the day of dosing and on day 15. Necropsies were not performed.

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries and held for approximately 2 weeks before the study began. Animals were approximately 8-9 weeks old when placed on study. Groups of five males and three or five females were fed diets containing 0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 1 for 14 days. Rats and mice were observed twice daily for mortality and were weighed on day 1 and day 15. Feed consumption was not measured. 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 toxicity of HC Blue No. 1 and to determine the concentrations to be used in the 2-year studies. Six-week-old male and female F344/N rats and B6C3F$_1$ mice were obtained from Harlan Industries, observed for 2 weeks, and then assigned to cages according to a table of random numbers. The cages were assigned to dosed and control groups according to another table of random numbers.

Diets containing 0, 750, 1,500, 3,000, 6,250, or 12,500 ppm HC Blue No. 1 were fed to groups of 10 rats and 10 mice of either sex. Diets consisted of Wayne Lab Blox® mash and the required amount of HC Blue No. 1. Control diets consisted of Wayne Lab Blox® mash. Formulated diets, control diets, and water (via an automatic watering system) were available freely. Further experimental details are summarized in Table 2.

Animals were checked twice daily for signs of moribundity and mortality; moribund animals were killed. Feed consumption by cage and individual animal weights were recorded weekly. At the end of the 13-week studies, survivors were killed. Necropsies were performed on all animals except those excessively autolyzed or cannibalized. Tissues and groups examined are listed in Table 2.

TWO-YEAR STUDIES

Study Design

Diets containing 0, 1,500, or 3,000 ppm HC Blue No. 1 were fed to groups of 50 male and female rats and 50 male mice for 103 weeks. Groups of 50 female mice were fed diets containing 0, 3,000, or 6,000 ppm. Rats and mice were approximately 8 weeks old when placed on study (Table 2).

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 Harlan Industries 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 6 weeks of age. The animals were quarantined at the testing facility for 2 weeks. Thereafter, a complete pathologic examination was performed on a selected number of animals to assess their health status. The rodents were 53 days old when placed on study. 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).

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF HC BLUE NO. 1

Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Two-Year Studies
EXPERIMENTAL DESIGN			
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	50 males and 50 females of each species
Doses			
Rats--31, 62, 125, 250, or 500 mg/kg HC Blue No. 1 in 1% carboxymethylcellulose (CMC) by gavage; mice--62, 125, 250, 500, or 1,000 mg/kg in 1% CMC by gavage	0, 3,100, 6,200, 12,500, 25,000, or 50,000 ppm HC Blue No. 1 in the diet	0, 750, 1,500, 3,000, 6,250, or 12,500 ppm HC Blue No. 1 in the diet	Rats and male mice--0, 1,500, or 3,000 ppm HC Blue No. 1 in the diet; female mice--0, 3,000, or 6,000 ppm in the diet
Date of First Dose			
1/24/78	2/15/78	5/24/78	5/1/79
Date of Last Dose			
N/A	2/28/78	8/22/78	4/20/81
Duration of Dosing			
Single dose	14 d	13 wk	103 wk
Type and Frequency of Observation			
Observed 2 × d for 14 additional days for signs of moribundity and mortality; weighed on d 0 and d 15	Observed 2 × d for 15 d for signs of moribundity and mortality; weighed on d 1 and d 15; feed consumption not measured	Observed 2 × d for signs of moribundity and mortality; body weight and feed consumption recorded 1 × wk	Observed 2 × d for signs of moribundity and mortality; weighed on d 1, 1 × wk for 13 wk, 1 × mo thereafter; feed consumption measured 1 wk/mo
Necropsy and Histologic Examination			
Necropsies not performed	Standard necropsies performed on all animals; no histopath exam performed	Necropsies performed on all animals; the following tissues were examined histopathologically in all control and high dose animals: skin, mandibular lymph node, mammary gland salivary gland, thigh muscle, femur including marrow, thymus, trachea, lungs and bronchi, heart, thyroid gland, parathyroids, stomach, small intestine, esophagus, colon, mesenteric lymph node, liver, gallbladder (mice), pancreas, spleen, kidneys, adrenal gland, brain, pituitary gland, urinary bladder, vesicular gland, prostate/testes, or ovaries/uterus; in addition, lungs of all mice, thyroid glands and lungs of 3,000- and 6,250-ppm rats and mice, and mesenteric masses of 1,500- and 6,250-ppm female rats were examined	Necropsies performed on all animals; histopathologic exam performed on the following: pituitary gland, brain, eyes, external and middle ear, spinal cord, mandibular lymph node, nasal cavity, thyroid gland, parathyroids, salivary glands, thymus, larynx, liver, gallbladder (mice), pancreas, spleen, heart, adrenal gland, kidneys, urinary bladder, inguinal lymph node, mesenteric lymph node, mammary gland, sciatic nerve, bone (femur), trachea, esophagus, lungs and bronchi, stomach, costochrondral junction (rib), duodenum, jejunum, ileum, cecum, colon, rectum, bone marrow (femur), thigh muscle, ovaries/fallopian tubes/uterus/vagina, or seminal vesicles/prostate/testes/epididymus, and skin

TABLE 2. EXPERIMENTAL DESIGN AND MATERIALS AND METHODS IN THE STUDIES OF HC BLUE NO. 1
(Continued)

Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Two-Year Studies
ANIMALS AND ANIMAL MAINTENANCE			
Species			
F344/N rats; B6C3F$_1$ mice	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Animal Source			
Harlan Industries (Indianapolis, IN)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Testing Laboratory			
Southern Research Institute	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Time Held Before Start of Test			
13 d	13 d	Rats--15 d; mice--14 d	14 d
Age When Placed on Study			
8-9 wk	8-9 wk	8-9 wk	53 d
Age When Killed			
10-11 wk	10-11 wk	21-22 wk	112-115 wk
Necropsy Dates			
None performed	Rats--3/2/78-3/5/78; mice--3/2/78-3/6/78	8/23/78-8/27/78	4/29/81-5/20/81
Method of Distribution			
Assigned to cages, then to groups according to two tables of random numbers	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); ad libitum	Wayne Lab Blox® mash (Allied Mills, Inc., Chicago, IL); ad libitum	Same as 14-d studies	Same as 14-d studies
Bedding			
Heat-treated hardwood chips (PWI, Inc., Lowville, NY)	Same as single-administration studies	Same as single-administration studies	Beta Chips® (Northeastern Products Corp., Warrensburg, NY)
Water			
Automatic watering system (Edstrom Industries, Waterford, WI); ad libitum	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Cages			
Polycarbonate (Lab Products, Garfield, NJ)	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. 1 (Continued)

Single-Administration Studies	Fourteen-Day Repeated-Exposure Studies	Thirteen-Week Studies	Two-Year Studies
Cage Filters			
Reemay® spun-bonded polyester disposable filters (Snow Filtration, Cincinnati, OH)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
Animals per Cage			
5	5	5	5
Animal Room Environment			
15 changes room air/h; fluorescent light 12 h/d; temp--21°-23° C; rel hum--40%-60%	Same as single-administration studies	Same as single-administration studies except humidity--30%-50%	Same as single-administration studies except humidity--30%-65%
Other Chemicals on Test in Same Room			
None	None	None	None
CHEMISTRY			
Lot Numbers Used			
3270877	3270877	3270877	3270877; 3670379
Date of Initial Use of Subsequent Lots			
N/A	N/A	N/A	3/3/80
Supplier			
Clairol Research Lab (Stamford, CT)	Same as single-administration studies	Same as single-administration studies	Same as single-administration studies
CHEMICAL/VEHICLE			
Preparation			
Appropriate amounts of chemical were weighed into a beaker and brought to volume with 1% CMC in saline and mixed using a sonifier with a probe for 10 min	Small portion of feed mixed with chemical in a specimen cup and manually shaken for 20 sec; premix and remaining feed were mixed for 15 min in a 4-qt Patterson-Kelly® (P-K) twin-shell blender equipped with intensifier bar	Equal amounts of feed and chemical were mixed in a Waring® blender for 2 min; equal portions of premix were layered between 1/3 portions of plain feed in a 16-qt P-K® blender equipped with intensifier bar and mixed for 15 min	Equal amounts of feed and chemical were manually shaken for 1 min; the premix was layered between equal amounts of remaining feed in a 16-qt P-K® blender equipped with an intensifer bar and mixed for 15 min
Maximum Storage Time			
3 h	7 d	14 d	13 d
Storage Conditions			
N/A	Sealed in plastic bag in plastic container at room temp	5° C for 7 d; room temp for 7 d	5° C prior to use; room temp thereafter

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 summarized in Table 2.

Clinical Examinations and Pathology

All animals were observed twice daily 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. Mean body weights then were calculated for each group. The average feed consumption per animal was calculated by dividing the total feed consumption for all cages in the dose groups by the number of surviving animals in the group. Moribund animals and animals that survived to the end of the study were killed with carbon dioxide, and necropsies were performed. Necropsies were also performed on all animals 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 PWG. Certain nonneoplastic findings are reviewed by the quality assurance pathologist and 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) life table test 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 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 interval. 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. These two tests are based on the overall proportion of tumor-bearing animals and do not adjust for survival differences.

III. RESULTS

RATS

SINGLE-ADMINISTRATION STUDIES
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
THIRTEEN-WEEK STUDIES
TWO-YEAR STUDIES
Body Weights and Clinical Signs
Survival
Pathology and Statistical Analyses of Results

SINGLE-ADMINISTRATION STUDIES

Survival and body weight data for male and female rats administered a single gavage dose of HC Blue No. 1 (31-500 mg/kg) are summarized in Table 3. No rats died, and mean body weights of all dosed groups were comparable.

The urine of dosed rats was blue for several days after the chemical was administered, indicating that HC Blue No. 1 or a degradation product was absorbed via the gastrointestinal tract.

TABLE 3. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF HC BLUE NO. 1

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams)		
		Initial	Final	Change (b)
MALE				
31	5/5	113 ± 3	173 ± 6	+ 60 ± 5
62	5/5	114 ± 3	186 ± 8	+ 72 ± 6
125	5/5	113 ± 4	160 ± 5	+ 47 ± 2
250	5/5	114 ± 2	166 ± 4	+ 52 ± 4
500	5/5	108 ± 1	164 ± 5	+ 56 ± 5
FEMALE				
31	5/5	94 ± 4	124 ± 6	+ 30 ± 3
62	5/5	100 ± 1	130 ± 2	+ 30 ± 2
125	5/5	90 ± 2	122 ± 5	+ 32 ± 4
250	5/5	94 ± 2	125 ± 2	+ 31 ± 1
500	5/5	95 ± 3	127 ± 6	+ 32 ± 3

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

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES

Dietary doses of HC Blue No. 1 used in the 14-day repeated-exposure studies ranged from 0 to 50,000 ppm. Survival and body weight data for these repeated-exposure studies are summarized in Table 4. Four of five males and 3/5 females receiving 50,000 ppm died. No other deaths occurred. Body weight gains were decreased in a dose-related manner, and animals in the 25,000-ppm and 50,000-ppm groups lost weight. Feed consumption was not measured. Animals in the 50,000-ppm groups had dark violet urine, dark feces, and sunken eyes suggestive of dehydration; they walked with an unusual gait and were generally inactive.

TABLE 4. SURVIVAL AND MEAN BODY WEIGHTS OF RATS IN THE FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 1

Dose (ppm)	Survival (a)	Mean Body Weights (grams)		
		Initial (b)	Final	Change (c)
MALE				
0	5/5	126 ± 9	176 ± 11	+ 50 ± 3
3,100	5/5	138 ± 5	179 ± 6	+ 41 ± 6
6,200	5/5	134 ± 6	178 ± 6	+ 44 ± 3
12,500	5/5	113 ± 10	134 ± 12	+ 21 ± 2
25,000	5/5	124 ± 14	110 ± 12	− 14 ± 4
50,000	(d) 1/5	96 ± 5	70	− 26
FEMALE				
0	3/3	101 ± 1	125 ± 2	+ 24 ± 1
3,100	5/5	112 ± 6	128 ± 5	+ 16 ± 2
6,200	5/5	109 ± 2	123 ± 4	+ 14 ± 4
12,500	5/5	111 ± 8	116 ± 8	+ 5 ± 1
25,000	5/5	104 ± 7	92 ± 5	− 12 ± 4
50,000	(e) 2/5	103 ± 3	74 ± 3	− 32 ± 1

(a) Number surviving/number initially per group
(b) Initial mean body weight for all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) Days of death: 9, 10, 12, 14
(e) Days of death: 9, 13, 14

III. RESULTS: RATS

THIRTEEN-WEEK STUDIES

Dietary concentrations (0-12,500 ppm) of HC Blue No. 1 used in the 13-week studies were selected on the basis of survival and body weight gains during the repeated-exposure studies. Survival, body weight, and feed consumption data for the 13-week studies are summarized in Table 5. All rats survived the entire experimental period. Final mean body weights were depressed by 10.8% or 17.3% in male rats fed diets containing 6,250 or 12,500 ppm and by 9.7% in female rats fed diets containing 12,500 ppm. Feed consumption for rats receiving the highest (12,500 ppm) dose was equal to or greater than that of the controls.

Brown-to-gold pigment granules (unidentified) were found in the cytoplasm of thyroid epithelial cells in 10/10 males and 10/10 females receiving 12,500 ppm and in 5/10 males and 10/10 females receiving 6,250 ppm. Additional pathologic changes were not observed, and other groups were not affected.

Doses of 1,500 and 3,000 ppm HC Blue No. 1 in feed were selected for rats in the 2-year studies.

TABLE 5. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF RATS IN THE THIRTEEN-WEEK FEED STUDIES OF HC BLUE NO. 1

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)	Average Daily Feed Consumption (grams)
MALE						
0	10/10	112 ± 3	295 ± 6	+ 183 ± 7	--	17.0
750	10/10	112 ± 4	289 ± 8	+ 177 ± 7	98.0	16.4
1,500	10/10	115 ± 3	312 ± 7	+ 197 ± 6	105.8	17.9
3,000	10/10	110 ± 5	286 ± 9	+ 176 ± 7	96.9	18.3
6,250	10/10	117 ± 4	263 ± 7	+ 146 ± 8	89.2	17.0
12,500	10/10	107 ± 3	244 ± 8	+ 137 ± 8	82.7	17.3
FEMALE						
0	10/10	97 ± 2	175 ± 4	+ 78 ± 3	--	12.1
750	10/10	99 ± 3	176 ± 4	+ 77 ± 3	100.6	11.6
1,500	10/10	97 ± 1	177 ± 4	+ 80 ± 4	101.1	12.4
3,000	10/10	102 ± 3	177 ± 3	+ 75 ± 2	101.1	11.6
6,250	10/10	99 ± 3	165 ± 5	+ 66 ± 3	94.3	10.7
12,500	10/10	102 ± 3	158 ± 4	+ 56 ± 2	90.3	14.4

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

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Body weights of dosed and control rats are summarized in Table 6 and Figure 1. The mean body weights of dosed males and females were decreased relative to those of the controls in a dose-related manner. The difference between dosed and control males appeared around week 16. Beginning at week 50, only the high dose female rats showed body weights that were more than 10% different from those of the controls.

The average daily feed consumption by high dose groups was 95% that of the controls; feed consumption by all other dosed groups was comparable to that of the controls (Appendix L, Tables L1 and L2). The feed consumption data were not corrected for scattering of feed. The calculated average dose per day during the 2-year studies was 66 and 129 mg/kg for low dose and high dose males and 74 and 154 mg/kg for low dose and high dose females (Appendix L, Tables L1 and L2).

TABLE 6. MEAN BODY WEIGHTS AND SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

Weeks on Study	Control		1,500 ppm			3,000 ppm		
	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								
0	99	50	104	105	50	105	106	50
1	128	50	128	100	50	131	102	50
2	154	50	154	100	50	153	99	50
3	172	50	170	99	50	171	99	50
4	188	50	184	98	50	186	99	50
5	197	50	193	98	50	192	97	50
6	205	50	201	98	50	199	97	50
7	215	50	208	97	50	208	97	50
8	224	50	217	97	50	217	97	50
9	232	50	226	97	50	228	98	50
10	245	50	238	97	50	240	98	50
11	254	50	250	98	50	253	100	50
12	265	50	261	98	50	263	99	50
16	303	50	275	91	50	272	90	50
21	332	50	320	96	50	315	95	50
26	356	50	344	97	50	335	94	50
29	365	50	355	97	50	345	95	50
33	375	50	362	97	50	354	94	50
38	391	50	377	96	50	369	94	50
42	394	50	382	97	50	371	94	50
46	405	50	390	96	50	378	93	50
50	409	50	393	96	50	382	93	50
55	420	50	402	96	50	392	93	50
60	419	50	407	97	49	396	95	50
65	426	49	409	96	49	399	94	50
69	425	48	406	96	49	395	93	50
74	425	48	407	96	49	394	93	50
77	429	48	406	95	47	393	92	48
81	420	48	400	95	46	392	93	47
85	419	44	399	95	44	390	93	44
89	421	43	393	93	44	384	91	44
93	422	43	404	96	42	393	93	42
96	424	43	399	94	37	394	93	42
101	411	41	390	95	34	378	92	42
104	402	39	382	95	33	371	92	41
FEMALE								
0	89	50	90	101	50	90	101	50
1	104	50	100	96	50	102	98	50
2	116	50	114	98	50	112	97	50
3	122	50	119	98	50	118	97	50
4	128	50	127	99	50	122	95	50
5	132	50	132	100	50	126	95	50
6	138	50	138	100	50	133	96	50
7	146	50	144	99	50	139	95	50
8	154	50	150	97	50	144	94	50
9	153	50	151	99	50	146	95	50
10	156	50	155	99	50	150	96	50
11	159	50	158	99	50	153	96	50
12	162	50	161	99	50	157	97	50
16	176	50	174	99	50	160	91	50
21	186	50	184	99	50	180	97	50
26	197	50	192	97	50	186	94	50
29	202	50	197	98	50	190	94	50
33	207	50	201	97	50	193	93	50
38	216	50	210	97	50	201	93	50
42	217	50	210	97	50	199	92	50
46	224	50	216	96	50	203	91	50
50	230	50	220	96	50	205	89	50
55	240	50	227	95	50	213	89	50
60	249	50	235	94	49	218	88	50
65	257	50	243	95	49	223	87	50
69	263	50	247	94	49	227	86	50
74	271	50	258	95	49	236	87	50
77	281	49	265	94	48	243	86	50
81	285	47	269	94	47	244	86	50
85	287	47	275	96	45	248	86	49
89	297	47	277	93	45	252	85	47
93	300	47	287	96	42	261	87	46
96	305	43	288	94	41	267	88	44
101	299	42	289	97	39	262	88	42
104	301	40	294	98	35	262	87	41

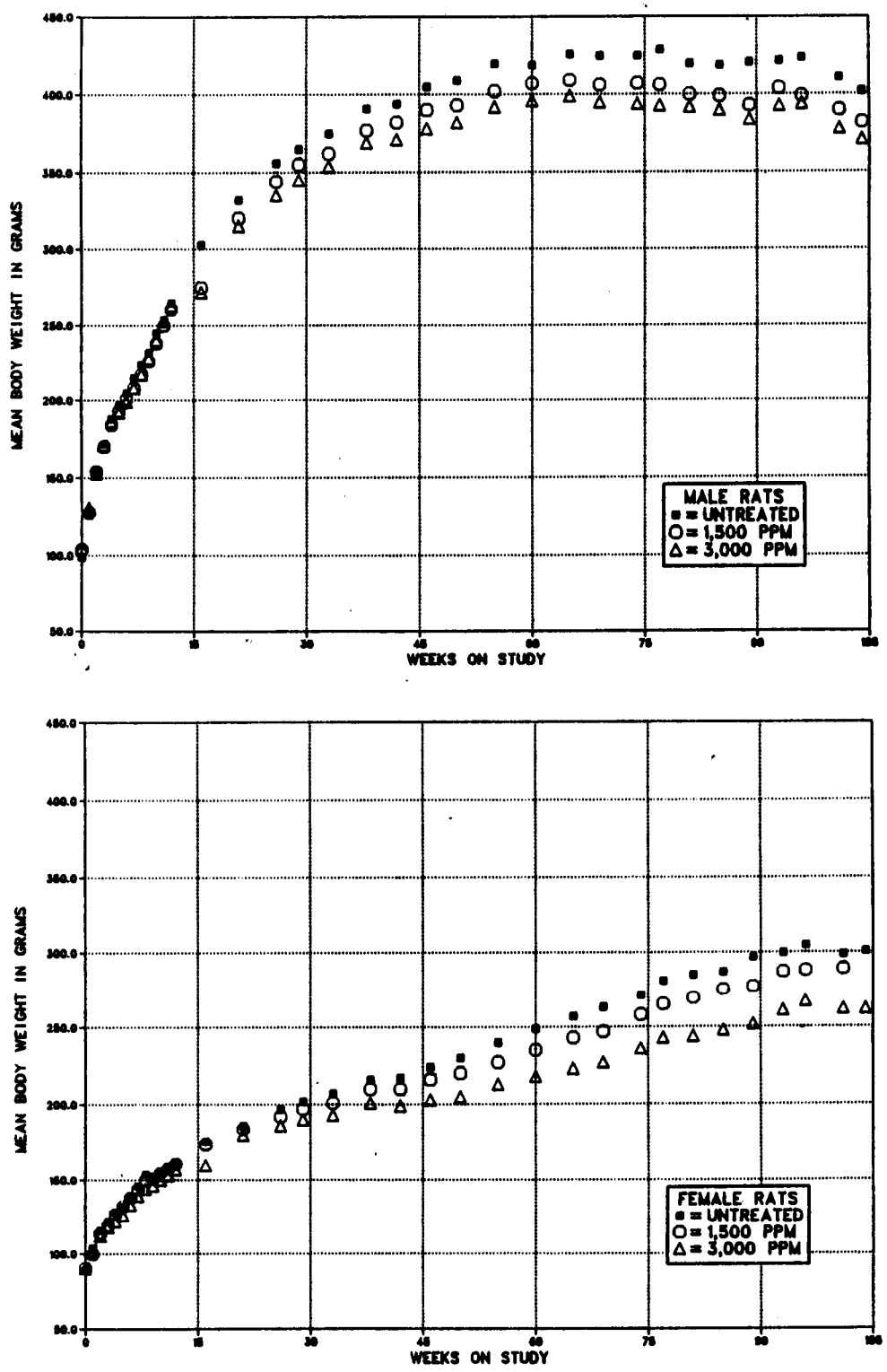

FIGURE 1. GROWTH CURVES FOR RATS ADMINISTERED HC BLUE NO. 1 IN FEED FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female rats fed diets containing HC Blue No. 1 at the concentrations used in these studies and those of the controls are shown in the Kaplan-Meier survival curves in Figure 2. No significant differences in survival were observed between any groups of either sex (Table 7).

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidence of animals 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, contain the statistical analyses of those primary tumors that occurred with an incidence of at least 5% in one of the three groups. Historical incidences of tumors in control animals are listed in Appendix F. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).

TABLE 7. SURVIVAL OF RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
MALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	11	16	9
Killed at termination	37	32	41
Died during termination period	2	2	0
Survival P values (c)	0.541	0.347	0.591
FEMALE (a)			
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	10	12	9
Killed at termination	40	34	40
Died during termination period	0	4	1
Survival P values (c)	0.930	0.262	0.985

(a) Terminal kill period: weeks 104-107
(b) Includes animals killed in a moribund condition
(c) The results of the life table trend test are in the control column, and those of the life table exact pairwise comparisons with the controls are in the dosed columns.

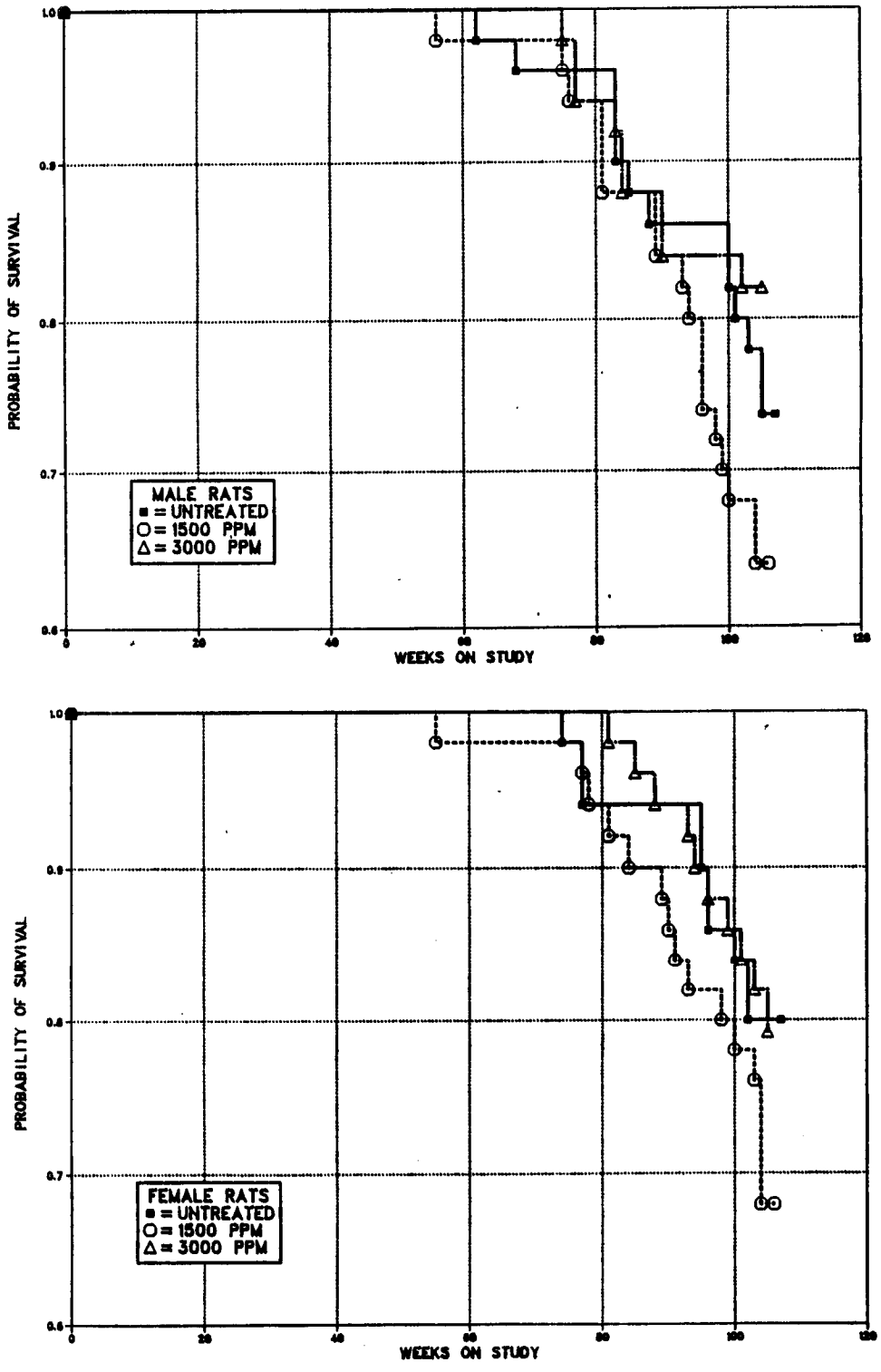

FIGURE 2. KAPLAN-MEIER SURVIVAL CURVES FOR RATS ADMINISTERED HC BLUE NO. 1 IN FEED FOR TWO YEARS

Liver: Neoplastic nodules were classified according to the recommendations of Squire and Levitt (1975) and the National Academy of Sciences (1980) with some modification; that is, small focal lesions causing only minimal compression, with little or no cytologic atypia in livers or with toxic or anoxic hepatic change (such as occurs with mononuclear cell leukemia), were classified in this study as nodular hyperplasia. Neoplastic nodules and neoplastic nodules or carcinomas (combined) occurred in male rats with significant positive trends, but the incidences in the dosed groups were not significantly higher than those in the controls (Table 8). One low dose female rat had a neoplastic nodule, and one high dose female rat had an hepatocellular carcinoma. No toxicologically significant nonneoplastic lesions were found.

TABLE 8. ANALYSIS OF LIVER TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (a)

	Control	1,500 ppm (b)	3,000 ppm (b)
Neoplastic Nodule			
Overall Rates	0/49 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates	0.0%	0.0%	6.8%
Terminal Rates	0/38 (0%)	0/34 (0%)	2/41 (5%)
Life Table Tests	P=0.045	(c)	P=0.135
Incidental Tumor Tests	P=0.050	(c)	P=0.157
Carcinoma			
Overall Rates	1/49 (2%)	0/50 (0%)	3/50 (6%)
Adjusted Rates	2.6%	0.0%	7.3%
Terminal Rates	1/38 (3%)	0/34 (0%)	3/41 (7%)
Life Table Tests	P=0.199	P=0.522N	P=0.333
Incidental Tumor Tests	P=0.199	P=0.522N	P=0.333
Neoplastic Nodule or Carcinoma (d)			
Overall Rates	1/49 (2%)	0/50 (0%)	6/50 (12%)
Adjusted Rates	2.6%	0.0%	14.0%
Terminal Rates	1/38 (3%)	0/34 (0%)	5/41 (12%)
Life Table Tests	P=0.023	P=0.522N	P=0.072
Incidental Tumor Tests	P=0.025	P=0.522N	P=0.082

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) The equivalent dose in milligrams per kilograms per day is given in Body Weights and Clinical Signs (page 33) and Appendix L.
(c) No P value is presented because no tumors were observed in 1,500-ppm and control groups.
(d) Historical incidence at testing laboratory--mean: 3%; range: 0%-5%; historical incidence in NTP studies--mean: 4%; range: 0%-14%

Lung: Adenomatous hyperplasia of the lung was found at increased incidences in dosed male and female rats (male: control, 3/50, 6%; low dose, 5/50, 10%; high dose, 7/50, 14%; female: control, 2/50, 4%; low dose, 5/49, 10%; high dose, 8/50, 16%). Alveolar/bronchiolar carcinomas and alveolar/bronchiolar adenomas or carcinomas (combined) occurred in female rats with significant positive trends (Table 9). The combined incidence of these tumors was significantly greater in the high dose group than in the controls. Alveolar/bronchiolar carcinomas occurred in dosed male rats (control, 0/50; low dose, 2/50, 4%; high dose, 1/50, 2%), but the increases were not significant.

The alveolar/bronchiolar proliferative lesions appear to represent a continuum with no sharp demarcations between hyperplasia, adenoma, and carcinoma. All are focal lesions, but they tend to merge gradually with the surrounding normal parenchyma. Hyperplasia is defined as a single layer of plump epithelial cells lining contiguous alveoli that sometimes contain macrophages. Adenomas are defined as proliferative lesions in which the epithelial cells form solid sheets that fill adjacent alveoli or line papillary structures that project into the alveoli. Carcinomas show features characteristic of adenomas; but the neoplastic cells show a greater degree of anaplasia and better defined patterns of growth, and they invade blood vessels or pleura.

TABLE 9. ANALYSIS OF LUNG LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Adenomatous Hyperplasia			
Overall Rates	2/50 (4%)	5/49 (10%)	8/50 (16%)
Alveolar/Bronchiolar Adenoma			
Overall Rates	1/50 (2%)	2/49 (4%)	3/50 (6%)
Adjusted Rates	2.5%	5.4%	7.3%
Terminal Rates	1/40 (3%)	2/37 (5%)	3/41 (7%)
Life Table Tests	P=0.233	P=0.473	P=0.314
Incidental Tumor Tests	P=0.233	P=0.473	P=0.314
Alveolar/Bronchiolar Carcinoma			
Overall Rates	0/50 (0%)	1/49 (2%)	4/50 (8%)
Adjusted Rates	0.0%	2.7%	9.4%
Terminal Rates	0/40 (0%)	1/37 (3%)	3/41 (7%)
Life Table Tests	P=0.030	P=0.484	P=0.068
Incidental Tumor Tests	P=0.029	P=0.484	P=0.062
Alveolar/Bronchiolar Adenoma or Carcinoma (a)			
Overall Rates	1/50 (2%)	3/49 (6%)	7/50 (14%)
Adjusted Rates	2.5%	8.1%	16.6%
Terminal Rates	1/40 (3%)	3/37 (8%)	6/41 (15%)
Life Table Tests	P=0.021	P=0.278	P=0.036
Incidental Tumor Tests	P=0.020	P=0.278	P=0.034

(a) Historical incidence at testing laboratory--mean: 2%; range: 0%-3%; historical incidence in NTP studies--mean: 1%; range: 0%-10%

Uterus: Endometrial stromal polyps occurred with a significant positive trend in female rats, and the incidence in the high dose group was significantly greater than that in the controls (Table 10). One sarcoma also was observed in each group.

Kidney: Pigmentation of the renal tubular epithelial cells occurred at increased incidences in dosed male and female rats (male: control, 0/50; low dose, 36/50, 72%; high dose, 42/50, 84%; female: control, 0/50; low dose, 39/50, 78%; high dose, 40/50, 80%). A renal tubular cell adenocarcinoma and a transitional cell papilloma were observed in 2/50 (4%) high dose male rats. An adenoma in the kidney cortex was observed in 1/50 (2%) control male rats. No kidney tumors were observed in dosed or control female rats or in low dose male rats.

Zymbal Gland: Carcinomas occurred in female rats with a significant negative trend, but the incidences in the dosed groups were not significantly lower than that of the controls (control, 3/50, 6%; low dose, 0/50; high dose, 0/50).

Thyroid Gland: Pigmentation of follicular cells in the thyroid gland was found at increased incidences in dosed male and female rats (male: control, 0/50; low dose, 42/48, 88%; high dose, 48/50, 96%; female: control, 0/40; low dose, 44/50, 88%; high dose, 47/50 (94%). C-Cell adenomas occurred in female rats with a significant negative trend (control, 5/49, 10%; low dose, 2/50, 4%; high dose, 0/50; P=0.015), and the incidence in the high dose group was significantly lower than that in the controls (P=0.029). The incidences of C-cell carcinomas and of adenomas or carcinomas (combined) were not significant (C-cell adenomas or carcinomas: control, 7/49, 14%; low dose, 6/50, 12%; high dose, 2/50, 4%).

Eye: Retinopathy and cataracts occurred at increased incidences in high dose males and low dose females (Table 11).

TABLE 10. ANALYSIS OF UTERINE TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Endometrial Stromal Polyp (a)			
Overall Rates	5/50 (10%)	9/50 (18%)	14/50 (28%)
Adjusted Rates	12.5%	21.0%	33.2%
Terminal Rates	5/40 (13%)	5/38 (13%)	13/41 (32%)
Life Table Tests	P=0.020	P=0.174	P=0.023
Incidental Tumor Tests	P=0.023	P=0.289	P=0.022

(a) Historical incidence at testing laboratory--mean: 15%; range: 8%-22%; historical incidence in NTP studies--mean: 18%; range: 4%-37%

TABLE 11. INCIDENCES OF EYE LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1 (a)

	Control	1,500 ppm	3,000 ppm
MALE			
Retinopathy	5	1	21
Cataracts	5	1	19
FEMALE			
Retinopathy	5	21	3
Cataracts	2	20	1

(a) Fifty animals in each group.

Hematopoietic System: Mononuclear cell leukemia occurred in male rats with a significant negative trend, and the incidence in the high dose group was significantly lower than that in the controls (Table 12). The incidences of mononuclear cell leukemia in dosed female rats were not significantly different from that in the controls (control, 4/50, 8%; low dose, 3/50, 6%; high dose, 5/50, 10%).

Pituitary Gland: Adenomas or carcinomas (combined) occurred in male rats with a significant negative trend (adenoma: control, 9/49, 18%; low dose, 8/49, 16%; high dose, 3/47, 6%; adenoma or carcinoma: control, 11/49, 22%; low dose, 9/49, 18%; high dose, 4/47, 9%; P=0.037), but the incidence in the high dose group was significantly lower than that of the controls only by the life table test (P=0.039).

Adrenal Gland: Cortical adenomas occurred in female rats with a significant negative trend (control, 5/50, 10%; low dose, 2/50, 4%; high dose, 0/50; P<0.02). The incidences of cortical adenomas in high dose females and of pheochromocytomas in low dose females (control, 8/50, 16%; low dose, 2/50, 4%; high dose, 3/50, 6%) were significantly lower than those in the controls.

TABLE 12. ANALYSIS OF HEMATOPOIETIC SYSTEM TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Mononuclear Cell Leukemia (a, b)			
Overall Rates	13/50 (26%)	9/50 (18%)	2/50 (4%)
Adjusted Rates	30.2%	20.9%	4.5%
Terminal Rates	9/39 (23%)	3/34 (9%)	0/41 (0%)
Life Table Tests	P=0.003N	P=0.343N	P=0.003N
Incidental Tumor Tests	P=0.003N	P=0.160N	P=0.008N

(a) Designated as monocytic leukemia in the Carcinogenesis Bioassay Data System and in Appendix A
(b) Historical incidence of leukemia at testing laboratory--mean: 27%; range: 12%-40%; historical incidence in NTP studies--mean: 28%; range: 0%-46%

SINGLE-ADMINISTRATION STUDIES

Survival and body weight data for male and female mice administered a single gavage dose of HC Blue No. 1 (62-1,000 mg/kg) are summarized in Table 13. No compound-related deaths occurred. The urine of dosed male and female mice was blue. No other compound-related clinical signs were observed.

TABLE 13. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE SINGLE-ADMINISTRATION GAVAGE STUDIES OF HC BLUE NO. 1

Dose (mg/kg)	Survival (a)	Mean Body Weights (grams)		
		Initial (b)	Final	Change (c)
MALE				
62	5/5	20.8 ± 0.9	22.6 ± 0.7	+1.8 ± 0.4
125	5/5	20.8 ± 0.7	23.6 ± 0.9	+2.8 ± 0.2
250	(d) 4/5	21.6 ± 0.4	24.3 ± 1.1	+2.7 ± 0.7
500	5/5	20.8 ± 0.2	23.4 ± 0.5	+2.6 ± 0.5
1,000	5/5	21.8 ± 1.0	24.2 ± 0.9	+2.4 ± 0.5
FEMALE				
62	5/5	16.4 ± 0.4	18.0 ± 0.4	+1.6 ± 0.4
125	5/5	18.0 ± 0.8	19.0 ± 0.6	+1.0 ± 0.3
250	5/5	17.6 ± 0.7	17.8 ± 1.4	+0.2 ± 0.9
500	5/5	17.6 ± 0.7	19.4 ± 0.9	+1.8 ± 0.4
1,000	5/5	18.6 ± 0.7	19.4 ± 0.5	+0.8 ± 0.4

(a) Number surviving/number initially in the group
(b) Initial mean body weight of all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean weight change of the group ± standard error of the mean
(d) One animal died on day 10 of unknown causes.

FOURTEEN-DAY REPEATED-EXPOSURE STUDIES

Dietary doses of HC Blue No. 1 used in the 14-day repeated-exposure studies ranged from 0 to 50,000 ppm. Survival and body weight data for these repeated-exposure studies are summarized in Table 14. One male receiving 50,000 ppm died; no other deaths occurred. Body weight gains were depressed in a dose-related manner in animals receiving the compound at the 6,200-ppm concentration or higher. Four of four male mice and 4/5 female mice receiving 50,000 ppm, 3/5 male mice and 2/5 female mice receiving 25,000 ppm, and 4/5 male mice receiving 12,500 ppm lost weight. Two of five female mice receiving 25,000 ppm and 3/5 female mice receiving 12,500 ppm gained no weight. (Feed consumption was not measured.) Violet urine and dark feces were observed for all dosed mice.

TABLE 14. SURVIVAL AND MEAN BODY WEIGHTS OF MICE IN THE FOURTEEN-DAY REPEATED-EXPOSURE FEED STUDIES OF HC BLUE NO. 1

Dose (ppm)	Survival (a)	Mean Body Weights (grams)		
		Initial (b)	Final	Change (c)
MALE				
0	5/5	21.2 ± 0.6	23.0 ± 0.5	+ 1.8 ± 0.2
3,100	5/5	21.0 ± 0.6	22.8 ± 0.7	+ 1.8 ± 0.4
6,200	5/5	21.6 ± 0.2	23.0 ± 0.3	+ 1.4 ± 0.2
12,500	5/5	20.4 ± 1.0	19.8 ± 0.6	− 0.6 ± 0.7
25,000	5/5	20.0 ± 0.5	19.2 ± 0.4	− 0.8 ± 0.7
50,000	(d) 4/5	20.8 ± 0.4	17.0 ± 1.6	− 3.5 ± 1.5
FEMALE				
0	5/5	17.6 ± 0.5	19.2 ± 0.5	+ 1.6 ± 0.2
3,100	5/5	17.8 ± 0.4	19.6 ± 0.4	+ 1.8 ± 0.2
6,200	5/5	17.2 ± 0.2	18.4 ± 0.2	+ 1.2 ± 0.4
12,500	5/5	18.6 ± 0.7	19.0 ± 0.7	+ 0.4 ± 0.2
25,000	5/5	18.0 ± 0.4	17.6 ± 0.2	− 0.4 ± 0.5
50,000	5/5	18.2 ± 0.4	17.0 ± 0.5	− 1.2 ± 0.6

(a) Number surviving/number initially in the group
(b) Initial mean body weight of all animals in the group. Subsequent calculations are based on those animals surviving to the end of the study.
(c) Mean body weight change of the survivors ± standard error of the mean
(d) One mouse died on day 15.

THIRTEEN-WEEK STUDIES

Dietary concentrations (0-12,500 ppm) of HC Blue No. 1 used in the 13-week studies were selected on the basis of survival and body weight gains during the repeated-exposure studies. Survival, body weight, and feed consumption data for the 13-week studies are summarized in Table 15. All mice survived for the entire experimental period. The final mean body weight relative to that of the controls was depressed by 12% for male mice administered 12,500 ppm and by 5% for female mice administered 12,500 ppm.

Brown-to-golden pigment granules were detected in the cytoplasm of thyroid gland epithelial cells in 10/10 male and female mice receiving 12,500 ppm and in 5/10 male mice receiving 6,250 ppm. Yellow crystals occurred extracellularly or in the phagocytes of some bronchioles or alveoli in all mice receiving 6,250 or 12,500 ppm and in 7/10 males and 8/10 females receiving 3,000 ppm. These crystals, although not identified, were not considered to be of toxicologic significance. No other histopathologic lesions were observed.

Doses of 1,500 or 3,000 ppm HC Blue No. 1 in feed were selected for male mice and doses of 3,000 or 6,000 ppm HC Blue No. 1 in feed were selected for female mice in the 2-year studies.

TABLE 15. SURVIVAL, MEAN BODY WEIGHTS, AND FEED CONSUMPTION OF MICE IN THE THIRTEEN-WEEK FEED STUDIES OF HC BLUE NO. 1

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Change (b)	Final Weight Relative to Controls (percent)	Average Daily Feed Consumption (grams)
MALE						
0	10/10	23.3 ± 0.5	31.8 ± 1.0	+8.5 ± 0.8	--	6.6
750	10/10	22.8 ± 0.8	29.8 ± 0.6	+7.0 ± 0.5	93.7	6.4
1,500	10/10	22.7 ± 0.5	31.2 ± 0.6	+8.5 ± 0.6	98.1	6.2
3,000	10/10	23.7 ± 0.4	31.3 ± 0.7	+7.6 ± 0.6	98.4	7.0
6,250	10/10	23.3 ± 0.6	29.3 ± 0.5	+6.0 ± 0.3	92.1	6.8
12,500	10/10	23.8 ± 0.6	27.9 ± 0.5	+4.1 ± 0.3	87.7	7.0
FEMALE						
0	10/10	18.4 ± 0.5	24.9 ± 0.7	+6.5 ± 0.4	--	6.0
750	10/10	18.7 ± 0.4	24.4 ± 0.4	+5.7 ± 0.3	98.0	6.3
1,500	10/10	19.2 ± 0.6	25.7 ± 0.6	+6.5 ± 0.2	103.2	6.2
3,000	10/10	18.7 ± 0.3	25.3 ± 0.3	+6.6 ± 0.3	101.6	6.7
6,250	10/10	18.6 ± 0.4	24.5 ± 0.5	+5.9 ± 0.5	98.4	6.1
12,500	10/10	19.3 ± 0.3	23.6 ± 0.4	+4.3 ± 0.4	94.8	6.0

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

TWO-YEAR STUDIES

Body Weights and Clinical Signs

Body weights of dosed and control mice are summarized in Table 16 and Figure 3. Final mean body weights of dosed male and female mice were lower (10%-12% and 21%-29%, respectively) than those of the controls, and the depressions in mean body weight gain were dose related. The average daily feed consumption relative to the control groups was approximately 100% for both dosed male groups and 105% for both dosed female groups (Appendix L, Tables L3 and L4). The feed consumption data were not corrected for scattering of feed. The average dose per day during the 2-year studies was 309 and 650 mg/kg for low dose and high dose males and 778 and 1,634 mg/kg for low dose and high dose females (Appendix L, Tables L3 and L4).

TABLE 16. MEAN BODY WEIGHTS AND SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

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			1,500 ppm			3,000 ppm		
0	25.2	50	24.3	96	50	25.0	99	50
1	25.1	50	24.8	99	50	24.4	97	50
2	25.6	50	25.2	98	50	25.7	100	50
3	26.7	50	25.4	95	50	26.7	100	50
4	28.5	50	27.7	97	50	28.7	101	50
5	28.9	50	28.1	97	50	29.8	103	50
6	30.2	50	29.5	98	50	30.7	102	50
7	31.2	50	30.5	98	50	31.4	101	50
8	32.6	50	30.1	92	50	31.6	97	50
9	32.5	50	31.5	97	50	31.7	98	50
10	32.9	50	32.1	98	50	32.0	97	50
11	33.8	50	32.2	95	50	33.1	98	50
12	34.0	50	33.6	99	50	33.1	97	50
16	35.3	50	31.3	89	50	33.6	95	50
21	37.3	49	33.8	91	50	35.4	95	50
26	38.1	49	35.5	93	50	36.4	96	50
29	39.4	49	37.7	96	50	36.9	94	50
33	40.2	48	38.0	95	50	37.2	93	50
38	41.6	48	39.0	94	50	38.7	93	50
42	41.8	48	39.0	93	50	39.2	94	50
46	42.1	48	39.6	94	50	39.3	93	50
49	42.1	48	40.0	95	50	39.6	94	50
55	43.4	48	40.0	92	50	40.0	92	50
59	43.3	48	40.8	94	50	40.3	93	50
65	43.6	48	40.5	93	50	40.7	93	49
69	43.6	48	40.8	94	50	40.5	93	49
74	43.8	47	41.4	95	50	39.9	91	49
77	43.0	47	41.2	96	50	40.1	93	48
81	42.7	46	40.3	94	49	39.7	93	47
85	42.0	44	40.0	95	48	39.5	94	43
88	41.9	44	39.9	95	48	39.2	94	42
92	43.1	41	40.2	93	44	40.2	93	38
96	42.6	36	40.6	95	42	39.6	93	35
101	42.8	33	39.6	93	38	39.4	92	31
104	45.3	33	40.8	90	37	39.8	88	30
FEMALE			3,000 ppm			6,000 ppm		
0	19.1	50	18.4	96	50	18.3	96	50
1	18.8	50	18.3	97	50	19.3	103	50
2	19.6	50	18.9	96	50	18.9	96	50
3	20.8	50	20.6	99	50	20.6	99	50
4	22.3	50	21.1	95	50	21.7	97	50
5	22.3	50	21.7	97	50	22.1	99	50
6	24.2	50	22.3	92	50	22.5	93	50
7	23.9	50	24.1	101	50	23.8	100	50
8	24.9	50	23.6	95	50	24.3	98	50
9	24.8	50	23.8	96	50	24.2	98	50
10	24.8	50	23.9	96	50	23.9	96	50
11	25.5	50	24.2	95	50	25.2	99	50
12	25.8	50	25.1	97	50	24.5	95	50
16	27.2	50	25.3	93	50	25.0	92	50
21	29.5	50	26.0	88	50	26.4	89	50
26	30.1	50	27.6	92	50	26.8	89	50
29	30.6	50	27.8	91	50	27.2	89	50
33	32.6	50	29.8	91	50	27.8	85	50
38	33.6	50	29.5	88	50	27.8	83	50
42	33.3	50	30.2	91	50	28.5	86	50
46	33.8	50	31.0	92	49	28.5	84	50
49	34.2	48	30.9	90	48	29.0	85	49
55	36.8	46	31.9	87	46	29.3	80	49
59	36.6	45	31.4	86	45	29.7	81	47
65	37.8	44	31.5	83	45	30.5	81	47
69	38.1	44	32.5	85	44	29.9	78	47
74	39.8	43	32.5	82	43	30.4	76	47
77	39.5	43	32.0	81	42	29.6	75	45
81	40.8	42	33.1	81	41	30.3	74	44
85	40.2	42	32.2	80	40	30.3	75	42
88	41.4	42	32.1	78	39	31.4	76	41
92	43.1	39	33.0	77	37	31.0	72	40
96	43.3	37	32.2	74	36	30.5	70	34
101	41.6	37	31.7	76	30	30.2	73	25
104	41.6	36	33.0	79	28	30.3	73	22

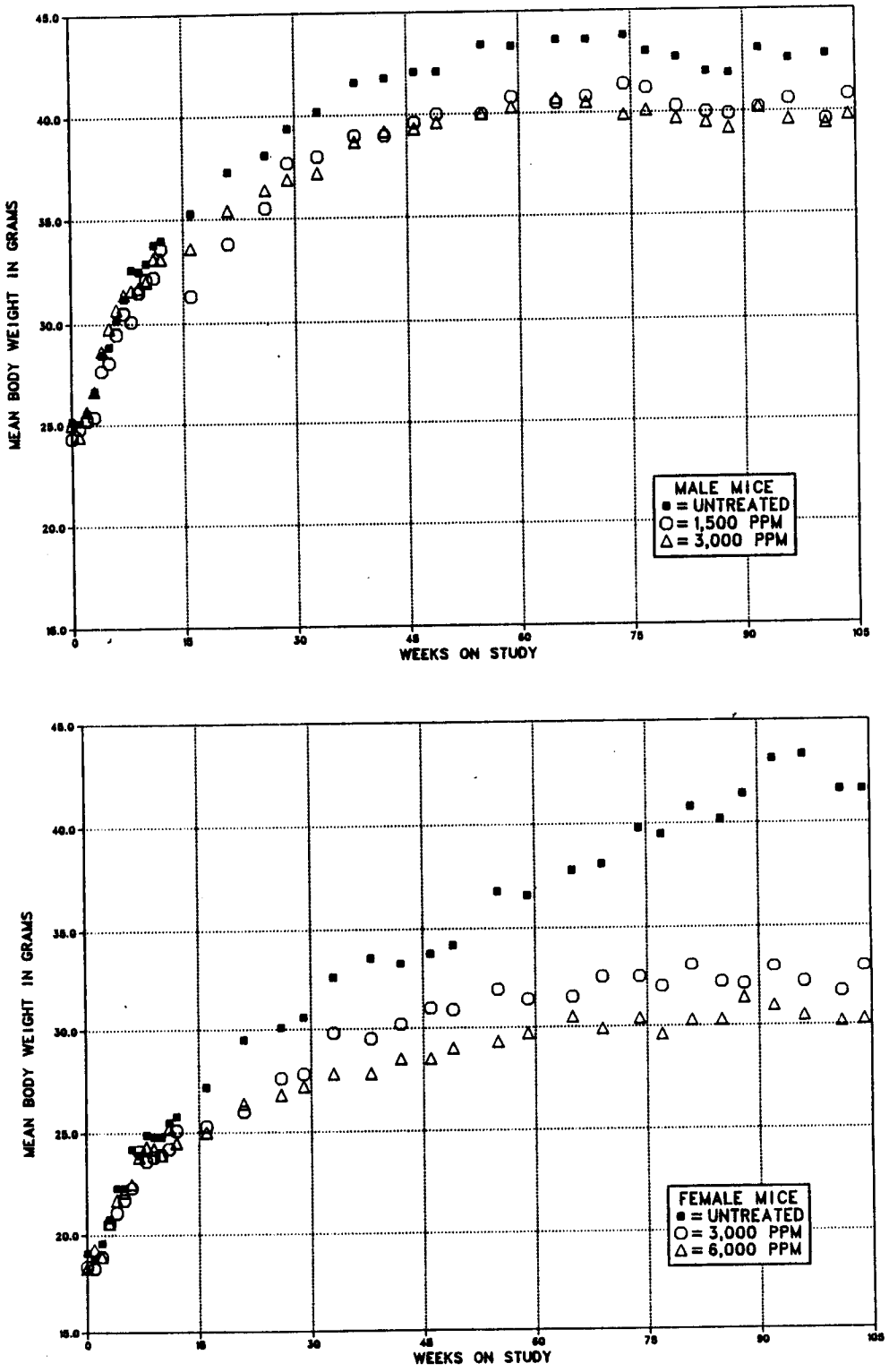

FIGURE 3. GROWTH CURVES FOR MICE ADMINISTERED HC BLUE NO. 1 IN FEED FOR TWO YEARS

Survival

Estimates of the probabilities of survival for male and female mice fed diets containing HC Blue No. 1 at the concentration of these studies and for the controls are shown in the Kaplan-Meier survival curves in Figure 4. The survival of high dose female mice was significantly less than that of the controls (P = 0.026) (Table 17).

Pathology and Statistical Analyses of Results

This section describes the significant or noteworthy changes in the incidence of animals 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. Historical incidences of tumors in control animals are listed in Appendix F. The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).

TABLE 17. SURVIVAL OF MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

	Control	Low Dose	High Dose
MALE (a)		**1,500 ppm**	**3,000 ppm**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	17	13	20
Killed at termination	32	35	30
Died during termination period	1	2	0
Survival P values (c)	0.601	0.515	0.734
FEMALE (a)		**3,000 ppm**	**6,000 ppm**
Animals initially in study	50	50	50
Nonaccidental deaths before termination (b)	14	22	26
Killed at termination	36	27	22
Died during termination period	0	1	2
Survival P values (c)	0.017	0.141	0.026

(a) Terminal kill period: weeks 104-107
(b) Includes animals killed in a moribund condition
(c) The results of the life table trend test are in the control column, and those of the life table exact pairwise comparisons with the controls are in the dosed columns.

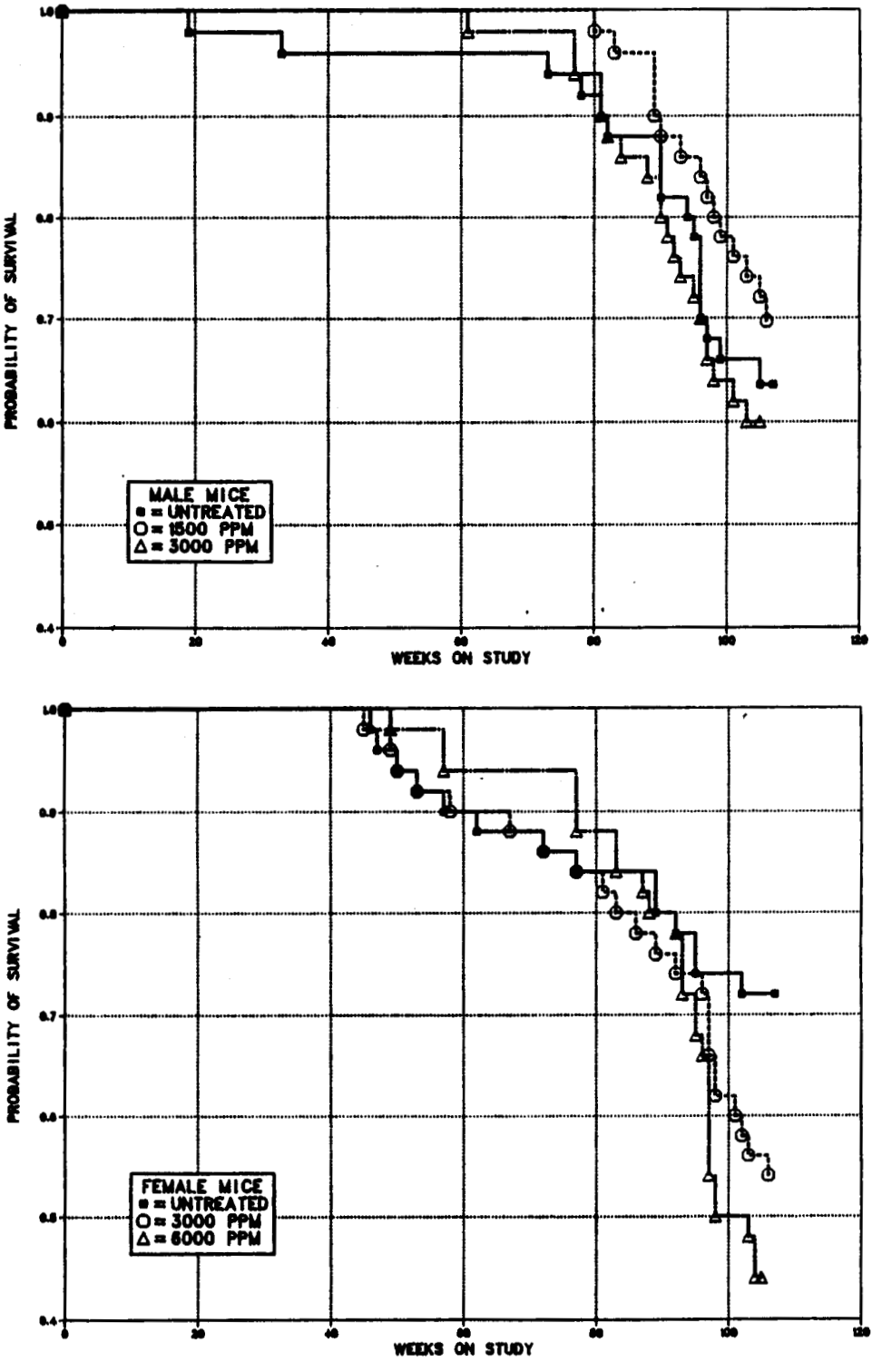

FIGURE 4. KAPLAN-MEIER SURVIVAL CURVES FOR MICE ADMINISTERED HC BLUE NO. 1 IN FEED FOR TWO YEARS

Liver: Hepatocellular adenomas in male mice and hepatocellular carcinomas in male and female mice occurred with significant positive trends (Table 18). The incidences of hepatocellular adenomas in low dose males and females and of hepatocellular carcinomas in dosed males and females were significantly greater than those in the controls. These hepatocellular tumors had the usual morphological pattern; that of carcinomas was trabecular.

TABLE 18. ANALYSIS OF LIVER TUMORS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1 (a)

	Control	Low Dose (b)	High Dose (b)
MALE		**1,500 ppm**	**3,000 ppm**
Hepatocellular Adenoma			
Overall Rates	4/50 (8%)	17/50 (34%)	10/50 (20%)
Adjusted Rates	12.1%	45.9%	30.5%
Terminal Rates	4/33 (12%)	17/37 (46%)	8/30 (27%)
Life Table Tests	P=0.046	P=0.003	P=0.051
Incidental Tumor Tests	P=0.052	P=0.003	P=0.062
Hepatocellular Carcinoma			
Overall Rates	11/50 (22%)	20/50 (40%)	30/50 (60%)
Adjusted Rates	25.4%	44.7%	67.2%
Terminal Rates	4/33 (12%)	13/37 (35%)	16/30 (53%)
Life Table Tests	P<0.001	P=0.107	P<0.001
Incidental Tumor Tests	P<0.001	P=0.040	P<0.001
Hepatocellular Adenoma or Carcinoma (c)			
Overall Rates	15/50 (30%)	31/50 (62%)	37/50 (74%)
Adjusted Rates	35.7%	70.0%	83.6%
Terminal Rates	8/33 (24%)	24/37 (65%)	23/30 (77%)
Life Table Tests	P<0.001	P=0.012	P<0.001
Incidental Tumor Tests	P<0.001	P=0.002	P<0.001
FEMALE		**3,000 ppm**	**6,000 ppm**
Hepatocellular Adenoma			
Overall Rates	2/50 (4%)	11/48 (23%)	4/49 (8%)
Adjusted Rates	5.6%	36.0%	15.2%
Terminal Rates	2/36 (6%)	9/28 (32%)	3/24 (13%)
Life Table Tests	P=0.127	P=0.002	P=0.190
Incidental Tumor Tests	P=0.259	P=0.004	P=0.276
Hepatocellular Carcinoma			
Overall Rates	1/50 (2%)	24/48 (50%)	47/49 (96%)
Adjusted Rates	2.5%	74.7%	100.0%
Terminal Rates	0/36 (0%)	20/28 (71%)	24/24 (100%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests	P<0.001	P<0.001	P<0.001
Hepatocellular Adenoma or Carcinoma (d)			
Overall Rates	3/50 (6%)	33/48 (69%)	47/49 (96%)
Adjusted Rates	7.9%	97.0%	100.0%
Terminal Rates	2/36 (6%)	27/28 (96%)	24/24 (100%)
Life Table Tests	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests	P<0.001	P<0.001	P<0.001

(a) The statistical analyses used are discussed in Chapter II (Statistical Methods) and Appendix E (footnotes).
(b) The equivalent dose in milligrams per kilograms per day is given in Body Weights and Clinical Signs (page 44) and Appendix L.
(c) Historical incidence at testing laboratory--mean: 32%; range: 24%-38%; historical incidence in NTP studies--mean: 31%; range: 16%-58%
(d) Historical incidence at testing laboratory--mean: 7%; range: 4%-18%; historical incidence in NTP studies--mean: 8%; range: 0%-20%

Hepatocellular carcinomas metastasized to the lungs in one control male, two low dose males, two high dose males, one control female, three low dose females, and three high dose females. An hepatocellular carcinoma metastasized to the kidney in another high dose female mouse.

There was no evidence of toxicologically significant nonneoplastic liver lesions induced by HC Blue No. 1 in either male or female mice.

Thyroid Gland: Follicular cell hyperplasia was observed at increased incidences in dosed male mice (Table 19). Pigmentation occurred at increased incidences in dosed male and female mice (male: control, 0/47; low dose, 49/49, 100%; high dose, 50/50, 100%; female: control, 0/47; low dose, 42/46, 83%; high dose, 44/44, 100%).

Follicular cell adenomas occurred in male mice with a significant positive trend, and the incidence in the high dose group was significantly greater than that in the controls (Table 19); no carcinomas were observed. The combined incidence of follicular cell adenomas or carcinomas in dosed female mice was lower than that in the controls (control, 3/47, 6%; low dose, 0/46; high dose, 2/44, 5%).

Integumentary System: Rhabdomyosarcomas of the skin occurred in male mice with a significant negative trend, but the combined incidence of this lesion in the skin or subcutaneous tissue was not statistically significant in dosed male mice.

Lung: Adenomatous hyperplasia was observed at increased incidences in all groups of mice (male: control, 22/50, 44%; low dose, 24/50, 48%; high dose, 25/50, 50%; female: control, 9/50, 18%; low dose, 13/48, 27%; high dose, 15/48, 32%). A positive Sendai titer was detected in sentinel animals at week 26.

Spleen: Hematopoiesis was seen at increased incidences in high dose female mice (control, 9/50, 18%; low dose, 10/47, 21%; high dose, 19/49, 39%) but not in dosed male mice (control, 8/50, 16%; low dose, 7/50, 14%; high dose, 7/49, 14%).

Reproductive System: Cystic disease of the mammary gland occurred at increased incidences in dosed female mice (control, 1/50, 2%; low dose, 5/48, 10%; high dose, 15/49, 31%).

Suppurative inflammation of the uterus, ovaries, or multiple organs was found in 7 control, 14 low dose, and 6 high dose female mice that died before the end of the study. This infection has been present in female $B6C3F_1$ mice at this and other laboratories. Both *Klebsiella oxytoca* and *K. pneumoniae* have been isolated from the ovarian abscesses; however, the exact etiology is not known.

TABLE 19. ANALYSIS OF THYROID GLAND LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Follicular Cell Hyperplasia			
Overall Rates	3/47 (6%)	7/49 (15%)	14/50 (28%)
Follicular Cell Adenoma (a)			
Overall Rates	0/47 (0%)	0/49 (0%)	5/50 (10%)
Adjusted Rates	0.0%	0.0%	16.7%
Terminal Rates	0/32 (0%)	0/37 (0%)	5/30 (17%)
Life Table Tests	P=0.004	(b)	P=0.027
Incidental Tumor Tests	P=0.004	(b)	P=0.027

(a) Historical incidence at testing laboratory--mean: 2%; range: 0%-6%; historical incidence in NTP studies--mean: 1%; range: 0%-6%
(b) *No P value is presented because no tumors were observed* in 1,500-ppm and control groups.

IV. DISCUSSION AND CONCLUSIONS

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Toxicology and carcinogenesis studies of HC Blue No. 1, a semipermanent hair dye, were conducted by administering the test chemical in the diet to groups of 50 male and 50 female F344/N rats and to groups of 50 male and 50 female B6C3F$_1$ mice for 103 weeks. Groups of 50 rats and 50 mice of each sex served as untreated controls. HC Blue No. 1 is absorbed through the skin of rats (strain unspecified) and rabbits in small (not quantitated) but detectable amounts, as evidenced by urinary and biliary excretion (Frenkel and Brody, 1973). Because a larger proportion of a dose would be absorbed through the gastrointestinal tract than through the skin, the dosed feed route of administration was selected for these studies to provide more systemic exposure of the chemical than would have been possible by dermal application.

The dietary concentrations of HC Blue No. 1 used in these 2-year studies (rats and male mice: 0, 1,500, and 3,000 ppm; female mice: 0, 3,000, and 6,000 ppm) were selected on the basis of survival, body weight gains, and histopathologic changes produced during 13-week studies in which dietary concentrations ranged from 0 to 12,500 ppm. The results of those studies showed that in both species the thyroid gland is a potential target organ. A brown-to-gold pigment was detected in the cytoplasm of thyroid epithelial cells in rats and mice of each sex. The pigment was not identified, and no other histopathologic lesions were observed in either species during these 90-day studies.

In the 2-year studies, the mean body weights of dosed rats were lower than those of the controls and the decreases were dose related. Female rats receiving the compound at the 3,000- ppm concentration had a mean body weight that was 12%-15% lower than that of the control group between weeks 50 and 104. Mean body weights of dosed mice were lower than those of controls, and females were more severely affected. Mean body weights of dosed male mice ranged from 89% to 103% that of controls. Mean body weights of low dose females were from 9% to 26% lower than that of controls, and those of high dose females were from 10% to 30% lower than that of controls. These body weight differences cannot be attributed to a reduction in feed consumption, as no consistent differences were observed among groups (Appendix L) and feed consumption data are considered to be generally useful but less than fully accurate.

The survival of dosed rats and of dosed male mice was not significantly different from that of controls. The survival of female mice dosed with HC Blue No. 1 at the 6,000-ppm concentration was reduced relative to that of the control group (24/50, 48%, vs 36/50, 72%). This reduced survival is not considered to be a compromising factor for the studies. Sixteen of the 26 early deaths in the high dose female mouse group occurred after week 91; therefore, most high dose females were at risk for the development of cancer. Further, 23/26 of the high dose female mice that died early had hepatocellular carcinomas. These early deaths were probably due to this hepatic lesion.

HC Blue No. 1 was carcinogenic in mice, causing increases in the incidences of hepatocellular carcinomas in both males and females. All 24 of the high dose female mice surviving to the end of the study had this lesion. Of the 49 high dose females that were examined microscopically, only one animal that died at week 49 and one that died at week 57 did not have hepatocellular carcinoma. The earliest carcinoma was diagnosed in a high dose animal that died during week 57. The only control female with an hepatocellular carcinoma died during week 92. The number of female mice in the 6,000-ppm group having hepatocellular carcinoma (47/49, 96%) represents one of the highest incidence rates seen in any chemically exposed group in the National Toxicology Program.

Significantly increased incidences of hepatocellular adenomas occurred in low dose mice (male: 1,500 ppm; female: 3,000 ppm). Hepatocellular carcinomas metastasized to the lungs in one control, two low dose, and two high dose males and in one control, three low dose, and three high dose females. In addition, a metastatic hepatocellular carcinoma was found in the kidney of one high dose female.

The administration of HC Blue No. 1 was associated with a marginally increased incidence of hepatocellular neoplasia in male rats, as shown by positive trends in the incidences of neoplastic nodules (control, 0/49; low dose, 0/50; high dose,

3/50) and of neoplastic nodules or hepatocellular carcinomas (control, 1/49; low dose, 0/50; high dose, 6/50). Pairwise comparisons did not reveal statistically significant differences between dosed and control groups (Table 8). The overall rate of hepatocellular carcinoma in the high dose male group (3/50) exceeds the mean historical rate for this tumor in untreated controls at this laboratory (12/438, 3%) or in the Carcinogenesis Program (96/2,306, 4%; Appendix F, Table F1). There was no evidence of an hepatocarcinogenic effect of HC Blue No. 1 in female rats.

Cytoplasmic pigmentation of the follicular epithelial cells of the thyroid gland occurred at incidences ranging from 83% to 100% in dosed rats and mice. None was observed in any control animal. This pigmentation also was detected during the 13-week studies in both species (rats and male mice dosed at 6,250 ppm or higher and female mice dosed at 12,500 ppm). The pigment was not identified. Since the pigment was not detected in the 13-week studies at the concentrations used in the 2-year experiments, its presence in the 2-year studies appears to be indicative of a cumulative effect, at least for periods longer than 13 weeks.

The administration of HC Blue No. 1 was associated with a significant positive trend in the incidence of thyroid gland follicular cell adenomas in male mice (control, 0/47; low dose, 0/49; high dose, 5/50), and the incidence in the high dose group was significantly increased ($P = 0.027$). In addition, a dose-related increase in hyperplasia of thyroid gland follicular cells (control, 3/47; low dose, 7/49; high dose, 14/50) was observed. The administration of HC Blue No. 1 was not associated with the production of proliferative lesions of the thyroid gland in male or female rats or in female mice. The proliferative changes in the thyroid glands of male mice were considered to be compound related.

Follicular cell pigmentation seems unrelated to production of thyroid gland proliferative changes. Pigmentation of tissues is commonly observed in studies of hair dye toxicity, and no consistent relationship between the presence of pigments per se and proliferation has been noted (C. Burnett, personal communication to NTP, 1984). Results from two other NTP studies of hair dye pigments, C.I. Disperse Blue 1 (tetraaminoanthraquinone) (NTP, 1985b) and HC Red No. 3 (1985c), indicate dose-related thyroid gland pigmentation without consistent hyperplasia of the thyroid gland.

Dose-related increases in lesions of the lung occurred in male and female rats. These changes--adenomatous hyperplasia, alveolar/ bronchiolar adenoma, and alveolar/bronchiolar carcinoma--represent a spectrum of proliferative lesions with no clear demarcation between stages. The lesions occurred in 5/50 control, 8/50 low dose, and 10/50 high dose males and in 3/50 control, 8/49 low dose, and 15/50 high dose females. The combined incidence of adenomas and carcinomas in high dose females is both significant and greater than concurrent and historical control rates (7/434, 2%, at the same laboratory; 27/2,354, 1.1%, throughout the Carcinogenesis Program; Appendix F, Table F2); the occurrence of adenomatous hyperplasia adds further support that these lesions are compound related.

The incidence of pulmonary neoplasms in dosed animals is not believed to have been significantly influenced by the Sendai infection that occurred during these studies. The NTP has compared the incidence of pulmonary neoplasms in Sendai-positive control animals with that in Sendai-negative controls. In studies completed before 1982, the incidence of positive Sendai reactions was 32% in rats and 25% in mice. No difference was found in the incidence of lung neoplasms in these control subgroups.

The pulmonary changes detected in female rats dosed with HC Blue No. 1 for 103 weeks are due to the administration of the chemical and are indicative of a carcinogenic effect. The marginally increased incidence of proliferative changes in male rats may have been related to HC Blue No. 1 administration. Bronchioles or alveoli of male and female mice administered 3,000 ppm HC Blue No. 1 in the 13-week studies contained unidentified yellow crystals; no compound-related changes were found in the lungs. The crystals were not seen in mice in the 2-year studies, and no evidence of pulmonary neoplasia was found in male or female mice.

Uterine stromal polyps occurred in female rats with a significant positive trend, and the

incidence in the high dose group was significantly greater (P=0.022) than that in the control group (control, 5/50; low dose, 9/50; high dose, 14/50). The incidence of this lesion in the untreated controls is lower than that observed historically at the same laboratory (66/438, 15%) and throughout the Carcinogenesis Program (424/2,318, 18%; Appendix F, Table F3). In addition, the incidence in the high dose group was within the range of incidences seen in untreated controls throughout the Carcinogenesis Program.

Uterine polyps generally represent benign tumors (Altman and Goodman, 1979; Baba and Von Haan, 1976; Burek, 1978); however, morphologic criteria have not been established to distinguish between small focal lesions that may represent hyperplasia and autonomous benign tumors. In the NTP, polypoid lesions of the uterine corpus are diagnosed as uterine polyps. Although this practice produces consistency throughout the laboratories, it may result in more lesions being diagnosed as polyps. In a review (Baba and Von Haan, 1976) of a series of chemically induced uterine tumors in rats, the tumors were found to be either epithelial (squamous cell carcinomas or adenocarcinomas) or mesenchymal (endometrial stromal sarcomas or sarcomas). Uterine polyps were not mentioned, and the NTP is not aware of any chemical reported to induce only uterine polyps. Thus, these uterine polyps were not considered to be related to the administration of HC Blue No. 1.

High dose male and low dose female rats exhibited increases in the incidences of retinopathy and cataracts. These lesions are believed to be related to cage placement relative to light source rather than to HC Blue No. 1 administration. This study was conducted before cages were routinely rotated during 2-year studies; therefore, the rats remained in their initially assigned cage positions for the full length of the study. High dose males and low dose females (the groups 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 were housed in the bottom rows. No relationship between cage position and tumor incidence was observed.

Zymbal gland carcinomas occurred in 3/50 (6%) control female rats. The incidence of these tumors in untreated females is 4/439 (0.9%) at this laboratory and 9/2,370 (0.4%) throughout the Carcinogenesis Program. The incidence in the present study is included in the laboratory and Program historical data (Appendix F, Table F11). Zymbal glands are examined microscopically only when a grossly observable lesion is detected in the external ear canal at necropsy. Thus, small tumors may go undetected. The actual incidence of this lesion in the present study and throughout the Carcinogenesis Program may be greater. The Zymbal glands from five control females and two high dose male rats were examined microscopically. Three control females and one high dose male had carcinomas. This lesion is frequently found in rats dosed with aromatic amines. The negative trend in female rats and the single carcinoma found in a high dose male rat are not considered to be due to administration of HC Blue No. 1.

Negative trends in the incidences of leukemia and pituitary gland adenomas in male rats and of pituitary gland adenomas or carcinomas and adrenal gland adenomas in female rats were not considered to be due to administration of HC Blue No. 1. Negative trends in the incidences of rhabdomyosarcomas in male mice were not considered to be the result of HC Blue No. 1 administration.

The carcinogenicity of HC Blue No. 1 in rats and mice is consistent with the mutagenicity of the same lot in Salmonella, positive results for HC Blue No. 1 in the L5178Y/TK$^{+/-}$ mouse lymphoma forward mutation assay, and the ability of this chemical to induce unscheduled DNA synthesis (UDS) in rat hepatocytes in vitro (Appendix M). These results contrast with the reported lack of genetic toxicity of a different lot of HC Blue No. 1 in eight of nine short-term tests (Darroudi et al., 1983; Loprieno et al., 1983; Shahin and Bugaut, 1983). The positive results in Salmonella and UDS assays produced by the lot used in the NTP studies and the negative results in the same two assays produced by the lot used by Shahin and Bugaut (1983) and Loprieno et al. (1983) suggest that the two lots are not identical and contain different components.

Frenkel and Brody (1973) reported that HC Blue No. 1 absorbed through the skin of rats and rabbits (or administered intraperitoneally or subcutaneously) is excreted unchanged in urine and bile. Although the dye administered by these routes may not undergo biotransformation, the earlier investigators did not demonstrate quantitative recovery of the administered doses. Therefore, the absence of metabolism of HC Blue No. 1 has not been conclusively demonstrated. In the present study, the excretion of a blue material in the urine provided evidence for absorption of the dye from the gastrointestinal tract; however, since the dye is blue, this observation cannot be taken to mean that any metabolism had occurred. No attempt was made to identify or quantitate the chemical(s) in the urine.

When administered orally, as in the present study, the dye is exposed to the bacterial flora of the gastrointestinal tract. Nitro reduction, N-demethylation, or N-dealkylation could be carried out by the anerobic flora of the intestinal tract. Any of these metabolic steps could result in the formation of a free aromatic amine that might be absorbed and then subjected to hepatic N-acetylation and hydroxylation (metabolic steps believed to be associated with the activation of carcinogenic aromatic amines). HC Blue No. 1 was negative in the in vivo mouse micronucleus test, in which chemicals are administered by intraperitoneal injection (Darroudi, 1983). This route of administration limits the amount of chemical available for metabolic reduction to a free aromatic amine by the intestinal flora.

Subsequent to the conduct of these studies, it was determined that the dye sample used for the 2-year studies contained approximately 45 ppm of nitrosamines. There were three discrete nitrosamines in the sample, and only one (N-nitrosodiethanolamine, 9 ppm) was identified. Based on the total nitrosamine content of the dye and the concentrations of dye in the diets, it is estimated that the high dose male and female rats and male mice received approximately 138 ppb of total nitrosamines and high dose female mice received approximately 276 ppb. These dietary concentrations correspond to approximately half the concentrations of nitrosamines received by rats and mice in the NTP studies of HC Blue No. 2 (NTP, 1985a). In the latter study, no compound-related neoplastic effects were observed in either sex or species. Therefore, it seems unlikely that the nitrosamines in the HC Blue No. 1 studies influenced the results of the present studies.

Conclusions: Under the conditions of these feed studies, there was *equivocal evidence of carcinogenicity** in male F344/N rats, since HC Blue No. 1 caused a marginal increase in the incidence of hepatocellular neoplastic nodules/carcinomas. For female F344/N rats, there was *some evidence of carcinogenicity* in that HC Blue No. 1 induced increased incidences of alveolar/bronchiolar neoplasms. There was *clear evidence of carcinogenicity* of HC Blue No. 1 for male and female B6C3F_1 mice as shown by increased incidences of hepatocellular carcinomas. The incidences of follicular cell adenomas of the thyroid gland were also increased in male mice receiving HC Blue No. 1.

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

V. REFERENCES

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. 1

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	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%)	
BASAL-CELL TUMOR		1 (2%)	1 (2%)
BASAL-CELL CARCINOMA			1 (2%)
SEBACEOUS ADENOCARCINOMA			1 (2%)
KERATOACANTHOMA	1 (2%)	1 (2%)	1 (2%)
NEURILEMOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROMA	2 (4%)	4 (8%)	
FIBROSARCOMA		1 (2%)	1 (2%)
NEURILEMOMA	1 (2%)	2 (4%)	
RESPIRATORY SYSTEM			
*NASAL CAVITY	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
#LUNG	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		2 (4%)	1 (2%)
C-CELL CARCINOMA, METASTATIC	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MONOCYTIC LEUKEMIA	13 (26%)	8 (16%)	2 (4%)
#LIVER	(49)	(50)	(50)
LEUKEMIA, NOS			1 (2%)
MONOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
#SPLEEN	(50)	(49)	(50)
HEMANGIOSARCOMA	2 (4%)		
#STOMACH	(50)	(49)	(50)
HEMANGIOSARCOMA		1 (2%)	
*MESENTERY	(50)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
#TESTIS	(50)	(50)	(50)
HEMANGIOSARCOMA		1 (2%)	
DIGESTIVE SYSTEM			
*TONGUE	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
#LIVER	(49)	(50)	(50)
NEOPLASTIC NODULE			3 (6%)
HEPATOCELLULAR CARCINOMA	1 (2%)		3 (6%)
#PANCREAS	(49)	(49)	(49)
ACINAR-CELL ADENOMA		1 (2%)	
#FORESTOMACH	(50)	(49)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
TUBULAR CELL ADENOCARCINOMA			1 (2%)
#KIDNEY/CORTEX	(50)	(50)	(50)
ADENOMA, NOS	1 (2%)		
#KIDNEY/PELVIS	(50)	(50)	(50)
TRANSITIONAL CELL PAPILLOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(49)	(47)
CARCINOMA, NOS	2 (4%)	2 (4%)	1 (2%)
ADENOMA, NOS	9 (18%)	7 (14%)	3 (6%)
ACIDOPHIL ADENOMA		1 (2%)	
#ADRENAL	(49)	(49)	(50)
CORTICAL ADENOMA	1 (2%)	1 (2%)	
PHEOCHROMOCYTOMA	20 (41%)	17 (35%)	17 (34%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (2%)	1 (2%)	2 (4%)
GANGLIONEUROMA	1 (2%)		
#THYROID	(50)	(48)	(50)
FOLLICULAR-CELL ADENOMA		2 (4%)	
C-CELL ADENOMA	9 (18%)	4 (8%)	5 (10%)
C-CELL CARCINOMA	1 (2%)	2 (4%)	2 (4%)
#PANCREATIC ISLETS	(49)	(49)	(49)
ISLET-CELL ADENOMA	5 (10%)	4 (8%)	1 (2%)
ISLET-CELL CARCINOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
FIBROADENOMA		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	2 (4%)	1 (2%)	2 (4%)
ADENOMA, NOS	2 (4%)	2 (4%)	2 (4%)
*SEMINAL VESICLE	(50)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
#TESTIS	(50)	(50)	(50)
INTERSTITIAL-CELL TUMOR	45 (90%)	44 (88%)	44 (88%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(49)	(50)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
#BRAIN	(50)	(49)	(50)
ASTROCYTOMA		1 (2%)	
SPECIAL SENSE ORGANS			
*EXTERNAL EAR	(50)	(50)	(50)
NEUROFIBROSARCOMA	1 (2%)		
*ZYMBAL GLAND	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*TUNICA VAGINALIS	(50)	(50)	(50)
MESOTHELIOMA, NOS		1 (2%)	1 (2%)

TABLE A1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)	1 (2%)
MESOTHELIOMA, NOS		1 (2%)	
MESOTHELIOMA, MALIGNANT		2 (4%)	
TAIL			
SQUAMOUS CELL PAPILLOMA		1	
KERATOACANTHOMA		1	
LEG			
FIBROSARCOMA		1	
# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY			
* NUMBER OF ANIMALS NECROPSIED			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	9	7	7
MORIBUND SACRIFICE	4	11	2
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	37	32	41
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	49	49	50
TOTAL PRIMARY TUMORS	124	125	105
TOTAL ANIMALS WITH BENIGN TUMORS	47	47	49
TOTAL BENIGN TUMORS	99	97	78
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	22	20
TOTAL MALIGNANT TUMORS	25	26	23
TOTAL ANIMALS WITH SECONDARY TUMORS##	2	1	
TOTAL SECONDARY TUMORS	2	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		2	4
TOTAL UNCERTAIN TUMORS		2	4
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. 1

	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%)		
KERATOACANTHOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROMA	2 (4%)	1 (2%)	4 (8%)
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
SQUAMOUS CELL CARCINOMA, METASTA	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	2 (4%)	3 (6%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG. LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
MONOCYTIC LEUKEMIA	4 (8%)	3 (6%)	5 (10%)
#THYMUS	(48)	(50)	(49)
THYMOMA			1 (2%)
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
#ENDOCARDIUM	(50)	(50)	(50)
ANITSCHKOW-CELL SARCOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
NEOPLASTIC NODULE		1 (2%)	
HEPATOCELLULAR CARCINOMA			1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(49)	(49)
CARCINOMA, NOS	6 (12%)	2 (4%)	7 (14%)
ADENOMA, NOS	25 (50%)	21 (43%)	18 (37%)
#ADRENAL	(50)	(50)	(50)
CORTICAL ADENOMA	5 (10%)	2 (4%)	
PHEOCHROMOCYTOMA	8 (16%)	2 (4%)	3 (6%)
#THYROID	(49)	(50)	(50)
FOLLICULAR-CELL ADENOMA	1 (2%)	2 (4%)	
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL ADENOMA	5 (10%)	2 (4%)	
C-CELL CARCINOMA	3 (6%)	4 (8%)	2 (4%)
#PARATHYROID	(48)	(49)	(48)
ADENOMA, NOS			1 (2%)

TABLE A2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#PANCREATIC ISLETS	(50)	(50)	(50)
ISLET-CELL ADENOMA		1 (2%)	
ISLET-CELL CARCINOMA	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS	3 (6%)		
MIXED TUMOR, MALIGNANT		1 (2%)	
FIBROADENOMA	13 (26%)	12 (24%)	8 (16%)
*CLITORAL GLAND	(50)	(50)	(50)
CARCINOMA, NOS	3 (6%)	5 (10%)	3 (6%)
SQUAMOUS CELL CARCINOMA		1 (2%)	
ADENOMA, NOS	1 (2%)		1 (2%)
#UTERUS	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
ENDOMETRIAL STROMAL POLYP	5 (10%)	9 (18%)	14 (28%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	1 (2%)	1 (2%)
#CERVIX UTERI	(50)	(50)	(50)
LEIOMYOMA		1 (2%)	
NERVOUS SYSTEM			
#CEREBRUM	(50)	(50)	(49)
GLIOMA, NOS		1 (2%)	
#BRAIN	(50)	(50)	(49)
CARCINOMA, NOS, INVASIVE			1 (2%)
SPECIAL SENSE ORGANS			
*ZYMBAL GLAND	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
ADENOSQUAMOUS CARCINOMA	2 (4%)		
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(50)	(50)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
CARCINOSARCOMA			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MUCINOUS ADENOCARCINOMA		1 (2%)	
FIBROSARCOMA		1 (2%)	
MIXED TUMOR, METASTATIC		1 (2%)	
CARCINOSARCOMA		1 (2%)	
MESOTHELIOMA, MALIGNANT			1 (2%)

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. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	4	6	4
MORIBUND SACRIFICE	6	10	6
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	40	34	40
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	45	43	45
TOTAL PRIMARY TUMORS	93	82	83
TOTAL ANIMALS WITH BENIGN TUMORS	37	35	37
TOTAL BENIGN TUMORS	68	55	53
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	23	25
TOTAL MALIGNANT TUMORS	25	26	30
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	1	1
TOTAL SECONDARY TUMORS	1	1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: 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	053	053	054	054	054	046	046	046	046	046	046	046	046	046	052	051	054	046	046	046	046	046	046	047	047
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
KERATOACANTHOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA		X				X													X						
NEURILEMOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA															X										
ALVEOLAR/BRONCHIOLAR ADENOMA											X														
C-CELL CARCINOMA, METASTATIC																									
TRACHEA	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NASAL CAVITY	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
SQUAMOUS CELL CARCINOMA																									
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																	X								
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA								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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS																									
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
CARCINOMA,NOS			X																X		X				X
ADENOMA, NOS	X																								
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA																									
PHEOCHROMOCYTOMA	X	X			X	X	X			X	X			X	X			X		X		X	X		
PHEOCHROMOCYTOMA, MALIGNANT														X											
GANGLIONEUROMA																									
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-CELL ADENOMA		X	X											X						X					
C-CELL CARCINOMA																									
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA													X						X						X
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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																			X					
ADENOMA, NOS																									X
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL CARCINOMA, METASTAT																									
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	N
NEUROFIBROSARCOMA																									
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
MONOCYTIC LEUKEMIA		X						X			X			X						X					

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

ANIMAL NUMBER	026	029	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	107	107	107	107	107	107	107	095	087	107	097	097	087	107	097	107	088	068	083	097	107	097	107	107	085	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
KERATOACANTHOMA																									X	1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROMA																										2
NEURILEMOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SQUAMOUS CELL CARCINOMA																										1
ALVEOLAR/BRONCHIOLAR ADENOMA																				X						2
C-CELL CARCINOMA, METASTATIC																									X	1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NASAL CAVITY	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	+	N	N	N	N	N	N	50
SQUAMOUS CELL CARCINOMA																			X							1
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMANGIOSARCOMA																			X							2
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR CARCINOMA																										1
BILE DUCT	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
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	50
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOMA, NOS									X																	1
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARCINOMA,NOS														X												2
ADENOMA, NOS					X	X			X				X												X	9
ADRENAL	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CORTICAL ADENOMA			X																							1
PHEOCHROMOCYTOMA		X	X			X		X												X	X			X		20
PHEOCHROMOCYTOMA, MALIGNANT												X														1
GANGLIONEUROMA																										1
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA			X		X											X			X			X				9
C-CELL CARCINOMA																									X	1
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	46
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ISLET-CELL ADENOMA											X	X														5
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	N	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	50
CARCINOMA,NOS													X													2
ADENOMA, NOS																										2
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SQUAMOUS CELL CARCINOMA, METASTAT																			X							1
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	50
NEUROFIBROSARCOMA								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	50
MONOCYTIC LEUKEMIA	X		X					X	X	X		X		X			X									13

* ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: 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	101	106	106	053	094	097	104	106	106	104	097	106	106	099	102	106	106	093	102	106	106	106	106	106	095
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA																									
BASAL-CELL TUMOR																									
KERATOACANTHOMA							X													X					
NEURILEMOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA					X																				
FIBROSARCOMA											X														X
NEURILEMOMA								X											X						
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA		X																							
ALVEOLAR/BRONCHIOLAR CARCINOMA												X													
TRACHEA	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MONOCYTIC 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	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ACINAR-CELL ADENOMA												X													
ESOPHAGUS	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA																						X			
SMALL INTESTINE	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA,NOS																									
ADENOMA, NOS					X					X								X				X			
ACIDOPHIL ADENOMA		X																							
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA			X																						
PHEOCHROMOCYTOMA			X						X			X	X		X	X	X	X		X		X			
PHEOCHROMOCYTOMA, MALIGNANT																									
THYROID	+	+	+	-	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																									
C-CELL ADENOMA		X										X							X						
C-CELL CARCINOMA																									
PARATHYROID	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
PANCREATIC ISLETS	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA								X	X																
ISLET-CELL CARCINOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+
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
HEMANGIOSARCOMA																									
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SEMINAL VESICLE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA, NOS, METASTATIC						X																			
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																			
ADENOMA, NOS																		X							
NERVOUS SYSTEM																									
BRAIN	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ASTROCYTOMA																				X					
BODY CAVITIES																									
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
SARCOMA, NOS																								X	
MESOTHELIOMA, NOS																	X								
MESOTHELIOMA, MALIGNANT					X								X										X		
MONOCYTIC LEUKEMIA																									
TAIL																									
SQUAMOUS CELL PAPILLOMA																							X		
KERATOACANTHOMA																									
LEG NOS																									
FIBROSARCOMA											X														

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

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL TISSUES TUMORS
INTEGUMENTARY SYSTEM	
SKIN	50
SQUAMOUS CELL PAPILLOMA	1
BASAL-CELL TUMOR	1
KERATOACANTHOMA	1
NEURILEMOMA	1
SUBCUTANEOUS TISSUE	50
FIBROMA	4
FIBROSARCOMA	1
NEURILEMOMA	2
RESPIRATORY SYSTEM	
LUNGS AND BRONCHI	50
ALVEOLAR/BRONCHIOLAR ADENOMA	1
ALVEOLAR/BRONCHIOLAR CARCINOMA	2
TRACHEA	49
HEMATOPOIETIC SYSTEM	
BONE MARROW	50
SPLEEN	49
LYMPH NODES	49
THYMUS	48
CIRCULATORY SYSTEM	
HEART	50
DIGESTIVE SYSTEM	
SALIVARY GLAND	50
LIVER	50
MONOCYTIC LEUKEMIA	1
BILE DUCT	50
GALLBLADDER & COMMON BILE DUCT	50
PANCREAS	49
ACINAR-CELL ADENOMA	1
ESOPHAGUS	49
STOMACH	49
HEMANGIOSARCOMA	1
SMALL INTESTINE	44
LARGE INTESTINE	49
URINARY SYSTEM	
KIDNEY	50
URINARY BLADDER	49
ENDOCRINE SYSTEM	
PITUITARY	49
CARCINOMA, NOS	2
ADENOMA, NOS	7
ACIDOPHIL ADENOMA	1
ADRENAL	49
CORTICAL ADENOMA	1
PHEOCHROMOCYTOMA	17
PHEOCHROMOCYTOMA, MALIGNANT	1
THYROID	48
FOLLICULAR-CELL ADENOMA	2
C-CELL ADENOMA	4
C-CELL CARCINOMA	2
PARATHYROID	42
PANCREATIC ISLETS	49
ISLET-CELL ADENOMA	4
ISLET-CELL CARCINOMA	1
REPRODUCTIVE SYSTEM	
MAMMARY GLAND	50
FIBROADENOMA	1
TESTIS	50
INTERSTITIAL-CELL TUMOR	44
HEMANGIOSARCOMA	1
PROSTATE	50
SEMINAL VESICLE	50
CARCINOMA, NOS, METASTATIC	1
PREPUTIAL/CLITORAL GLAND	50
CARCINOMA, NOS	1
ADENOMA, NOS	2
NERVOUS SYSTEM	
BRAIN	49
ASTROCYTOMA	1
BODY CAVITIES	
TUNICA VAGINALIS	50
MESOTHELIOMA, NOS	1
ALL OTHER SYSTEMS	
MULTIPLE ORGANS NOS	50
SARCOMA, NOS	1
MESOTHELIOMA, NOS	1
MESOTHELIOMA, MALIGNANT	2
MONOCYTIC LEUKEMIA	8
TAIL	1
SQUAMOUS CELL PAPILLOMA	1
KERATOACANTHOMA	1
LEG NOS	1
FIBROSARCOMA	1

* ANIMALS NECROPSIED

TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: HIGH DOSE

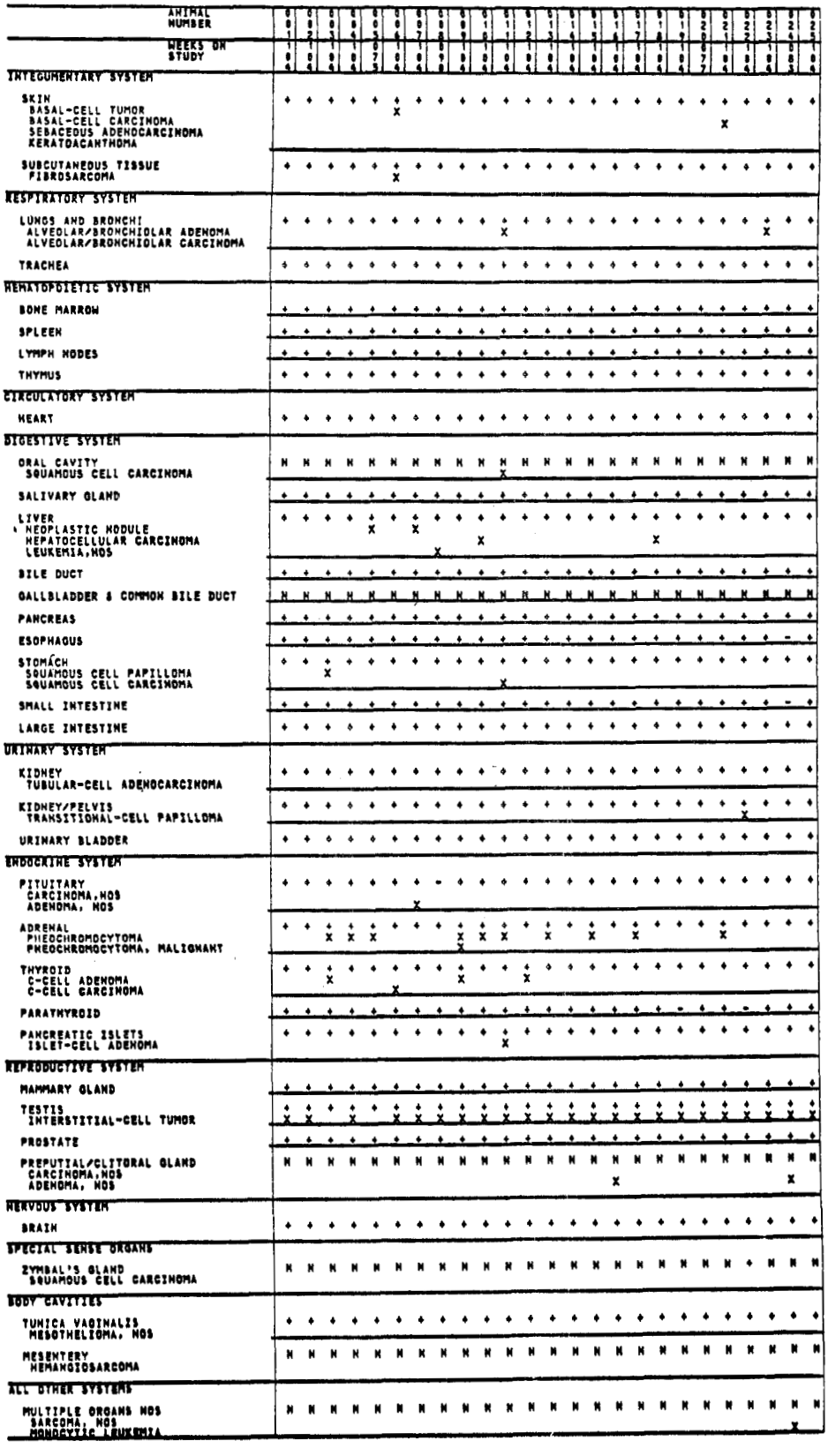


TABLE A3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE RATS: HIGH DOSE (Continued)

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	0 5	0 5	7 7	0 5	0 5	0 4	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	0 5	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
BASAL-CELL TUMOR																										1
BASAL-CELL CARCINOMA																										1
SEBACEOUS ADENOCARCINOMA														X												1
KERATOACANTHOMA										X																1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROSARCOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA														X												2
ALVEOLAR/BRONCHIOLAR CARCINOMA																										1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
ORAL CAVITY	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	50
SQUAMOUS CELL CARCINOMA																										1
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR NODULE												X														3
HEPATOCELLULAR CARCINOMA							X																			3
LEUKEMIA,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	N	50
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	47
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SQUAMOUS CELL PAPILLOMA																										1
SQUAMOUS CELL CARCINOMA																										1
SMALL INTESTINE	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	47
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TUBULAR-CELL ADENOCARCINOMA							X																			1
KIDNEY/PELVIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
TRANSITIONAL-CELL PAPILLOMA																										1
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	47
CARCINOMA,NOS								X		X										X						1
ADENOMA, NOS																										3
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA	X						X				X		X								X			X	X	17
PHEOCHROMOCYTOMA, MALIGNANT				X																						2
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-CELL ADENOMA					X								X													3
C-CELL CARCINOMA													X													2
PARATHYROID	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	49
ISLET-CELL ADENOMA																										1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	X	44
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	50
CARCINOMA,NOS											X										X					2
ADENOMA, NOS																										2
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
ZYMBAL'S 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	50
SQUAMOUS CELL CARCINOMA			X																							1
BODY CAVITIES																										
TUNICA VAGINALIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MESOTHELIOMA, NOS							X																			1
MESENTERY	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	50
HEMANGIOSARCOMA																				N 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	50
SARCOMA, NOS																								X		1
MONOCYTIC LEUKEMIA																							X			2

N ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: UNTREATED CONTROL

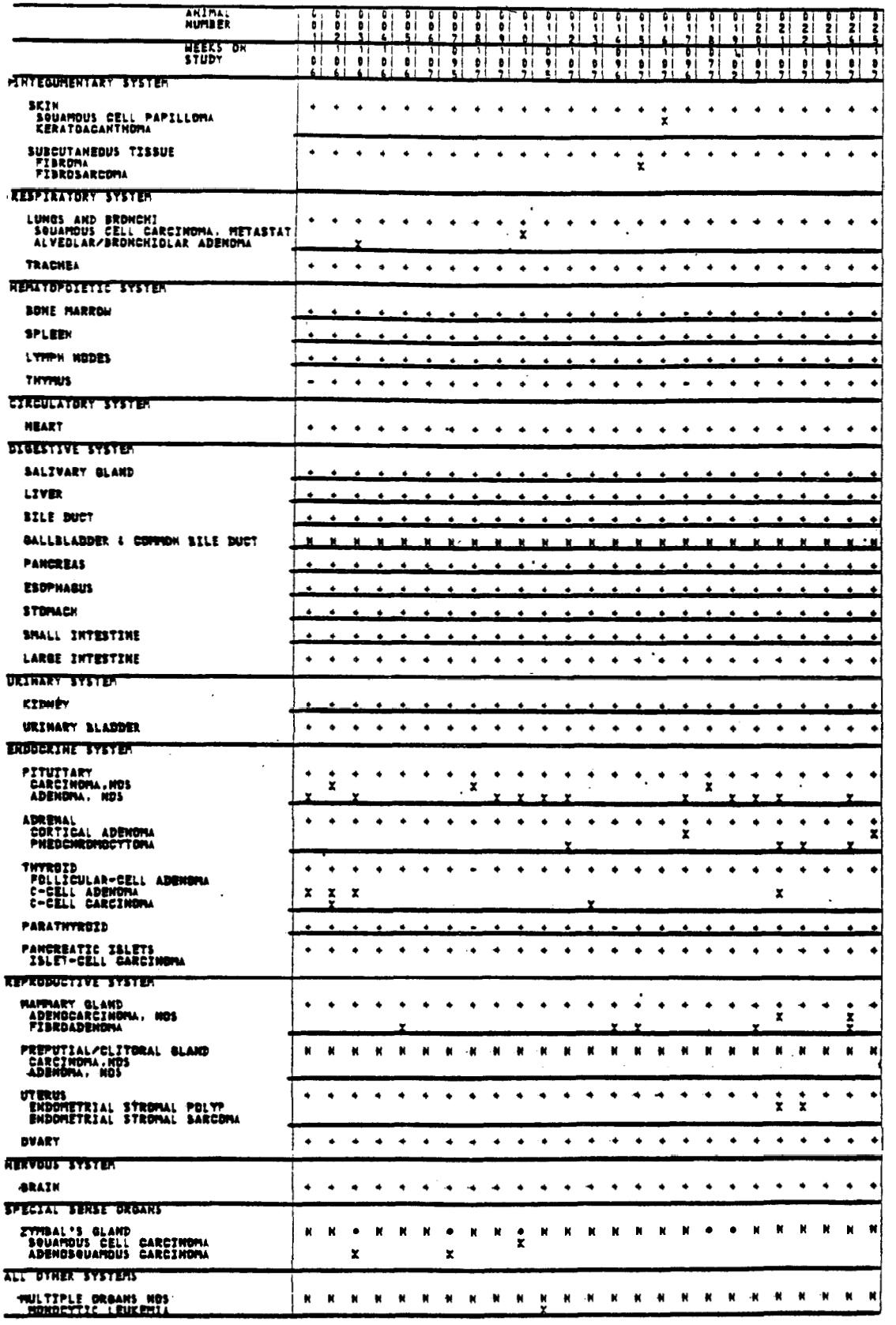


TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS: UNTREATED CONTROL (Continued)

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	107	107	107	107	107	107	107	107	107	076	107	107	107	107	107	107	107	108	107	107	107	107	107	112	107	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
SQUAMOUS CELL PAPILLOMA																										1
KERATOACANTHOMA							X																			1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
FIBROMA		X																								2
FIBROSARCOMA																				X						1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SQUAMOUS CELL CARCINOMA, METASTAT																										1
ALVEOLAR/BRONCHIOLAR ADENOMA																										1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	49
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CARCINOMA,NOS														X		X								X		6
ADENOMA, NOS	X	X	X		X	X			X	X		X	X		X			X	X			X	X			25
ADRENAL	+	•	•	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CORTICAL ADENOMA							X													X					X	5
PHEOCHROMOCYTOMA	X											X						X						X		5
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
FOLLICULAR-CELL ADENOMA	X																									1
C-CELL ADENOMA																									X	5
C-CELL CARCINOMA					X																					3
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL CARCINOMA														X												1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
ADENOCARCINOMA, NOS													X													3
FIBROADENOMA						X	X		X		X	X										X	X	X		13
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		X	X					3
ADENOMA, NOS													X													1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOMETRIAL STROMAL POLYP						X		X				X														5
ENDOMETRIAL STROMAL SARCOMA																						X				1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPECIAL SENSE ORGANS																										
ZYMBAL'S 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	50M
SQUAMOUS CELL CARCINOMA																										1
ADENOSQUAMOUS CARCINOMA																										2
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
MONOCYTIC LEUKEMIA	X	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. 1: 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	106	061	106	100	061	093	061	061	086	061	041	061	061	061	061	061	061	089	053	061	051	061	061	055	106
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROMA								X																	
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																									
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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CORTICAL ADENOMA		X																							
PHEOCHROMOCYTOMA													X												
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																									
C-CELL ADENOMA																							X		
C-CELL CARCINOMA	X												X												
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MIXED TUMOR, MALIGNANT											X														
FIBROADENOMA		X			X			X	X		X						X	X		X	X			
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		X									X		
SQUAMOUS CELL CARCINOMA																						X			
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS				X																					
LEIOMYOMA																									
ENDOMETRIAL STROMAL POLYP						X											X	X		X					
ENDOMETRIAL STROMAL SARCOMA																								X	
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GLIOMA, NOS						X																			
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
MUCINOUS ADENOCARCINOMA									X																
FIBROSARCOMA																		X							
MIXED TUMOR, METASTATIC											X														
CARCINOSARCOMA																									
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																									
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									
MONOCYTIC LEUKEMIA													X							X					

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

ANIMAL NUMBER	0216	0227	0228	0229	0310	0311	0312	0313	0314	0315	0316	0317	0318	0319	0320	0321	0322	0323	0324	0325	0326	0327	0328	0329	0330	TOTAL
WEEKS ON STUDY	106	106	078	106	090	077	105	106	106	098	106	106	106	105	106	106	106	105	104	106	106	106	091	106	081	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
FIBROMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ALVEOLAR/BRONCHIOLAR ADENOMA																								X		2
ALVEOLAR/BRONCHIOLAR CARCINOMA																				X						1
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NEOPLASTIC NODULE																									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	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	48
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
CARCINOMA,NOS																		X								2
ADENOMA, NOS	X	X		X		X	X							X	X		X			X	X			X		21
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
CORTICAL ADENOMA																								+		2
PHEOCHROMOCYTOMA														X										X		2
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL ADENOMA					X	X																				2
C-CELL ADENOMA																								X		2
C-CELL CARCINOMA							X															X				4
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA													X													1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
MIXED TUMOR, MALIGNANT																										1
FIBROADENOMA								X											X	X						12
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						X		5
SQUAMOUS CELL CARCINOMA																										1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOCARCINOMA, NOS																										1
LEIOMYOMA														X												1
ENDOMETRIAL STROMAL POLYP					X						X		X	X										X		9
ENDOMETRIAL STROMAL SARCOMA																										1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GLIOMA, NOS																										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
MUCINOUS ADENOCARCINOMA																										1
FIBROSARCOMA																										1
MIXED TUMOR, METASTATIC																										1
CARCINOSARCOMA								X																		1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																				X						1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																							X			1
MONOCYTIC LEUKEMIA					X																					1

M ANIMALS NECROPSIED

TABLE A4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: HIGH DOSE

ANIMAL NUMBER	0001	0002	0003	0004	0005	0006	0007	0008	0009	0010	0011	0012	0013	0014	0015	0016	0017	0018	0019	0020	0021	0022	0023	0024	0025
WEEKS ON STUDY	104	104	104	104	104	104	104	104	104	088	104	104	104	105	105	105	081	105	094	105	105	105	085	105	105
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	•	+	+	+	+	+	+	+	+
FIBROMA																									
HEMANGIOSARCOMA			X							X		X													
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ALVEOLAR/BRONCHIOLAR ADENOMA								X																	
ALVEOLAR/BRONCHIOLAR CARCINOMA		X												X											
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMOMA																									
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ANITSCHKOW-CELL SARCOMA								X																	
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA																									
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			X					X																	X
ADENOMA, NOS					X	X	X		X		X					X		X	X		X	X			
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA													X								X				
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL CARCINOMA		X																							
C-CELL CARCINOMA																									
PARATHYROID	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
ADENOMA, NOS																X									
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL CARCINOMA																									
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROADENOMA						X						X				X					X				
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																									
ADENOMA, NOS																									
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOMETRIAL STROMAL POLYP							X		X								X		X		X		X		
ENDOMETRIAL STROMAL SARCOMA																									
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
CARCINOMA, NOS, INVASIVE																									X
BODY CAVITIES																									
MEDIASTINUM	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
CARCINOSARCOMA																						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
MESOTHELIOMA, MALIGNANT																									
MALIGNANT LYMPHOMA, MIXED TYPE																									
MONOCYTIC LEUKEMIA													X						X						

+: TISSUE EXAMINED MICROSCOPICALLY
-: REQUIRED TISSUE NOT EXAMINED MICROSCOPICALLY
X: TUMOR INCIDENCE
N: NECROPSY, NO AUTOLYSIS, NO MICROSCOPIC EXAMINATION
$: 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: HIGH DOSE (Continued)

ANIMAL NUMBER	0262	0271	0281	0291	0301	0311	0321	0331	0341	0351	0361	0371	0381	0391	0401	0411	0421	0431	0441	0451	0461	0471	0481	0491	0501	TOTAL
WEEKS ON STUDY	105	051	099	055	055	055	051	031	093	055	055	051	055	051	055	051	055	055	055	055	055	096	105	055	055	TISSUES TUMORS
INTEGUMENTARY SYSTEM																										
SUBCUTANEOUS TISSUE	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
FIBROMA			X																							4
HEMANGIOSARCOMA																						X				1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ALVEOLAR/BRONCHIOLAR ADENOMA															X	X										3
ALVEOLAR/BRONCHIOLAR CARCINOMA			X															X								4
TRACHEA	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMOMA												X														1
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ANITSCHKOW-CELL SARCOMA																										1
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA																										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	50N
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SMALL INTESTINE	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
CARCINOMA,NOS				X	X												X								X	7
ADENOMA, NOS	X	X	X									X		X					X	X	X					18
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA				X																						5
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL CARCINOMA																										1
C-CELL CARCINOMA																							X	X		2
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ADENOMA, NOS																										1
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL CARCINOMA																					X					1
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50N
FIBROADENOMA					X								X							X	X					3
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	50N
CARCINOMA,NOS	X			X											X						X					3
ADENOMA, NOS																										1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOMETRIAL STROMAL POLYP									X	X	X	X			X		X							X	X	14
ENDOMETRIAL STROMAL SARCOMA				X																						1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
CARCINOMA, NOS, INVASIVE																										1
BODY CAVITIES																										
MEDIASTINUM	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
CARCINOSARCOMA																										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
MESOTHELIOMA, MALIGNANT						X																				1
MALIGNANT LYMPHOMA, MIXED TYPE																	X									1
MONOCYTIC LEUKEMIA								X	X					X												5

N ANIMALS NECROPSIED

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	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			
*EAR	(50)	(50)	(50)
FIBROUS HISTIOCYTOMA, MALIGNANT		1 (2%)	
*SKIN	(50)	(50)	(50)
SARCOMA, NOS	1 (2%)	2 (4%)	1 (2%)
FIBROMA	2 (4%)	7 (14%)	1 (2%)
FIBROSARCOMA	2 (4%)	2 (4%)	
RHABDOMYOSARCOMA	3 (6%)	1 (2%)	
NEUROFIBROMA	1 (2%)	1 (2%)	1 (2%)
NEUROFIBROSARCOMA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
SARCOMA, NOS		1 (2%)	
FIBROMA	1 (2%)		
FIBROSARCOMA	1 (2%)	2 (4%)	1 (2%)
LEIOMYOSARCOMA		1 (2%)	
RHABDOMYOSARCOMA	1 (2%)	1 (2%)	1 (2%)
FIBROADENOMA		1 (2%)	
NEUROFIBROSARCOMA	1 (2%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	2 (4%)	2 (4%)
ALVEOLAR/BRONCHIOLAR ADENOMA	3 (6%)	5 (10%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	1 (2%)	1 (2%)
ACINAR-CELL CARCINOMA, METASTATI	1 (2%)		
FIBROSARCOMA, METASTATIC	1 (2%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		1 (2%)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
#SPLEEN	(50)	(50)	(49)
MALIGNANT LYMPHOMA, MIXED TYPE	1 (2%)		
#AXILLARY LYMPH NODE	(49)	(50)	(50)
FIBROSARCOMA, METASTATIC		1 (2%)	
#LIVER	(50)	(50)	(50)
MALIG. LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
#THYMUS	(36)	(41)	(37)
THYMOMA	1 (3%)		
CIRCULATORY SYSTEM			
*LEG	(50)	(50)	(50)
HEMANGIOSARCOMA			1 (2%)
#BONE MARROW	(50)	(48)	(50)
HEMANGIOSARCOMA		1 (2%)	
#SPLEEN	(50)	(50)	(49)
HEMANGIOSARCOMA			1 (2%)

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(50)	(50)	(50)
HEPATOCELLULAR ADENOMA	4 (8%)	17 (34%)	10 (20%)
HEPATOCELLULAR CARCINOMA	11 (22%)	20 (40%)	30 (60%)
MIXED HEPATO/CHOLANGIO CARCINOMA	1 (2%)		
ACINAR-CELL CARCINOMA, INVASIVE	1 (2%)		
#PANCREAS	(50)	(48)	(49)
ACINAR-CELL CARCINOMA	1 (2%)		
#FORESTOMACH	(49)	(49)	(49)
SQUAMOUS CELL PAPILLOMA		1 (2%)	
#S. INTESTINE/MUCOSA	(46)	(46)	(47)
ADENOCARCINOMA, NOS			1 (2%)
#DUODENAL MUCOSA	(46)	(46)	(47)
ADENOMA, NOS		1 (2%)	
#JEJUNUM	(46)	(46)	(47)
ADENOMATOUS POLYP, NOS	1 (2%)		
*RECTUM	(50)	(50)	(50)
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(46)	(46)
ADENOMA, NOS			1 (2%)
#ADRENAL	(49)	(48)	(50)
PHEOCHROMOCYTOMA	2 (4%)	7 (15%)	1 (2%)
#ADRENAL/CAPSULE	(49)	(48)	(50)
CARCINOMA, NOS		1 (2%)	
ADENOMA, NOS	2 (4%)	1 (2%)	
#THYROID	(47)	(49)	(50)
FOLLICULAR-CELL ADENOMA			5 (10%)
#PANCREATIC ISLETS	(50)	(48)	(49)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*SCROTUM	(50)	(50)	(50)
NEUROFIBROSARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(50)	(50)
ADENOMA, NOS	2 (4%)	2 (4%)	1 (2%)
CYSTADENOMA, NOS	2 (4%)		
*ZYMBAL GLAND	(50)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

TABLE B1. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
CARCINOMA, NOS	1 (2%)		
MIXED HEPATO/CHOLANGIOCA, INVASI	1 (2%)		
MIXED HEPATO/CHOLANGIOCA, METAST	1 (2%)		
MESOTHELIOMA, MALIGNANT	1 (2%)		
NEUROFIBROSARCOMA		1 (2%)	
LUMBAR REGION			
FIBROSARCOMA			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	10	10
MORIBUND SACRIFICE	8	5	10
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	32	35	30
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
OTHER CASES			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	36	44	43
TOTAL PRIMARY TUMORS	56	80	64
TOTAL ANIMALS WITH BENIGN TUMORS	18	29	17
TOTAL BENIGN TUMORS	22	43	22
TOTAL ANIMALS WITH MALIGNANT TUMORS	22	34	37
TOTAL MALIGNANT TUMORS	34	37	42
TOTAL ANIMALS WITH SECONDARY TUMORS##	4	4	2
TOTAL SECONDARY TUMORS	6	4	2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(48)	(49)
SQUAMOUS CELL PAPILLOMA			1 (2%)
SEBACEOUS ADENOMA	1 (2%)		
FIBROSARCOMA	1 (2%)		1 (2%)
*SUBCUT TISSUE	(50)	(48)	(49)
NEURILEMOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(48)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	3 (6%)	3 (6%)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (4%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (4%)		1 (2%)
OSTEOSARCOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)	3 (6%)	
MALIG. LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	2 (4%)	1 (2%)
LYMPHOCYTIC LEUKEMIA		1 (2%)	4 (8%)
*ABDOMINAL CAVITY	(50)	(48)	(49)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
*SKIN	(50)	(48)	(49)
MAST-CELL TUMOR	1 (2%)		
#SPLEEN	(50)	(47)	(49)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)
#THYMUS	(41)	(39)	(29)
MALIG. LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(48)	(49)
HEMANGIOSARCOMA			1 (2%)
#URINARY BLADDER	(50)	(48)	(48)
HEMANGIOMA	1 (2%)		
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(49)
HEPATOCELLULAR ADENOMA	2 (4%)	11 (23%)	4 (8%)
HEPATOCELLULAR CARCINOMA	1 (2%)	24 (50%)	47 (96%)
#GASTRIC MUCOSA	(49)	(48)	(48)
ADENOMATOUS POLYP, NOS		1 (2%)	
#FORESTOMACH	(49)	(48)	(48)
SQUAMOUS CELL PAPILLOMA	2 (4%)		
#DUODENUM	(49)	(44)	(47)
ADENOMATOUS POLYP, NOS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(49)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(42)	(44)
ADENOMA, NOS	4 (9%)	1 (2%)	1 (2%)
#ADRENAL	(50)	(48)	(47)
PHEOCHROMOCYTOMA	2 (4%)		3 (6%)
#THYROID	(47)	(46)	(44)
FOLLICULAR-CELL ADENOMA	2 (4%)		2 (5%)
FOLLICULAR-CELL CARCINOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(48)	(49)
ADENOCARCINOMA, NOS	1 (2%)		1 (2%)
ADENOSQUAMOUS CARCINOMA		1 (2%)	
#UTERUS	(50)	(48)	(49)
ADENOCARCINOMA, NOS	1 (2%)		
LEIOMYOSARCOMA		1 (2%)	
ENDOMETRIAL STROMAL POLYP		1 (2%)	1 (2%)
ENDOMETRIAL STROMAL SARCOMA	1 (2%)	1 (2%)	
#OVARY/PAROVARIAN	(47)	(42)	(48)
ENDOMETRIAL STROMAL SARCOMA, INV		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(50)	(48)	(49)
ADENOMA, NOS	2 (4%)	2 (4%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(48)	(49)
LEIOMYOSARCOMA, INVASIVE		1 (2%)	
*PELVIS	(50)	(48)	(49)
OSTEOSARCOMA		1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(48)	(49)
ADENOCA/SQUAMOUS METAPLASIA LUMBAR REGION	1 (2%)		
RHABDOMYOSARCOMA		1	

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

TABLE B2. SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH	12	18	14
MORIBUND SACRIFICE	2	5	14
SCHEDULED SACRIFICE			
TERMINAL SACRIFICE	36	27	22
DOSING ACCIDENT			
ACCIDENTALLY KILLED, NDA			
ACCIDENTALLY KILLED, NOS			
ANIMAL MISSING			
ANIMAL MISSEXED			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS**	29	35	47
TOTAL PRIMARY TUMORS	36	55	71
TOTAL ANIMALS WITH BENIGN TUMORS	16	16	11
TOTAL BENIGN TUMORS	19	19	14
TOTAL ANIMALS WITH MALIGNANT TUMORS	15	31	47
TOTAL MALIGNANT TUMORS	16	36	57
TOTAL ANIMALS WITH SECONDARY TUMORS##	1	6	4
TOTAL SECONDARY TUMORS	1	6	4
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		
TOTAL UNCERTAIN TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: UNTREATED CONTROL

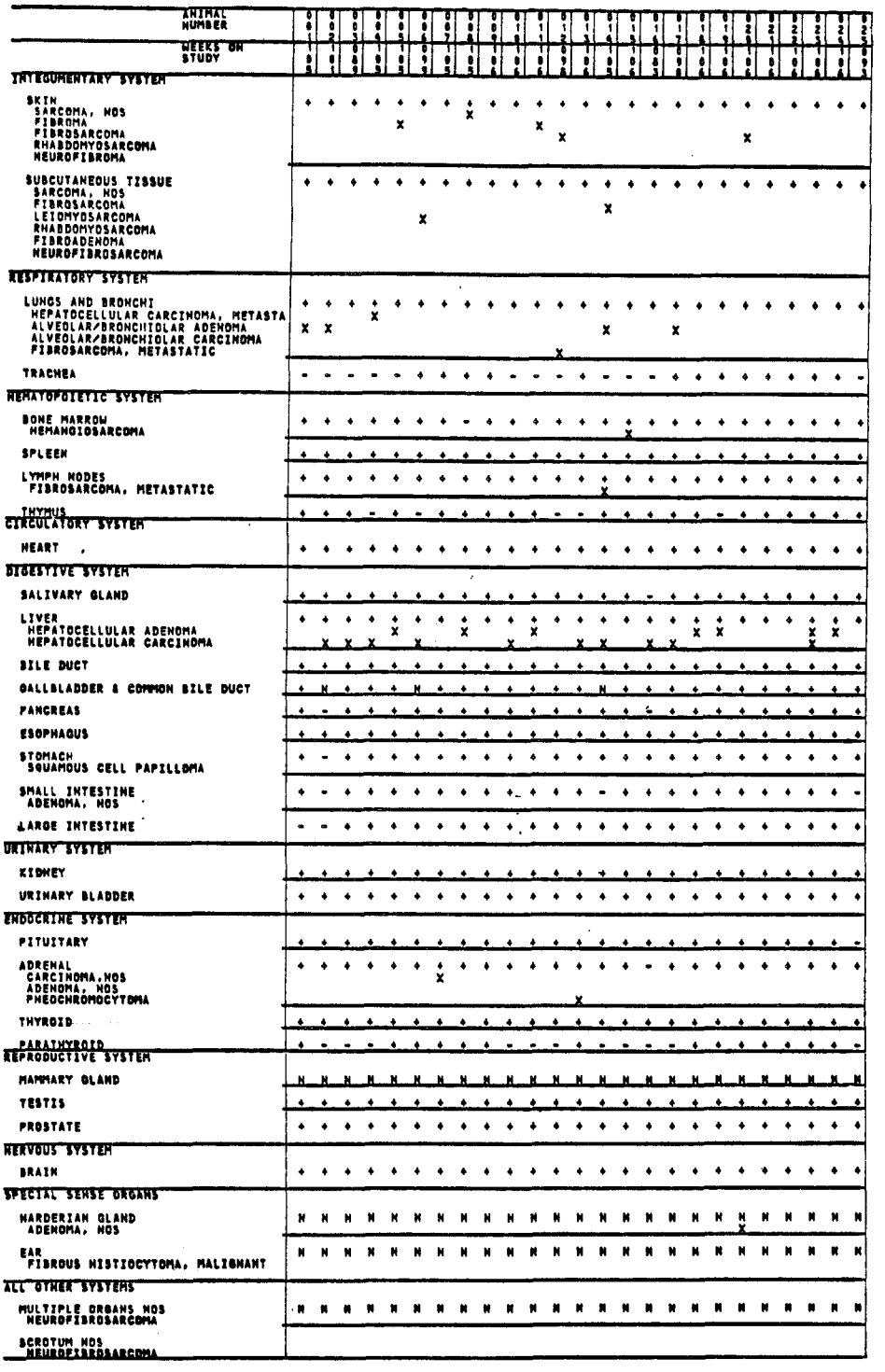
ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	099	084	094	098	093	095	095	095	095	095	095	095	091	091	091	082	084	097	096	096	078	096	096	088	095
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
SARCOMA, NOS																									X
FIBROMA																									
FIBROSARCOMA																									
RHABDOMYOSARCOMA	X			X																					
NEUROFIBROMA																								X	
NEUROFIBROSARCOMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
FIBROMA																									
FIBROSARCOMA																									
RHABDOMYOSARCOMA									X								X								
NEUROFIBROSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA					X																				
ALVEOLAR/BRONCHIOLAR ADENOMA		X																							
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
ACINAR-CELL CARCINOMA, METASTATIC																									
FIBROSARCOMA, METASTATIC																X									
TRACHEA	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+	-	+	-	-	+	-	-	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MALIGNANT LYMPHOMA, MIXED TYPE																									
LYMPH NODES	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	-	+	+	-	-	+	+	+	-	-	+	+	+	-	-	+	+	+	+	+	+	+	-
THYMOMA																									
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA											X														
HEPATOCELLULAR CARCINOMA	X				X					X				X		X					X	X			
MIXED HEPATO/CHOLANGIO CARCINOMA																							X		
ACINAR-CELL CARCINOMA, INVASIVE																X									
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	N	N	+	N	+	+	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ACINAR-CELL CARCINOMA																X									
ESOPHAGUS	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
ADENOMATOUS POLYP, NOS																			X						
LARGE INTESTINE	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
RECTUM	+	+	N	N	+	N	+	+	+	N	N	+	+	+	+	N	N	N	+	+	N	N	+	+	
ADENOCARCINOMA, NOS							X																		
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	-	-	+	+	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
ADRENAL	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMA, NOS															X										
PHEOCHROMOCYTOMA																				X					
THYROID	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+
PARATHYROID	+	+	-	+	-	+	+	+	-	-	+	+	+	+	+	+	+	-	+	-	-	+	-	-	+
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ISLET-CELL ADENOMA																									
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	N	N	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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															X									X	
CYSTADENOMA, NOS																									
ZYMBAL'S 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
SQUAMOUS CELL CARCINOMA																									
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	R	N
CARCINOMA,NOS																						X			
MIXED HEPATO/CHOLANGIOCA, INVASIV																						X			
MIXED HEPATO/CHOLANGIOCA, METASTA										X															
MESOTHELIOMA, MALIGNANT																									
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																					X				
MALIGNANT LYMPHOMA, MIXED TYPE																									

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 5	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0 1 9	1 0 7	1 0 7	1 0 7	1 0 5	1 0 4	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 8	1 0 7	1 0 9	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 7	1 0 8	1 0 7	1 0 7	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SARCOMA, NOS																										1
FIBROMA		X								X			X													2
FIBROSARCOMA										X																2
RHABDOMYOSARCOMA					X																					3
NEUROFIBROMA																										1
NEUROFIBROSARCOMA				X																						1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROMA															X											1
FIBROSARCOMA														X												1
RHABDOMYOSARCOMA																										1
NEUROFIBROSARCOMA																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA																										1
ALVEOLAR/BRONCHIOLAR ADENOMA									X														X			3
ALVEOLAR/BRONCHIOLAR CARCINOMA						X				X													X			3
ACINAR-CELL CARCINOMA, METASTATIC																										1
FIBROSARCOMA, METASTATIC														X												1
TRACHEA	-	+	+	+	+	+	+	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	-	37
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
MALIGNANT LYMPHOMA, MIXED TYPE		X																								1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
THYMUS	+	+	+	-	-	+	-	-	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	36
THYMOMA			X																							1
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA									X																	4
HEPATOCELLULAR CARCINOMA		X						X					X				X						X			11
MIXED HEPATO/CHOLANGIO CARCINOMA																										1
ACINAR-CELL CARCINOMA, INVASIVE																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	N	N	+	+	+	+	+	N	+	+	+	+	+	N	+	+	+	+	N	+	+	+	+	+	48
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ACINAR-CELL CARCINOMA																										1
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SMALL INTESTINE	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
ADENOMATOUS POLYP, NOS																										1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
RECTUM	N	+	+	+	N	+	+	+	+	+	+	+	+	N	+	+	+	+	N	+	+	+	+	+	+	48
ADENOCARCINOMA, NOS																										1
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	43
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMA, NOS																				X						2
PHEOCHROMOCYTOMA							X																			2
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
PARATHYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	-	38
PANCREATIC ISLETS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ISLET-CELL ADENOMA										X																1
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	N	N	N	N	48
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	50
ADENOMA, NOS									X																	2
CYSTADENOMA, NOS																			X							2
ZYMBAL'S 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	+	50
SQUAMOUS CELL CARCINOMA																									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	50
CARCINOMA,NOS																							X			1
MIXED HEPATO/CHOLANGIOCA, INVASIV																										1
MIXED HEPATO/CHOLANGIOCA, METASTA																										1
MESOTHELIOMA, MALIGNANT																										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE										X																1
MALIGNANT LYMPHOMA, MIXED TYPE																									X	2

N ANIMALS NECROPSIED

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: LOW DOSE



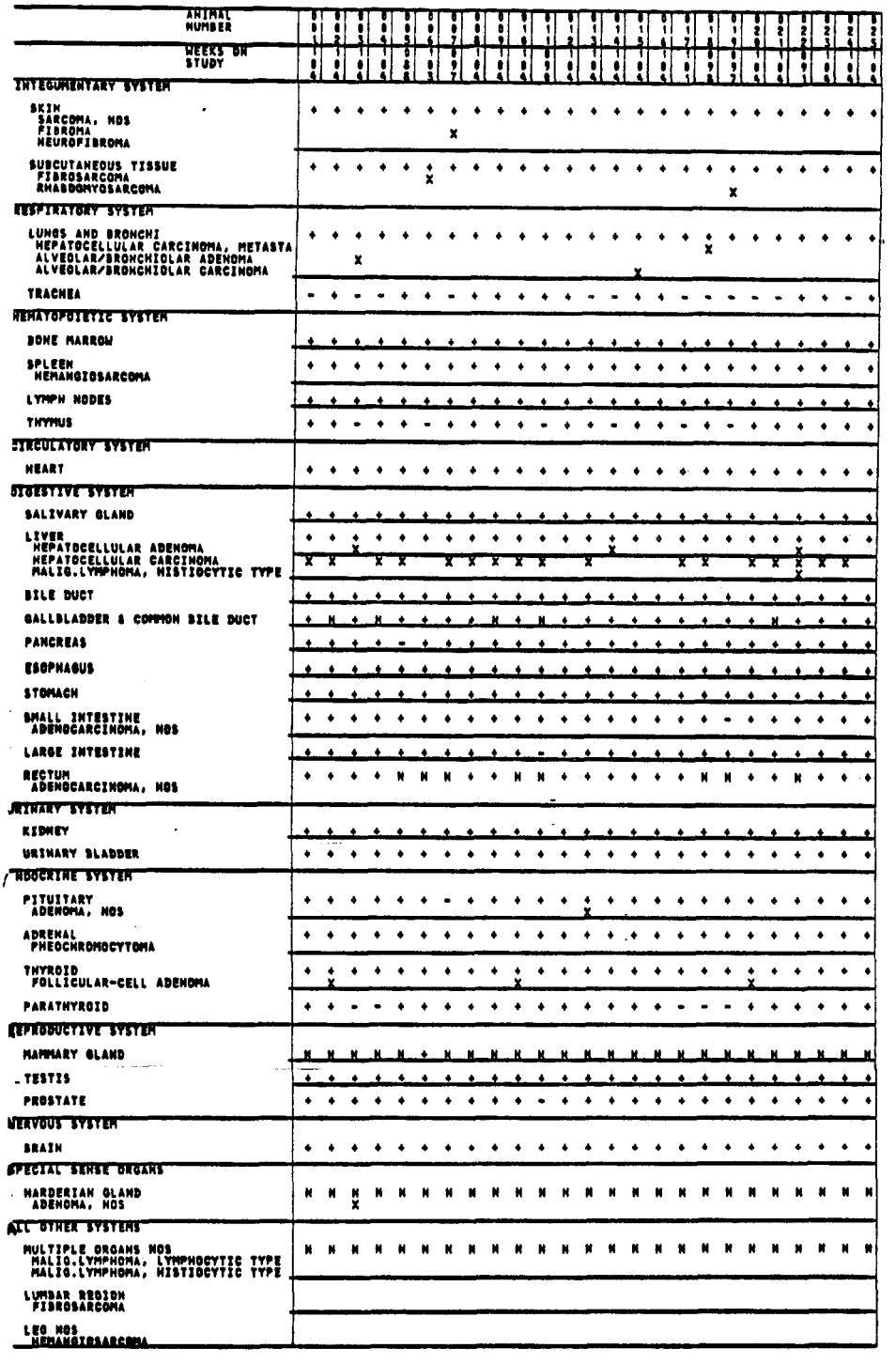
ANIMAL NUMBER	001	002	003	004	005	006	007	008	009	010	011	012	013	014	015	016	017	018	019	020	021	022	023	024	025
WEEKS ON STUDY	000	001	009	013	013	019	019	019	020	020	020	020	020	020	020	020	020	020	020	020	020	020	020	020	019
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS					X			X				X	X												
FIBROMA																									
FIBROSARCOMA																				X					
RHABDOMYOSARCOMA																									
NEUROFIBROMA																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SARCOMA, NOS																									
FIBROSARCOMA														X											
LEIOMYOSARCOMA						X																			
RHABDOMYOSARCOMA																									
FIBROADENOMA																									
NEUROFIBROSARCOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA				X																					
ALVEOLAR/BRONCHIOLAR ADENOMA	X	X													X			X							
ALVEOLAR/BRONCHIOLAR CARCINOMA													X												
FIBROSARCOMA, METASTATIC																									
TRACHEA	-	-	-	-	-	+	+	+	+	-	-	-	-	+	-	-	-	+	+	+	+	+	+	+	-
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOSARCOMA															X										
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FIBROSARCOMA, METASTATIC														X											
THYMUS	+	+	+	-	+	-	+	+	+	+	+	-	-	+	+	+	+	+	-	+	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA					X			X				X							X	X				X	
HEPATOCELLULAR CARCINOMA		X	X	X		X				X			X	X		X	X							X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	N	+	+	+	N	+	+	+	+	+	+	+	N	+	+	+	+	+	+	+	+	+	+	+
PANCREAS	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA																									
SMALL INTESTINE	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-
ADENOMA, NOS																									
LARGE INTESTINE	-	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
CARCINOMA, NOS							X																		
ADENOMA, NOS																									
PHEOCHROMOCYTOMA													X												
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PARATHYROID	+	-	-	-	+	+	+	+	+	-	-	-	+	-	-	-	-	+	+	+	+	+	+	+	-
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	N	N	N	N
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
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																				X					
EAR	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																									
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
NEUROFIBROSARCOMA																									
SCROTUM NOS																									
NEUROFIBROSARCOMA																									

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

ANIMAL NUMBER / WEEKS ON STUDY	TOTAL TISSUES TUMORS
INTEGUMENTARY SYSTEM	
SKIN	50
SARCOMA, NOS	2
FIBROMA	7
FIBROSARCOMA	2
RHABDOMYOSARCOMA	1
NEUROFIBROMA	1
SUBCUTANEOUS TISSUE	50
SARCOMA, NOS	1
FIBROSARCOMA	2
LEIOMYOSARCOMA	1
RHABDOMYOSARCOMA	1
FIBROADENOMA	1
NEUROFIBROSARCOMA	1
RESPIRATORY SYSTEM	
LUNGS AND BRONCHI	50
HEPATOCELLULAR CARCINOMA, METASTA	2
ALVEOLAR/BRONCHIOLAR ADENOMA	5
ALVEOLAR/BRONCHIOLAR CARCINOMA	1
FIBROSARCOMA, METASTATIC	1
TRACHEA	24
HEMATOPOIETIC SYSTEM	
BONE MARROW	48
HEMANGIOSARCOMA	1
SPLEEN	50
LYMPH NODES	50
FIBROSARCOMA, METASTATIC	1
THYMUS	41
CIRCULATORY SYSTEM	
HEART	50
DIGESTIVE SYSTEM	
SALIVARY GLAND	49
LIVER	50
HEPATOCELLULAR ADENOMA	17
HEPATOCELLULAR CARCINOMA	23
BILE DUCT	50
GALLBLADDER & COMMON BILE DUCT	50
PANCREAS	49
ESOPHAGUS	49
STOMACH	49
SQUAMOUS CELL PAPILLOMA	1
SMALL INTESTINE	46
ADENOMA, NOS	1
LARGE INTESTINE	47
URINARY SYSTEM	
KIDNEY	50
URINARY BLADDER	49
ENDOCRINE SYSTEM	
PITUITARY	46
ADRENAL	48
CARCINOMA, NOS	1
ADENOMA, NOS	1
PHEOCHROMOCYTOMA	7
THYROID	49
PARATHYROID	33
REPRODUCTIVE SYSTEM	
MAMMARY GLAND	50
TESTIS	49
PROSTATE	50
NERVOUS SYSTEM	
BRAIN	50
SPECIAL SENSE ORGANS	
HARDERIAN GLAND	50
ADENOMA, NOS	2
EAR	50
FIBROUS HISTIOCYTOMA, MALIGNANT	1
ALL OTHER SYSTEMS	
MULTIPLE ORGANS NOS	50
NEUROFIBROSARCOMA	1
SCROTUM NOS	1
NEUROFIBROSARCOMA	1

M ANIMALS NECROPSIED

TABLE B3. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: HIGH DOSE



ANIMAL NUMBER	0256	0257	0258	0259	0330	0331	0332	0334	0335	0336	0337	0338	0339	0340	0341	0342	0343	0344	0345	0346	0347	0348	0349	0350	TOTAL
WEEKS ON STUDY	104	096	084	082	084	093	084	084	092	084	092	085	085	085	093	081	095	095	093	081	092	087	087	093	TISSUES TUMORS
INTEGUMENTARY SYSTEM																									
SKIN	+	+	+	+	+	+	+	+	X	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SARCOMA, NOS																									1
FIBROMA																									1
NEUROFIBROMA																								X	1
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FIBROSARCOMA																									1
RHABDOMYOSARCOMA																									1
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA					X																				2
ALVEOLAR/BRONCHIOLAR ADENOMA																								X	2
ALVEOLAR/BRONCHIOLAR CARCINOMA																									1
TRACHEA	+	-	+	-	+	+	+	+	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	32
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEMANGIOSARCOMA																		X							1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	-	+	+	+	-	+	+	+	+	+	+	-	-	-	+	-	+	+	-	37
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA	X	X	X					X	X															X	10
HEPATOCELLULAR CARCINOMA		X		X	X					X	X		X			X			X	X		X	X	X	20
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	M	+	+	M	M	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	M	M	+	39
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
STOMACH	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SMALL INTESTINE	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	47
ADENOCARCINOMA, NOS				X																					1
LARGE INTESTINE	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	44
RECTUM	+	+	+	M	M	M	+	+	M	+	M	+	+	+	+	M	+	+	+	M	M	+	M	M	50
ADENOCARCINOMA, NOS																			X						1
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	49
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	46
ADENOMA, NOS																									1
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA																								X	1
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
FOLLICULAR-CELL ADENOMA																		X						X	3
PARATHYROID	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+	+	+	+	41
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	+	M	M	+	M	39
TESTIS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PROSTATE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	39
ADENOMA, NOS																									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	50
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE									X						X										1
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									1
LUMBAR REGION																									
FIBROSARCOMA																							X		1
LEG NOS																									
HEMANGIOSARCOMA											X														1

M ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: UNTREATED CONTROL

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	104	046	046	053	047	062	046	046	046	071	077	057	071	081	077	077	077	071	077	095	077	077	071	092	077
INTEGUMENTARY SYSTEM																									
SKIN	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SEBACEOUS ADENOMA																	X								
FIBROSARCOMA												X													
MAST-CELL TUMOR																						X			
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA																								X	
ALVEOLAR/BRONCHIOLAR ADENOMA								X																	
ALVEOLAR/BRONCHIOLAR CARCINOMA																									
TRACHEA	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+	+	+	+	+	+
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	-	+	+	-	+	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																	X								
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA																						X			
HEPATOCELLULAR CARCINOMA																								X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	+	N	N	+	+	+	+	+	N	N	+	+	+	+	+	+	+	N	+	+	+	+	+
PANCREAS	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
STOMACH	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SQUAMOUS CELL PAPILLOMA											X														
SMALL INTESTINE	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOMATOUS POLYP, NOS																									
LARGE INTESTINE	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEMANGIOMA																			X						
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+
ADENOMA, NOS																			X						
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA													X						X						
THYROID	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
FOLLICULAR-CELL ADENOMA																	X								
FOLLICULAR-CELL CARCINOMA																									X
PARATHYROID	+	-	+	-	-	+	+	+	+	+	+	-	+	-	+	+	+	+	-	+	+	+	+	-	+
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+
ADENOCARCINOMA, NOS																									
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ADENOCARCINOMA, NOS																									
ENDOMETRIAL STROMAL SARCOMA																									
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									X										X						
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
LYMPHOCYTIC LEUKEMIA																									
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
ADENOCA/SQUAMOUS METAPLASIA						X																			
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																			X	X					
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE: 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	107	105	102	121	107	107	107	095	107	105	107	107	107	107	107	089	107	107	107	107	107	107	107	107	107	
INTEGUMENTARY SYSTEM																										
SKIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
SEBACEOUS ADENOMA																										1
FIBROSARCOMA																										1
MAST-CELL TUMOR																										1
RESPIRATORY SYSTEM																										
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR CARCINOMA, METASTA																										1
ALVEOLAR/BRONCHIOLAR ADENOMA															X											2
ALVEOLAR/BRONCHIOLAR CARCINOMA	X																		X							2
TRACHEA	+	-	+	-	+	-	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	38
HEMATOPOIETIC SYSTEM																										
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
THYMUS	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	41
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																										1
CIRCULATORY SYSTEM																										
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
DIGESTIVE SYSTEM																										
SALIVARY GLAND	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEPATOCELLULAR ADENOMA																				X						2
HEPATOCELLULAR CARCINOMA																										1
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
GALLBLADDER & COMMON BILE DUCT	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	49
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SQUAMOUS CELL PAPILLOMA									X																	2
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ADENOMATOUS POLYP, NOS					X																					1
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																										
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
HEMANGIOMA																										1
ENDOCRINE SYSTEM																										
PITUITARY	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	44
ADENOMA, NOS										X					X							X				4
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
PHEOCHROMOCYTOMA																										2
THYROID	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	47
FOLLICULAR-CELL ADENOMA																										2
FOLLICULAR-CELL CARCINOMA												X														1
PARATHYROID	+	-	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
REPRODUCTIVE SYSTEM																										
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50M
ADENOCARCINOMA, NOS								X																		1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
ADENOCARCINOMA, NOS									X																	1
ENDOMETRIAL STROMAL SARCOMA																		X								1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	47
NERVOUS SYSTEM																										
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
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	M	50M
ADENOMA, NOS																										2
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	M	50M
LYMPHOCYTIC LEUKEMIA												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
ADENOCA/SQUAMOUS METAPLASIA																										1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																										2
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				X																		X	X			1

M ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: 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	1 0 6	0 6 7	1 0 6	1 0 6	1 0 6	0 5 3	0 5 8	0 8 6	0 5 0	0 4 5	1 0 6	0 8 1	1 0 6	0 9 7	1 0 6	1 0 6	0 9 7	1 0 6	1 0 6	0 9 6	1 0 6	1 0 6	1 0 6	1 0 8	0 4 9
INTEGUMENTARY SYSTEM																									
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
NEURILEMOMA																									
RESPIRATORY SYSTEM																									
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA	X																					X			
ALVEOLAR/BRONCHIOLAR ADENOMA											X							X							
OSTEOSARCOMA, METASTATIC																									
TRACHEA	-	-	+	+	+	-	+	-	-	-	+	+	+	+	+	-	-	+	+	A	+	-	-	+	-
HEMATOPOIETIC SYSTEM																									
BONE MARROW	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	A	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	A	+	+	+	+	+
THYMUS	+	+	+	+	+	+	-	-	-	+	+	-	+	+	+	+	+	+	+	A	+	+	+	+	+
CIRCULATORY SYSTEM																									
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
DIGESTIVE SYSTEM																									
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	-	+	+	+	A	+	+	+	+	+
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
HEPATOCELLULAR ADENOMA					X													X			X		X		
HEPATOCELLULAR CARCINOMA	X		X	X							X		X		X	X	X		X			X		X	
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	N	+	+	+	+	+	N	+	+	+	+	+	+	+	+	N	N	+	+	A	+	+	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	A	+	+	+	+	+
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
ADENOMATOUS POLYP, NOS																		X							
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
URINARY SYSTEM																									
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
ENDOCRINE SYSTEM																									
PITUITARY	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	A	+	+	+	+	+
ADENOMA, NOS													X												
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	A	+	+	+	+	+
PARATHYROID	-	+	+	+	+	-	-	+	-	-	+	-	+	+	+	-	+	-	+	A	+	+	-	-	-
REPRODUCTIVE SYSTEM																									
MAMMARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
ADENOSQUAMOUS CARCINOMA															X										
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+
LEIOMYOSARCOMA																X									
ENDOMETRIAL STROMAL POLYP	X																								
ENDOMETRIAL STROMAL SARCOMA																									
OVARY	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	-	+	+	+
ENDOMETRIAL STROMAL SARCOMA, INVA																X									
NERVOUS SYSTEM																									
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	A	+	+	+	+	+
SPECIAL SENSE ORGANS																									
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N
ADENOMA, NOS																			X						
BODY CAVITIES																									
PERITONEUM	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	A	N	N	N	N	N
LEIOMYOSARCOMA, INVASIVE																									
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	A	N	N	N	N	N
MALIGNANT LYMPHOMA, NOS																		X	X						
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE																									
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																									
LYMPHOCYTIC LEUKEMIA																									
LUMBAR REGION																				A					
RHABDOMYOSARCOMA																									

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

ANIMAL NUMBER	0 2 4 6	0 2 7 8	0 2 8 9	0 3 0 1	0 3 1 2	0 3 3 4	0 3 5 6	0 3 7 8	0 3 9 0	0 4 0 1	0 4 1 2	0 4 3 4	0 4 4 5	0 4 5 6	0 4 6 7	0 4 8 9	0 5 1 5	TOTAL TISSUES TUMORS
WEEKS ON STUDY	0 7 7	0 5 3	0 8 3	0 8 5	0 8 5	0 9 7	0 8 5	0 8 5	0 9 3	0 7 7	0 9 7	0 8 5	1 0 5	0 8 5	0 8 5	0 8 7	0 8 3	
INTEGUMENTARY SYSTEM																		
SKIN	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
SQUAMOUS CELL PAPILLOMA		X																1
FIBROSARCOMA																		1
SUBCUTANEOUS TISSUE	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
HEMANGIOSARCOMA									X									1
RESPIRATORY SYSTEM																		
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEPATOCELLULAR CARCINOMA, METASTA											X		X			X		3
ALVEOLAR/BRONCHIOLAR ADENOMA																		1
ALVEOLAR/BRONCHIOLAR CARCINOMA																		1
TRACHEA	-	+	-	+	+	-	+	+	+	-	-	-	+	+	+	+	-	27
HEMATOPOIETIC SYSTEM																		
BONE MARROW	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	47
SPLEEN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
MALIGNANT LYMPHOMA, MIXED TYPE																		1
LYMPH NODES	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
THYMUS	-	-	-	+	+	+	-	+	-	+	-	+	+	+	+	+	+	29
CIRCULATORY SYSTEM																		
HEART	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
DIGESTIVE SYSTEM																		
SALIVARY GLAND	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LIVER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR ADENOMA									X						X			4
HEPATOCELLULAR CARCINOMA	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	47
BILE DUCT	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
GALLBLADDER & COMMON BILE DUCT	+	+	+	+	+	+	+	+	N	+	+	+	N	+	N	N	+	49M
PANCREAS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ESOPHAGUS	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	45
STOMACH	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
SMALL INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	47
LARGE INTESTINE	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY SYSTEM																		
KIDNEY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
HEPATOCELLULAR CARCINOMA, METASTA																		1
URINARY BLADDER	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																		
PITUITARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
ADENOMA, NOS				X														1
ADRENAL	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
PHEOCHROMOCYTOMA		X										X						1
THYROID	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
FOLLICULAR-CELL ADENOMA											X							2
PARATHYROID	+	+	+	+	+	-	+	+	+	-	-	+	+	+	+	+	-	32
REPRODUCTIVE SYSTEM																		
MAMMARY GLAND	+	+	N	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49M
ADENOCARCINOMA, NOS																		1
UTERUS	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
ENDOMETRIAL STROMAL POLYP																		1
OVARY	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
NERVOUS SYSTEM																		
BRAIN	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
SPECIAL SENSE ORGANS																		
HARDERIAN GLAND	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	49M
ADENOMA, NOS															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	49M
MALIG.LYMPHOMA, HISTIOCYTIC TYPE												X						1
LYMPHOCYTIC LEUKEMIA						X												4

M ANIMALS NECROPSIED

TABLE B4. INDIVIDUAL ANIMAL TUMOR PATHOLOGY OF FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1: 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 4	1 0 4	1 0 4	0 9 7	1 0 4	1 0 4	0 9 7	0 9 3	0 9 7	1 0 4	0 9 5	0 9 7	1 0 4	0 9 2	0 9 7	1 0 4	0 9 8	0 9 8	1 0 5	0 9 7	1 0 5	0 9 5	0 9 3	0 9 6	1 0 5
INTEGUMENTARY SYSTEM																								
SKIN	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+
SQUAMOUS CELL PAPILLOMA	X																							
FIBROSARCOMA																								
SUBCUTANEOUS TISSUE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	N	+
HEMANGIOSARCOMA																								
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA																								
ALVEOLAR/BRONCHIOLAR ADENOMA																		X						
ALVEOLAR/BRONCHIOLAR CARCINOMA		X																						
TRACHEA	-	+	-	-	+	+	+	-	A	+	-	-	+	+	-	+	+	+	+	-	+	+	-	-
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	+	+	+	+	+	-	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPLEEN	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MALIGNANT LYMPHOMA, MIXED TYPE																X								
LYMPH NODES	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
THYMUS	+	+	+	-	+	+	-	+	A	+	+	+	+	-	+	+	+	+	+	-	+	-	-	+
CIRCULATORY SYSTEM																								
HEART	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+
LIVER	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR ADENOMA							X												X					
HEPATOCELLULAR CARCINOMA	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BILE DUCT	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
GALLBLADDER & COMMON BILE DUCT	+	+	N	+	+	+	+	N	A	+	+	N	N	+	N	+	+	N	N	+	N	+	+	+
PANCREAS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ESOPHAGUS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+
STOMACH	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SMALL INTESTINE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
LARGE INTESTINE	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
URINARY SYSTEM																								
KIDNEY	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HEPATOCELLULAR CARCINOMA, METASTA				X																				
URINARY BLADDER	+	+	+	+	+	+	+	+	A	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+
ENDOCRINE SYSTEM																								
PITUITARY	+	+	+	+	+	+	+	-	A	+	+	+	+	+	+	+	+	+	-	+	+	-	+	+
ADENOMA, NOS																								
ADRENAL	+	+	+	+	+	+	-	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PHEOCHROMOCYTOMA																								X
THYROID	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-
FOLLICULAR-CELL ADENOMA					X																			
PARATHYROID	+	+	+	-	+	+	+	-	A	+	+	-	+	-	-	+	+	+	+	-	+	-	-	-
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	N	+	+	+	+	+	+	N	A	+	N	+	+	+	+	+	+	+	+	+	+	+	N	+
ADENOCARCINOMA, NOS																			X					
UTERUS	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
ENDOMETRIAL STROMAL POLYP					X																			
OVARY	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+
NERVOUS SYSTEM																								
BRAIN	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
SPECIAL SENSE ORGANS																								
HARDERIAN GLAND	N	N	N	N	N	N	N	N	A	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	A	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
MALIG.LYMPHOMA, HISTIOCYTIC TYPE																								
LYMPHOCYTIC LEUKEMIA							X				X										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: HIGH DOSE (Continued)

ANIMAL NUMBER	0216	0217	0218	0219	0311	0312	0313	0314	0315	0316	0317	0318	0319	0401	0402	0403	0404	0405	0406	0407	0408	0409	0410	TOTAL
WEEKS ON STUDY	072	101	106	106	106	097	106	106	108	106	102	106	218	103	106	106	106	106	098	106	106	106	776	TISSUES TUMORS
INTEGUMENTARY SYSTEM																								
SUBCUTANEOUS TISSUE	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48M
NEURILEMOMA										X														1
RESPIRATORY SYSTEM																								
LUNGS AND BRONCHI	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEPATOCELLULAR CARCINOMA, METASTA											X													3
ALVEOLAR/BRONCHIOLAR ADENOMA																								2
OSTEOSARCOMA, METASTATIC	X																							1
TRACHEA	-	A	+	-	-	-	+	+	+	+	+	+	+	-	-	+	+	+	-	-	+	+	+	29
HEMATOPOIETIC SYSTEM																								
BONE MARROW	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SPLEEN	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
LYMPH NODES	+	A	+	-	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	45
THYMUS	-	A	+	+	+	-	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	39
CIRCULATORY SYSTEM																								
HEART	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
DIGESTIVE SYSTEM																								
SALIVARY GLAND	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
LIVER	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
HEPATOCELLULAR ADENOMA						X			X	X	X						X					X	X	11
HEPATOCELLULAR CARCINOMA			X	X			X	X			X	X			X	X	X	X	X	X		X		24
BILE DUCT	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
GALLBLADDER & COMMON BILE DUCT	M	A	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	M	46M
PANCREAS	-	A	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	46
ESOPHAGUS	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
STOMACH	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ADENOMATOUS POLYP, NOS																								1
SMALL INTESTINE	-	A	+	+	+	-	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	44
LARGE INTESTINE	-	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
URINARY SYSTEM																								
KIDNEY	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
URINARY BLADDER	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
ENDOCRINE SYSTEM																								
PITUITARY	-	A	+	+	+	-	+	+	+	+	+	+	-	+	+	+	+	+	+	-	+	+	+	42
ADENOMA, NOS																								1
ADRENAL	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
THYROID	+	A	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
PARATHYROID	-	A	+	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	-	-	-	-	+	28
REPRODUCTIVE SYSTEM																								
MAMMARY GLAND	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48M
ADENOSQUAMOUS CARCINOMA																								1
UTERUS	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
LEIOMYOSARCOMA														X										1
ENDOMETRIAL STROMAL POLYP																								1
ENDOMETRIAL STROMAL SARCOMA																								1
OVARY	-	A	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	+	+	+	+	42
ENDOMETRIAL STROMAL SARCOMA, INVA																								1
NERVOUS SYSTEM																								
BRAIN	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
SPECIAL SENSE ORGANS																								
HARDERIAN GLAND	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	48M
ADENOMA, NOS								X																2
BODY CAVITIES																								
PERITONEUM	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	48M
LEIOMYOSARCOMA, INVASIVE														X										1
OSTEOSARCOMA	X																							1
ALL OTHER SYSTEMS																								
MULTIPLE ORGANS NOS	M	A	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	48M
MALIGNANT LYMPHOMA, NOS										X														1
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE								X																3
MALIG.LYMPHOMA, HISTIOCYTIC TYPE						X									X									2
LYMPHOCYTIC LEUKEMIA							X																	1
LUMBAR REGION		A																						
RHABDOMYOSARCOMA																							X	1

M ANIMALS NECROPSIED

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	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%)	1 (2%)	
HYPERKERATOSIS	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
EDEMA, NOS	1 (2%)		
HEMORRHAGE	1 (2%)		
INFLAMMATION, ACUTE	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(50)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
PIGMENTATION, NOS			1 (2%)
HYPERPLASIA, ADENOMATOUS	3 (6%)	5 (10%)	7 (14%)
METAPLASIA, OSSEOUS	1 (2%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(50)	(50)
MYELOFIBROSIS	1 (2%)		
#SPLEEN	(50)	(49)	(50)
CONGESTION, NOS		1 (2%)	
ATROPHY, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
HEMATOPOIESIS	2 (4%)		
#MEDIASTINAL L. NODE	(50)	(49)	(50)
HEMOSIDEROSIS	1 (2%)		
#LIVER	(49)	(50)	(50)
LEUKOCYTOSIS, NOS			1 (2%)
#THYMUS	(46)	(48)	(50)
CYST, NOS	1 (2%)	1 (2%)	
LYMPHOID DEPLETION			1 (2%)
#THYMIC LYMPHOCYTES	(46)	(48)	(50)
NECROSIS, NOS		1 (2%)	
CIRCULATORY SYSTEM			
#BONE MARROW	(49)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
#RENAL LYMPH NODE	(50)	(49)	(50)
LYMPHANGIECTASIS	1 (2%)		
#HEART	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)		
FIBROSIS, FOCAL	2 (4%)	1 (2%)	
#AURICULAR APPENDAGE	(50)	(50)	(50)
THROMBUS, MURAL		1 (2%)	
#MYOCARDIUM	(50)	(50)	(50)
MINERALIZATION			1 (2%)
INFLAMMATION, CHRONIC	42 (84%)	38 (76%)	39 (78%)
INFLAMMATION, CHRONIC FOCAL		1 (2%)	
#PANCREAS	(49)	(49)	(49)
PERIARTERITIS	1 (2%)		1 (2%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(49)	(50)	(50)
CONGESTION, ACUTE PASSIVE		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
CHOLANGIOFIBROSIS			1 (2%)
DEGENERATION, CYSTIC			1 (2%)
NECROSIS, COAGULATIVE		1 (2%)	2 (4%)
CYTOPLASMIC VACUOLIZATION	4 (8%)	5 (10%)	9 (18%)
BASOPHILIC CYTO CHANGE			1 (2%)
FOCAL CELLULAR CHANGE	2 (4%)	2 (4%)	1 (2%)
CYTOLOGIC ALTERATION, NOS	3 (6%)		
HYPERPLASIA, NOS		1 (2%)	
ANGIECTASIS			1 (2%)
NODULAR REGENERATION			1 (2%)
#LIVER/CENTRILOBULAR	(49)	(50)	(50)
NECROSIS, NOS	1 (2%)		
CYTOPLASMIC VACUOLIZATION	1 (2%)		
#BILE DUCT	(49)	(50)	(50)
HYPERPLASIA, NOS	11 (22%)	6 (12%)	3 (6%)
#PANCREAS	(49)	(49)	(49)
ATROPHY, NOS	6 (12%)	1 (2%)	3 (6%)
ATROPHY, FOCAL	1 (2%)	2 (4%)	2 (4%)
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(49)	(49)	(49)
ATROPHY, NOS		1 (2%)	
HYPERPLASIA, FOCAL			2 (4%)
#GASTRIC SUBMUCOSA	(50)	(49)	(50)
EDEMA, NOS	1 (2%)		
#FORESTOMACH	(50)	(49)	(50)
CYST, NOS		1 (2%)	
EDEMA, NOS		1 (2%)	
ULCER, NOS	4 (8%)	1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)	1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
ULCER, CHRONIC		1 (2%)	
HYPERPLASIA, EPITHELIAL	2 (4%)	2 (4%)	
#COLON	(50)	(49)	(50)
PARASITISM			2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
CYST, NOS			1 (2%)
INFLAMMATION, CHRONIC	4 (8%)	11 (22%)	1 (2%)
INFLAMMATION, CHRONIC DIFFUSE			2 (4%)
NEPHROPATHY	41 (82%)	36 (72%)	40 (80%)
NEPHROSIS, NOS			2 (4%)
#KIDNEY/TUBULE	(50)	(50)	(50)
PIGMENTATION, NOS		36 (72%)	42 (84%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(49)	(49)	(47)
FOCAL CELLULAR CHANGE	1 (2%)		1 (2%)
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	4 (8%)		1 (2%)
ANGIECTASIS	6 (12%)	4 (8%)	2 (4%)
#ADRENAL	(49)	(49)	(50)
CYST, NOS	1 (2%)		
NECROSIS, ZONAL	1 (2%)		
CYTOPLASMIC VACUOLIZATION	1 (2%)		1 (2%)
ANGIECTASIS	2 (4%)		1 (2%)
METAPLASIA, OSSEOUS	1 (2%)		
#ADRENAL CORTEX	(49)	(49)	(50)
CYST, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	2 (4%)	9 (18%)	7 (14%)
FOCAL CELLULAR CHANGE	1 (2%)	2 (4%)	2 (4%)
CYTOLOGIC ALTERATION, NOS	1 (2%)		
CYTOLOGIC DEGENERATION		1 (2%)	
ANGIECTASIS		1 (2%)	
#ADRENAL MEDULLA	(49)	(49)	(50)
CYTOPLASMIC VACUOLIZATION			1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, FOCAL	2 (4%)	2 (4%)	8 (16%)
#THYROID	(50)	(48)	(50)
THYROGLOSSAL DUCT CYST	2 (4%)	1 (2%)	
CYSTIC FOLLICLES	2 (4%)	3 (6%)	1 (2%)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, CYSTIC	1 (2%)		
HYPERPLASIA, C-CELL	5 (10%)	3 (6%)	3 (6%)
#THYROID FOLLICLE	(50)	(48)	(50)
PIGMENTATION, NOS		42 (88%)	48 (96%)
#PARATHYROID	(48)	(42)	(47)
HYPERPLASIA, NOS			2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS	2 (4%)	2 (4%)	3 (6%)
FIBROSIS			1 (2%)
*MAMMARY LOBULE	(50)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS	4 (8%)	2 (4%)	2 (4%)
INFLAMMATION, SUPPURATIVE	1 (2%)		2 (4%)
INFLAMMATION, CHRONIC	2 (4%)		1 (2%)
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)		3 (6%)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, NOS	3 (6%)		
HYPERPLASIA, FOCAL	1 (2%)		
#PROSTATE	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	2 (4%)	8 (16%)	8 (16%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
INFLAMMATION, CHRONIC SUPPURATIV	2 (4%)	2 (4%)	
*SEMINAL VESICLE	(50)	(50)	(50)
HEMORRHAGE	1 (2%)		
INFLAMMATION, SUPPURATIVE	1 (2%)		
INFLAMMATION, CHRONIC		1 (2%)	
#TESTIS	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
NECROSIS, NOS		1 (2%)	
NECROSIS, ISCHEMIC		1 (2%)	
ATROPHY, NOS	1 (2%)	3 (6%)	2 (4%)
HYPERPLASIA, INTERSTITIAL CELL	10 (20%)	5 (10%)	3 (6%)

TABLE C1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
*SCROTUM	(50)	(50)	(50)
ULCER, NOS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(49)	(50)
HEMORRHAGE			1 (2%)
#CEREBELLUM	(50)	(49)	(50)
HEMORRHAGE	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
RETINOPATHY	5 (10%)	1 (2%)	21 (42%)
CATARACT	4 (8%)	1 (2%)	18 (36%)
*EYE/CRYSTALLINE LENS	(50)	(50)	(50)
CATARACT	1 (2%)		1 (2%)
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(50)	(50)
FIBROUS OSTEODYSTROPHY			1 (2%)
OSTEOSCLEROSIS		1 (2%)	
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
STEATITIS	4 (8%)	6 (12%)	7 (14%)
NECROSIS, FAT	1 (2%)	1 (2%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MINERALIZATION			1 (2%)
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	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)
ABSCESS, CHRONIC		1 (2%)	
HYPERKERATOSIS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(50)
EMPHYSEMA, ALVEOLAR	1 (2%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	3 (6%)		
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
ALVEOLAR MACROPHAGES		1 (2%)	
HYPERPLASIA, ADENOMATOUS	2 (4%)	5 (10%)	8 (16%)
#LUNG/ALVEOLI	(50)	(49)	(50)
HISTIOCYTOSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(50)	(50)
ATROPHY, NOS			1 (2%)
MYELOFIBROSIS		1 (2%)	
#SPLEEN	(50)	(50)	(50)
FIBROSIS, DIFFUSE		1 (2%)	
HEMOSIDEROSIS	2 (4%)	1 (2%)	1 (2%)
HEMATOPOIESIS		1 (2%)	1 (2%)
#SPLENIC CAPSULE	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
#LYMPH NODE	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
ANGIECTASIS		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	
#CERVICAL LYMPH NODE	(50)	(50)	(50)
ANGIECTASIS	1 (2%)		
#MESENTERIC L. NODE	(50)	(50)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#INGUINAL LYMPH NODE	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(50)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
#MYOCARDIUM	(50)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC	29 (58%)	24 (48%)	17 (34%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)	1 (2%)	
*HEPATIC VEIN	(50)	(50)	(50)
THROMBUS, ORGANIZED	1 (2%)		
#LIVER	(50)	(50)	(50)
THROMBOSIS, 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. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(50)	(50)
INFLAMMATION, CHRONIC SUPPURATIVE	1 (2%)		
#LIVER	(50)	(50)	(50)
DEFORMITY, NOS	1 (2%)	1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
DEGENERATION, NOS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	6 (12%)	4 (8%)	5 (10%)
BASOPHILIC CYTO CHANGE			1 (2%)
FOCAL CELLULAR CHANGE	1 (2%)		4 (8%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#PANCREAS	(50)	(50)	(50)
ATROPHY, NOS	3 (6%)	2 (4%)	1 (2%)
ATROPHY, FOCAL	2 (4%)	1 (2%)	1 (2%)
ATROPHY, DIFFUSE		1 (2%)	
#PANCREATIC ACINUS	(50)	(50)	(50)
HYPERPLASIA, FOCAL		1 (2%)	
#STOMACH	(50)	(50)	(50)
MINERALIZATION		1 (2%)	
EPIDERMAL INCLUSION CYST			1 (2%)
#GASTRIC SUBMUCOSA	(50)	(50)	(50)
EDEMA, NOS	1 (2%)		
#FORESTOMACH	(50)	(50)	(50)
CYST, NOS		2 (4%)	
EDEMA, NOS	1 (2%)		1 (2%)
ULCER, NOS		2 (4%)	3 (6%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC			1 (2%)
ULCER, CHRONIC	2 (4%)	1 (2%)	
HYPERPLASIA, EPITHELIAL		2 (4%)	2 (4%)
HYPERKERATOSIS			1 (2%)
#COLON	(50)	(50)	(50)
PARASITISM	1 (2%)	1 (2%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
INFLAMMATION, CHRONIC	1 (2%)	3 (6%)	1 (2%)
INFLAMMATION, CHRONIC DIFFUSE	1 (2%)	1 (2%)	
NEPHROPATHY	25 (50%)	13 (26%)	16 (32%)
ATROPHY, FATTY		1 (2%)	
#KIDNEY/CORTEX	(50)	(50)	(50)
METAMORPHOSIS FATTY		1 (2%)	
#KIDNEY/TUBULE	(50)	(50)	(50)
PIGMENTATION, NOS		39 (78%)	40 (80%)
#KIDNEY/PELVIS	(50)	(50)	(50)
MINERALIZATION			1 (2%)
#URINARY BLADDER	(50)	(49)	(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. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM			
#PITUITARY	(50)	(49)	(49)
CYST, NOS	2 (4%)	2 (4%)	6 (12%)
MULTIPLE CYSTS		1 (2%)	1 (2%)
HEMOSIDEROSIS	2 (4%)	1 (2%)	1 (2%)
CYTOPLASMIC VACUOLIZATION			1 (2%)
FOCAL CELLULAR CHANGE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
HYPERPLASIA, FOCAL	3 (6%)		2 (4%)
HYPERPLASIA, DIFFUSE		1 (2%)	
ANGIECTASIS	25 (50%)	14 (29%)	8 (16%)
#ADRENAL	(50)	(50)	(50)
PIGMENTATION, NOS			1 (2%)
CYTOPLASMIC VACUOLIZATION	1 (2%)	1 (2%)	1 (2%)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(50)	(50)	(50)
ACCESSORY STRUCTURE	1 (2%)		1 (2%)
DEFORMITY, NOS		1 (2%)	
CYST, NOS	2 (4%)	1 (2%)	
CYTOPLASMIC VACUOLIZATION	7 (14%)	7 (14%)	7 (14%)
FOCAL CELLULAR CHANGE	2 (4%)	1 (2%)	2 (4%)
ANGIECTASIS		1 (2%)	1 (2%)
#ADRENAL MEDULLA	(50)	(50)	(50)
HYPERPLASIA, FOCAL		2 (4%)	
#THYROID	(49)	(50)	(50)
THYROGLOSSAL DUCT CYST		1 (2%)	
CYSTIC FOLLICLES	2 (4%)	1 (2%)	
HYPERPLASIA, CYSTIC	1 (2%)	1 (2%)	
HYPERPLASIA, C-CELL	5 (10%)	13 (26%)	4 (8%)
#THYROID FOLLICLE	(49)	(50)	(50)
PIGMENTATION, NOS		44 (88%)	47 (94%)
#PARATHYROID	(48)	(49)	(48)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
CYSTIC DUCTS	28 (56%)	19 (38%)	17 (34%)
HYPERPLASIA, CYSTIC	1 (2%)	1 (2%)	1 (2%)
ADENOSIS		1 (2%)	
*MAMMARY LOBULE	(50)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	4 (8%)
HYPERPLASIA, FOCAL			1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS		1 (2%)	2 (4%)
INFLAMMATION, ACUTE SUPPURATIVE		1 (2%)	1 (2%)
ABSCESS, CHRONIC		1 (2%)	
HYPERPLASIA, NOS			2 (4%)
*CLITORAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS	4 (8%)	5 (10%)	3 (6%)
INFLAMMATION, SUPPURATIVE	1 (2%)	3 (6%)	1 (2%)
INFLAMMATION, CHRONIC	3 (6%)	1 (2%)	
INFLAMMATION, CHRONIC SUPPURATIV	1 (2%)	2 (4%)	2 (4%)
ATROPHY, NOS	1 (2%)	2 (4%)	
HYPERPLASIA, NOS	1 (2%)		
*VAGINA	(50)	(50)	(50)
HYPERPLASIA, EPITHELIAL			1 (2%)
#UTERUS/ENDOMETRIUM	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	
HYPERPLASIA, NOS	2 (4%)	2 (4%)	
HYPERPLASIA, CYSTIC	3 (6%)	2 (4%)	4 (8%)
#OVARY	(50)	(50)	(50)
CYSTIC FOLLICLES	1 (2%)		4 (8%)

TABLE C2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(49)
HEMORRHAGE		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
HEMORRHAGE		1 (2%)	1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
RETINOPATHY	5 (10%)	21 (42%)	3 (6%)
CATARACT	2 (4%)	20 (40%)	
PHTHISIS BULBI		2 (4%)	
*EYE/CRYSTALLINE LENS	(50)	(50)	(50)
CATARACT			1 (2%)
*HARDERIAN GLAND	(50)	(50)	(50)
ECTOPIA		2 (4%)	
*MIDDLE EAR	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*FEMUR	(50)	(50)	(50)
FIBROUS OSTEODYSTROPHY		1 (2%)	
BODY CAVITIES			
*MESENTERY	(50)	(50)	(50)
STEATITIS	5 (10%)	6 (12%)	7 (14%)
NECROSIS, FAT	3 (6%)	2 (4%)	3 (6%)
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1		

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	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)
INFLAMMATION, NOS	1 (2%)	2 (4%)	1 (2%)
ULCER, NOS	1 (2%)	5 (10%)	3 (6%)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC	9 (18%)	8 (16%)	8 (16%)
ULCER, CHRONIC	3 (6%)	1 (2%)	6 (12%)
EROSION		1 (2%)	
FIBROSIS	4 (8%)		3 (6%)
ATROPHY, NOS		1 (2%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)		1 (2%)
HYPERPLASIA, EPITHELIAL	1 (2%)		
POLYPOID HYPERPLASIA	1 (2%)		
*SUBCUT TISSUE	(50)	(50)	(50)
EDEMA, NOS	1 (2%)		
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, SUPPURATIVE	2 (4%)	3 (6%)	1 (2%)
LIPOGRANULOMA	1 (2%)		
INFECTION, FUNGAL	1 (2%)		
RESPIRATORY SYSTEM			
*BRONCHIAL LUMEN	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#BRONCHIAL GLAND	(50)	(50)	(50)
HYPERPLASIA, ADENOMATOUS			1 (2%)
#LUNG	(50)	(50)	(50)
CONGESTION, NOS			2 (4%)
HEMORRHAGE			1 (2%)
INFLAMMATION, FOCAL	16 (32%)	22 (44%)	23 (46%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
INFLAMMATION, FOCAL GRANULOMATOUS	3 (6%)	3 (6%)	4 (8%)
HYPERPLASIA, ADENOMATOUS	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	2 (4%)	6 (12%)	3 (6%)
HISTIOCYTOSIS	1 (2%)		
#LUNG/ALVEOLI	(50)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOUS		1 (2%)	
HYPERPLASIA, ADENOMATOUS	5 (10%)	7 (14%)	5 (10%)
HISTIOCYTOSIS	3 (6%)	3 (6%)	8 (16%)
#ALVEOLAR EPITHELIUM	(50)	(50)	(50)
HYPERPLASIA, ADENOMATOUS	16 (32%)	17 (34%)	20 (40%)
HISTIOCYTOSIS	1 (2%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
HYPERPLASIA, PLASMA CELL	1 (2%)		
HYPERPLASIA, LYMPHOID		2 (4%)	1 (2%)
HEMATOPOIESIS		1 (2%)	
#BONE MARROW	(50)	(48)	(50)
HYPERPLASIA, GRANULOCYTIC	6 (12%)		3 (6%)
#SPLEEN	(50)	(50)	(49)
METAMORPHOSIS FATTY	1 (2%)		
ANGIECTASIS	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	8 (16%)	7 (14%)	7 (14%)

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#LYMPH NODE	(49)	(50)	(50)
INFLAMMATION, NOS	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
#MANDIBULAR L. NODE	(49)	(50)	(50)
PIGMENTATION, NOS			1 (2%)
ANGIECTASIS	1 (2%)		
#MEDIASTINAL L. NODE	(49)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		
#MESENTERIC L. NODE	(49)	(50)	(50)
CONGESTION, NOS	1 (2%)	5 (10%)	1 (2%)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	1 (2%)
ANGIECTASIS	3 (6%)	6 (12%)	2 (4%)
HYPERPLASIA, GRANULOCYTIC		1 (2%)	
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
#AXILLARY LYMPH NODE	(49)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#INGUINAL LYMPH NODE	(49)	(50)	(50)
HYPERPLASIA, NOS	2 (4%)		1 (2%)
HYPERPLASIA, LYMPHOID		1 (2%)	
#LUNG	(50)	(50)	(50)
LEUKOCYTOSIS, NOS		1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		
#LIVER	(50)	(50)	(50)
LEUKOCYTOSIS, NOS	3 (6%)	1 (2%)	1 (2%)
#STOMACH	(49)	(49)	(49)
LEUKOCYTOSIS, NOS	1 (2%)		
#PEYER'S PATCH	(46)	(46)	(47)
HYPERPLASIA, LYMPHOID		2 (4%)	
#URINARY BLADDER	(50)	(49)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#THYMUS	(36)	(41)	(37)
CYST, NOS	1 (3%)		
CIRCULATORY SYSTEM			
*SKIN	(50)	(50)	(50)
LYMPHANGIECTASIS	1 (2%)		
#INGUINAL LYMPH NODE	(49)	(50)	(50)
LYMPHANGIECTASIS		1 (2%)	
#LIVER	(50)	(50)	(50)
THROMBOSIS, NOS	1 (2%)		1 (2%)
*MESENTERY	(50)	(50)	(50)
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(49)	(50)
BASEMENT MEMBRANE, ALTERATION			1 (2%)
#SUBLINGUAL GLAND	(50)	(49)	(50)
FIBROSIS, DIFFUSE	1 (2%)		
ATROPHY, NOS	1 (2%)		
#LIVER	(50)	(50)	(50)
DEFORMITY, NOS		1 (2%)	
INFLAMMATION, FOCAL	1 (2%)		
CIRRHOSIS, NOS	1 (2%)		
DEGENERATION, CYSTIC			1 (2%)
NECROSIS, NOS		3 (6%)	
NECROSIS, FOCAL	4 (8%)	3 (6%)	5 (10%)
INFARCT, NOS			2 (4%)
METAMORPHOSIS FATTY	4 (8%)		
ANISOKARYOSIS		1 (2%)	
CYTOPLASMIC VACUOLIZATION	1 (2%)	1 (2%)	

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER (Continued)	(50)	(50)	(50)
FOCAL CELLULAR CHANGE	3 (6%)		2 (4%)
ATROPHY, NOS			1 (2%)
ATROPHY, FOCAL		1 (2%)	
ANGIECTASIS	1 (2%)	1 (2%)	
#LIVER/CENTRILOBULAR	(50)	(50)	(50)
NECROSIS, NOS	1 (2%)		1 (2%)
#LIVER/HEPATOCYTES	(50)	(50)	(50)
NUCLEAR ALTERATION			1 (2%)
**GALLBLADDER	(50)	(50)	(50)
HEMORRHAGE			1 (2%)
#BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	1 (2%)		
#PANCREAS	(50)	(48)	(49)
ATROPHY, FOCAL		1 (2%)	
#STOMACH	(49)	(49)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#GASTRIC FUNDAL GLAND	(49)	(49)	(49)
CYST, NOS		1 (2%)	
#FORESTOMACH	(49)	(49)	(49)
ULCER, NOS		1 (2%)	
EROSION			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
DYSPLASIA, EPITHELIAL			1 (2%)
#DUODENUM	(46)	(46)	(47)
DYSPLASIA, EPITHELIAL	1 (2%)		
ADENOMYOSIS	1 (2%)		
#ILEUM	(46)	(46)	(47)
DYSPLASIA, EPITHELIAL	1 (2%)		
ADENOMYOSIS	1 (2%)		
#COLONIC SUBMUCOSA	(49)	(47)	(46)
EDEMA, NOS			1 (2%)
*RECTUM	(50)	(50)	(50)
PROLAPSE	1 (2%)		1 (2%)
ULCER, NOS			1 (2%)
INFLAMMATION, CHRONIC	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(50)	(50)
INFLAMMATION, FOCAL		1 (2%)	
NEPHROSIS, NOS	11 (22%)	4 (8%)	9 (18%)
ATROPHY, NOS	1 (2%)		
#KIDNEY/TUBULE	(50)	(50)	(50)
PIGMENTATION, NOS			1 (2%)
#KIDNEY/PELVIS	(50)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(43)	(46)	(46)
HYPERPLASIA, FOCAL		2 (4%)	
#ADRENAL	(49)	(48)	(50)
CYST, NOS	1 (2%)		
#ADRENAL CORTEX	(49)	(48)	(50)
FOCAL CELLULAR CHANGE	1 (2%)		1 (2%)
HYPERPLASIA, NODULAR		2 (4%)	1 (2%)
#ADRENAL MEDULLA	(49)	(48)	(50)
FIBROSIS	1 (2%)	1 (2%)	
HYPERPLASIA, NOS	1 (2%)		

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM (Continued)			
#THYROID	(47)	(49)	(50)
CYSTIC FOLLICLES		1 (2%)	
INFLAMMATION, FOCAL	1 (2%)		
DEGENERATION, CYSTIC	16 (34%)	12 (24%)	16 (32%)
PIGMENTATION, NOS		49 (100%)	47 (94%)
HYPERPLASIA, CYSTIC			1 (2%)
HYPERPLASIA, FOLLICULAR-CELL		5 (10%)	2 (4%)
#THYROID FOLLICLE	(47)	(49)	(50)
PIGMENTATION, NOS			3 (6%)
HYPERPLASIA, CYSTIC	3 (6%)	2 (4%)	12 (24%)
#PARATHYROID	(38)	(23)	(41)
EMBRYONAL DUCT CYST		1 (4%)	1 (2%)
CYST, NOS		1 (4%)	
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
CYSTIC DUCTS	3 (6%)	3 (6%)	3 (6%)
INFLAMMATION, NOS	1 (2%)	2 (4%)	3 (6%)
INFLAMMATION, SUPPURATIVE	4 (8%)	7 (14%)	5 (10%)
DEGENERATION, CYSTIC		1 (2%)	
CYSTIC DISEASE	1 (2%)		
#PROSTATE	(50)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)		
#TESTIS	(50)	(49)	(50)
CALCIFICATION, NOS		1 (2%)	
ATROPHY, NOS		1 (2%)	
*EPIDIDYMIS	(50)	(50)	(50)
GRANULOMA, SPERMATIC	1 (2%)	1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(50)	(50)	(50)
PSAMMOMA BODIES		1 (2%)	
#BRAIN/THALAMUS	(50)	(50)	(50)
PSAMMOMA BODIES	16 (32%)	19 (38%)	16 (32%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(50)	(50)
DEFORMITY, NOS	1 (2%)		
RETINOPATHY	1 (2%)		
CATARACT	1 (2%)		
*EYEBALL TUNICA FIBRO	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
*HARDERIAN GLAND	(50)	(50)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
*ZYMBAL'S GLAND	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
*MIDDLE EAR	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			

TABLE D1. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
*MESENTERY	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE			1 (2%)
NECROSIS, FAT	4 (8%)	1 (2%)	1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE		1 (2%)	
ORBITAL REGION			
INFLAMMATION, NOS		1	
LEG			
INFLAMMATION, CHRONIC SUPPURATIVE			1
OMENTUM			
NECROSIS, FAT	1		
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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. 1

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	48	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	48	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(48)	(49)
EPIDERMAL INCLUSION CYST		1 (2%)	
INFLAMMATION, NOS			1 (2%)
ULCER, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(48)	(48)
HEMORRHAGE	1 (2%)		
INFLAMMATION, FOCAL	9 (18%)	18 (38%)	11 (23%)
INFLAMMATION, INTERSTITIAL	1 (2%)		
INFLAMMATION, SUPPURATIVE		1 (2%)	
BRONCHOPNEUMONIA, ACUTE	1 (2%)		
INFLAMMATION, FOCAL GRANULOMATOUS	1 (2%)	1 (2%)	4 (8%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	5 (10%)	3 (6%)	8 (17%)
HISTIOCYTOSIS	1 (2%)		
#LUNG/ALVEOLI	(50)	(48)	(48)
INFLAMMATION, FOCAL GRANULOMATOUS			1 (2%)
HYPERPLASIA, ADENOMATOUS	1 (2%)	2 (4%)	6 (13%)
HISTIOCYTOSIS	6 (12%)	8 (17%)	8 (17%)
#ALVEOLAR EPITHELIUM	(50)	(48)	(48)
HYPERPLASIA, ADENOMATOUS	8 (16%)	11 (23%)	9 (19%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
LEUKOCYTOSIS, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID	4 (8%)	1 (2%)	
HEMATOPOIESIS			1 (2%)
*ABDOMINAL CAVITY	(50)	(48)	(49)
LEUKOCYTOSIS, NOS			1 (2%)
*BLOOD	(50)	(48)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
LEUKOCYTOSIS, NEUTROPHILIC			1 (2%)
#BONE MARROW	(50)	(47)	(47)
CONGESTION, NOS			1 (2%)
ATROPHY, NOS			1 (2%)
HYPERPLASIA, HEMATOPOIETIC	1 (2%)		
HYPERPLASIA, GRANULOCYTIC	5 (10%)	3 (6%)	1 (2%)
#SPLEEN	(50)	(47)	(49)
HEMATOMA, NOS	1 (2%)		
HYPERPLASIA, RETICULUM CELL			1 (2%)
HYPERPLASIA, LYMPHOID	6 (12%)		
HEMATOPOIESIS	9 (18%)	10 (21%)	19 (39%)
#LYMPH NODE	(50)	(45)	(48)
HYPERPLASIA, PLASMA CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)		
#MANDIBULAR L. NODE	(50)	(45)	(48)
HEMOSIDEROSIS	1 (2%)		
ANGIECTASIS			1 (2%)
#BRONCHIAL LYMPH NODE	(50)	(45)	(48)
HYPERPLASIA, NOS	1 (2%)		
#MEDIASTINAL L. NODE	(50)	(45)	(48)
HYPERPLASIA, NOS	1 (2%)	1 (2%)	
#ABDOMINAL LYMPH NODE	(50)	(45)	(48)
ANGIECTASIS	1 (2%)		

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
HEMATOPOIETIC SYSTEM (Continued)			
#MESENTERIC L. NODE	(50)	(45)	(48)
CONGESTION, NOS	1 (2%)		1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, NOS		1 (2%)	
ANGIECTASIS	2 (4%)	1 (2%)	1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)		
#RENAL LYMPH NODE	(50)	(45)	(48)
HYPERPLASIA, NOS	2 (4%)	1 (2%)	
#ILIAC LYMPH NODE	(50)	(45)	(48)
HYPERPLASIA, NOS	1 (2%)		
#LUNG	(50)	(48)	(48)
LEUKOCYTOSIS, NOS	3 (6%)	2 (4%)	2 (4%)
#LIVER	(50)	(48)	(49)
LEUKOCYTOSIS, NOS	7 (14%)	5 (10%)	3 (6%)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	2 (4%)	2 (4%)	
#PEYER'S PATCH	(49)	(44)	(47)
HYPERPLASIA, LYMPHOID	3 (6%)		
#KIDNEY	(50)	(48)	(49)
LEUKOCYTOSIS, NOS		1 (2%)	
HYPERPLASIA, LYMPHOID	2 (4%)	2 (4%)	
#ADRENAL	(50)	(48)	(47)
HEMATOPOIESIS			1 (2%)
#THYMUS	(41)	(39)	(29)
HYPERPLASIA, NOS		1 (3%)	
CIRCULATORY SYSTEM			
*MULTIPLE ORGANS	(50)	(48)	(49)
PERIARTERITIS	1 (2%)		1 (2%)
#MESENTERIC L. NODE	(50)	(45)	(48)
LYMPHANGIECTASIS		1 (2%)	
#HEART	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#MYOCARDIUM	(50)	(48)	(49)
INFLAMMATION, FOCAL	1 (2%)		1 (2%)
PERIARTERITIS		1 (2%)	
PIGMENTATION, NOS			1 (2%)
#PANCREAS	(49)	(46)	(48)
PERIARTERITIS			1 (2%)
*MESENTERY	(50)	(48)	(49)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(49)
CONGESTION, NOS	1 (2%)		1 (2%)
HEMORRHAGE	1 (2%)		
SEQUESTRATION		1 (2%)	
INFLAMMATION, FOCAL	2 (4%)	2 (4%)	
FIBROSIS, FOCAL	1 (2%)		
CHOLANGIOFIBROSIS		1 (2%)	1 (2%)
NECROSIS, FOCAL	2 (4%)		3 (6%)
NECROSIS, ISCHEMIC			1 (2%)
FOCAL CELLULAR CHANGE	2 (4%)	2 (4%)	
NODULAR REGENERATION		1 (2%)	
#LIVER/HEPATOCYTES	(50)	(48)	(49)
ANISOKARYOSIS			2 (4%)
*GALLBLADDER	(50)	(48)	(49)
EDEMA, NOS			1 (2%)
#BILE DUCT	(50)	(48)	(49)
HYPERPLASIA, NOS		2 (4%)	

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM (Continued)			
#PANCREAS	(49)	(46)	(48)
CYSTIC DUCTS	1 (2%)	1 (2%)	
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL		1 (2%)	
#GASTRIC CARDIAC GLAN	(49)	(48)	(48)
METAPLASIA, SQUAMOUS			1 (2%)
#FORESTOMACH	(49)	(48)	(48)
ULCER, NOS	1 (2%)		
INFLAMMATION, FOCAL	4 (8%)	4 (8%)	1 (2%)
INFLAMMATION, SUPPURATIVE			1 (2%)
HYPERPLASIA, EPITHELIAL	4 (8%)	6 (13%)	2 (4%)
#LARGE INTESTINE	(48)	(46)	(48)
EDEMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(49)
HYDRONEPHROSIS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)	1 (2%)	1 (2%)
NEPHROSIS, NOS	3 (6%)	2 (4%)	2 (4%)
INFARCT, HEALED	1 (2%)		
METAPLASIA, OSSEOUS	2 (4%)		
#KIDNEY/TUBULE	(50)	(48)	(49)
DILATATION, NOS			1 (2%)
CALCINOSIS, NOS			1 (2%)
PIGMENTATION, NOS	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(44)	(42)	(44)
FOCAL CELLULAR CHANGE	1 (2%)		
HYPERPLASIA, FOCAL	5 (11%)	3 (7%)	2 (5%)
ANGIECTASIS	1 (2%)		
#ADRENAL	(50)	(48)	(47)
ACCESSORY STRUCTURE		1 (2%)	
ANGIECTASIS		2 (4%)	
#ADRENAL CORTEX	(50)	(48)	(47)
CYTOPLASMIC VACUOLIZATION	1 (2%)		
FOCAL CELLULAR CHANGE	1 (2%)	2 (4%)	
HYPERPLASIA, NODULAR	1 (2%)		
#THYROID	(47)	(46)	(44)
EMBRYONAL DUCT CYST			1 (2%)
CYSTIC FOLLICLES	2 (4%)		1 (2%)
INFLAMMATION, FOCAL	2 (4%)	1 (2%)	1 (2%)
DEGENERATION, CYSTIC	10 (21%)	8 (17%)	7 (16%)
PIGMENTATION, NOS		38 (83%)	40 (91%)
HYPERPLASIA, CYSTIC	1 (2%)		1 (2%)
#THYROID FOLLICLE	(47)	(46)	(44)
PIGMENTATION, NOS		4 (9%)	4 (9%)
HYPERPLASIA, CYSTIC	2 (4%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(48)	(49)
CYSTIC DUCTS		2 (4%)	
CYSTIC DISEASE	1 (2%)	5 (10%)	15 (31%)

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM (Continued)			
#UTERUS	(50)	(48)	(49)
HYDROMETRA	1 (2%)		
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	5 (10%)	7 (15%)	4 (8%)
ADENOMYOSIS	1 (2%)		
#UTERUS/ENDOMETRIUM	(50)	(48)	(49)
CYST, NOS		1 (2%)	2 (4%)
HYPERPLASIA, CYSTIC	33 (66%)	27 (56%)	19 (39%)
#FALLOPIAN TUBE	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE		1 (2%)	
#OVARY/PAROVARIAN	(47)	(42)	(48)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, SUPPURATIVE			1 (2%)
#OVARY	(47)	(42)	(48)
CYST, NOS	1 (2%)		
FOLLICULAR CYST, NOS	10 (21%)	7 (17%)	12 (25%)
PAROVARIAN CYST		1 (2%)	
HEMORRHAGIC CYST	1 (2%)		
INFLAMMATION, NOS		1 (2%)	1 (2%)
INFLAMMATION, SUPPURATIVE	3 (6%)	1 (2%)	2 (4%)
INFLAMMATION, CHRONIC SUPPURATIVE		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
CHOLESTEROL DEPOSIT			1 (2%)
NERVOUS SYSTEM			
#BRAIN/THALAMUS	(50)	(47)	(49)
PSAMMOMA BODIES	21 (42%)	13 (28%)	21 (43%)
SPECIAL SENSE ORGANS			
*EYE	(50)	(48)	(49)
PHTHISIS BULBI	1 (2%)		
*EAR CANAL	(50)	(48)	(49)
HYPERPLASIA, EPITHELIAL	1 (2%)		
*MIDDLE EAR	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKULL	(50)	(48)	(49)
EXOSTOSIS		1 (2%)	
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(48)	(49)
INFECTION, BACTERIAL	1 (2%)		
*PERITONEUM	(50)	(48)	(49)
INFLAMMATION, NOS	1 (2%)		
INFLAMMATION, SUPPURATIVE	3 (6%)		
INFLAMMATION, CHRONIC SUPPURATIVE		1 (2%)	
*MESENTERY	(50)	(48)	(49)
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
INFLAMMATION, CHRONIC SUPPURATIVE			1 (2%)
NECROSIS, FAT	5 (10%)	1 (2%)	

TABLE D2. SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	CONTROL (UNTR)	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(48)	(49)
INFLAMMATION, SUPPURATIVE	5 (10%)	10 (21%)	5 (10%)
LEG			
FIBROSIS			1
SPECIAL MORPHOLOGY SUMMARY			
AUTOLYSIS/NO NECROPSY		2	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. 1

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	2/50 (4%)	4/50 (8%)	0/50 (0%)
Adjusted Rates (b)	5.0%	10.5%	0.0%
Terminal Rates (c)	1/39 (3%)	2/34 (6%)	0/41 (0%)
Life Table Tests (d)	P=0.223N	P=0.290	P=0.232N
Incidental Tumor Tests (d)	P=0.307N	P=0.464	P=0.370N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Tests		P=0.339	P=0.247N
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	2/50 (4%)	5/50 (10%)	1/50 (2%)
Adjusted Rates (b)	5.0%	12.8%	2.4%
Terminal Rates (c)	1/39 (3%)	2/34 (6%)	1/41 (2%)
Life Table Tests (d)	P=0.406N	P=0.182	P=0.486N
Incidental Tumor Tests (d)	P=0.573N	P=0.355	P=0.633N
Cochran-Armitage Trend Test (d)	P=0.412N		
Fisher Exact Tests		P=0.218	P=0.500N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	2/50 (4%)	3/50 (6%)	3/50 (6%)
Adjusted Rates (b)	4.9%	7.9%	7.3%
Terminal Rates (c)	1/39 (3%)	2/34 (6%)	3/41 (7%)
Life Table Tests (d)	P=0.433	P=0.444	P=0.520
Incidental Tumor Tests (d)	P=0.390	P=0.523	P=0.407
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Tests		P=0.500	P=0.500
Hematopoietic System: Mononuclear Cell Leukemia (e)			
Overall Rates (a)	13/50 (26%)	9/50 (18%)	2/50 (4%)
Adjusted Rates (b)	30.2%	20.9%	4.5%
Terminal Rates (c)	9/39 (23%)	3/34 (9%)	0/41 (0%)
Life Table Tests (d)	P=0.003N	P=0.343N	P=0.003N
Incidental Tumor Tests (d)	P=0.003N	P=0.160N	P=0.008N
Cochran-Armitage Trend Test (d)	P=0.002N		
Fisher Exact Tests		P=0.235N	P=0.002N
Hematopoietic System: Leukemia			
Overall Rates (a)	13/50 (26%)	9/50 (18%)	3/50 (6%)
Adjusted Rates (b)	30.2%	20.9%	6.6%
Terminal Rates (c)	9/39 (23%)	3/34 (9%)	0/41 (0%)
Life Table Tests (d)	P=0.008N	P=0.343N	P=0.009N
Incidental Tumor Tests (d)	P=0.007N	P=0.160N	P=0.020N
Cochran-Armitage Trend Test (d)	P=0.005N		
Fisher Exact Tests		P=0.235N	P=0.007N
Liver: Neoplastic Nodule			
Overall Rates (a)	0/49 (0%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	0.0%	0.0%	6.8%
Terminal Rates (c)	0/38 (0%)	0/34 (0%)	2/41 (5%)
Life Table Tests (d)	P=0.045	(f)	P=0.135
Incidental Tumor Tests (d)	P=0.050	(f)	P=0.157
Cochran-Armitage Trend Test (d)	P=0.038		
Fisher Exact Tests		(f)	P=0.125
Liver: Carcinoma			
Overall Rates (a)	1/49 (2%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	2.6%	0.0%	7.3%
Terminal Rates (c)	1/38 (3%)	0/34 (0%)	3/41 (7%)
Life Table Tests (d)	P=0.199	P=0.522N	P=0.333
Incidental Tumor Tests (d)	P=0.199	P=0.522N	P=0.333
Cochran-Armitage Trend Test (d)	P=0.180		
Fisher Exact Tests		P=0.495N	P=0.316

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	1,500 ppm	3,000 ppm
Liver: Neoplastic Nodule or Carcinoma			
Overall Rates (a)	1/49 (2%)	0/50 (0%)	6/50 (12%)
Adjusted Rates (b)	2.6%	0.0%	14.0%
Terminal Rates (c)	1/38 (3%)	0/34 (0%)	5/41 (12%)
Life Table Tests (d)	P=0.023	P=0.522N	P=0.072
Incidental Tumor Tests (d)	P=0.025	P=0.522N	P=0.082
Cochran-Armitage Trend Test (d)	P=0.017		
Fisher Exact Tests		P=0.495N	P=0.059
Pituitary: Adenoma			
Overall Rates (a)	9/49 (18%)	8/49 (16%)	3/47 (6%)
Adjusted Rates (b)	21.3%	20.5%	7.3%
Terminal Rates (c)	6/38 (16%)	5/34 (15%)	3/41 (7%)
Life Table Tests (d)	P=0.051N	P=0.582N	P=0.055N
Incidental Tumor Tests (d)	P=0.088N	P=0.459N	P=0.110N
Cochran-Armitage Trend Test (d)	P=0.061N		
Fisher Exact Tests		P=0.500N	P=0.070N
Pituitary: Adenoma or Carcinoma			
Overall Rates (a)	11/49 (22%)	9/49 (18%)	4/47 (9%)
Adjusted Rates (b)	26.2%	22.3%	9.8%
Terminal Rates (c)	8/38 (21%)	5/34 (15%)	4/41 (10%)
Life Table Tests (d)	P=0.037N	P=0.492N	P=0.039N
Incidental Tumor Tests (d)	P=0.069N	P=0.373N	P=0.077N
Cochran-Armitage Trend Test (d)	P=0.045N		
Fisher Exact Tests		P=0.401N	P=0.054N
Adrenal: Pheochromocytoma			
Overall Rates (a)	20/49 (41%)	17/49 (35%)	17/50 (34%)
Adjusted Rates (b)	48.5%	46.9%	40.2%
Terminal Rates (c)	17/38 (45%)	15/34 (44%)	16/41 (39%)
Life Table Tests (d)	P=0.218N	P=0.507N	P=0.249N
Incidental Tumor Tests (d)	P=0.262N	P=0.378N	P=0.317N
Cochran-Armitage Trend Test (d)	P=0.275N		
Fisher Exact Tests		P=0.339N	P=0.311N
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant			
Overall Rates (a)	21/49 (43%)	18/49 (37%)	18/50 (36%)
Adjusted Rates (b)	51.0%	49.7%	42.6%
Terminal Rates (c)	18/38 (47%)	16/34 (47%)	17/41 (41%)
Life Table Tests (d)	P=0.215N	P=0.516N	P=0.245N
Incidental Tumor Tests (d)	P=0.258N	P=0.387N	P=0.312N
Cochran-Armitage Trend Test (d)	P=0.276N		
Fisher Exact Tests		P=0.340N	P=0.311N
Thyroid: C-Cell Adenoma			
Overall Rates (a)	9/50 (18%)	4/48 (8%)	5/50 (10%)
Adjusted Rates (b)	21.7%	11.8%	12.2%
Terminal Rates (c)	7/39 (18%)	4/34 (12%)	5/41 (12%)
Life Table Tests (d)	P=0.128N	P=0.178N	P=0.173N
Incidental Tumor Tests (d)	P=0.139N	P=0.144N	P=0.218N
Cochran-Armitage Trend Test (d)	P=0.142N		
Fisher Exact Tests		P=0.133N	P=0.194N
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	10/50 (20%)	5/48 (10%)	6/50 (12%)
Adjusted Rates (b)	23.4%	14.7%	14.6%
Terminal Rates (c)	7/39 (18%)	5/34 (15%)	6/41 (15%)
Life Table Tests (d)	P=0.143N	P=0.200N	P=0.188N
Incidental Tumor Tests (d)	P=0.153N	P=0.163N	P=0.229N
Cochran-Armitage Trend Test (d)	P=0.158N		
Fisher Exact Tests		P=0.150N	P=0.207N

TABLE E1. ANALYSIS OF PRIMARY TUMORS IN MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	1,500 ppm	3,000 ppm
Pancreatic Islets: Islet Cell Adenoma			
Overall Rates (a)	5/49 (10%)	4/49 (8%)	1/49 (2%)
Adjusted Rates (b)	12.8%	11.8%	2.4%
Terminal Rates (c)	5/39 (13%)	4/34 (12%)	1/41 (2%)
Life Table Tests (d)	P=0.074N	P=0.586N	P=0.092N
Incidental Tumor Tests (d)	P=0.074N	P=0.586N	P=0.092N
Cochran-Armitage Trend Test (d)	P=0.080N		
Fisher Exact Tests		P=0.500N	P=0.102N
Pancreatic Islets: Islet Cell Adenoma or Carcinoma			
Overall Rates (a)	5/49 (10%)	5/49 (10%)	1/49 (2%)
Adjusted Rates (b)	12.8%	14.7%	2.4%
Terminal Rates (c)	5/39 (13%)	5/34 (15%)	1/41 (2%)
Life Table Tests (d)	P=0.082N	P=0.543	P=0.092N
Incidental Tumor Tests (d)	P=0.082N	P=0.543	P=0.092N
Cochran-Armitage Trend Test (d)	P=0.090N		
Fisher Exact Tests		P=0.630	P=0.102N
Testis: Interstitial Cell Tumor			
Overall Rates (a)	45/50 (90%)	44/50 (88%)	44/50 (88%)
Adjusted Rates (b)	100.0%	97.8%	91.7%
Terminal Rates (c)	39/39 (100%)	33/34 (97%)	37/41 (90%)
Life Table Tests (d)	P=0.295N	P=0.186	P=0.316N
Incidental Tumor Tests (d)	P=0.531N	P=0.633N	P=0.598N
Cochran-Armitage Trend Test (d)	P=0.437N		
Fisher Exact Tests		P=0.500N	P=0.500N
Preputial Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	4/50 (8%)
Adjusted Rates (b)	9.6%	7.0%	9.3%
Terminal Rates (c)	3/39 (8%)	0/34 (0%)	3/41 (7%)
Life Table Tests (d)	P=0.561N	P=0.543N	P=0.624N
Incidental Tumor Tests (d)	P=0.523	P=0.427N	P=0.623N
Cochran-Armitage Trend Test (d)	P=0.576		
Fisher Exact Tests		P=0.500N	P=0.643
All Sites: Mesothelioma			
Overall Rates (a)	0/50 (0%)	4/50 (8%)	1/50 (2%)
Adjusted Rates (b)	0.0%	10.4%	2.4%
Terminal Rates (c)	0/39 (0%)	2/34 (6%)	1/41 (2%)
Life Table Tests (d)	P=0.401	P=0.053	P=0.510
Incidental Tumor Tests (d)	P=0.349	P=0.077	P=0.510
Cochran-Armitage Trend Test (d)	P=0.390		
Fisher Exact Tests		P=0.059	P=0.500

(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) Diagnosed as monocytic leukemia in Carcinogenesis Bioassay Data System
(f) No P value is presented because no tumors were observed in the 1,500-ppm and control groups.

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Subcutaneous Tissue: Fibroma			
Overall Rates (a)	2/50 (4%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	5.0%	2.6%	9.0%
Terminal Rates (c)	2/40 (5%)	1/38 (3%)	2/41 (5%)
Life Table Tests (d)	P=0.252	P=0.518N	P=0.351
Incidental Tumor Tests (d)	P=0.291	P=0.518N	P=0.495
Cochran-Armitage Trend Test (d)	P=0.238		
Fisher Exact Tests		P=0.500N	P=0.339
Subcutaneous Tissue: Fibroma or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	4/50 (8%)
Adjusted Rates (b)	6.9%	2.6%	9.0%
Terminal Rates (c)	2/40 (5%)	1/38 (3%)	2/41 (5%)
Life Table Tests (d)	P=0.425	P=0.321N	P=0.512
Incidental Tumor Tests (d)	P=0.382	P=0.321N	P=0.495
Cochran-Armitage Trend Test (d)	P=0.412		
Fisher Exact Tests		P=0.309N	P=0.500
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	1/50 (2%)	2/49 (4%)	3/50 (6%)
Adjusted Rates (b)	2.5%	5.4%	7.3%
Terminal Rates (c)	1/40 (3%)	2/37 (5%)	3/41 (7%)
Life Table Tests (d)	P=0.233	P=0.473	P=0.314
Incidental Tumor Tests (d)	P=0.233	P=0.473	P=0.314
Cochran-Armitage Trend Test (d)	P=0.223		
Fisher Exact Tests		P=0.492	P=0.309
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	0/50 (0%)	1/49 (2%)	4/50 (8%)
Adjusted Rates (b)	0.0%	2.7%	9.4%
Terminal Rates (c)	0/40 (0%)	1/37 (3%)	3/41 (7%)
Life Table Tests (d)	P=0.030	P=0.484	P=0.068
Incidental Tumor Tests (d)	P=0.029	P=0.484	P=0.062
Cochran-Armitage Trend Test (d)	P=0.026		
Fisher Exact Tests		P=0.495	P=0.059
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	1/50 (2%)	3/49 (6%)	7/50 (14%)
Adjusted Rates (b)	2.5%	8.1%	16.6%
Terminal Rates (c)	1/40 (3%)	3/37 (8%)	6/41 (15%)
Life Table Tests (d)	P=0.021	P=0.278	P=0.036
Incidental Tumor Tests (d)	P=0.020	P=0.278	P=0.034
Cochran-Armitage Trend Test (d)	P=0.018		
Fisher Exact Tests		P=0.301	P=0.030
Hematopoietic System: Mononuclear Cell Leukemia (e)			
Overall Rates (a)	4/50 (8%)	3/50 (6%)	5/50 (10%)
Adjusted Rates (b)	9.2%	7.3%	10.9%
Terminal Rates (c)	2/40 (5%)	1/38 (3%)	1/41 (2%)
Life Table Tests (d)	P=0.438	P=0.534N	P=0.505
Incidental Tumor Tests (d)	P=0.420	P=0.482N	P=0.433
Cochran-Armitage Trend Test (d)	P=0.427		
Fisher Exact Tests		P=0.500N	P=0.500
Pituitary: Adenoma			
Overall Rates (a)	25/50 (50%)	21/49 (43%)	18/49 (37%)
Adjusted Rates (b)	55.3%	53.5%	41.6%
Terminal Rates (c)	20/40 (50%)	19/37 (51%)	15/40 (38%)
Life Table Tests (d)	P=0.105N	P=0.404N	P=0.129N
Incidental Tumor Tests (d)	P=0.152N	P=0.490N	P=0.178N
Cochran-Armitage Trend Test (d)	P=0.109N		
Fisher Exact Tests		P=0.305N	P=0.130N

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	1,500 ppm	3,000 ppm
Pituitary: Carcinoma			
Overall Rates (a)	6/50 (12%)	2/49 (4%)	7/49 (14%)
Adjusted Rates (b)	13.9%	5.4%	17.5%
Terminal Rates (c)	4/40 (10%)	2/37 (5%)	7/40 (18%)
Life Table Tests (d)	P=0.436	P=0.161N	P=0.501
Incidental Tumor Tests (d)	P=0.362	P=0.182N	P=0.370
Cochran-Armitage Trend Test (d)	P=0.421		
Fisher Exact Tests		P=0.141N	P=0.484
Pituitary: Adenoma or Carcinoma			
Overall Rates (a)	31/50 (62%)	23/49 (47%)	25/49 (51%)
Adjusted Rates (b)	65.8%	58.7%	58.0%
Terminal Rates (c)	24/40 (60%)	21/37 (57%)	22/40 (55%)
Life Table Tests (d)	P=0.149N	P=0.173N	P=0.180N
Incidental Tumor Tests (d)	P=0.238N	P=0.228N	P=0.310N
Cochran-Armitage Trend Test (d)	P=0.159N		
Fisher Exact Tests		P=0.096N	P=0.185N
Adrenal: Cortical Adenoma			
Overall Rates (a)	5/50 (10%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	12.0%	5.3%	0.0%
Terminal Rates (c)	4/40 (10%)	2/38 (5%)	0/41 (0%)
Life Table Tests (d)	P=0.017N	P=0.242N	P=0.033N
Incidental Tumor Tests (d)	P=0.019N	P=0.269N	P=0.036N
Cochran-Armitage Trend Test (d)	P=0.016N		
Fisher Exact Tests		P=0.218N	P=0.028N
Adrenal: Pheochromocytoma			
Overall Rates (a)	8/50 (16%)	2/50 (4%)	3/50 (6%)
Adjusted Rates (b)	19.0%	5.3%	7.3%
Terminal Rates (c)	6/40 (15%)	2/38 (5%)	3/41 (7%)
Life Table Tests (d)	P=0.055N	P=0.060N	P=0.098N
Incidental Tumor Tests (d)	P=0.060N	P=0.078N	P=0.104N
Cochran-Armitage Trend Test (d)	P=0.055N		
Fisher Exact Tests		P=0.046N	P=0.100N
Thyroid: C-Cell Adenoma			
Overall Rates (a)	5/49 (10%)	2/50 (4%)	0/50 (0%)
Adjusted Rates (b)	12.8%	5.3%	0.0%
Terminal Rates (c)	5/39 (13%)	2/38 (5%)	0/41 (0%)
Life Table Tests (d)	P=0.015N	P=0.226N	P=0.029N
Incidental Tumor Tests (d)	P=0.015N	P=0.226N	P=0.029N
Cochran-Armitage Trend Test (d)	P=0.015N		
Fisher Exact Tests		P=0.210N	P=0.027N
Thyroid: C-Cell Carcinoma			
Overall Rates (a)	3/49 (6%)	4/50 (8%)	2/50 (4%)
Adjusted Rates (b)	7.7%	10.5%	4.9%
Terminal Rates (c)	3/39 (8%)	4/38 (11%)	2/41 (5%)
Life Table Tests (d)	P=0.392N	P=0.486	P=0.477N
Incidental Tumor Tests (d)	P=0.392N	P=0.486	P=0.477N
Cochran-Armitage Trend Test (d)	P=0.407N		
Fisher Exact Tests		P=0.511	P=0.490N
Thyroid: C-Cell Adenoma or Carcinoma			
Overall Rates (a)	7/49 (14%)	6/50 (12%)	2/50 (4%)
Adjusted Rates (b)	17.9%	15.8%	4.9%
Terminal Rates (c)	7/39 (18%)	6/38 (16%)	2/41 (5%)
Life Table Tests (d)	P=0.056N	P=0.520N	P=0.069N
Incidental Tumor Tests (d)	P=0.056N	P=0.520N	P=0.069N
Cochran-Armitage Trend Test (d)	P=0.062N		
Fisher Exact Tests		P=0.484N	P=0.075N

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	1,500 ppm	3,000 ppm
Mammary Gland: Adenocarcinoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	7.5%	0.0%	0.0%
Terminal Rates (c)	3/40 (7%)	0/38 (0%)	0/41 (0%)
Life Table Tests (d)	P=0.037N	P=0.130N	P=0.117N
Incidental Tumor Tests (d)	P=0.037N	P=0.130N	P=0.117N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Tests		P=0.121N	P=0.121N
Mammary Gland: Fibroadenoma			
Overall Rates (a)	13/50 (26%)	12/50 (24%)	8/50 (16%)
Adjusted Rates (b)	30.8%	29.8%	19.5%
Terminal Rates (c)	11/40 (28%)	10/38 (26%)	8/41 (20%)
Life Table Tests (d)	P=0.129N	P=0.558N	P=0.150N
Incidental Tumor Tests (d)	P=0.117N	P=0.534N	P=0.158N
Cochran-Armitage Trend Test (d)	P=0.139N		
Fisher Exact Tests		P=0.500N	P=0.163N
Clitoral Gland: Carcinoma			
Overall Rates (a)	3/50 (6%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	6.9%	15.8%	7.3%
Terminal Rates (c)	2/40 (5%)	6/38 (16%)	3/41 (7%)
Life Table Tests (d)	P=0.558N	P=0.222	P=0.651N
Incidental Tumor Tests (d)	P=0.514	P=0.222	P=0.511
Cochran-Armitage Trend Test (d)	P=0.573		
Fisher Exact Tests		P=0.243	P=0.661
Clitoral Gland: Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	6/50 (12%)	4/50 (8%)
Adjusted Rates (b)	9.4%	15.8%	9.8%
Terminal Rates (c)	3/40 (7%)	6/38 (16%)	4/41 (10%)
Life Table Tests (d)	P=0.552N	P=0.341	P=0.629N
Incidental Tumor Tests (d)	P=0.515	P=0.341	P=0.514
Cochran-Armitage Trend Test (d)	P=0.568		
Fisher Exact Tests		P=0.370	P=0.643
Uterus: Endometrial Stromal Polyp			
Overall Rates (a)	5/50 (10%)	9/50 (18%)	14/50 (28%)
Adjusted Rates (b)	12.5%	21.0%	33.2%
Terminal Rates (c)	5/40 (13%)	5/38 (13%)	13/41 (32%)
Life Table Tests (d)	P=0.020	P=0.174	P=0.023
Incidental Tumor Tests (d)	P=0.023	P=0.289	P=0.022
Cochran-Armitage Trend Test (d)	P=0.015		
Fisher Exact Tests		P=0.194	P=0.020
Uterus: Endometrial Stromal Polyp or Sarcoma			
Overall Rates (a)	6/50 (12%)	10/50 (20%)	15/50 (30%)
Adjusted Rates (b)	15.0%	22.6%	35.5%
Terminal Rates (c)	6/40 (15%)	5/38 (13%)	14/41 (34%)
Life Table Tests (d)	P=0.025	P=0.188	P=0.027
Incidental Tumor Tests (d)	P=0.021	P=0.301	P=0.026
Cochran-Armitage Trend Test (d)	P=0.018		
Fisher Exact Tests		P=0.207	P=0.024
Zymbal Gland: Carcinoma			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	7.0%	0.0%	0.0%
Terminal Rates (c)	2/40 (5%)	0/38 (0%)	0/41 (0%)
Life Table Tests (d)	P=0.040N	P=0.138N	P=0.123N
Incidental Tumor Tests (d)	P=0.045N	P=0.162N	P=0.129N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Tests		P=0.121N	P=0.121N

TABLE E2. ANALYSIS OF PRIMARY TUMORS IN FEMALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

(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) Diagnosed as monocytic leukemia in Carcinogenesis Bioassay Data System

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	1,500 ppm	3,000 ppm
Skin: Rhabdomyosarcoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	0/50 (0%)
Adjusted Rates (b)	7.4%	2.7%	0.0%
Terminal Rates (c)	1/33 (3%)	1/37 (3%)	0/30 (0%)
Life Table Tests (d)	P=0.066N	P=0.283N	P=0.135N
Incidental Tumor Tests (d)	P=0.036N	P=0.252N	P=0.062N
Cochran-Armitage Trend Test (d)	P=0.060N		
Fisher Exact Tests		P=0.309N	P=0.121N
Integumentary System: Rhabdomyosarcoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	10.3%	5.4%	2.9%
Terminal Rates (c)	2/33 (6%)	2/37 (5%)	0/30 (0%)
Life Table Tests (d)	P=0.130N	P=0.302N	P=0.202N
Incidental Tumor Tests (d)	P=0.092N	P=0.276N	P=0.126N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Tests		P=0.339N	P=0.181N
Skin: Fibroma			
Overall Rates (a)	2/50 (4%)	7/50 (14%)	1/50 (2%)
Adjusted Rates (b)	6.1%	18.9%	2.9%
Terminal Rates (c)	2/33 (6%)	7/37 (19%)	0/30 (0%)
Life Table Tests (d)	P=0.462N	P=0.108	P=0.522N
Incidental Tumor Tests (d)	P=0.462N	P=0.108	P=0.522N
Cochran-Armitage Trend Test (d)	P=0.421N		
Fisher Exact Tests		P=0.080	P=0.500N
Integumentary System: Fibroma			
Overall Rates (a)	3/50 (6%)	7/50 (14%)	1/50 (2%)
Adjusted Rates (b)	9.1%	18.9%	2.9%
Terminal Rates (c)	3/33 (9%)	7/37 (19%)	0/30 (0%)
Life Table Tests (d)	P=0.317N	P=0.205	P=0.332N
Incidental Tumor Tests (d)	P=0.318N	P=0.205	P=0.332N
Cochran-Armitage Trend Test (d)	P=0.283N		
Fisher Exact Tests		P=0.159	P=0.309N
Skin: Fibroma or Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	9/50 (18%)	1/50 (2%)
Adjusted Rates (b)	9.1%	23.5%	2.9%
Terminal Rates (c)	3/33 (9%)	8/37 (22%)	0/30 (0%)
Life Table Tests (d)	P=0.335N	P=0.092	P=0.332N
Incidental Tumor Tests (d)	P=0.335N	P=0.084	P=0.332N
Cochran-Armitage Trend Test (d)	P=0.297N		
Fisher Exact Tests		P=0.061	P=0.309N
Integumentary System: Fibrosarcoma			
Overall Rates (a)	3/50 (6%)	4/50 (8%)	1/50 (2%)
Adjusted Rates (b)	8.1%	10.1%	3.2%
Terminal Rates (c)	2/33 (6%)	2/37 (5%)	0/30 (0%)
Life Table Tests (d)	P=0.274N	P=0.565	P=0.328N
Incidental Tumor Tests (d)	P=0.240N	P=0.529	P=0.273N
Cochran-Armitage Trend Test (d)	P=0.252N		
Fisher Exact Tests		P=0.500	P=0.309N
Integumentary System: Fibroma or Fibrosarcoma			
Overall Rates (a)	5/50 (10%)	11/50 (22%)	2/50 (4%)
Adjusted Rates (b)	14.0%	28.1%	6.0%
Terminal Rates (c)	4/33 (12%)	9/37 (24%)	0/30 (0%)
Life Table Tests (d)	P=0.260N	P=0.136	P=0.246N
Incidental Tumor Tests (d)	P=0.235N	P=0.121	P=0.207N
Cochran-Armitage Trend Test (d)	P=0.221N		
Fisher Exact Tests		P=0.086	P=0.218N

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	1,500 ppm	3,000 ppm
Integumentary System: Neurofibroma or Neurofibrosarcoma			
Overall Rates (a)	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	8.5%	5.4%	3.3%
Terminal Rates (c)	2/33 (6%)	2/37 (5%)	1/30 (3%)
Life Table Tests (d)	P=0.244N	P=0.450N	P=0.339N
Incidental Tumor Tests (d)	P=0.242N	P=0.471N	P=0.332N
Cochran-Armitage Trend Test (d)	P=0.222N		
Fisher Exact Tests		P=0.500N	P=0.309N
Integumentary System: All Fibromas or Fibrosarcomas			
Overall Rates (a)	8/50 (16%)	11/50 (22%)	3/50 (6%)
Adjusted Rates (b)	22.0%	28.1%	9.1%
Terminal Rates (c)	6/33 (18%)	9/37 (24%)	1/30 (3%)
Life Table Tests (d)	P=0.130N	P=0.411	P=0.131N
Incidental Tumor Tests (d)	P=0.112N	P=0.375	P=0.104N
Cochran-Armitage Trend Test (d)	P=0.102N		
Fisher Exact Tests		P=0.306	P=0.100N
Lung: Alveolar/Bronchiolar Adenoma			
Overall Rates (a)	3/50 (6%)	5/50 (10%)	2/50 (4%)
Adjusted Rates (b)	8.2%	12.3%	6.7%
Terminal Rates (c)	2/33 (6%)	2/37 (5%)	2/30 (7%)
Life Table Tests (d)	P=0.458N	P=0.416	P=0.539N
Incidental Tumor Tests (d)	P=0.386N	P=0.396	P=0.483N
Cochran-Armitage Trend Test (d)	P=0.421N		
Fisher Exact Tests		P=0.357	P=0.500N
Lung: Alveolar/Bronchiolar Carcinoma			
Overall Rates (a)	3/50 (6%)	1/50 (2%)	1/50 (2%)
Adjusted Rates (b)	8.2%	2.7%	3.3%
Terminal Rates (c)	2/33 (6%)	1/37 (3%)	1/30 (3%)
Life Table Tests (d)	P=0.219N	P=0.275N	P=0.336N
Incidental Tumor Tests (d)	P=0.185N	P=0.261N	P=0.279N
Cochran-Armitage Trend Test (d)	P=0.202N		
Fisher Exact Tests		P=0.309N	P=0.309N
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	5/50 (10%)	6/50 (12%)	3/50 (6%)
Adjusted Rates (b)	14.1%	14.8%	10.0%
Terminal Rates (c)	4/33 (12%)	3/37 (8%)	3/30 (10%)
Life Table Tests (d)	P=0.346N	P=0.571	P=0.406N
Incidental Tumor Tests (d)	P=0.289N	P=0.556	P=0.362N
Cochran-Armitage Trend Test (d)	P=0.303N		
Fisher Exact Tests		P=0.500	P=0.358N
Hematopoietic System: Malignant Lymphoma, Mixed Type			
Overall Rates (a)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted Rates (b)	8.7%	0.0%	0.0%
Terminal Rates (c)	2/33 (6%)	0/37 (0%)	0/30 (0%)
Life Table Tests (d)	P=0.037N	P=0.100N	P=0.134N
Incidental Tumor Tests (d)	P=0.040N	P=0.117N	P=0.134N
Cochran-Armitage Trend Test (d)	P=0.037N		
Fisher Exact Tests		P=0.122N	P=0.122N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	4/50 (8%)	0/50 (0%)	3/50 (6%)
Adjusted Rates (b)	11.7%	0.0%	8.2%
Terminal Rates (c)	3/33 (9%)	0/37 (0%)	1/30 (3%)
Life Table Tests (d)	P=0.432N	P=0.049N	P=0.535N
Incidental Tumor Tests (d)	P=0.355N	P=0.058N	P=0.441N
Cochran-Armitage Trend Test (d)	P=0.406N		
Fisher Exact Tests		P=0.059N	P=0.500N

TABLE E3. ANALYSIS OF PRIMARY TUMORS IN MALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (*Continued*)

	Control	1,500 ppm	3,000 ppm
Liver: Adenoma			
Overall Rates (a)	4/50 (8%)	17/50 (34%)	10/50 (20%)
Adjusted Rates (b)	12.1%	45.9%	30.5%
Terminal Rates (c)	4/33 (12%)	17/37 (46%)	8/30 (27%)
Life Table Tests (d)	P=0.046	P=0.003	P=0.051
Incidental Tumor Tests (d)	P=0.052	P=0.003	P=0.062
Cochran-Armitage Trend Test (d)	P=0.087		
Fisher Exact Tests		P=0.001	P=0.074
Liver: Carcinoma			
Overall Rates (a)	11/50 (22%)	20/50 (40%)	30/50 (60%)
Adjusted Rates (b)	25.4%	44.7%	67.2%
Terminal Rates (c)	4/33 (12%)	13/37 (35%)	16/30 (53%)
Life Table Tests (d)	P<0.001	P=0.107	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.040	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.041	P<0.001
Liver: Adenoma or Carcinoma			
Overall Rates (a)	15/50 (30%)	31/50 (62%)	37/50 (74%)
Adjusted Rates (b)	35.7%	70.0%	83.6%
Terminal Rates (c)	8/33 (24%)	24/37 (65%)	23/30 (77%)
Life Table Tests (d)	P<0.001	P=0.012	P<0.001
Incidental Tumor Tests (d)	P<0.001	P=0.002	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P=0.001	P<0.001
Adrenal: Pheochromocytoma			
Overall Rates (a)	2/49 (4%)	7/48 (15%)	1/50 (2%)
Adjusted Rates (b)	6.1%	18.9%	2.6%
Terminal Rates (c)	2/33 (6%)	7/37 (19%)	0/30 (0%)
Life Table Tests (d)	P=0.466N	P=0.108	P=0.531N
Incidental Tumor Tests (d)	P=0.455N	P=0.108	P=0.507N
Cochran-Armitage Trend Test (d)	P=0.410N		
Fisher Exact Tests		P=0.075	P=0.492N
Thyroid: Follicular Cell Adenoma			
Overall Rates (a)	0/47 (0%)	0/49 (0%)	5/50 (10%)
Adjusted Rates (b)	0.0%	0.0%	16.7%
Terminal Rates (c)	0/32 (0%)	0/37 (0%)	5/30 (17%)
Life Table Tests (d)	P=0.004	(e)	P=0.027
Incidental Tumor Tests (d)	P=0.004	(e)	P=0.027
Cochran-Armitage Trend Test (d)	P=0.007		
Fisher Exact Tests		(e)	P=0.033
Harderian Gland: Adenoma			
Overall Rates (a)	4/50 (8%)	2/50 (4%)	1/50 (2%)
Adjusted Rates (b)	12.1%	5.4%	3.3%
Terminal Rates (c)	4/33 (12%)	2/37 (5%)	1/30 (3%)
Life Table Tests (d)	P=0.130N	P=0.284N	P=0.207N
Incidental Tumor Tests (d)	P=0.130N	P=0.284N	P=0.207N
Cochran-Armitage Trend Test (d)	P=0.118N		
Fisher Exact Tests		P=0.339N	P=0.181N

(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 1,500-ppm and control groups.

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

	Control	3,000 ppm	6,000 ppm
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma			
Overall Rates (a)	4/50 (8%)	2/48 (4%)	2/48 (4%)
Adjusted Rates (b)	11.1%	7.1%	7.9%
Terminal Rates (c)	4/36 (11%)	2/28 (7%)	1/23 (4%)
Life Table Tests (d)	P=0.437N	P=0.457N	P=0.544N
Incidental Tumor Tests (d)	P=0.334N	P=0.457N	P=0.400N
Cochran-Armitage Trend Test (d)	P=0.269N		
Fisher Exact Tests		P=0.359N	P=0.359N
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type			
Overall Rates (a)	3/50 (6%)	3/48 (6%)	0/49 (0%)
Adjusted Rates (b)	8.0%	10.7%	0.0%
Terminal Rates (c)	2/36 (6%)	3/28 (11%)	0/24 (0%)
Life Table Tests (d)	P=0.180N	P=0.559	P=0.179N
Incidental Tumor Tests (d)	P=0.115N	P=0.630	P=0.074N
Cochran-Armitage Trend Test (d)	P=0.106N		
Fisher Exact Tests		P=0.641	P=0.125N
Hematopoietic System: Malignant Lymphoma, Histiocytic Type			
Overall Rates (a)	3/50 (6%)	2/48 (4%)	1/49 (2%)
Adjusted Rates (b)	7.7%	6.9%	4.2%
Terminal Rates (c)	2/36 (6%)	1/28 (4%)	1/24 (4%)
Life Table Tests (d)	P=0.333N	P=0.587N	P=0.412N
Incidental Tumor Tests (d)	P=0.239N	P=0.495N	P=0.441N
Cochran-Armitage Trend Test (d)	P=0.229N		
Fisher Exact Tests		P=0.520N	P=0.316N
Hematopoietic System: Lymphoma, All Malignant			
Overall Rates (a)	6/50 (12%)	6/48 (13%)	2/49 (4%)
Adjusted Rates (b)	15.4%	19.7%	8.3%
Terminal Rates (c)	4/36 (11%)	4/28 (14%)	2/24 (8%)
Life Table Tests (d)	P=0.258N	P=0.477	P=0.253N
Incidental Tumor Tests (d)	P=0.107N	P=0.600N	P=0.183N
Cochran-Armitage Trend Test (d)	P=0.122N		
Fisher Exact Tests		P=0.591	P=0.141N
Hematopoietic System: Lymphocytic Leukemia			
Overall Rates (a)	1/50 (2%)	1/48 (2%)	4/49 (8%)
Adjusted Rates (b)	2.8%	2.8%	11.3%
Terminal Rates (c)	1/36 (3%)	0/28 (0%)	0/24 (0%)
Life Table Tests (d)	P=0.079	P=0.730	P=0.140
Incidental Tumor Tests (d)	P=0.443	P=0.704N	P=0.610
Cochran-Armitage Trend Test (d)	P=0.098		
Fisher Exact Tests		P=0.742	P=0.175
Hematopoietic System: Lymphoma or Leukemia			
Overall Rates (a)	7/50 (14%)	7/48 (15%)	6/49 (12%)
Adjusted Rates (b)	18.0%	22.0%	18.7%
Terminal Rates (c)	5/36 (14%)	4/28 (14%)	2/24 (8%)
Life Table Tests (d)	P=0.455	P=0.463	P=0.546
Incidental Tumor Tests (d)	P=0.189N	P=0.563N	P=0.273N
Cochran-Armitage Trend Test (d)	P=0.458N		
Fisher Exact Tests		P=0.581	P=0.516N
Liver: Adenoma			
Overall Rates (a)	2/50 (4%)	11/48 (23%)	4/49 (8%)
Adjusted Rates (b)	5.6%	36.0%	15.2%
Terminal Rates (c)	2/36 (6%)	9/28 (32%)	3/24 (13%)
Life Table Tests (d)	P=0.127	P=0.002	P=0.190
Incidental Tumor Tests (d)	P=0.259	P=0.004	P=0.276
Cochran-Armitage Trend Test (d)	P=0.306		
Fisher Exact Tests		P=0.006	P=0.329

TABLE E4. ANALYSIS OF PRIMARY TUMORS IN FEMALE MICE IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1 (Continued)

	Control	3,000 ppm	6,000 ppm
Liver: Carcinoma			
Overall Rates (a)	1/50 (2%)	24/48 (50%)	47/49 (96%)
Adjusted Rates (b)	2.5%	74.7%	100.0%
Terminal Rates (c)	0/36 (0%)	20/28 (71%)	24/24 (100%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Liver: Adenoma or Carcinoma			
Overall Rates (a)	3/50 (6%)	33/48 (69%)	47/49 (96%)
Adjusted Rates (b)	7.9%	97.0%	100.0%
Terminal Rates (c)	2/36 (6%)	27/28 (96%)	24/24 (100%)
Life Table Tests (d)	P<0.001	P<0.001	P<0.001
Incidental Tumor Tests (d)	P<0.001	P<0.001	P<0.001
Cochran-Armitage Trend Test (d)	P<0.001		
Fisher Exact Tests		P<0.001	P<0.001
Pituitary: Adenoma			
Overall Rates (a)	4/44 (9%)	1/42 (2%)	1/44 (2%)
Adjusted Rates (b)	11.8%	3.8%	4.5%
Terminal Rates (c)	4/34 (12%)	1/26 (4%)	1/22 (5%)
Life Table Tests (d)	P=0.200N	P=0.267N	P=0.329N
Incidental Tumor Tests (d)	P=0.200N	P=0.267N	P=0.329N
Cochran-Armitage Trend Test (d)	P=0.102N		
Fisher Exact Tests		P=0.195N	P=0.180N
Adrenal: Pheochromocytoma			
Overall Rates (a)	2/50 (4%)	0/48 (0%)	3/47 (6%)
Adjusted Rates (b)	5.6%	0.0%	12.0%
Terminal Rates (c)	2/36 (6%)	0/28 (0%)	2/24 (8%)
Life Table Tests (d)	P=0.258	P=0.295N	P=0.324
Incidental Tumor Tests (d)	P=0.369	P=0.295N	P=0.470
Cochran-Armitage Trend Test (d)	P=0.368		
Fisher Exact Tests		P=0.258N	P=0.470
Thyroid: Follicular Cell Adenoma or Carcinoma			
Overall Rates (a)	3/47 (6%)	0/46 (0%)	2/44 (5%)
Adjusted Rates (b)	8.3%	0.0%	9.1%
Terminal Rates (c)	3/36 (8%)	0/27 (0%)	2/22 (9%)
Life Table Tests (d)	P=0.573N	P=0.176N	P=0.648
Incidental Tumor Tests (d)	P=0.573N	P=0.176N	P=0.648
Cochran-Armitage Trend Test (d)	P=0.414N		
Fisher Exact Tests		P=0.125N	P=0.532N

(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).

APPENDIX F

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

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

Study	Incidence in Controls		
	Neoplastic Nodule	Hepatocellular Carcinoma	Neoplastic Nodule or Hepatocellular Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	0/49	0/49	0/49
Cytembena	0/50	1/50	1/50
Eugenol	2/40	0/40	2/40
Stannous chloride	2/50	0/50	2/50
Mannitol	0/50	0/50	0/50
Ziram	2/50	0/50	2/50
Propyl gallate	2/50	0/50	2/50
Zearalenone	2/50	0/50	2/50
HC Blue No. 1	0/49	1/49	1/49
TOTAL	10/438 (2.3%)	2/438 (0.5%)	12/438 (2.7%)
SD (b)	2.24%	0.89%	1.85%
Range (c)			
High	2/40	1/49	2/40
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	78/2,306 (3.4%)	18/2,306 (0.8%)	96/2,306 (4.2%)
SD (b)	3.46%	1.09%	3.86%
Range (c)			
High	6/49	2/49	7/49
Low	0/50	0/90	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 F2. HISTORICAL INCIDENCE OF ALVEOLAR/BRONCHIOLAR TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	0/48	0/48	0/48
Cytembena	0/49	1/49	1/49
Eugenol	0/39	1/39	1/39
Stannous chloride	0/50	1/50	1/50
Mannitol	0/50	1/50	1/50
Ziram	1/50	0/50	1/50
Propyl gallate	0/50	0/50	0/50
Zearalenone	1/48	0/48	1/48
HC Blue No. 1	1/50	0/50	1/50
TOTAL	3/434 (0.7%)	4/434 (0.9%)	7/434 (1.6%)
SD (b)	1.01%	1.15%	0.94%
Range (c)			
High	1/48	1/39	1/39
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	18/2,354 (0.8%)	9/2,354 (0.4%)	27/2,354 (1.1%)
SD (b)	1.36%	0.93%	1.77%
Range (c)			
High	3/50	2/50	5/50
Low	0/88	0/88	0/88

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

Study	Incidence of Endometrial Stromal Polyp in Controls
Historical Incidence at Southern Research Institute	
Reserpine	10/50
Cytembena	9/48
Eugenol	6/40
Stannous chloride	11/50
Mannitol	10/50
Ziram	5/50
Propyl gallate	6/50
Zearalenone	4/50
HC Blue No. 1	5/50
TOTAL	66/438 (15.1%)
SD (b)	5.26%
Range (c)	
High	11/50
Low	4/50
Overall Historical Incidence	
TOTAL	424/2,318 (18.3%)
SD (b)	8.09%
Range (c)	
High	18/49
Low	2/47

(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 F4. HISTORICAL INCIDENCE OF STOMACH EPITHELIAL TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	No. of Animals Examined	No. of Tumors	Site	Diagnosis
Historical Incidence at Southern Research Institute				
Reserpine	49	1	Stomach, NOS	Squamous cell papilloma
All others	389	0		
TOTAL	438	1 (0.2%)		
Overall Historical Incidence				
	2,276	2	Stomach, NOS	Squamous cell papilloma
		1	Stomach, NOS	Squamous cell carcinoma
		2	Forestomach	Squamous cell papilloma
		1	Cardiac stomach	Squamous cell papilloma
TOTAL		(b) 6 (0.3%)		

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) No more than one tumor was observed in any control group.

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

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	7/50	6/50	12/50
Cytembena	4/47	13/47	17/47
Mannitol	3/50	11/50	14/50
Ziram	6/49	13/49	19/49
Eugenol	4/50	10/50	14/50
Propyl gallate	3/50	14/50	17/50
Zearalenone	4/50	15/50	19/50
HC Blue No. 1	4/50	11/50	15/50
Stannous chloride	7/50	10/50	16/50
TOTAL	42/446 (9.4%)	103/446 (23.1%)	143/446 (32.1%)
SD (b)	3.17%	5.58%	5.04%
Range (c)			
High	7/50	15/50	19/49
Low	3/50	6/50	12/50
Overall Historical Incidence			
TOTAL	240/2,334 (10.3%)	498/2,334 (21.3%)	725/2,334 (31.1%)
SD (b)	4.98%	6.95%	7.47%
Range (c)			
High	11/50	18/50	29/50
Low	0/49	4/50	7/44

(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 THYROID GLAND FOLLICULAR CELL TUMORS IN MALE B6C3F$_1$ MICE RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	0/48	0/48	0/48
Cytembena	1/44	0/44	1/44
Mannitol	0/50	0/50	0/50
Ziram	2/49	0/49	2/49
Eugenol	0/48	0/48	0/48
Propyl gallate	3/49	0/49	3/49
Zearalenone	2/50	0/50	2/50
HC Blue No. 1	0/47	0/47	0/47
Stannous chloride	0/48	0/48	0/48
TOTAL	8/433 (1.8%)	0/433 (0.0%)	8/433 (1.8%)
SD (b)	2.38%	0.00%	2.38%
Range (c)			
High	3/49	0/50	3/49
Low	0/50	0/50	0/50
Overall Historical Incidence			
TOTAL	(d) 21/2,178 (1.0%)	5/2,178 (0.2%)	26/2,178 (1.2%)
SD (b)	1.61%	0.68%	1.73%
Range (c)			
High	3/49	1/44	3/49
Low	0/50	0/50	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) One adenoma, NOS and one papillary adenoma were also observed. The inclusion of these tumors does not affect the reported range for adenoma.

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

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at Southern Research Institute			
Reserpine	2/50	0/50	2/50
Cytembena	0/48	2/48	2/48
Mannitol	0/48	3/48	3/48
Ziram	7/50	2/50	9/50
Eugenol	0/50	2/50	2/50
Propyl gallate	0/50	3/50	3/50
Zearalenone	0/50	3/50	3/50
HC Blue No. 1	2/50	1/50	3/50
Stannous chloride	3/49	0/49	3/49
TOTAL	14/445 (3.1%)	16/445 (3.6%)	30/445 (6.7%)
SD (b)	4.71%	2.44%	4.34%
Range (c)			
High	7/50	3/48	9/50
Low	0/50	0/50	2/50
Overall Historical Incidence			
TOTAL	98/2,469 (4.0%)	(d) 101/2,469 (4.1%)	196/2,469 (7.9%)
SD (b)	3.90%	3.01%	4.58%
Range (c)			
High	9/49	7/48	10/49
Low	0/50	0/50	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) One hepatoblastoma was also observed.

TABLE F8. HISTORICAL INCIDENCE OF HEMATOPOIETIC SYSTEM TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence of Leukemia in Controls
Historical Incidence at Southern Research Institute	
Reserpine	18/49
Cytembena	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 F9. HISTORICAL INCIDENCE OF PITUITARY GLAND TUMORS IN MALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls		
	All Adenomas (b)	All Carcinomas (c)	All Adenomas or Carcinomas
Historical Incidence at Southern Research Institute			
Reserpine	14/49	4/49	18/49
Cytembena	3/50	0/50	3/50
Eugenol	2/39	0/39	2/39
Stannous chloride	11/50	1/50	12/50
Mannitol	9/46	0/46	9/46
Ziram	13/50	2/50	15/50
Propyl gallate	5/49	0/49	5/49
Zearalenone	5/46	1/46	6/46
HC Blue No. 1	9/49	2/49	11/49
TOTAL	71/428 (16.6%)	10/428 (2.3%)	81/428 (18.9%)
SD (d)	8.59%	2.78%	10.87%
Range (e)			
High	14/49	4/49	18/49
Low	2/39	0/50	2/39
Overall Historical Incidence			
TOTAL	466/2,158 (21.6%)	50/2,158 (2.3%)	516/2,158 (23.9%)
SD (d)	11.63%	2.97%	11.85%
Range (e)			
High	24/46	5/45	25/46
Low	1/47	0/50	1/47

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

TABLE F10. HISTORICAL INCIDENCE OF ADRENAL GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	Incidence in Controls			
	Cortical Adenoma	Cortical Adenoma or Carcinoma	Pheochromocytoma	Pheochromocytoma or Pheochromocytoma, Malignant
Historical Incidence at Southern Research Institute				
Reserpine	1/49	1/49	1/49	1/49
Cytembena	0/49	0/49	0/49	0/49
Eugenol	1/40	1/40	1/40	2/40
Stannous chloride	1/50	1/50	1/50	1/50
Mannitol	1/49	1/49	2/49	2/49
Ziram	2/50	2/50	1/50	1/50
Propyl gallate	1/50	1/50	4/50	4/50
Zearalenone	0/50	0/50	0/50	0/50
HC Blue No. 1	5/50	5/50	8/50	8/50
TOTAL	12/437 (2.7%)	12/437 (2.7%)	18/437 (4.1%)	19/437 (4.3%)
SD (b)	2.99%	2.99%	5.08%	5.05%
Range (c)				
High	5/50	5/50	8/50	8/50
Low	0/50	0/50	0/50	0/50
Overall Historical Incidence				
TOTAL	(d) 74/2,338 (3.2%)	(d) 81/2,338 (3.5%)	(e) 79/2,338 (3.4%)	90/2,338 (3.8%)
SD (b)	4.03%	4.09%	2.96%	2.83%
Range (c)				
High	12/50	12/50	8/50	8/50
Low	0/50	0/50	0/50	0/50

(a) Data as of March 16, 1983, for studies of at least 104 weeks. In no case were benign and malignant tumors of the same type observed in a single animal.
(b) Standard deviation
(c) Range and SD are presented for groups of 35 or more animals.
(d) Includes one adenoma, NOS
(e) Two pheochromocytomas of the adrenal medulla were also observed.

TABLE F11. HISTORICAL INCIDENCE OF ZYMBAL GLAND TUMORS IN FEMALE F344/N RATS RECEIVING NO TREATMENT (a)

Study	No. of Animals Examined	No. of Tumors	Diagnosis
Historical Incidence at Southern Research Institute			
Zearalenone	50	1	Squamous cell carcinoma
HC Blue No. 1	50	1	Squamous cell carcinoma
		2	Adenosquamous carcinoma
All Others	339	0	
TOTAL	439	4 (0.9%)	
Overall Historical Incidence			
	2,370	2	Carcinoma, NOS
		4	Squamous cell carcinoma
		1	Adenocarcinoma, NOS
		2	Adenosquamous carcinoma
TOTAL		(b) 9 (0.4%)	

(a) Data as of March 16, 1983, for studies of at least 104 weeks
(b) The greatest incidence of combined Zymbal gland tumors is the 3/50 (6%) observed in the current study. No other untreated control group had more than one of these tumors.

APPENDIX G

CHEMICAL CHARACTERIZATION

OF HC BLUE NO. 1

APPENDIX G. CHEMICAL CHARACTERIZATION

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

A. Lot No. 3270877

1. Physical Properties

a. Appearance: Dark blue microcrystals

b. Melting Point:

Determined	Literature Values
101.5°-104° C (visual melting point, capillary), small endotherm at 52.0°-54.0° C. Three large overlapping endotherms at 98.0°-107° C (Dupont 900 DTA)	102.5°-103° C (Clairol Research Labs, personal communication)

2. Spectral Data

a. Infrared

	Determined	Literature Values
(1) Instrument:	Beckman IR-12	
(2) Cell:	1% Potassium bromide pellet	
(3) Results:	See Figure 5	Consistent with spectrum obtained from Clairol Research Labs

b. Ultraviolet/Visible

	Determined	Literature Values
(1) Instrument:	Cary 118	
(2) Concentration:	(261 nm) 0.0002% (535 nm) 0.006%	0.0002%
(3) Solvent:	Methanol	Methanol

(4) Results:

Determined λ_{max} (nm)	Determined $\varepsilon \times 10^{-4}$	Literature Values λ_{max} (nm)	Literature Values $\varepsilon \times 10^{-4}$
261	2.57 ± 0.03 (δ)		
535	0.376 ± 0.003 (δ)	530	0.4365 (Clairol)
		540 (Frenkel and Brody, 1973)	

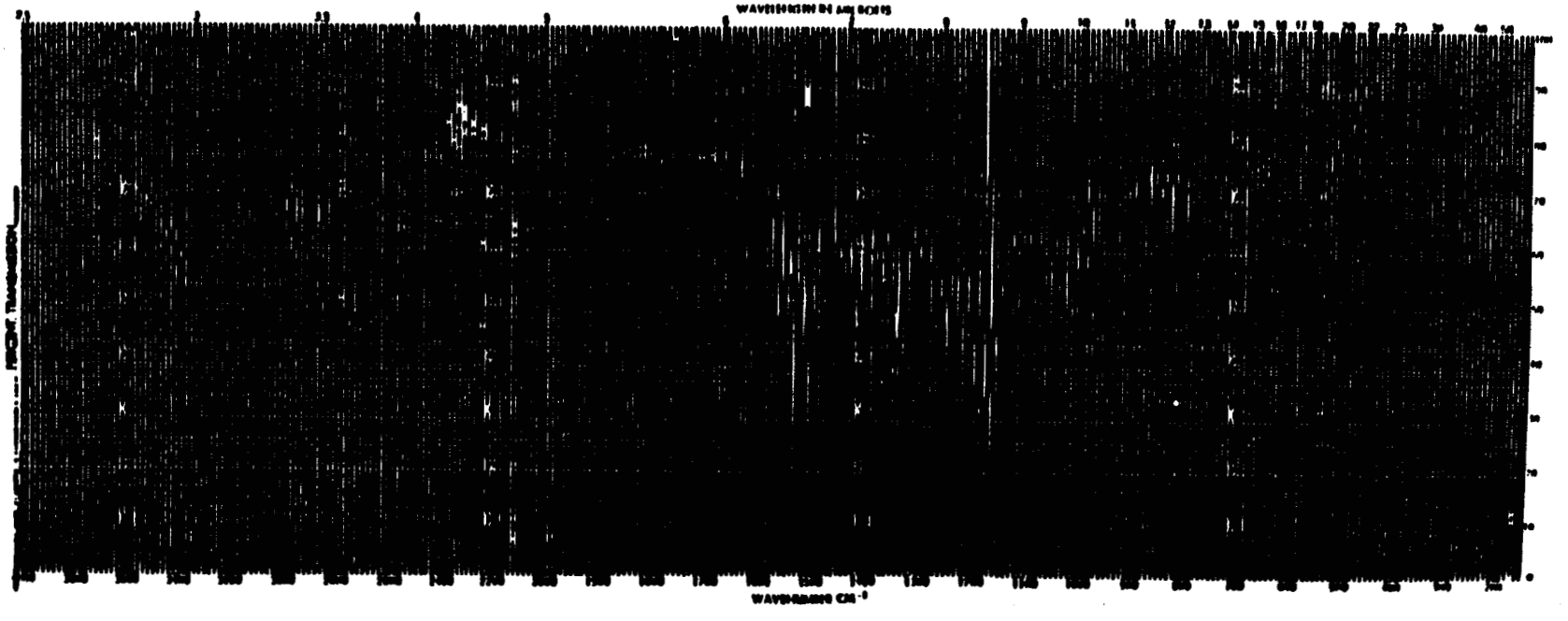


FIGURE 5. INFRARED ABSORPTION SPECTRUM OF HC BLUE NO. 1 (LOT NO. 3270877)

c. Nuclear Magnetic Resonance

	Determined	Literature Values
(1) Instrument:	Varian EM-360-A	
(2) Solvent:	Deuterated methanol with internal tetramethylsilane	
(3) Assignments:	See Figure 6	No literature reference found. Spectrum is consistent with that expected for structure.
(4) Chemical Shift (δ):	a s, 2.96 ppm b d, 3.48 ppm c d, 3.66 ppm d d, 6.88 ppm e d of d, 7.28 ppm f d, 7.45 ppm g s, 4.80 ppm (H_2O and exchangeable protons) h s, (impurity) 1.00 ppm	
(5) Coupling Constant:	$J_{b-c} = 4$ Hz $J_{d-f} = 9$ Hz $J_{e-f} = 3$ Hz	
(6) Integration Ratios:	a 2.93 b, c ⌐ 8.23 ⌙ d 0.93 e, f ⌐ 1.92 ⌙ g H_2O and exchangeable protons h 0.05	

3. Titration: Titration of one amine function with perchloric acid, 100.2% ± 0.1(δ)%

4. Water Analysis (Karl Fischer): 0.136% ± 0.021(δ)%

5. Elemental Analysis:

Element	C	H	N
Theory (T)	51.56	6.74	16.52
Determined (D)	51.46 51.63	7.02 6.98	16.31 16.49
Percent D/T	99.98	103.86	99.27

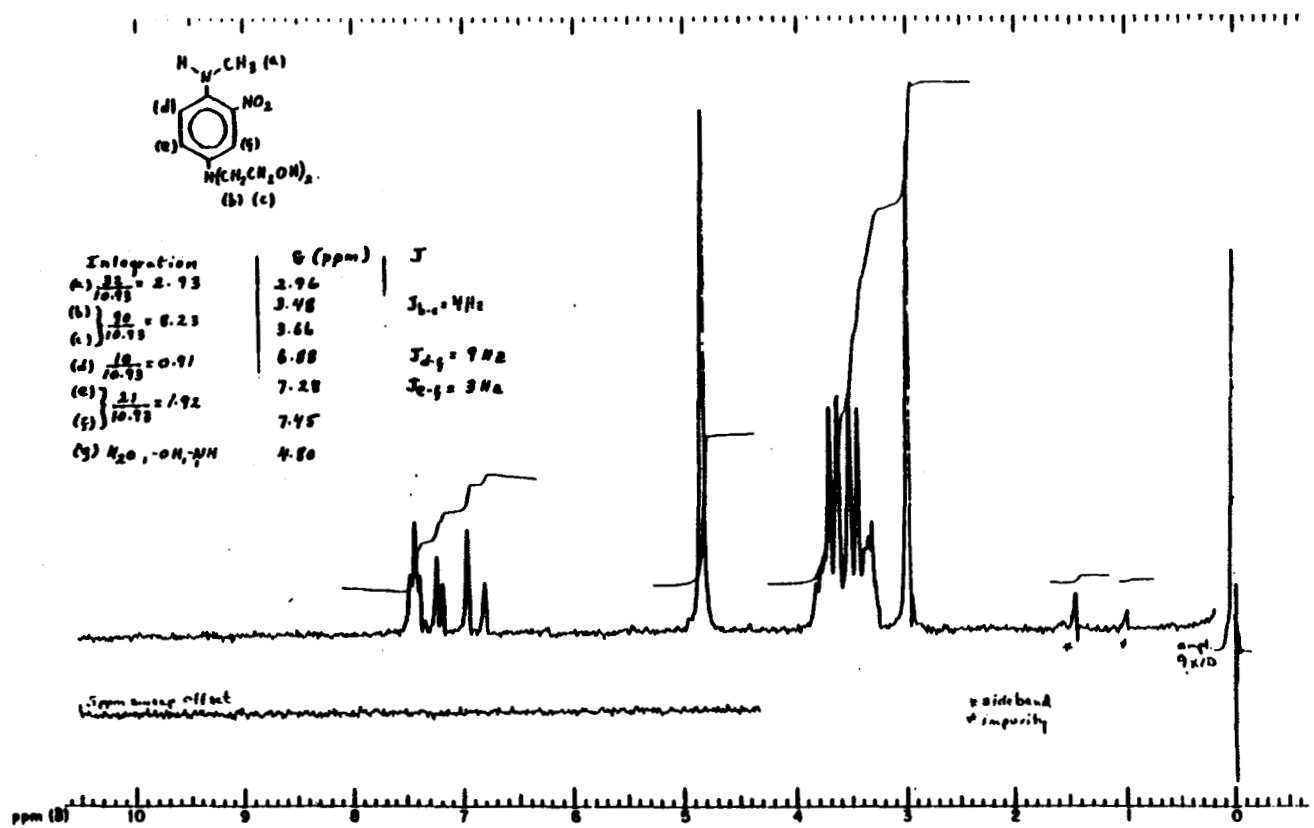


FIGURE 6. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HC BLUE NO. 1 (LOT NO. 3270877)

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

System 1: Chloroform:methanol (78:22)

(a) R_f: 0.79 (slight trace), 0.59 (major), 0.17 (trace, 366 nm only), origin (slight trace)

(b) R_{st}: 1.41, 0.95, 0.30, origin

System 2: Ethyl acetate:ethanol (90:10)

(a) R_f: 0.65 (trace), 0.45 (trace), 0.33 (major), 0.25 (trace, 366 nm only), origin (trace)

(b) R_{st}: 1.48, 1.02, 0.75, 0.54, 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) Flow Rate: 1.0 ml/min
(5) Chart Speed: 0.1 in/min
(6) Sample Injected: 10 µl of 1.0 mg/ml in methanol

System 1:
(a) Solvent Program: Acetonitrile:water (10:90), isocratic

(b) Results: Major peak and eight impurities. Four impurities had areas of 0.59%, 0.82%, 0.29%, and 0.99% of the major peak area. The other four impurities had areas totaling less than 0.4% of the major peak area.

Peak	Retention Time (min.)	RetentionTime Relative to Major Peak	Area (percent of major peak)
1	2.0	0.09	0.10
2	3.5	0.16	0.08
3	5.8	0.26	0.59
4	9.0	0.41	0.02
5 (a)	12.5	0.57	0.82
6	15.3	0.70	0.29
7	21.9	1.00	100
8	26.0	1.19	0.15
9	37.0	1.69	0.99

(a) Unresolved multiple peaks

After this system was completed, the solvent was programmed from 10% acetonitrile up to 100% acetonitrile, and only one other peak was observed. This peak was isolated and quantitated through the use of System 2 (below).

System 2:

(a) Solvent Program: Acetonitrile:water (23:77), isocratic

(b) Results: Major peak and one impurity

Peak	Retention Time (min.)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	12.3	1.00	100
2	46.7	3.81	0.27

7. Conclusions: Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values. Titration of one amine function with perchloric acid indicated a purity of 100.2% ± 0.1(δ)%. High-performance liquid chromatography by one system indicated eight impurities. Four impurities had areas of 0.59%, 0.82%, 0.29%, and 0.99% of the major peak area. The other four impurities had areas totaling less than 0.4% of the major peak area. A second high-pressure liquid chromatography system was used to isolate and quantitate one other impurity. Its area was 0.27% of the major peak area. Thin-layer chromatography by one system indicated two slight trace impurities and one trace impurity. A second thin-layer chromatography system indicated four trace impurities. The infrared and nuclear magnetic resonance spectra are consistent with the structure. The ultraviolet spectrum indicated absorbances between 261 nm and 535 nm with ε_{max} of 25,700 and 3,760, respectively. A corresponding absorbance (530 nm) in the spectrum of the purified dye obtained from Clairol had an ε_{max} of 4,365. The ε value for lot no. 3270877 at 530 nm was 86.1% of that for the purified dye.

B. Lot No. 3670379

1. Physical Properties

Appearance:	Blue-black amorphous powder

2. Spectral Data

a. Infrared	**Determined**	**Literature Values**
(1) Instrument:	Beckman IR-12	
(2) Cell:	1% in potassium bromide pellet	
(3) Results:	See Figure 7	Consistent with spectrum from Clairol Research Labs and identical to that for lot no. 3270877

b. Ultraviolet/Visible	**Determined**		**Literature Values**	
(1) Instrument:	Cary 118			
(2) Solvent:	Methanol		Methanol	
(3). Results:	λ_{max} (nm)	$\varepsilon \times 10^{-4}$	λ_{max} (nm)	$\varepsilon \times 10^{-4}$
	263	2.17 ± 0.04 (δ)		
	538	0.399 ± 0.007 (δ)	530 (Clairol Research Labs)	0.4365

c. Nuclear Magnetic Resonance	**Determined**	**Literature Values**
(1) Instrument:	Varian EM-360A	
(2) Solvent:	Deuterated methanol with internal tetramethylsilane	
(3) Assignments:	See Figure 8	No literature reference found. Spectrum consistent with structure and identical to that for lot no. 3270877

(4) Chemical Shift (δ):

a s, 2.95 ppm
b d, 3.47 ppm
c d, 3.64 ppm
d d, 6.86 ppm
e d of d, 7.18 ppm
f d, 7.39 ppm
g s, 4.81 ppm (H_2O and exchangeable protons)

(5) Coupling Constant:

J_{b-c} = 4 Hz
J_{d-e} = 9 Hz
J_{e-f} = 3 Hz

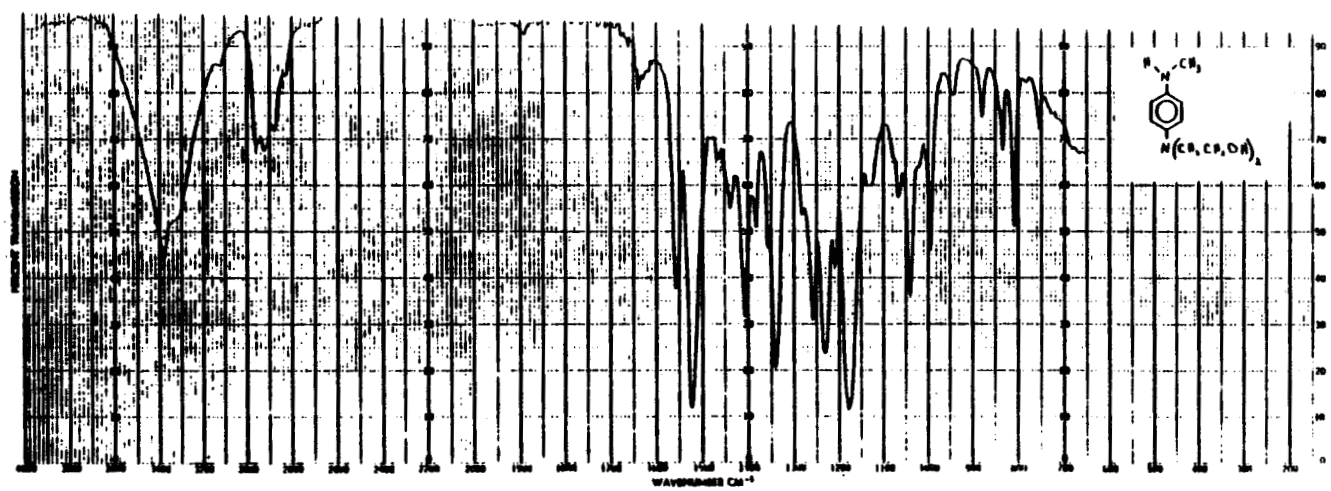


FIGURE 7. INFRARED ABSORPTION SPECTRUM OF HC BLUE NO. 1 (LOT NO. 3670379)

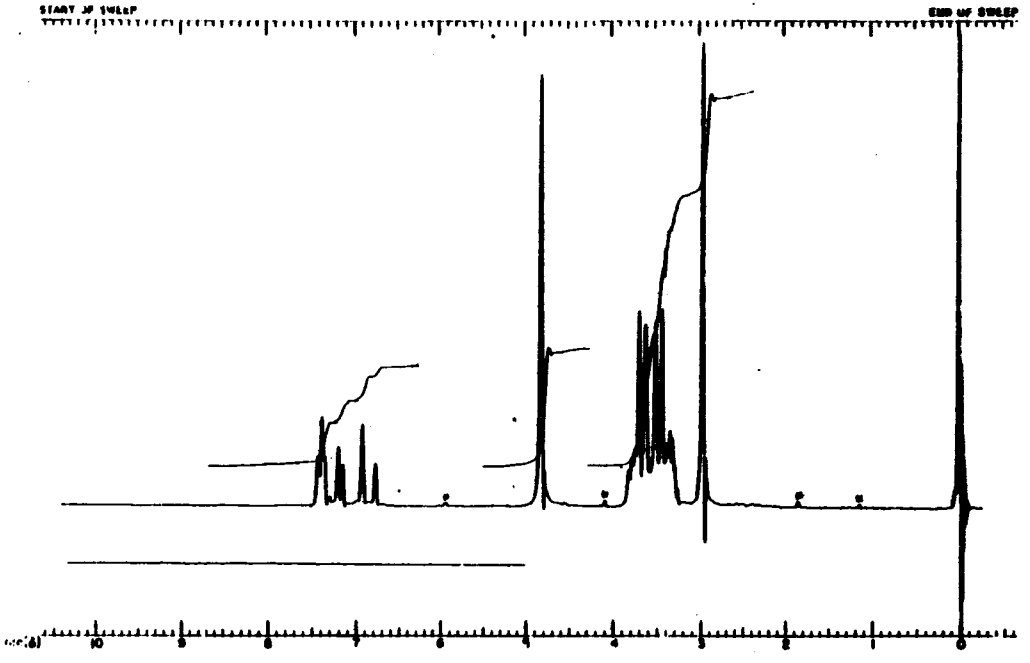


FIGURE 8. NUCLEAR MAGNETIC RESONANCE SPECTRUM OF HC BLUE NO. 1 (LOT NO. 3670379)

(6) Integration Ratios:

a	2.93	
b, c	8.08	
d	1.03	
e, f	1.97	
g	H_2O and exchangeable protons	

3. Titration: Titration of one amine function with 0.1N perchloric acid, monitored potentiometrically with a combination electrode: 99.28% ± 0.30(δ)%

4. Water Analysis (Karl Fischer): 0.35% ± 0.11(δ)%

5. Elemental Analysis:

Element	C	H	N
Theory (T)	51.75	6.71	16.46
Determined (D)	51.33 51.57	6.71 6.67	16.29 16.11
Percent D/T	99.37	99.70	98.42
Av. Dev. from Theoretical (percent)	0.30	0.02	0.26

6. Chromatographic Analyses

a. Thin-Layer Chromatography

(1) Plates: Silica Gel 60 F-254
(2) Reference Standard: 1 µl of a solution (10 mg/ml) of *p*-phenylenediamine in methanol
(3) Amount Spotted: 1, 10, and 30 µl of a solution (10 mg/ml) in methanol
(4) Visualization: Visible and ultraviolet light (254 nm); furfural:glacial acetic acid (10 drops:10 ml) (Feigl, 1966)

Spot Intensity	R_f	R_{st}	Visualization: Vis. Light	Visualization: Spray	Visualization: 254 nm UV
System 1: Ethyl acetate:ethanol (90:10)					
Trace	0.28	1.17	Rose	+	+
Major	0.35	1.46	Purple	+	+
Trace	0.45	1.88	Pink	+	+
Trace	Origin		Brown	+	+
Reference	0.24		Tan	+	+
System 2: Chloroform:methanol (100:10)					
Trace	0.19	1.86	Rose	+	+
Major	0.28	1.27	Purple	+	+
Slight Trace	0.36	1.64	Pink	+	+
Trace	Origin		Brown	+	+
Reference	0.22		Tan	+	+

b. High-Peformance Liquid Chromatography:

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

(A) Water containing 0.005 M heptanesulfonic acid, sodium salt, and added 1% (v/v) phosphoric acid
(B) Methanol containing 0.005 M heptanesulfonic acid, sodium salt, and added 1% (v/v) phosphoric acid

System 1:

Solvent Program: 74% (A):26% (B), isocratic
Flow Rate: 2 ml/min
Samples Injected: 10 μl of a solution (1 mg/ml) of HC Blue No. 1 in solvent (A), filtered

Results: A major peak and three impurities. One impurity eluted before the major peak with an area of 0.1% relative to the major peak area. Two other impurities eluting after the major peak had a combined area of 1.6% of the major peak area.

Peak	Retention Time (min.)	Retention Time Relative to Major Peak	Area (percent of major peak)
1	3.4	0.85	0.1
2	4.0	1.00	100
3	5.8	1.45	0.8
4	15.8	3.95	0.8

System 2: A comparison was made between the previous lot (3270877) and the present lot using a different solvent delivery system (Varian 5020 Liquid Chromatograph) and solvent ratio.

Solvent Program: 80%(A):20%(B)
Flow Rate: 1 ml/min
Samples Injected: Solutions of HC Blue No. 1 in methanol, filtered

Lot no. 3270877	0.892 mg/ml
Lot no. 3670379	0.952 mg/ml

Results: The chromatographic profiles of the two lots were very similar. In both lots the largest impurities were those eluting after the major peak (0.8% and 0.8% in lot no. 3670379 compared with 0.5% and 0.3% in lot no. 3270877). Two small impurities appeared before the major peak in lot no. 3270877 and three before the major peak in lot no. 3670379; all of these impurities were less than 0.1% except one in lot no. 3670379 which was 0.1%. The major peak area comparison of the two batches was identical within the error of the analysis.

Major Peak Comparison

	Peak Height (mm)	Concentration (µg/µl)	mm ÷ µg/µl	Relative Response (a)
Lot no. 3270877	171	0.892	192	99.0 ± 1.0
Lot no. 3670379	185	0.952	194	

(a) Lot no. 3270877 /Lot no. 3670379

Impurity Profile

Peak	Retention Time Relative to MajorPeak	Area (percent of major peak) Lot no. 3270877	Lot no. 3670379
1	0.9	<0.1	0.1
2	1.0	100	100
3	1.5	0.5	0.8
4	3.5	0.3	0.8

7. Conclusions: Elemental analyses for carbon, hydrogen, and nitrogen were in agreement with the theoretical values. Titration of one amine group with perchloric acid indicated a purity of 99.28% ± 0.30(δ)%. (Lot no. 3270877 was 100.2% ± 0.1% pure by titration.) Karl Fischer water analysis indicated 0.35% ± 0.1(δ)%. Thin-layer chromatography indicated a major spot and three trace impurities by one system. Another system indicated a major spot, two trace impurities, and a slight trace impurity. High-performance liquid chromatography by one system indicated three impurities with a combined area of 1.7% relative to the major peak area. A lot comparison using high-performance liquid chromatography indicated a major peak ratio of 100:99 (lot no. 3670379/lot no. 3270877). The ultraviolet, visible, infrared, and nuclear magnetic resonance spectra were consistent with the structure of HC Blue No. 1 and with that obtained on lot no. 3270877 of the same compound.

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

A. Sample Preparation and Storage: HC Blue No. 1 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 (degrees centigrade)	Percent Purity
−20	100.50 ± 0.685 (δ)
5	100.24 ± 0.050 (δ)
25	102.16 ± 1.60 (δ)
60	101.91 ± 0.728 (δ)

D. Conclusion: HC Blue No. 1 is stable as the bulk chemical when stored for 2 weeks at temperatures of up to 60° C.

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 535 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 using a Perkin-Elmer 621.

C. Results:

1. Purity:

Date of Analysis	Lot No.	Absorptivity (a) Bulk	Absorptivity (a) Reference	Percent Purity (b)
05/23/78	3270877	4.03	3.95	102
08/24/78	3270877	3.93	3.90	101
12/12/78	3270877	(c) 3.32	3.98	83
04/27/79	3270877	3.82	3.89	98
09/07/79	3270877	3.74	3.74	100
01/14/80	3270877	4.04	3.96	102
03/27/80	3670379	3.92	--	
05/05/80	3670379	3.96	4.05	98
09/16/80	3670379	3.78	3.73	101
01/16/81	3670379	3.86	3.86	100
05/11/81	3670379	3.93	3.94	100

Mean Percent Purity

Lot no. 3270877	97.7% ± 7.5%
Lot no. 3670379	99.8% ± 1.3%

(a) (1/g-cm) × 10^{-3}
(b) Purity of material stored at 5° C relative to reference standard stored at – 20° C.
(c) Low value possibly due to poor solubility of compound

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

APPENDIX H. PREPARATION AND CHARACTERIZATION

I. Studies Conducted at the Analytical Chemistry Laboratory

A. Preparation Procedure

1. Premix: HC Blue No. 1 (1.650 g) was dissolved in 100 ml of reagent-grade acetone in a volumetric flask. Wayne Lab Blox® Rodent Feed (200 g) was placed in a 2-liter round bottom flask and covered with 500 ml of reagent grade acetone. The 100-ml solution was then added to the mixture, and it was thoroughly swirled to distribute the HC Blue No. 1 evenly. The acetone was removed on a rotary evaporator (water aspirator vacuum, water bath at 35° C).

2. Bulk Mixing: The premix and 1,300 g more feed were mixed in a Patterson-Kelly® Twin Shell Blender for 15 minutes. The blender was loaded from the top of the shells as follows: 650 g of feed was poured in and allowed to settle and level at the bottom (the vertex of the "V"); then the dried premix was poured on top of the premix from each side; this layer was covered with the the remaining 650 g of feed poured in from each side. When the mixture had been blended for 15 minutes, duplicate 5-g samples were removed from the top of each shell and from the bottom trap of the blender for analysis.

3. Extraction and Analysis: Each sample was placed in a 200-ml centrifuge bottle (quantitative transfer) and triturated with 45 ml of absolute methanol in a Brinkmann Polytron® high-speed blender for 1 minute. The mixture was then placed in an ultrasonic vibratory bath for 2 minutes and centrifuged for 10 minutes. The supernatant solution was decanted through Whatman No. 1 filter paper into a 100-ml volumetric flask. The feed residue was mixed with an additional 45 ml of methanol and extracted again. The combined supernatant solutions were brought to volume with additional methanol. The visible absorbance of this solution was measured at 535 nm with a Cary 118 spectrophotometer to determine the HC Blue No. 1 content.

4. Quality Control : Blank (undosed) feed samples and individual spiked mixtures (at the 0.11% level) were extracted and prepared for analysis in the manner described above. Standard solutions of HC Blue No. 1 in methanol were used to determine the extinction coef ficient 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. 1.

B. Homogeneity

1. Results:

Sample Location	Average Percent Found in Chemical/Vehicle Mixture (a)
Right 1	0.111 ± 0.003
Right 2	0.113 ± 0.003
Left 1	0.111 ± 0.003
Left 2	0.108 ± 0.003
Bottom 1	0.108 ± 0.003
Bottom 2	0.105 ± 0.003

(a) Mean ± standard deviation; corrected for a spiked recovery yield of 92% ± 3%. The theoretical concentration of chemical in feed was 0.110% ± 0.001%.

2. Conclusion: HC Blue No. 1 mixed with stock rodent feed at the 0.11% concentration is homogeneous to within the experimental error of the average position sample determination (i.e., right, left, or bottom) when mixed for 15 minutes in a Patterson-Kelly® 4 quart twin-shell blender equipped with an intensifier bar.

C. Stability

1. Sample Mixing and Storage: A stock solution of HC Blue No. 1 in methanol (1.0 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°, and 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-μ 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: 28 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 a small feed interference, which was subtracted from the area of the compound peaks of the test samples.

4. Results:

Storage Temperature	Average Percent Chemical Found in Chemical/Vehicle Mixture (a)
−20° C	0.101 ± 0.003
5° C	0.099 ± 0.003
25° C	0.098 ± 0.003
45° C	0.076 ± 0.003

(a) Mean ± standard deviation; corrected for a spiked recovery yield of 99% ± 3%. The theoretical concentration of chemical in feed was 0.100% ± 0.001%.

5. Conclusions: HC Blue No. 1 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 25° C or below. Samples stored at 45° C for 2 weeks showed a significant loss of the major component when analyzed.

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 13 days.

B. Homogeneity

1. Procedure: Five-gram feed samples were weighed, placed in a large test tube, and triturated with 25 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 re-extracted with 25 ml of methanol in a Polytron blender (1 minute) and filtered through a Millipore filter. The feed residue was rinsed twice with washings from the sample tube. The residue was then rinsed with additional aliquots of methanol until there was no trace of the dye in the feed residue. The combined filtrates were then placed in a 100-ml volumetric flask and brought to volume with additional methanol. The absorbance of these solutions was measured at 535 nm to determine the HC Blue No. 1 content. These absorbances were compared to a standard absorption curve of HC Blue No. 1.

2. Results:

Sample Location	Target Concentration (percent wt/wt)	Determined Concentration (percent wt/wt)	Percent of Target Value
Top left	0.075	0.076	101
Top right	0.075	0.074	99
Bottom	0.075	0.084	112
Top left	1.25	1.22	98
Top right	1.25	1.24	99
Bottom	1.25	1.23	98

C. Conclusion: The homogeneity of the high dose mixtures was excellent. The concentration at one of the three sampling positions of the low dose was slightly greater than 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. 1 as a chemical/feed mixture were received for analysis.
2. Duplicate 5-g samples were weighed to the nearest 0.1 g into 50-ml test tubes.
3. Four 5-g samples of plain feed were weighed out, and two of these were spiked with 25 mg of HC Blue No. 1.
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. 1 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. 1 in each sample and spike was calculated from the measured absorbance at 530 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. 1 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 Procedure

A. Preparation of Spiked Feed Standards: Two standard solutions of HC Blue No. 1 were prepared independently in methanol. These solutions were diluted with methanol to span a range of 2-10 mg/ml. 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.

B. 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.

C. Analysis: Ninety milliliters of methanol was pipetted into each blank, standard, and sample bottle and the bottles were shaken at maximum stroke for 20 minutes on a Burrell Model 75 Wrist-Action® shaker. After being centrifuged for 10 minutes, a 6-ml aliquot from each extract was diluted to 100 ml with methanol and thoroughly mixed. The absorbance of each solution was measured at 535 nm versus methanol in 1-cm quartz cells on a Cary 118 spec-trophotometer.

The total milligrams of HC Blue No. 1 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 milligrams of chemical in the respective spiked feed.

APPENDIX J

ANALYSES OF FORMULATED DIETS: DATA

APPENDIX J. ANALYSES: DATA

I. Thirteen-Week Studies: Formulated diets were analyzed once during the 13-week studies. The results ranged from 97% to 113% of the target concentrations.

Target Concentration (ppm)				
750	1,500	3,000	6,250	12,500
Determined Concentration (ppm)				
780	1,700	2,920	6,560	12,300

II. Two-Year Studies: Samples of diet formulations were analyzed monthly. The results of the initial mixes ranged from 83% to 113% 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 1,500-, 3,000-, and 6,000-ppm mixes were out of specification 12%, 8%, and 0% 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. 1 IN FEED IN THE TWO-YEAR STUDIES

Date Mixed	Determined Concentration for Target Concentration of 1,500 ppm	3,000 ppm	6,000 ppm
05/21/79	1,400	3,000	
	1,400		
06/18/79	1,600	3,200	5,900
		3,100	
07/16/79	1,400	3,300	6,200
08/13/79	1,600	2,900	
09/10/79	1,600	2,800	5,900
10/08/79	1,500	3,300	
11/05/79	1,400	3,100	5,800
12/03/79	1,500	3,000	
12/31/79	1,400	2,800	6,000
01/28/80	1,600	2,900	
02/25/80	1,600	2,900	5,600
03/24/80	1,400	2,800	
04/21/80	(a) 1,340	2,800	5,610
05/19/80	1,400	2,730	
06/16/80	1,390	(b) 2,470	5,750
06/20/80		(c) 3,000	
07/14/80	1,490	2,870	
08/11/80	1,350	3,240	6,050
09/08/80	(b) 1,250	2,700	
09/12/80	(c) 1,450		
10/06/80	1,360	2,830	5,850
11/03/80	1,660	(b) 2,620	
11/06/80	(c) 1,370	(c) 3,250	
12/01/80	1,530	2,770	5,640
01/05/81	1,390	3,220	
01/26/81	1,440	2,750	5,970
02/23/81	1,420	3,000	
03/31/81	(b) 1,640	2,880	5,980
04/02/81	(c) 1,520		
Experimental mean (d)	1,464	2,926	5,865
Standard deviation (d)	108.9	208.5	181.4
Coefficent of variation (percent)	6.7	6.9	3.4
Range	1,250-1,660	2,470-3,300	5,600-6,200
No. of samples	26	26	13

(a) Dose out of specifications but not remixed
(b) Dose remixed
(c) Remix
(d) The statistical analyses of the data reflect only the initial mixes. They do not include the data from the remixes. This procedure should more accurately portray the doses given to the animals throughout the 2-year studies.

TABLE J2. REFEREE SAMPLE DATA FOR FEED IN THE TWO-YEAR STUDIES OF HC BLUE NO. 1

Date Mixed	Target Concentration (ppm)	Determined Concentration Testing Laboratory	Determined Concentration Analytical Laboratory
07/16/79	3,000	3,300	3,110
12/03/79	1,500	1,500	1,500
04/21/80	3,000	2,800	3,050
07/14/80	1,500	1,490	1,570
03/30/81	6,000	5,980	5,670

APPENDIX K

SENTINEL ANIMAL PROGRAM

IN THE TWO-YEAR FEED STUDIES OF

HC BLUE NO. 1

APPENDIX K. SENTINEL ANIMAL PROGRAM

A. 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)
Rats	PVM KRV (Kilham rat virus) H-1 (Toolan's H-1 virus) Sendai	RCV (rat coronavirus)	

B. 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. 1

	Interval	No. of Animals	Positive Serologic Reaction for
RAT			
	6 months	6/10	Sendai
		10/10	PVM
	12 months	9/10	Sendai
		5/10	KRV
		9/10	PVM
	18 months	5/10	Sendai
		6/10	PVM
	24 months	6/10	Sendai
		5/10	KRV
		6/10	PVM
		1/10	RCV
MICE			
	6 months	10/10	Sendai
		9/10	PVM
	12 months	9/10	Sendai
		2/10	PVM
	18 months	7/7	Sendai
	24 months	6/10	Sendai

APPENDIX L

FEED AND COMPOUND CONSUMPTION BY RATS AND MICE IN THE TWO-YEAR FEED STUDIES OF HC BLUE NO. 1

TABLE L1. FEED AND COMPOUND CONSUMPTION BY MALE RATS IN THE TWO-YEAR FEED STUDY OF HC BLUE NO. 1

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)
4	17	188	17	184	1.0	139	16	186	0.9	258
8	15	224	14	217	0.9	97	14	217	0.9	194
12	15	265	15	261	1.0	86	15	263	1.0	171
16	15	303	15	275	1.0	82	15	272	1.0	165
21	15	332	15	320	1.0	70	15	315	1.0	143
26	16	356	15	344	0.9	65	15	335	0.9	134
29	16	365	15	355	0.9	63	13	345	0.8	113
33	15	375	15	362	1.0	62	15	354	1.0	127
38	15	391	16	377	1.1	64	15	369	1.0	122
42	15	394	16	382	1.1	63	15	371	1.0	121
46	15	405	15	390	1.0	58	14	378	0.9	111
50	16	409	15	393	0.9	57	15	382	0.9	118
55	15	420	15	402	1.0	56	15	392	1.0	115
60	14	419	16	407	1.1	59	16	396	1.1	121
65	16	426	16	409	1.0	59	15	399	0.9	113
69	16	425	15	406	0.9	55	9	395	0.6	68
74	17	425	16	407	0.9	59	15	394	0.9	114
77	15	429	15	406	1.0	55	15	393	1.0	115
81	15	420	14	400	0.9	53	14	392	0.9	107
85	15	419	15	399	1.0	56	14	390	0.9	108
89	17	421	15	393	0.9	57	15	384	0.9	117
93	16	422	15	404	0.9	56	16	393	1.0	122
96	14	424	14	399	1.0	53	13	394	0.9	99
101	14	411	14	390	1.0	54	14	378	1.0	111
Mean	15.4	378	15.1	362	1.0	66	14.5	354	0.9	129
SD (d)	0.9		0.7		0.1	19	1.4		0.1	37
CV (e)	5.8		4.6		10.0	28.8	9.7		11.1	28.7

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of HC Blue No. 1 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. 1

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)
4	12	128	11	127	0.9	130	11	122	0.9	270
8	10	154	10	150	1.0	100	10	144	1.0	208
12	11	162	9	161	0.8	84	10	157	0.9	191
16	11	176	11	174	1.0	95	11	160	1.0	206
21	10	186	10	184	1.0	82	10	180	1.0	167
26	11	197	10	192	0.9	78	10	186	0.9	161
29	10	202	10	197	1.0	76	8	190	0.8	126
33	10	207	9	201	0.9	67	10	193	1.0	155
38	10	216	9	210	0.9	64	9	201	0.9	134
42	10	217	10	210	1.0	71	9	199	0.9	136
46	10	224	10	216	1.0	69	9	203	0.9	133
50	10	230	10	220	1.0	68	9	205	0.9	132
55	11	240	10	227	0.9	66	10	213	0.9	141
60	11	249	11	235	1.0	70	10	218	0.9	138
65	11	257	11	243	1.0	68	11	223	1.0	148
69	11	263	11	247	1.0	67	13	227	1.2	172
74	12	271	12	258	1.0	70	12	236	1.0	153
77	12	281	11	265	0.9	62	11	243	0.9	136
81	12	285	12	269	1.0	67	11	244	0.9	135
85	12	287	11	275	0.9	60	11	248	0.9	133
89	13	297	12	277	0.9	65	12	252	0.9	143
93	13	300	13	287	1.0	68	12	261	0.9	138
96	11	305	12	288	1.1	63	11	267	1.0	124
101	12	299	11	289	0.9	57	11	262	0.9	126
Mean	11.1	235	10.7	225	1.0	74	10.5	210	0.9	154
SD (d)	1.0		1.0		0.1	16	1.2		0.1	34
CV (e)	9.0		9.3		10.0	21.6	11.4		11.1	22.1

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of HC Blue No. 1 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. 1

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)
4	6	28.5	7	27.7	1.2	379	7	28.7	1.2	732
8	7	32.6	7	30.1	1.0	349	7	31.6	1.0	665
12	8	34.0	7	33.6	0.9	313	8	33.1	1.0	725
16	8	35.3	8	31.3	1.0	383	9	33.6	1.1	804
21	9	37.3	8	33.8	0.9	355	8	35.4	0.9	678
26	8	38.1	8	35.5	1.0	338	8	36.4	1.0	659
29	8	39.4	8	37.7	1.0	318	8	36.9	1.0	650
33	8	40.2	8	38.0	1.0	316	8	37.2	1.0	645
38	8	41.6	8	39.0	1.0	308	8	38.7	1.0	620
42	7	41.8	7	39.0	1.0	269	7	39.2	1.0	536
46	7	42.1	7	39.6	1.0	265	7	39.3	1.0	534
49	7	42.1	7	40.0	1.0	263	7	39.6	1.0	530
55	8	43.4	8	40.0	1.0	300	8	40.0	1.0	600
59	8	43.3	8	40.8	1.0	294	8	40.3	1.0	596
65	8	43.6	8	40.5	1.0	296	8	40.7	1.0	590
69	8	43.6	7	40.8	0.9	257	9	40.5	1.1	667
74	8	43.8	7	41.4	0.9	254	8	39.9	1.0	602
77	11	43.0	9	41.2	0.8	328	10	40.1	0.9	748
81	8	42.7	8	40.3	1.0	298	9	39.7	1.1	680
85	8	42.0	8	40.0	1.0	300	8	39.5	1.0	608
88	8	41.9	8	39.9	1.0	301	8	39.2	1.0	612
92	9	43.1	9	40.2	1.0	336	10	40.2	1.1	746
96	10	42.6	8	40.6	0.8	296	10	39.6	1.0	758
101	9	42.8	8	39.6	0.9	303	8	39.4	0.9	609
Mean	8.1	40.4	7.8	37.9	1.0	309	8.2	37.9	1.0	650
SD (d)	1.0		0.6		0.1	35	0.9		0.1	74
CV (e)	12.3		7.7		10.0	11.3	11.0		10.0	11.4

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of HC Blue No. 1 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. 1

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)
4	5	22.3	6	21.1	1.2	853	6	21.7	1.2	1,659
8	5	24.9	6	23.6	1.2	763	6	24.3	1.2	1,481
12	6	25.8	6	25.1	1.0	717	6	24.5	1.0	1,469
16	7	27.2	11	25.3	1.6	1,304	7	25.0	1.0	1,680
21	8	29.5	7	26.0	0.9	808	7	26.4	0.9	1,591
26	7	30.1	7	27.6	1.0	761	7	26.8	1.0	1,567
29	7	30.6	7	27.8	1.0	755	7	27.2	1.0	1,544
33	8	32.6	7	29.8	0.9	705	8	27.8	1.0	1,727
38	8	33.6	8	29.5	1.0	814	8	27.8	1.0	1,727
42	5	33.3	6	30.2	1.2	596	6	28.5	1.2	1,263
46	6	33.8	7	31.0	1.2	677	6	28.5	1.0	1,263
49	7	34.2	7	30.9	1.0	680	6	29.0	0.9	1,241
55	8	36.8	8	31.9	1.0	752	7	29.3	0.9	1,433
59	8	36.6	8	31.4	1.0	764	8	29.7	1.0	1,616
65	8	37.8	8	31.5	1.0	762	7	30.5	0.9	1,377
69	8	38.1	8	32.5	1.0	738	9	29.9	1.1	1,806
74	8	39.8	7	32.5	0.9	646	7	30.4	0.9	1,382
77	11	39.5	8	32.0	0.7	750	8	29.6	0.7	1,622
81	8	40.8	9	33.1	1.1	816	9	30.3	1.1	1,782
85	7	40.2	8	32.2	1.1	745	8	30.3	1.1	1,584
88	8	41.4	8	32.1	1.0	748	9	31.4	1.1	1,720
92	9	43.1	9	33.0	1.0	818	10	31.0	1.1	1,935
96	8	43.3	10	32.2	1.3	932	12	30.5	1.5	2,361
101	7	41.6	8	31.7	1.1	757	12	30.2	1.7	2,384
Mean	7.4	34.9	7.7	29.8	1.1	778	7.8	28.4	1.1	1,634
SD (d)	1.3		1.2		0.2	132	1.7		0.2	289
CV (e)	17.6		15.6		18.2	17.0	21.8		18.2	17.7

(a) Grams of feed removed from feed hopper per animal per day. Not corrected for scatter.
(b) Grams of feed per day for the dosed group divided by that for the controls
(c) Milligrams of HC Blue No. 1 consumed per day per kilogram of body weight
(d) Standard deviation
(e) Coefficient of variation = (standard deviation/mean) × 100

APPENDIX M

GENETIC TOXICOLOGY OF HC BLUE NO. 1

TABLE M1. MUTAGENICITY OF HC BLUE NO. 1 IN SALMONELLA

Strain	Dose (µg/plate)	Revertants/plate (a)		
		− S9	+ S9 (rat)	+ S9 (hamster)
TA100	0	122 ± 5.0	122 ± 6.1	93 ± 3.8
	100	142 ± 3.2	134 ± 10.5	130 ± 5.0
	333	183 ± 4.3	180 ± 7.4	236 ± 10.0
	1,000	252 ± 9.8	282 ± 11.5	312 ± 9.5
	3,333	416 ± 8.5	327 ± 21.9	324 ± 5.2
	5,000	432 ± 10.4	340 ± 10.2	313 ± 25.7
TA1535	0	16 ± 1.5	10 ± 2.6	11 ± 1.3
	100	16 ± 0.9	10 ± 0.6	12 ± 2.5
	333	16 ± 1.8	10 ± 2.0	13 ± 1.5
	1,000	16 ± 3.5	10 ± 0.9	14 ± 3.9
	3,333	19 ± 2.3	17 ± 1.0	16 ± 1.2
	5,000	21 ± 2.6	15 ± 1.8	18 ± 1.5
TA97	0	97 ± 3.7	115 ± 8.4	131 ± 9.2
	10	107 ± 5.7	131 ± 4.0	125 ± 5.8
	33	122 ± 9.8	159 ± 11.3	142 ± 5.6
	100	185 ± 1.2	193 ± 3.8	215 ± 11.1
	333	290 ± 1.2	322 ± 19.9	347 ± 15.1
	1,000	459 ± 15.0	523 ± 21.9	872 ± 19.8
TA98	0	15 ± 2.6	36 ± 5.7	26 ± 3.8
	100	63 ± 3.2	80 ± 11.3	123 ± 2.7
	333	167 ± 15.7	176 ± 13.8	378 ± 20.4
	1,000	328 ± 4.6	448 ± 28.2	1,088 ± 21.8
	3,333	764 ± 7.0	774 ± 8.7	871 ± 37.1
	5,000	855 ± 6.3	789 ± 72.8	863 ± 44.5

(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 (H_2O) were incubated for 20 min at 37° C in the presence of either S9 or buffer (Yahagi et al., 1975). 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 h (Ames et al., 1975). The experiment was performed twice, each in triplicate; because the results were similar, data from only one experiment are shown.

TABLE M2. INDUCTION OF UNSCHEDULED DNA SYNTHESIS IN RAT HEPATOCYTES BY HC BLUE NO. 1 (a)

Compound	Dose (μg/ml)	Net Grains per Nucleus ± Standard Error
DMSO	1%	−1.70 ± 0.40
2-Acetylaminofluorene	10	18.87 ± 0.42
HC Blue No. 1	1	−2.73 ± 0.22
	5	−1.48 ± 0.33
	10	0.67 ± 0.35
	25	1.32 ± 0.31
	50	11.07 ± 0.80
	100	9.58 ± 0.64
	250	13.93 ± 0.97
	500	7.60 ± 0.76
	1,000	Toxic

(a) Unscheduled DNA synthesis was determined essentially by the method of Williams (1977). Hepatocytes from male Fischer-344 rats were isolated according to the procedure of Williams et al. (1977); inoculated into Williams' Medium E supplemented with 2 mM glutamine, 50 μg/ml gentamicin, and 10% fetal bovine serum; and allowed to attach for 2 h. After incubation, the cells were washed, and serum-free medium was added. Three cultures were used per dose of compound (and for controls), and cultures were exposed simultaneously to the test compound and to tritiated thymidine (10μCi/ml) for 18 h. After exposure, cultures were washed, swelled in a hypotonic solution, fixed, and washed with water. The coverslips were mounted to slides, dipped in Kodak NTB-2 emulsion, and exposed at 20° C for 6 d. Cells were stained with methyl-green Pyronin. The grains over 50 morphologically unaltered cells were counted, and the highest count from two nuclear-sized areas over the most heavily labeled cytoplasmic areas adjacent to the nucleus was subtracted from the nuclear count to obtain the net grains per nucleus.

TABLE M3. MUTAGENICITY OF HC BLUE NO. 1 IN L5178Y/TK$^{+/-}$ MOUSE LYMPHOMA CELLS IN THE ABSENCE 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					
		50	78	100	21
		82	101	100	27
		80	93	100	29
		84	78	100	36
Methyl methanesulfonate					
	5	358	68	73	176
		567	92	82	205
		484	87	90	186
HC Blue No. 1					
	5	47	80	55	20
		77	81	67	32
	10	79	67	55	39
		113	108	32	35
		127	95	60	44
	20	198	86	20	76
		141	95	32	49
		169	89	20	63
	30	194	90	17	72
		238	78	7	102
		220	59	9	124
	40	239	38	4	209
		190	49	7	129
		288	77	7	125
	50	265	49	0.2	181
		297	60	6	165

(a) Experiments were performed twice, all doses were tested in duplicate, except the solvent control (DMSO), which was tested in triplicate. Because the results were similar, data from only one experiment are shown. 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.

APPENDIX N

DATA AUDIT SUMMARY

APPENDIX N. DATA AUDIT SUMMARY

The experimental data and draft tables for the NTP Technical Report of the toxicology and carcinogenesis studies on HC Blue No. 1 were examined September 6-23, 1983 for Good Laboratory Practice compliance and scientific procedures by the following persons: National Toxicology Program--Dr. D. Bristol, Ms. C. Davies, Dr. B. Gupta, Dr. J. Mennear, Dr. B. Schwetz, Dr. C. Whitmire, and Dr. M. Wolfe; Experimental Pathology Laboratories--Dr. D. Banas and Ms. H. Cooke; and Tracor Jitco--Ms. P. Errico.

The report of the audit of HC Blue No. 1 is on file at the National Toxicology Program. The only discrepancy that could have influenced the outcome of this study was a problem with the identity of control male and female mice. Animals in this study were identified by ear punches in groups of 1 through 50 for each dosed group of animals; control mice were identified as repeating groups of 1 through 10. The laboratory's explanation for this discrepancy was that as ear punches became difficult to read from growing closed, both the digit and the 10's identification were repunched in the dosed mice and only the unit designation was repunched in the control mice. Examination of the data throughout the study confirmed that dosed mice were not mixed up with control mice because animals receiving HC Blue No. 1 in the diet had distinctively stained fur. Thus, control mice were easily distinguished from the dosed mice.

Other discrepancies were not considered to influence the final interpretation of these studies. Some of the minor problems that were not considered to affect the outcome of the studies were not necessarily pursued to final resolution but are identified in the NTP audit report.

In conclusion, no data discrepancies were found that significantly influenced the final interpretation of these experiments.

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