NATIONAL TOXICOLOGY PROGRAM
Technical Report Series
No. 419

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF

HC YELLOW 4

(CAS NO. 59820-43-8)

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

FOREWORD

The National Toxicology Program (NTP) is made up of four charter agencies of the U.S. Department of Health and Human Services (DHHS): 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. The NTP coordinates the relevant programs, staff, and resources from these Public Health Service agencies relating to basic and applied research and to biological assay development and validation.

The NTP develops, evaluates, and disseminates scientific information about potentially toxic and hazardous chemicals. This knowledge is used for protecting the health of the American people and for the primary prevention of disease.

The studies described in this Technical Report were performed under the direction of the NIEHS and were conducted in compliance with NTP laboratory health and safety requirements and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The prechronic and chronic studies were conducted in compliance with Food and Drug Administration (FDA) Good Laboratory Practice Regulations, and all aspects of the chronic studies were subjected to retrospective quality assurance audits before being presented for public review.

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 NTP toxicology and carcinogenesis studies 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.

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NTP TECHNICAL REPORT

ON THE

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NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
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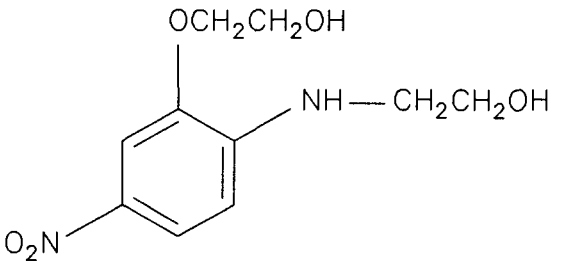
ABSTRACT



HC YELLOW 4

CAS No. 59820-43-8

Chemical Formula: $C_{10}H_{14}N_2O_5$ Molecular Weight: 242.2

Synonym: N,O-di(2-hydroxyethyl)-2-amino-5-nitrophenol

HC Yellow 4 is used in semipermanent hair dyes. Toxicology and carcinogenesis studies were conducted by administering HC Yellow 4 (greater than 93% pure) in feed to groups of F344/N rats and B6C3F$_1$ mice of each sex for 14 days, 13 weeks, and 2 years. Genetic toxicology studies were conducted in *Salmonella typhimurium*, cultured Chinese hamster ovary cells, and *Drosophila melanogaster*.

14-Day Studies: Groups of five rats of each sex were given 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm and groups of five mice of each sex were given 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm HC Yellow 4 in feed for 14 days. All animals survived to the end of the studies. Final mean body weights of male rats that received 20,000 ppm or more, female rats that received 10,000 ppm or more, and female mice that received 20,000 ppm were significantly lower than those of the controls. The mean body weights of exposed and control groups of male mice were similar. No chemical-related decrease in feed consumption was observed. No chemical-related clinical findings or changes in absolute or relative organ weights occurred in rats or mice. No gross or microscopic changes were related to HC Yellow 4 administration in rats or mice.

13-Week Studies: Groups of 10 rats of each sex were fed diets containing 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm and groups of 10 mice of each sex were fed diets containing 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm HC Yellow 4 for 13 weeks. All rats survived to study termination. Chemical-related deaths occurred at the two highest dose levels in male and female mice. Final mean body weights of male rats that received 10,000 ppm or greater, female rats that received 20,000 or 40,000 ppm, and mice that received 10,000 ppm or greater were significantly lower than those of the controls. There were no biologically significant changes in absolute or relative organ weights. Mineralization of the renal papilla occurred in all male rats in the 40,000 ppm group. Thyroid pigmentation occurred in rats receiving 40,000 ppm and in mice at all dose levels. Uterine atrophy occurred in female rats in the 20,000 and 40,000 ppm groups and female mice in the 40,000 and 80,000 ppm groups. Lymphoid depletion and atrophy of the spleen occurred in male mice that received 40,000 or 80,000 ppm and female mice that received 80,000 ppm. Atrophy of the thymus occurred in male and female mice that received 40,000 or 80,000 ppm.

2-Year Studies: Groups of 70 male rats were fed diets containing 0, 2,500, or 5,000 ppm and groups of 70 female rats and 70 mice of each sex were fed diets containing 0, 5,000, or 10,000 ppm HC Yellow 4 for up to 2 years. Interim evaluations were performed on 10 rats and 10 mice from each dose group at 6 and 15 months. No biologically significant changes in absolute or relative organ weight or hematology or clinical chemistry values were found in these rats or mice. No compound-related lesions were seen in exposed rats. In exposed mice, pigmentation of the thyroid gland was observed at the 6-month interim evaluations; pigmentation and hyperplasia of the thyroid gland were seen at the 15-month interim evaluations.

Body Weight, Survival, and Feed Consumption in the 2-Year Studies: The mean body weight of female rats that received 10,000 ppm was significantly lower than that of the controls. The mean body weights of mice receiving 10,000 ppm were 20% to 30% lower than those of the controls during the second year of the studies. The survival of exposed rats and mice was similar to that of the controls.

Neoplasms and Nonneoplastic Lesions in the 2-Year Studies: Pituitary gland pars distalis adenomas were marginally increased in exposed male rats (0 ppm, 17/45; 2,500 ppm, 20/49; 5,000 ppm, 28/49), and there was a concomitant dose-related increase in the incidence of hyperplasia (8/45, 13/49, 18/49). There was no increase in the incidence of pituitary gland adenomas or carcinomas in female rats (34/49, 35/48, 30/49).

In mice, no neoplasms were considered related to chemical administration. However, a dose-related increased incidence of thyroid gland pigmentation and follicular cell hyperplasia occurred in both sexes of mice.

Genetic Toxicology: HC Yellow 4 was mutagenic in *Salmonella typhimurium* strains TA100, TA1537, and TA98 with and without exogenous metabolic activation (S9); the response in strain TA1535 without S9 was equivocal. HC Yellow 4 induced sister chromatid exchanges in Chinese hamster ovary cells in the absence but not the presence of S9 activation; no induction of chromosomal aberrations occurred in Chinese hamster ovary cells, with or without S9. HC Yellow 4 induced sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* when administered by injection; results of a reciprocal translocation test in *D. melanogaster* were negative.

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of HC Yellow 4 in male F344/N rats based on the increased incidence of pituitary gland adenomas and hyperplasia. The male rats may have been able to tolerate a slightly higher dose of the chemical. There was *no evidence of carcinogenic activity* of HC Yellow 4 in female F344/N rats given 5,000 or 10,000 ppm. There was *no evidence of carcinogenic activity* of HC Yellow 4 in male or female $B6C3F_1$ mice given 5,000 or 10,000 ppm.

There was a chemical-related increase in the incidence of thyroid gland pigmentation and follicular cell hyperplasia in mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

Summary of the 2-Year Carcinogenicity and Genetic Toxicology Studies of HC Yellow 4

Male F344/N Rats	Female F344/N Rats	Male B6C3F$_1$ Mice	Female B6C3F$_1$ Mice
Doses			
0, 2,500, or 5,000 ppm in feed	0, 5,000, or 10,000 ppm in feed	0, 5,000, or 10,000 ppm in feed	0, 5,000, or 10,000 ppm in feed
Body weights			
Dosed groups similar to controls	High-dose group lower than controls	Dosed groups lower than controls	Dosed groups lower than controls
2-Year survival rates			
21/50, 29/50, 28/50	27/50, 31/50, 34/50	28/50, 29/50, 35/50	43/50, 38/50, 43/50
Nonneoplastic effects			
None	None	Thyroid gland: follicular cell pigmentation (0/47, 44/48, 49/49), follicular cell hyperplasia (0/47, 27/48, 41/49)	Thyroid gland: follicular cell pigmentation (0/48, 49/49, 50/50), follicular cell hyperplasia (0/48, 3/49, 13/50)
Neoplastic effects			
None	None	None	None
Uncertain findings			
Pituitary gland pars distalis: adenoma (17/45, 20/49, 28/49), hyperplasia (8/45, 13/49, 18/49)	None	None	None
Level of evidence of carcinogenic activity			
Equivocal evidence	No evidence	No evidence	No evidence
Genetic toxicology			
Salmonella typhimurium gene mutation:	Positive with and without S9 in strains TA100, TA1537, and TA98; equivocal without S9 in strain TA1535		
Sister chromatid exchanges			
Chinese hamster ovary cells *in vitro*:	Negative with S9, positive without S9		
Chromosomal aberrations			
Chinese hamster ovary cells *in vitro*:	Negative with and without S9		
Sex-linked recessive lethal mutations			
Drosophila melanogaster:	Positive by injection; negative by feeding		
Reciprocal translations			
Drosophila melanogaster:	Negative		

EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

The National Toxicology Program describes the results of individual experiments on a chemical agent and notes the strength of the evidence for conclusions regarding each study. Negative results, in which the study animals do not have a greater incidence of neoplasia than control animals, do not necessarily mean that a chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of conditions. Positive results demonstrate that a chemical is carcinogenic for laboratory animals under the conditions of the study and indicate that exposure to the chemical has the potential for hazard to humans. Other organizations, such as the International Agency for Research on Cancer, assign a strength of evidence for conclusions based on an examination of all available evidence, including animal studies such as those conducted by the NTP, epidemiologic studies, and estimates of exposure. Thus, the actual determination of risk to humans from chemicals found to be carcinogenic in laboratory animals requires a wider analysis that extends beyond the purview of these studies.

Five categories of evidence of carcinogenic activity are used in the Technical Report series to summarize the strength of the evidence observed in each experiment: two categories for positive results (**clear evidence** and **some evidence**); one category for uncertain findings (**equivocal evidence**); one category for no observable effects (**no evidence**); and one category for experiments that cannot be evaluated because of major flaws (**inadequate study**). These categories of interpretative conclusions were first adopted in June 1983 and then revised in March 1986 for use in the Technical Report series to incorporate more specifically the concept of actual weight of evidence of carcinogenic activity. For each separate experiment (male rats, female rats, male mice, female mice), one of the following five categories is selected to describe the findings. These categories refer to the strength of the experimental evidence and not to potency or mechanism.

- **Clear evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a dose-related (i) increase of malignant neoplasms, (ii) increase of a combination of malignant and benign neoplasms, or (iii) marked increase of benign neoplasms if there is an indication from this or other studies of the ability of such tumors to progress to malignancy.
- **Some evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a chemical-related increased incidence of neoplasms (malignant, benign, or combined) in which the strength of the response is less than that required for clear evidence.
- **Equivocal evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing a marginal increase of neoplasms that may be chemical related.
- **No evidence** of carcinogenic activity is demonstrated by studies that are interpreted as showing no chemical-related increases in malignant or benign neoplasms.
- **Inadequate study** of carcinogenic activity is demonstrated by studies that, because of major qualitative or quantitative limitations, cannot be interpreted as valid for showing either the presence or absence of carcinogenic activity.

When a conclusion statement for a particular experiment is selected, consideration must be given to key factors that would extend the actual boundary of an individual category of evidence. Such consideration should allow for incorporation of scientific experience and current understanding of long-term carcinogenesis studies in laboratory animals, especially for those evaluations that may be on the borderline between two adjacent levels. These considerations should include:

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS TECHNICAL REPORTS REVIEW SUBCOMMITTEE

The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on HC Yellow 4, NTP TR 419 on 9 July 1991 are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, panel members have five major responsibilities in reviewing NTP studies:

- to ascertain that all relevant literature data have been adequately cited and interpreted,
- to determine if the design and conditions of the NTP studies were appropriate,
- to ensure that the Technical Report presents the experimental results and conclusions fully and clearly,
- to judge the significance of the experimental results by scientific criteria, and
- to assess the evaluation of the evidence of carcinogenic activity and other observed toxic responses.

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SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On 9 July 1991, the draft Technical Report on the toxicology and carcinogenesis studies of HC Yellow 4 received public review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. J.K. Dunnick, NIEHS, introduced the toxicology and carcinogenesis studies of HC Yellow 4 by discussing the uses, describing the experimental design, reporting on survival and body weight effects, and commenting on neoplastic and nonneoplastic lesions in male rats and in mice. The proposed conclusions were *equivocal evidence of carcinogenic activity* of HC Yellow 4 in male rats and *no evidence of carcinogenic activity* of HC Yellow 4 in female rats and male and female mice.

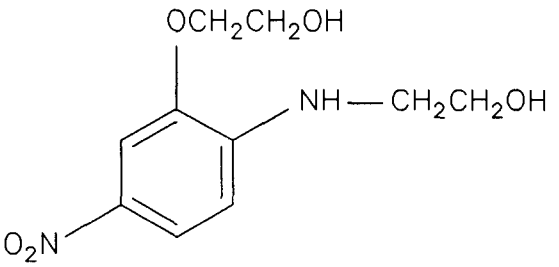
Dr. Zeise, a principal reviewer, agreed in principle with the proposed conclusions. She thought the conclusions should note that male and female rats could have tolerated significantly higher doses. Dr. Zeise said that the increased incidence of uterine stromal polyps in female rats should be considered "may have been related to chemical administration," unless there are better reasons for discounting them than that the incidence in treated animals falls within the range of overall NTP historical controls. Dr. Dunnick commented that more historical control data would be added and that there were no supporting nonneoplastic effects, providing further evidence that these lesions probably were not chemical related. Dr. J.K. Haseman, NIEHS, added that the rate of uterine polyps in the high-dose group was similar to the historical control mean from previous studies at this laboratory. Further, based on the results of previous NCI/NTP studies, it would be unusual for a chemical to induce only uterine polyps.

Dr. Carlson, the second principal reviewer, agreed with the proposed conclusions.

Dr. Garman, the third principal reviewer, agreed with the proposed conclusions. Because of the prominent chemical-related increased frequency of thyroid follicular cell hyperplasia in the 2-year studies in mice, he thought it appropriate to add frequency figures to the summary table in the Abstract.

Dr. Zeise moved that the Technical Report on HC Yellow 4 be accepted with the revisions discussed and with the conclusions as written, *equivocal evidence of carcinogenic activity* in male rats and *no evidence of carcinogenic activity* in female rats and male and female mice, and with the addition of a statement that "male and female rats may have been able to tolerate higher doses." Dr. Garman seconded the motion. Dr. Goodman offered an amendment that the added statement be removed. Dr. Klaassen seconded the amendment, which was accepted by seven yes to three no votes (Drs. Carlson, McKnight, and Zeise). Dr. McKnight offered an amendment to add a statement to the conclusions that "male rats may have been able to tolerate a higher dose." Dr. Zeise seconded the amendment, which was accepted by seven yes to three no votes (Mr. Beliczky and Drs. Goodman and Hayden). Dr. Zeise's amended motion was then accepted unanimously with ten votes.

INTRODUCTION



HC YELLOW 4

CAS No. 59820-43-8

Chemical Formula: $C_{10}H_{14}N_2O_5$ Molecular Weight: 242.2

Synonym: N,O-di(2-hydroxyethyl)-2-amino-5-nitrophenol

Chemical and Physical Properties, Production, Use, and Exposure

HC Yellow 4, a semipermanent dye, is a fluffy, yellow powder with a melting point of 145° to 147° C. HC Yellow 4 is used as an ingredient in hair dyes at concentrations ranging from 0.1% to 1.0% (USFDA, 1975, 1976). Production of HC Yellow 4 in the United States was estimated to be 2.3 x 10^6 g in 1976. The dye was not produced commercially in the United States in 1979 (HSDB, 1990). Human exposure is believed to occur primarily in department stores and beauty shops. An estimated 4,000 workers were exposed in 1974 (NIOSH, 1990). This estimate does not appear to include the general public and may be low for the cosmetology industry. No more recent information on occupational exposure to this dye was found.

Confusion has existed over the structure of HC Yellow 4. In the second edition of the Cosmetic, Toiletry and Fragrance Association's Cosmetic Ingredient Dictionary, the structure was shown with both hydroxyethyl groups on the amine and an assigned Chemical Abstracts Service number of 52551-67-4 (CTFA, 1977). Subsequently, based on additional analysis, the structure was corrected in the third edition to show one hydroxyethyl group on the amine and the other on the phenol. The Chemical Abstracts Service number for this structure is 59820-43-8. Analysis of the chemical used in these studies confirmed that the chemical has the structure given in the third edition of the Cosmetic Ingredient Dictionary (CTFA, 1982).

Toxicity and Metabolism in Animals

The oral LD_{50} for rats is presumed to be greater than 1.2 g/kg, although specific data are not available (Wernick *et al.*, 1975). In eye irritation tests of a composite mixture of hair dyes that included HC Yellow 4, a slight, transient conjunctival erythema was noted in rabbits (Draize, 1959). Percutaneous application of HC Yellow 4 to rabbits caused a mild epidermal irritation but no systemic toxicity (Burnett *et al.*, 1976). In teratology and reproduction studies, a composite mixture of dyes that included HC Yellow 4 was given to rats dermally or as a dietary admixture and was given to rabbits by gavage. The dye mixture did not cause any teratogenic or toxic effects. In a 2-year feed study, a composite dye mixture that contained 0.31% HC Yellow 4 (an equivalent of 0.06 or 0.3 mg HC Yellow 4 per kg body weight per day) was given to purebred beagle dogs. No gross or microscopic changes that could be attributed to dye mixture

administration were observed (Wernick *et al.*, 1975). These studies were considered inadequate to assess the toxicologic or carcinogenic potential of HC Yellow 4 because the doses were low, the tests were not lifetime studies, and mixtures of dyes were used. No information was found on the metabolism of HC Yellow 4.

Toxicity And Carcinogenicity In Humans

No information or epidemiological evidence on the toxicity or the carcinogenicity of HC Yellow 4 in humans was found in the literature. Dark urine, indicative of dermal absorption, has been reported occasionally by women using the dye (Wernick *et al.*, 1975).

Genetic Toxicity

No genotoxicity data were available for HC Yellow 4 other than the NTP-sponsored tests reported in Appendix E of this report. HC Yellow 4 has been shown to be mutagenic in *Salmonella typhimurium*, with and without S9 activation (Table E1; Mortelmans *et al.*, 1986). Administered by injection, the dye induced sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* (Table E4; Woodruff *et al.*, 1985). However, results of a reciprocal translocation test in *D. melanogaster* were negative (Table E5; Woodruff *et al.*, 1985).

Study Rationale

HC Yellow 4 is the last of six semipermanent hair dyes nominated by the Food and Drug Administration for toxicology and carcinogenicity assessment in a class study of hair color materials. The other dyes that have been studied and reported are HC Blue No. 1 (NTP, 1985a), HC Blue No. 2 (NTP, 1985b), C.I. Disperse Blue 1 (NTP, 1986a), HC Red No. 3 (NTP, 1986b), and C.I. Acid Orange 3 (NTP, 1988). HC Yellow 4 was recommended for testing because of the high potential for exposure of cosmetology industry workers and the general population through its use as a hair dye, lack of published toxicology data on this dye, and its possible enzymatic reduction to a potential tumor promoter, an aromatic *N*-hydroxy derivative. Although human exposure occurs primarily via the dermal route, the oral route was selected to ensure systemic exposure.

MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

HC Yellow 4 was obtained from Southland Corporation (lots 0-218 and 3-074) and Prochimie International (lot 81031). Lot 0-218 was used for the 14-day and 13-week studies and for the first 11 months of the 2-year study. Lot 3-074 was used for the next 7 months of the 2-year study, and lot 81031 was used for the final 6 months. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO), and are described in Appendix H. The study chemical, a fluffy, yellow powder, was identified as HC Yellow 4 by infrared, ultraviolet/visible, and nuclear magnetic resonance spectroscopy.

Purity was determined by weight loss on drying, Karl Fischer water analysis, thin-layer chromatography, high-performance liquid chromatography, ultraviolet/visible spectroscopy, titration, and elemental analysis. Lot 0-218 was greater than 93% pure, lot 3-074 was greater than 97% pure, and lot 81031 was greater than 98% pure. The largest impurity was tentatively identified as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline. The concentration of the impurity was determined to be 7% in lot 0-218, 2.5% in lot 3-074, and 0.3% in lot 81031. Stability studies performed by high-performance liquid chromatography indicated that HC Yellow 4 was stable as a bulk chemical for 2 weeks at temperatures up to 60° C when protected from light. To ensure stability, the bulk chemical was stored in the dark at 4° C throughout the studies. The stability of the bulk chemical was monitored periodically by high-performance liquid chromatography, titration, and infrared spectroscopy during all phases of the studies. No change in the dye was detected.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared by mixing HC Yellow 4 with feed (Table H1). Studies were conducted by the analytical chemistry laboratory to determine the homogeneity and stability of 10,000 ppm HC Yellow 4 in feed. Homogeneity was confirmed using an ultraviolet spectroscopic method for sample analysis; stability of the dose formulations for at least 14 days when stored in the dark at temperatures up to 25° C was confirmed using a high-performance liquid chromatographic method. During the 14-day and 13-week studies, the dose formulations were stored in the dark at 0° ± 5° C for no longer than 2 weeks. During the 2-year studies, the dose formulations were prepared weekly and stored protected from light at 0° ± 5° C for no longer than 2 weeks. The study laboratory conducted periodic analyses of the HC Yellow 4 dose formulations using ultraviolet spectroscopy as described in Appendix H. All dose formulations analyzed for the 14-day and 13-week studies were within 10% of the target concentrations (Tables H2 and H3). In the 2-year studies, the first set of dose formulations and one of every eight subsequent sets were analyzed and all were within 10% of the target concentrations (Table H4). Results of periodic referee analyses of the dose formulations performed by the analytical chemistry laboratory were in agreement with the results from the study laboratory (Table H5).

14-DAY STUDIES

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Charles River Breeding Laboratories (Kingston, NY) and observed for 13 to 15 days (rats) or 14 to 16 days (mice) before the studies began. Rats were 7 weeks old and mice were 8 weeks old when the studies began. Groups of five rats of each sex received feed with 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm and groups of five mice of each sex received feed with 0, 1,250, 2,500, 5,000, 10,000, or 20,000 ppm HC Yellow 4 (Table 1). All groups received dosed feed for 14 days, followed by a 1-day observation period when the animals were given only undosed feed. Animals were housed five per cage; water and feed were available *ad libitum*. Animals were observed twice daily for signs of toxicity. Clinical observations were recorded on the day of necropsy. Animals were weighed at the start of the study, on

days 7 and 14, and at necropsy. Feed consumption per cage was determined weekly. Complete necropsies were performed on all animals. The brain, heart, right kidney, liver, lung, right testis, and thymus of survivors were weighed at necropsy. Histopathology was performed on selected tissues from all rats in the 0, 20,000, 40,000, and 80,000 ppm dose groups, and mice in the 20,000 ppm dose groups. Further experimental details are presented in Table 1.

13-Week Studies

The 13-week studies were conducted to determine the cumulative toxic effects of repeated exposure to HC Yellow 4 and to determine appropriate concentrations for use in the 2-year studies. The experimental design of the 13-week studies is summarized in Table 1.

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility (Frederick, MD) and were observed for 13 to 14 days before the studies began. Rats were 7 to 8 weeks old and mice were 8 weeks old when the studies began. Groups of 10 rats of each sex were given 0, 2,500, 5,000, 10,000, 20,000, or 40,000 ppm HC Yellow 4 in feed 7 days a week for 13 weeks. Groups of 10 mice of each sex were given 0, 5,000, 10,000, 20,000, 40,000, or 80,000 ppm HC Yellow 4 in feed for 13 weeks. Animals were housed five per cage; water and feed were available *ad libitum*. Animals were observed twice each day and clinical observations were recorded daily. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K). Animals were weighed at the start of the study and weekly thereafter. Feed consumption per cage was measured weekly. Further experimental details are presented in Table 1.

Necropsies were performed on all study animals. The brain, heart, right kidney, liver, lung, right testis, and thymus of survivors were weighed at necropsy. Complete histopathology was performed on all animals that died or were killed moribund prior to the end of the studies, all control animals, all rats that received 40,000 ppm, and all mice that received 80,000 ppm. Tissues examined for rats in the 2,500, 5,000, 10,000 and 20,000 ppm dose groups were the kidney, thyroid gland, and uterus. The thyroid gland was examined for mice in the 5,000, 10,000, 20,000, and 40,000 ppm dose groups. Additional information is provided in Table 1.

2-Year Studies

Study Design

Groups of 70 rats and 70 mice of each sex were administered HC Yellow 4 in feed 7 days a week for up to 105 weeks. Male rats received doses of 0, 2,500, or 5,000 ppm; female rats and male and female mice received doses of 0, 5,000, or 10,000 ppm. After 6 months and again after 15 months of HC Yellow 4 administration, 10 rats and 10 mice of each sex were randomly selected from each group for interim evaluations.

Source and Specification of Animals

Male and female F344/N rats and B6C3F$_1$ mice were obtained from Frederick Cancer Research Facility for use in the 2-year studies. Rats were quarantined 14 days and mice were quarantined 13 to 14 days. Five rats and five mice of each sex were randomly selected and killed for parasite evaluation and gross observation of disease. Blood samples were collected for viral screens. Rats and mice were approximately 6 weeks old when the studies began. The health of the animals was monitored during the course of the studies according to the protocols of the NTP Sentinel Animal Program (Appendix K).

Animal Maintenance

Rats and mice were initially housed five per cage. Male mice were housed individually beginning 27 July 1984 (15 months after the studies began). Feed and water were available *ad libitum*. Cages were rotated every 2 weeks during the studies. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix J.

Clinical Examinations and Pathology

All animals were observed twice daily and findings were recorded monthly or as necessary. Animals were weighed at the beginning of the studies, weekly for 13 weeks, and monthly thereafter. Feed consumption per cage was measured once a month (Appendix I).

Ten rats and 10 mice from each group were randomly selected after 6 months and again after 15 months of HC Yellow 4 administration for interim evaluations. Blood was drawn from the tail

of rats and mice for hematology evaluations and from the external jugular of anesthetized animals for determining thyroid hormone levels. The brain, right kidney, and liver of each animal selected for the 15-month interim evaluations were weighed at necropsy. Further details of the interim evaluations are presented in Table 1.

Necropsies were performed on all animals. At necropsy, all organs and tissues were examined for gross lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for microscopic examination. Histopathologic examinations were performed on the thyroid gland of animals from the 6-month interim evaluations. At the 15-month interim evaluations, complete histopathology was performed on male rats that received 0 or 5,000 ppm, female rats that received 0 or 10,000 ppm, and male and female mice that received 0 or 10,000 ppm. Only gross lesions were examined in male rats receiving 2,500 ppm and female rats receiving 5,000 ppm; gross lesions and thyroid glands were examined in male and female mice that received 5,000 ppm. Complete histopathology was performed at the end of the studies on all animals that died or were killed moribund, all rats, and all control and high-dose mice. The thyroid gland and ovary of low-dose mice were examined. Tissues examined are listed in Table 1.

Upon completion of the microscopic evaluation by the study laboratory pathologist, the pathology data were entered into the Toxicology Data Management System. The microscope slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet-tissue audit. The slides, individual animal data records, and pathology tables were sent to an independent pathology quality assessment laboratory. The liver, pancreas, and pituitary gland of male rats, the liver and uterus of female rats, the thyroid gland of male mice, and the thyroid gland and ovary of female mice were reviewed microscopically by the quality assessment pathologist for neoplasms or nonneoplastic lesions. All parathyroid glands of male rats in which hyperplasia or adenoma had been diagnosed were also reviewed.

The quality assessment report and slides were submitted to the NTP Pathology Working Group (PWG) chair, who reviewed the selected tissues and any other tissues for which there was a disagreement in diagnosis between the laboratory and quality assessment pathologists. Representative histopathology slides of the uterus, liver, male pituitary gland, male pancreas, and male parathyroid gland for rats and thyroid gland, ovary, mammary gland, and epididymis for mice; examples of disagreements in diagnosis between the laboratory and quality assessment pathologists; and lesions of general interest were presented by the chair to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without knowledge of dose groups or previously rendered diagnoses. When the consensus opinion of the PWG differed from that of the laboratory pathologist, the diagnosis was changed. Thus, the final diagnoses represent a consensus of contractor pathologists and the PWG. Details of these review procedures have been described by Maronpot and Boorman (1982) and Boorman *et al.* (1985). For subsequent analysis of pathology data, the diagnosed lesions for each tissue type are evaluated separately or combined according to the guidelines of McConnell *et al.* (1986).

Statistical Methods

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 to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence

The incidence of neoplasms 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 had multiple potential

sites of occurrence (e.g., mononuclear cell leukemia), the denominators consist of the number of animals on which a necropsy was performed.

Analysis of Tumor Incidence

The majority of tumors in these studies were considered to be incidental to the cause of death or not rapidly lethal. Thus, the primary statistical method used was a logistic regression analysis, which assumed that the diagnosed tumors were discovered as the result of death from an unrelated cause and thus did not affect the risk of death. In this approach, tumor prevalence was modeled as a logistic function of chemical exposure and time. Both linear and quadratic terms in time were incorporated initially, and the quadratic term was eliminated if it did not significantly enhance the fit of the model. The dosed and control groups were compared on the basis of the likelihood score test for the regression coefficient of dose. This method of adjusting for intercurrent mortality is the prevalence analysis of Dinse and Lagakos (1983), further described and illustrated by Dinse and Haseman (1986).

In addition to logistic regression, alternative methods of statistical analysis were used, and the results of these tests are summarized in the appendixes. These include the life table test (Cox, 1972; Tarone, 1975), appropriate for rapidly lethal tumors, and the Fisher exact test and the Cochran-Armitage trend test (Armitage, 1971; Gart *et al.*, 1979), procedures based on the overall proportion of tumor-bearing animals.

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-response trend. Continuity-corrected tests were used in the analysis of tumor incidence, and reported P values are one sided. The procedures described above also were used to evaluate selected nonneoplastic lesions. (For further discussion of these statistical methods, see Haseman, 1984.)

Historical Control Data

Although the concurrent control group is always the first and most appropriate control group used for evaluation, there are certain instances in which historical control data can be helpful in the overall assessment of tumor incidence. Consequently, control tumor incidences from the NTP historical control database (Haseman *et al.*, 1984, 1985) are included in the NTP reports for tumors appearing to show compound-related effects.

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Clinical chemistry and hematology data, which typically have skewed distributions, were analyzed using the multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test).

QUALITY ASSURANCE METHODS

The 13-week and 2-year studies were conducted in compliance with FDA Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as study records were submitted to the NTP Archives, they were audited retrospectively by an independent quality assurance contractor. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and preliminary review draft of the NTP Technical Report were conducted. Audit procedures and findings are presented in the reports, which are on file at the NIEHS. The audit findings were reviewed and assessed by NTP staff so that all discrepancies had been resolved or were otherwise addressed during the preparation of this Technical Report.

GENETIC TOXICITY

The genetic toxicity of HC Yellow 4 was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium*, sister chromatid exchanges and chromosomal aberrations in Chinese hamster ovary cells, and sex-linked recessive lethal mutations and reciprocal translocations in *Drosophila melanogaster*. The protocols for these studies and tabular presentations of their findings are in Appendix E.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of HC Yellow 4

14-Day Studies	13-Week Studies	2-Year Studies
Study Laboratory		
EG&G Mason Research Institute (Worcester, MA)	EG&G Mason Research Institute (Worcester, MA)	EG&G Mason Research Institute (Worcester, MA)
Strain and Species		
Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$	Rats: F344/N Mice: B6C3F$_1$
Animal Source		
Charles River Breeding Laboratories (Kingston, NY)	Frederick Cancer Research Facility (Frederick, MD)	Frederick Cancer Research Facility (Frederick, MD)
Size of Study Groups		
5 males and 5 females	10 males and 10 females	70 males and 70 females
Doses		
Rats: 0, 5,000, 10,000, 20,000, 40,000, and 80,000 ppm HC Yellow 4 in feed Mice: 0, 1,250, 2,500, 5,000, 10,000, and 20,000 ppm HC Yellow 4 in feed	Rats: 0, 2,500, 5,000, 10,000, 20,000, and 40,000 ppm HC Yellow 4 in feed Mice: 0, 5,000, 10,000, 20,000, 40,000, and 80,000 ppm HC Yellow 4 in feed	Rats: Male - 0, 2,500, and 5,000 ppm HC Yellow 4 in feed; Female - 0, 5,000, and 10,000 ppm HC Yellow in feed Mice: 0, 5,000, and 10,000 ppm HC Yellow 4 in feed
Time Held Before Study		
Rats: 13-15 days Mice: 14-16 days	13-14 days	Rats: 14 days Mice: 13-14 days
Average Age When Placed on Study		
Rats: 7 weeks Mice: 8 weeks	Rats: 7-8 weeks Mice: 8 weeks	6 weeks
Date of First Dose		
Rats: Male - 13 July 1981 Female - 15 July 1981 Mice: Male - 21 July 1981 Female - 23 July 1981	Rats: Male - 17 February 1982 Female - 24 February 1982 Mice: Male - 10 March 1982 Female - 3 March 1982	Rats: Male - 12 April 1983, Female - 20 April 1983; Mice: Male - 16 March 1983, Female - 29 March 1983
Duration of Dosing		
14 days	13 weeks (7 days/week)	104 weeks (7 days/week)
Necropsy Dates		
Rats: Male - 28 July 1981 Female - 30 July 1981 Mice: Male - 5 August 1981 Female - 7 August 1981	Rats: Male - 19-21 May 1982 Female - 26-27 May 1982 Mice: Male - 9-10 June 1982 Female - 2-5 June 1982	6-month interim – Male Rats: 3-4 October 1983; Female Rats: 18-19 October 1983; Mice: 20-22 September 1983 15-month interim - Rats: 24-26 July 1984; Male Mice: 12-13 June 1984; Female Mice: 26 June 1984 2-year studies – Male Rats: 9-12 April 1985; Female Rats: 17-24 April 1985; Male Mice: 14-18 March 1985; Female Mice: 27-29 March 1985

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of HC Yellow 4 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Average Age at Necropsy		
Rats: 9 weeks Mice: 10 weeks	Rats: 20-21 weeks Mice: 21 weeks	110-111 weeks
Method of Animal Distribution		
Animals assigned to groups by weight, so that cage weights were approximately equal (± 2 g)	Same as 14-day studies	Animals of each sex randomized in cage groups, then cages randomized to dose and control groups using random number table
Animals per Cage		
5	5	5 (male mice housed individually beginning 27 July 1984)
Method of Animal Identification		
Ear punch	Same as 14-day studies	Same as 14-day studies
Diet		
NIH-07 Rat and Mouse Ration, Open formula, mash (Zeigler Bros., Inc., Gardners, PA), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Water		
Tap water (Worcester Public Water Supply) via outside-the-cage automatic watering system (Edstrom Industries, Inc., Waterford, WI), available *ad libitum*	Same as 14-day studies	Same as 14-day studies
Cages		
Polycarbonate cages (Lab Products, Inc., Rochelle Park, NJ)	Same as 14-day studies	Same as 14-day studies
Bedding		
Aspen Bed, heat-treated hardwood chips (American Excelsior Co., Baltimore, MD), changed twice weekly	Same as 14-day studies	Aspen Bed (American Excelsior Co., Baltimore, MD) or BetaChips (Northeastern Products Corp., Warrensburg, NY); changed twice weekly
Cage Filters		
Non-woven polyester filters (Snow Filtration, Cincinnati, OH)	Non-woven polyester filters (Lab Products, Rochelle Park, NJ or Snow Filtration, Cincinnati, OH)	Same as 13-week studies

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of HC Yellow 4 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Animal Room Environment		
Average temperature: 22.2° C; Relative humidity: 69% Fluorescent light: 12 hours/day Room air changes: 12-15/hour	Rats: Male - Average temperature 22.2° C; Relative humidity 34%; Female - Average temperature 22.3° C; Relative humidity 35.3% Mice: Male - Average temperature 22.8°-23.6° C; Relative humidity 38.1%-42%; Female - Average temperature 22.3° C; Relative humidity 37.2% Fluorescent light: 12 hours/day Room air changes: >12/hour	Rats: Average temperature 22.5° ± 1.3° C; Relative humidity 47.5% ± 5.7%; Mice: Average temperature 22.7° ± 2.2° C; Relative humidity 44.4% ± 6.0% Fluorescent light: 12 hours/day
Type and Frequency of Observation		
Observed twice/day; weighed initially and once/week; clinical observations recorded at necropsy; feed consumption once/week by cage	Observed twice/day; weighed initially and once/week; clinical observations recorded daily; feed consumption once/week by cage	Observed twice/day; weighed and clinical observations recorded initially, once/week for 13 weeks, once/month thereafter; feed consumption per cage measured once/month
Necropsy		
Necropsy performed on all animals. The following organs were weighed: brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. The following organs were weighed for all survivors: brain, heart, right kidney, liver, lung, right testis, and thymus.	Necropsy performed on all animals. The following organs were weighed for all animals at 15-month interim evaluation: brain, right kidney, and liver.
Clinical Pathology		
None	None	Clinical pathology studies were performed at 6 months on control and high-dose rats and on mice from each dose group and at 15 months on rats and mice from each dose group. *Hematology:* None at 6 months. 15 months: hematocrit, hemoglobin, erythrocyte count, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, leukocyte count and differential *Clinical chemistry:* 6 months: thyroid stimulating hormone (rats), triiodothyronine, and thyroxine. 15 months: blood urea nitrogen, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, sorbitol dehydrogenase, thyroid stimulating hormone (rats), triiodothyronine, and thyroxine.

TABLE 1
Experimental Design and Materials and Methods in the Feed Studies of HC Yellow 4 (continued)

14-Day Studies	13-Week Studies	2-Year Studies
Histopathology Histopathology performed on all rats that received 0, 20,000, 40,000, or 80,000 ppm. Tissues examined included: bone and marrow, Peyer's patch, spleen, and thymus. Tissues examined only for the 80,000 ppm dose group included: brain, clitoral gland, and kidney. Tissues examined only for the 0, 20,000, and 40,000 ppm dose groups included: mediastinal lymph node and testis. The lung, skin, and urinary bladder were examined from mice that received 20,000 ppm.	Complete histopathology on all animals that died or were killed moribund during study, all rats that received 0 or 40,000 ppm, and all mice that received 0 or 80,000 ppm. Tissues examined included: adrenal gland, bone and marrow (sternum), brain, clitoral or preputial gland (rats), epididymis, esophagus, heart, kidney, large intestine, liver, lung, lymph node (mandibular and mesenteric), mammary gland, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skin, small intestine, spleen, stomach, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Tissues examined from rats in the 2,500, 5,000, 10,000, and 20,000 ppm dose groups were kidney, thyroid gland, and uterus. Thyroid gland was examined for all mice in the 5,000, 10,000, 20,000, and 40,000 ppm dose groups.	Histopathology of thyroid glands performed on rats and mice at 6-month interim evaluation. Complete histopathology performed at 15-month interim evaluation on all control animals, male rats that received 5,000 ppm, and mice and female rats that received 10,000 ppm. At the 15-month evaluation, only gross lesions were examined in male rats receiving 2,500 ppm and female rats receiving 5,000 ppm, and gross lesions and thyroid gland were examined in male and female mice that received 5,000 ppm. Complete histopathology performed on all animals that died or were killed moribund during 2-year studies, all controls, all rats, and high-dose mice at the end of the studies. Tissues examined: adrenal gland, bone, bone marrow, brain, cecum, clitoral or preputial gland (rats), colon, duodenum, epididymis, esophagus, forestomach, gallbladder (mice), glandular stomach, heart, ileum, jejunum, kidney, liver, lung, mammary gland, mandibular and mesenteric lymph nodes, nasal cavity, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, rectum, salivary gland, seminal vesicle, skin, spleen, testis, thymus, thyroid gland, trachea, urinary bladder, and uterus. Organs examined from low-dose mice were thyroid gland and ovary.

RESULTS

RATS

14-Day Studies

All animals survived to the end of the studies. The final mean body weights and mean body weight changes of males that received doses of 20,000 ppm and above and females that received doses of 10,000 ppm and above were significantly lower than those of the controls (Table 2). Feed consumption by males that received doses of 20,000 ppm or greater and females that received doses of 10,000 ppm or greater was lower than that of the controls during the first week. During the second week, feed consumption by males in the 40,000 ppm dose group was lower than controls; feed consumption by other male and female dose groups was similar to or higher than that of the controls. Because rats that received 40,000 ppm did not gain weight, and the final mean body weights of rats that received 80,000 ppm were decreased approximately 30%, it was concluded that the feed consumption values were high and may have included feed scattered by animals searching for unadulterated feed.

No clinical findings were attributed to HC Yellow 4 administration. Significant changes in absolute and relative organ weights were observed but were considered to be secondary to decreases in body weights (Table F1).

TABLE 2
Survival, Mean Body Weights, and Feed Consumption of Rats in the 14-Day Feed Studies of HC Yellow 4

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	107 ± 6	178 ± 7	71 ± 3		15.3	16.7
5,000	5/5	108 ± 5	173 ± 6	66 ± 3	97	15.6	16.7
10,000	5/5	107 ± 5	170 ± 5	63 ± 2	95	14.9	16.9
20,000	5/5	107 ± 4	148 ± 6**	40 ± 5**	83	10.7	14.4
40,000	5/5	108 ± 3	107 ± 4**	-1 ± 2**	60	5.9	11.6
80,000	5/5	108 ± 4	75 ± 3**	-32 ± 3**	42	8.7	16.3
Female							
0	5/5	101 ± 3	145 ± 2	44 ± 1		16.0	11.6
5,000	5/5	101 ± 3	138 ± 5	37 ± 2	95	15.0	10.4
10,000	5/5	101 ± 3	131 ± 1**	29 ± 3**	90	12.6	10.1
20,000	5/5	101 ± 2	130 ± 3**	29 ± 4**	90	7.2	11.7
40,000	5/5	101 ± 2	102 ± 3**	0 ± 3**	70	10.6	15.4
80,000	5/5	102 ± 3	72 ± 3**	-29 ± 4**	50	9.4	12.1

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Grams per animal per day, based on average consumption data per group per week for weeks 1 and 2

13-Week Studies

All rats survived to study termination. Final mean body weights of males that received doses of 10,000 ppm or greater and females that received 20,000 or 40,000 ppm were significantly lower than those of the controls (Table 3). Feed consumption by males in all dose groups was generally higher than that of the controls throughout the study (Table 4). Feed consumption by females that received 40,000 ppm was generally higher than that of the controls; feed consumption by females in other dose groups was lower than that of the controls. The values for feed consumption by rats receiving 40,000 ppm were nearly twice that of other groups and are probably due to spillage of unpalatable diet.

There were no biologically significant clinical findings. Statistically significant changes in absolute and relative organ weights were observed but were considered to reflect the changes in body weights and were not considered to be related to chemical administration (Table F2).

TABLE 3
Survival and Mean Body Weights of Rats in the 13-Week Feed Studies of HC Yellow 4

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	144 ± 3	354 ± 7	210 ± 5	
2,500	10/10	143 ± 4	364 ± 6	221 ± 6	103
5,000	10/10	144 ± 3	346 ± 6	202 ± 6	98
10,000	10/10	143 ± 3	326 ± 5**	183 ± 6**	92
20,000	10/10	143 ± 3	287 ± 5**	144 ± 8**	81
40,000	10/10	143 ± 3	250 ± 5**	108 ± 5**	71
Female					
0	10/10	135 ± 2	204 ± 4	69 ± 4	
2,500	10/10	135 ± 2	214 ± 3	79 ± 3	105
5,000	10/10	135 ± 2	203 ± 3	67 ± 3	99
10,000	10/10	135 ± 2	205 ± 3	70 ± 3	101
20,000	10/10	134 ± 2	194 ± 2*	61 ± 2	95
40,000	10/10	134 ± 2	181 ± 2**	47 ± 3**	89

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error.

TABLE 4
Feed Consumption of Rats in the 13-Week Feed Studies of HC Yellow 4[a]

Week of Study	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Male						
1	91.2	96.4	94.6	96.3	85.8	106.9
2	78.4	81.3	79.0	79.5	89.6	119.6
3	71.8	73.0	73.5	81.3	78.7	106.3
4	69.6	73.3	73.4	75.6	78.5	159.6
5	63.0	65.4	66.8	71.3	72.8	147.2
6	57.5	58.7	60.6	64.0	73.7	136.7
7	55.0	60.7	60.1	58.4	67.4	131.0
8	53.9	62.4	58.0	59.1	67.3	127.6
9	59.1	57.3	60.0	60.9	70.4	104.8
10	57.4	55.8	55.2	58.8	65.4	115.6
11	52.9	52.6	55.4	55.5	63.8	108.2
12	44.2	47.7	48.5	49.5	56.7	104.8
13	45.5	45.6	51.5	48.2	55.3	103.8
Mean ± SD	61.5 ± 13.3	63.9 ± 14.2	64.4 ± 12.8	66.0 ± 14.0	71.2 ± 10.2	120.9 ± 18.3
Female						
1	87.1	86.8	85.1	81.6	65.5	99.3
2	84.5	89.5	83.3	74.3	68.4	86.9
3	85.3	87.8	83.7	68.3	66.1	125.5
4	86.2	75.7	75.7	80.4	55.4	117.8
5	82.0	77.0	72.3	79.0	66.8	123.3
6	86.0	75.6	70.3	82.0	87.7	136.5
7	87.6	74.2	77.0	79.0	78.4	152.3
8	81.3	71.7	73.5	69.0	70.2	143.1
9	78.9	76.7	53.3	70.9	61.8	135.2
10	70.4	79.3	68.7	71.6	56.0	143.8
11	72.6	69.6	64.4	65.5	52.8	143.6
12	70.2	64.1	60.3	86.9	54.7	155.3
13	70.7	58.5	58.2	59.4	52.0	174.5
Mean ± SD	80.2 ± 6.9	75.9 ± 8.9	71.2 ± 10.1	74.5 ± 7.8	64.3 ± 10.6	133.6 ± 23.4

[a] Feed consumption given in grams per kilogram body weight per day

Lesions related to chemical administration were seen in the thyroid gland of males and females, the kidney in males, and the uterus in females (Table 5). The severity of all lesions ranged from minimal to mild except for uterine atrophy in the 40,000 ppm female group which ranged from mild to moderate. Thyroid gland pigmentation was present in males and females in the 40,000 ppm dose groups and appeared as a golden brown, granular pigment scattered within the cytoplasm of follicular epithelial cells; occasionally a sloughed cell containing pigment was seen within the colloid. The nature of the pigment was undetermined. Special histologic stains (Perl's stain and acid fast) showed that the pigment was not hemosiderin or ceroid, and the periodic acid-Schiff method indicated that the pigment was not colloid. Mineralization of the renal papilla was observed in males that received 40,000 ppm. Mineralization consisted of small numbers of minute basophilic crystalline foci diffusely scattered within the renal papilla and usually located within tubule lumens.

Uterine atrophy, observed in females that received 20,000 or 40,000 ppm, was characterized by a decrease in uterine size, a decrease in the myometrium and endometrium, and a decrease in the size and number of endometrial glands as compared with the uteri of control females.

Dose Selection Rationale: The decreases in mean body weights of male rats in the 10,000, 20,000, and 40,000 ppm dose groups were quite dramatic (8%, 19%, and 29%) and suggested that for male rats, 10,000 ppm may exceed an exposure compatible with long-term survival in the 2-year study. There were no significant histological findings in males receiving doses of 20,000 ppm or less in the 13-week study; thus, doses of 2,500 and 5,000 ppm were selected for the males in the 2-year study. In the females, the weight decreases were much less dramatic (20,000 ppm, 5%; 40,000 ppm, 11%), but all females receiving 20,000 ppm or more had uterine atrophy. Thus, doses of 5,000 and 10,000 ppm were selected for the female rats in the 2-year study.

TABLE 5
Incidences of Treatment-Related Lesions in Rats in the 13-Week Feed Studies of HC Yellow 4[a]

	0 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm
Male						
Kidney, papilla						
Mineralization	0/10	0/10	0/10	0/10	0/10	9/10** (1.3)[b]
Thyroid gland						
Pigmentation	0/10	0/10	0/10	0/10	0/10	8/10** (1.0)
Female						
Thyroid gland						
Pigmentation	0/10	0/10	0/10	0/10	0/10	2/10 (1.0)
Uterus						
Atrophy	0/10	0/10	0/10	0/10	10/10** (1.0)	10/10** (2.3)

** Significantly different ($P \leq 0.01$) from the control group by the Fisher exact test
[a] Incidences given as number of lesions/number of tissues examined
[b] Average severity grades for affected animals. Minimal = 1, Mild = 2, Moderate = 3

2-Year Studies

6-Month Interim Evaluations

There were no biologically significant changes in thyroid hormone levels (Table G1) or histopathology observations that were related to administration of HC Yellow 4 at 6 months.

15-Month Interim Evaluations

There were no biologically significant changes in hematology or clinical chemistry values (Table G2). The apparent increases in blood urea nitrogen reported in the high-dose males and in dosed females may have been an artifact of the assay, caused by the presence of HC Yellow 4 in the urine. Statistically significant changes in absolute and relative organ weights observed in females were considered to reflect differences in body weight (Table F3). Neoplasms were observed in control and dosed rats (Table 6); neoplasms in dosed rats were not attributed to administration of HC Yellow 4.

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of low-dose males and females were similar to those of the controls throughout the studies; mean body weights of dosed males were slightly higher than those of the controls after week 61 (Tables 7 and 8 and Figure 1). Mean body weights of high-dose females were lower than controls after week 29. Feed consumption by high-dose males and dosed females was lower than that of the controls through week 53 and similar to that of the controls thereafter (Tables I1 and I2). Feed consumption by low-dose males was similar to that of the controls throughout the study. No clinical findings were attributed to the administration of HC Yellow 4.

TABLE 6
Incidences of Neoplasms in Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

	0 ppm	2,500 ppm[b]	5,000 ppm
Male			
Adrenal gland, medulla			
Pheochromocytoma, malignant	0/9	1/10	0/10
Brain			
Astrocytoma	1/9	0/10	0/10
Lung			
Alveolar/bronchiolar adenoma	0/9	1/10	0/10
Alveolar/bronchiolar carcinoma	0/9	1/10	0/10
Pituitary gland, pars distalis			
Adenoma	5/9	0/10	4/9
Preputial gland			
Adenoma	1/9	0/10	0/10
Carcinoma	1/9	0/10	1/10
Testis, interstitial cell			
Adenoma	7/9	5/10	3/10
Thyroid gland, C-cell			
Adenoma	0/9	0/10	2/10
Zymbal's gland			
Papilloma	0/9	0/10	1/10
	0 ppm	**5,000 ppm[b]**	**10,000 ppm**
Female			
Clitoral gland			
Adenoma	0/10	1/10	0/10
Pituitary gland, pars distalis			
Adenoma	1/10	3/10	5/10

[a] Incidences given as number of lesions/number of tissues examined
[b] Only gross lesions were examined microscopically. The denominator is the number of tissues examined grossly.

TABLE 7
Mean Body Weights and Survival of Male Rats in the 2-Year Feed Study of HC Yellow 4

Weeks on Study	0 ppm		2,500 ppm			5,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	107	70	103	97	70	104	97	70
2	159	70	153	96	70	152	96	70
3	193	70	188	97	70	184	95	70
4	222	70	216	97	70	211	95	70
5	246	70	242	99	70	239	97	70
6	262	70	261	99	70	257	98	70
7	278	70	278	100	70	273	98	70
8	291	70	286	98	70	283	97	70
9	308	70	305	99	70	301	98	69
10	315	70	310	98	70	307	97	69
11	329	70	326	99	70	323	98	69
12	337	70	334	99	70	329	98	69
13	343	70	342	100	70	338	99	69
14	352	70	349	99	70	347	99	69
17	367	70	367	100	70	363	99	69
21	388	70	384	99	70	380	98	69
25	406	70	410	101	70	405	100	69
29[a]	420	60	426	101	60	421	100	59
33	433	60	434	100	60	431	100	59
37	439	60	439	100	60	431	98	59
41	449	60	449	100	60	446	99	59
45	461	59	458	99	60	454	98	59
49	465	59	467	101	60	463	100	59
53	465	59	465	100	60	465	100	59
57	472	59	473	100	59	473	100	58
61	478	59	482	101	58	478	100	58
65	473	59	481	102	58	479	102	56
69[a]	472	49	480	102	47	488	103	46
73	473	48	487	103	46	489	103	46
77	466	47	479	103	46	481	103	46
81	464	47	477	103	46	481	104	44
85	456	46	472	104	45	483	106	42
89	445	43	454	102	44	473	106	41
93	435	38	441	101	39	463	106	41
97	434	31	439	101	34	445	103	38
101	416	29	430	104	34	439	106	31
104	413	24	417	101	33	440	107	28
Terminal sacrifice		21			29			28
Mean for weeks								
1-13	261		257	98		254	97	
14-52	418		418	100		414	99	
53-104	454		463	102		470	104	

[a] Interim evaluations occurred during weeks 25 and 68.

TABLE 8
Mean Body Weights and Survival of Female Rats in the 2-Year Feed Study of HC Yellow 4

Weeks on Study	0 ppm		5,000 ppm			10,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	99	70	99	100	70	98	99	70
2	131	70	134	102	70	128	98	70
3	144	70	150	104	70	143	99	70
4	154	70	162	105	70	156	102	70
5	165	70	171	104	70	165	100	70
6	177	70	180	102	70	174	99	70
7	180	70	185	103	70	179	100	70
8	190	70	191	101	70	184	97	70
9	196	70	197	101	70	189	97	70
10	200	70	202	101	70	196	98	70
11	204	70	206	101	70	199	98	70
12	206	70	208	101	70	200	97	70
13	209	70	212	102	70	204	98	70
14	212	70	216	102	70	208	98	70
17	221	70	225	102	70	217	98	70
21	228	70	229	101	70	222	98	70
25	237	70	238	101	70	230	97	70
29[a]	247	60	247	100	60	239	97	60
33	254	60	250	99	60	239	94	60
37	264	60	258	98	60	245	93	60
41	276	60	266	96	60	250	91	60
45	277	60	271	98	60	255	92	60
49	292	60	279	95	60	262	90	60
53	300	60	288	96	60	272	91	60
57	315	60	299	95	60	281	89	60
61	317	58	300	95	60	280	88	60
65	334	57	318	95	60	297	89	60
69[a]	336	47	321	96	49	298	89	50
73	349	47	335	96	49	309	89	49
77	355	46	342	96	49	314	88	48
81	360	46	352	98	47	322	90	46
85	360	45	348	96	47	321	89	46
89	358	43	351	98	45	326	91	46
93	356	41	356	100	43	324	91	43
97	356	36	351	99	39	327	92	40
101	355	31	351	99	37	330	93	38
104	354	29	353	100	31	330	93	35
Terminal sacrifice		27			31			34
Mean for weeks								
1-13	173		177	102		170	98	
14-52	251		248	99		237	94	
53-104	343		333	97		309	90	

[a] Interim evaluations occurred during weeks 26 and 67.

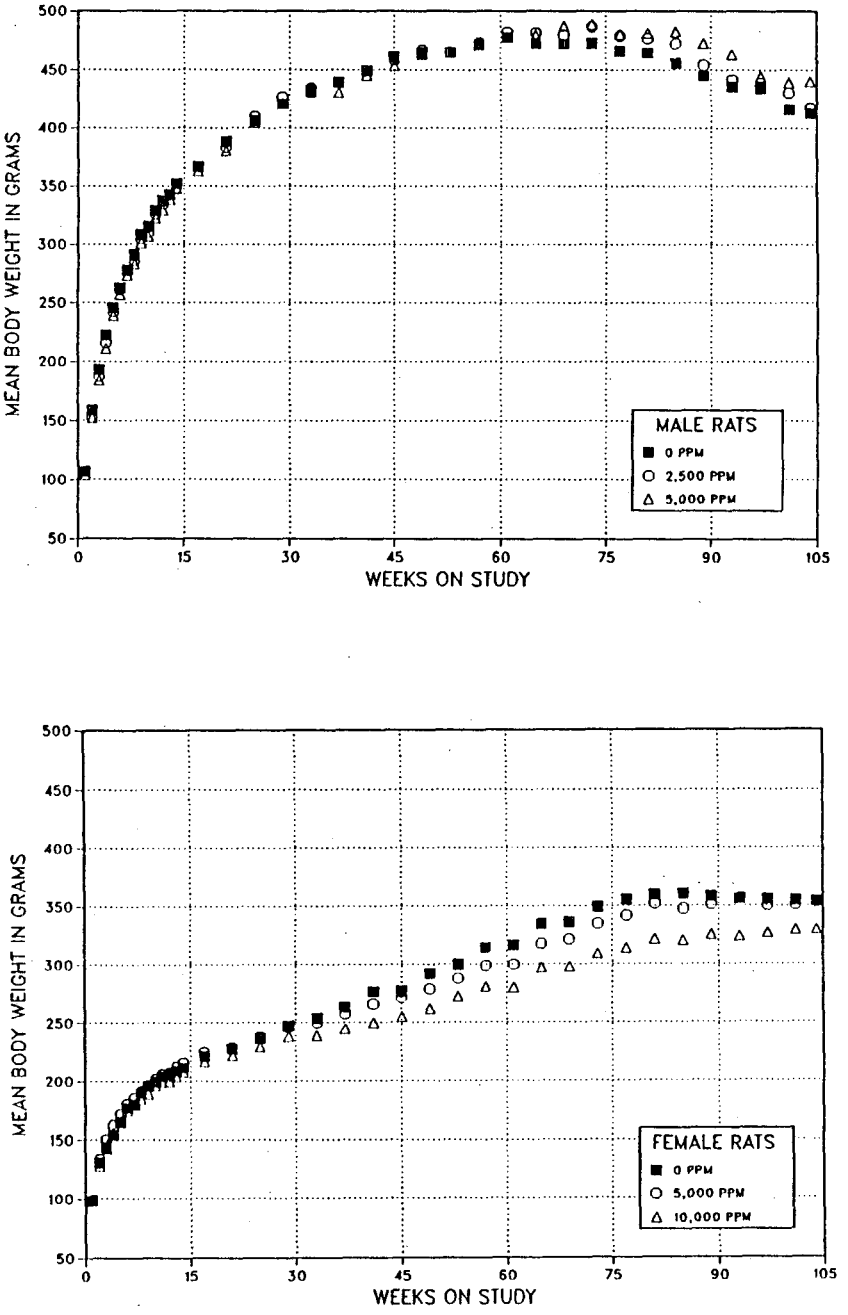


FIGURE 1
Growth Curves for Rats Administered HC Yellow 4 in Feed for 2 Years

Survival

Survival of dosed males and females was similar to those of the controls (Table 9 and Figure 2).

Pathology and Statistical Analyses of Results

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplastic or nonneoplastic lesions of the pituitary gland, uterus, and mammary gland in rats. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendixes A for male rats and B for female rats.

TABLE 9
Survival of Rats in the 2-Year Feed Studies of HC Yellow 4

	0 ppm	2,500 ppm	5,000 ppm
Male			
Animals initially in study	70	70	70
6-month interim evaluation[a]	10	10	10
15-month interim evaluation[a]	9	10	10
Natural deaths	6	4	3
Moribund kills	24	17	18
Missexed[a]	0	0	1
Animals surviving to study termination	21	29	28
Percent survival at end of study[b]	41	59	58
Mean survival (days)[c]	576	580	579
Survival analyses[d]	P=0.141N	P=0.139N	P=0.174N

	0 ppm	5,000 ppm	10,000 ppm
Female			
Animals initially in study	70	70	70
6-month interim evaluation[a]	10	10	10
15-month interim evaluation[a]	10	10	10
Natural deaths	2	4	1
Moribund kills	21	15	15
Animals surviving to study termination	27[e]	31[e]	34
Percent survival at end of study[b]	55	62	68
Mean survival (days)[c]	581	592	593
Survival analyses[d]	P=0.168N	P=0.453N	P=0.210N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[c] Mean of all deaths (uncensored, censored, terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.
[e] Includes one animal that was sacrificed moribund during the last week of study

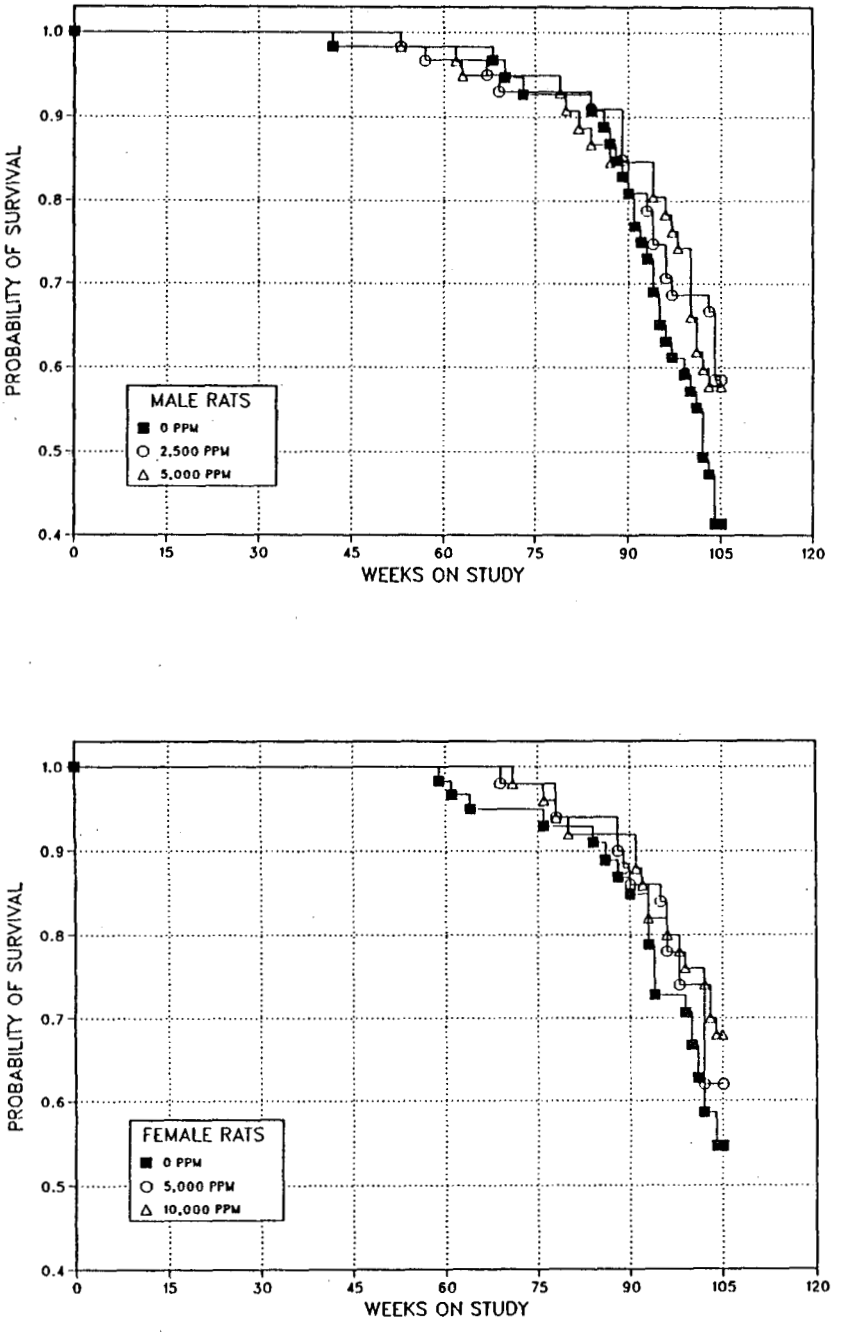


FIGURE 2
Kaplan-Meier Survival Curves for Rats Administered HC Yellow 4 in Feed for 2 Years

Pituitary Gland: Adenomas of the pars distalis occurred at greater incidences in dosed male rats than in the controls (Table 10). The increased incidence was significant in the high-dose group. The incidences of hyperplasia of the pars distalis, a lesion generally considered to be a precursor to adenoma, were also increased in dosed males, and the increase was significant in the high-dose group. The incidence of adenomas in dosed males was within the range of 12% to 60% for historical control incidences for untreated male F344/N rats from NTP 2-year feed studies (230/785 or 29.3%; Table A4). Because adenomas and hyperplasia were seen in the pituitary gland of male rats at the 15-month interim evaluation, the results of the 15-month evaluation were combined and analyzed with the 2-year study results (Table 10). The low-dose male rat group was not included in the analysis, because no pituitary glands from this group were examined microscopically at the 15-month evaluation. The combined incidence of adenoma in high-dose males was greater than the combined incidence in the control group, but the difference was not significant. The combined incidence of hyperplasia was significantly greater in the high-dose group than in the control group.

Adenomas of the pars distalis were discrete nodular masses which compressed and sometimes replaced adjacent parenchyma. They were composed of pale-staining polygonal cells which formed sheets or trabecular patterns and which often contained multiple cystic vascular spaces. Hyperplasias were composed of cells similar to those of adenomas; however, hyperplasias were smaller lesions which blended smoothly with adjacent parenchyma and usually caused no compression.

Uterus: Stromal polyps occurred with a positive trend, and the incidence in the high-dose females was significantly greater than that in the controls (0 ppm, 4/48; 5,000 ppm, 8/50; 10,000 ppm, 12/50; Table B3). One low-dose female had a uterine stromal sarcoma. The incidence of stromal polyps in the high-dose group was within the range (8%-30%) of historical control incidences in untreated female F344/N rats from 2-year NTP feed studies (142/800 or 17.8%, Table B4a). It was also similar to the mean historical incidence from three previous feed studies at this laboratory (30/150 or 20%, Table B4a). The incidence in the control group of the present study is at the low end of the historical control range, so the significant difference between the control and high-dose incidences may be due to an unusually low control group incidence. Consequently, the higher incidence of stromal polyps in treated females as compared with controls was not considered to be due to the administration of HC Yellow 4.

Mammary Gland: In females, fibroadenomas occurred with a significant negative trend (28/48, 19/37, 18/47; Table B3). The incidence in the control group was at the upper end of the historical control range, while the incidences in the dosed groups were near the mean historical control incidence for female rats from 2-year feed studies (314/800 or 39.3%, range 8%-58%; Table B4b). Thus, the significance of this negative trend was considered to be due to the high incidence in the control group and is not considered to be related to the administration of HC Yellow 4.

TABLE 10
Lesions of the Pituitary Gland Pars Distalis in Male Rats in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	2,500 ppm	5,000 ppm
Adenoma (2-year incidence)[a]			
Overall rates[b]	17/45 (38%)	20/49 (41%)	28/49 (57%)
Adjusted rates[c]	59.1%	53.0%	67.4%
Terminal rates[d]	10/20 (50%)	12/29 (41%)	15/28 (54%)
First incidence (days)	598	626	367
Logistic regression tests[e]	P=0.034	P=0.489	P=0.047
Adenoma (combined 15-month and 2-year incidence)			
Overall rates	22/54 (41%)	–[f]	32/58 (55%)
Adjusted rates	62.6%		69.7%
Terminal rates	5/9 (56%)		4/9 (44%)
First incidence (days)	470 (I)		367
Logistic regression tests			P=0.091
Hyperplasia (2-year incidence)			
Overall rates	8/45 (18%)	13/49 (27%)	18/49 (37%)
Logistic regression tests	P=0.026	P=0.209	P=0.035
Hyperplasia (combined 15-month and 2-year incidence)			
Overall rates	11/54 (20%)	–	25/58 (43%)
Logistic regression tests			P=0.008

(I) Interim evaluation
[a] Historical incidence for 2-year NTP feed studies of untreated control groups (mean ± standard deviation): 230/785 (29.3% ± 11.5%), range 12%–60%
[b] Number of lesion-bearing animals/number of animals necropsied or examined microscopically for this lesion
[c] Number of lesion-bearing animals/effective number of animals, i.e., number of animals alive at first occurrence of this tumor type in any of the groups
[d] Observed incidence at terminal kill
[e] 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 the controls and that dosed group. The logistic regression tests regard tumors in animals dying prior to terminal kill as nonfatal.
[f] Not examined at the 15-month interim evaluation

MICE

14-Day Studies

All mice survived to the end of the studies. The final mean body weight and mean body weight change of females and the mean body weight change of males that received 20,000 ppm were significantly lower than those of the controls (Table 11). Final mean body weights and mean body weight changes of other dose groups were similar to those of the controls. Feed consumption by dosed groups was generally similar to that of the controls during the first week of the studies; during the second week, feed consumption by males and females in the 10,000 and 20,000 ppm dose groups was higher than that of the controls.

No clinical findings in mice were related to HC Yellow 4 administration. No biologically significant changes in absolute or relative organ weights were noted (Table F4). No gross or microscopic lesions were related to HC Yellow 4 administration.

TABLE 11
Survival, Mean Body Weights, and Feed Consumption of Mice in the 14-Day Feed Studies of HC Yellow 4

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)	Feed Consumption[c]	
		Initial	Final	Change		Week 1	Week 2
Male							
0	5/5	25.4 ± 0.5	27.8 ± 0.6	2.4 ± 0.3		4.3	3.3
1,250	5/5	24.9 ± 0.3	29.0 ± 0.4	4.1 ± 0.3	104	4.1	3.1
2,500	5/5	25.7 ± 0.2	29.3 ± 0.3	3.6 ± 0.3	106	4.1	3.0
5,000	5/5	25.5 ± 0.4	28.3 ± 0.3	2.9 ± 0.2	102	4.0	2.6
10,000	5/5	25.2 ± 0.3	27.5 ± 0.3	2.3 ± 0.2	99	4.5	4.7
20,000	5/5	25.2 ± 0.8	26.4 ± 0.8	1.2 ± 0.3**	95	3.7	4.7
Female							
0	5/5	18.4 ± 0.4	21.1 ± 0.3	2.8 ± 0.2		8.0	2.3
1,250	5/5	18.3 ± 0.5	22.1 ± 0.4	3.7 ± 0.8	104	7.8	3.3
2,500	5/5	18.2 ± 0.4	20.3 ± 0.4	2.0 ± 0.1	96	6.2	2.3
5,000	5/5	18.3 ± 0.3	20.3 ± 0.1	2.0 ± 0.2	96	7.0	2.1
10,000	5/5	18.2 ± 0.4	20.3 ± 0.4	2.1 ± 0.6	96	6.6	4.6
20,000	5/5	18.3 ± 0.6	19.6 ± 0.4**	1.3 ± 0.4*	93	8.4	5.3

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Number of animals surviving at 14 days/number initially in group
[b] Weights and weight changes are given as mean ± standard error.
[c] Grams per animal per day, based on average consumption data per group per week for weeks 1 and 2

13-Week Studies

Eight males and seven females in the 80,000 ppm dose groups died; nine of these deaths occurred during week 1, five occurred during week 2, and one occurred during week 11 (Table 12). One male that received 40,000 ppm died during week 7. Final mean body weights and mean body weight changes of male and female mice that received doses of 10,000 ppm or greater were significantly lower than those of the controls. Feed consumption by dosed and control mice is shown in Table 13. The high feed consumption values for dosed animals, particularly those receiving the three highest dose levels, may be due to spillage of unpalatable feed and therefore might not reflect the actual amount of feed consumed.

No biologically significant clinical findings were observed that were related to HC Yellow 4 administration. Statistically significant changes in absolute and relative organ weights were considered to reflect decreases in body weight (Table F5).

TABLE 12
Survival and Mean Body Weights of Mice in the 13-Week Feed Studies of HC Yellow 4

Concentration (ppm)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	21.8 ± 0.4	32.4 ± 0.7	10.6 ± 0.5	
5,000	10/10	21.7 ± 0.4	32.4 ± 0.7	10.7 ± 0.8	100
10,000	10/10	22.0 ± 0.5	29.2 ± 0.6**	7.2 ± 0.6**	90
20,000	10/10	22.0 ± 0.4	30.0 ± 0.5**	8.0 ± 0.4**	92
40,000	9/10[c]	21.8 ± 0.5	27.9 ± 0.5**	6.2 ± 0.5**	86
80,000	2/10[d]	21.9 ± 0.5	22.5 ± 1.1**	-0.2 ± 1.6**	69
Female					
0	10/10	18.1 ± 0.1	25.1 ± 0.6	6.9 ± 0.6	
5,000	10/10	18.4 ± 0.3	24.6 ± 0.4	6.2 ± 0.3	98
10,000	10/10	18.2 ± 0.1	23.3 ± 0.3**	5.1 ± 0.4**	93
20,000	10/10	18.4 ± 0.2	21.6 ± 0.3**	3.2 ± 0.2**	86
40,000	9/10[e]	18.4 ± 0.2	19.3 ± 0.2**	0.9 ± 0.3**	77
80,000	3/10[f]	18.0 ± 0.2	17.6 ± 0.8**	0.2 ± 0.7**	70

** Significantly different (P≤0.01) from the control group by Williams' or Dunnett's test
[a] Number of animals surviving at 13 weeks/number initially in group
[b] Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the studies.
[c] Week of death: 7
[d] Week of death: 1, 1, 1, 1, 1, 1, 2, 11
[e] Week of death: 2. Animal was missing from cage, sacrificed when captured.
[f] Week of death: 1, 1, 1, 2, 2, 2, 2

TABLE 13
Feed Consumption of Mice in the 13-Week Feed Studies of HC Yellow 4[a]

Week of Study	0 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male						
1	258	299	273	393	476	845
2	223	275	299	462	520	1,715
3	207	247	280	385	425	1,328
4	232	266	291	372	376	1,654
5	230	255	294	384	393	1,722
6	219	253	326	373	455	1,711
7	198	196	287	343	444	2,122
8	242	206	323	453	568	2,035
9	226	193	296	344	396	1,407
10	208	214	273	290	384	1,523
11	246	194	266	413	545	1,371
12	221	172	280	391	512	1,360
13	200	185	247	327	398	1,114
Mean ± SD	224 ± 18	227 ± 40	287 ± 22	379 ± 48	453 ± 66	1,531 ± 351
Female						
1	205	256	375	462	510	1,100
2	233	327	421	526	697	1,405
3	216	336	399	510	651	998
4	227	312	422	539	555	964
5	246	336	404	470	556	1,007
6	246	337	403	462	576	1,114
7	231	297	409	504	604	1,099
8	251	253	382	471	577	1,066
9	192	285	426	549	732	1,238
10	223	298	375	423	508	925
11	235	280	330	325	460	854
12	203	270	314	559	743	1,229
13	192	268	416	532	704	1,136
Mean ± SD	233 ± 20	297 ± 31	390 ± 35	487 ± 63	606 ± 92	1,087 ± 147

[a] Feed consumption given in grams per kilogram body weight per day

Treatment-related lesions were observed in the thyroid gland, spleen, thymus, and uterus of dosed mice (Table 14). Pigmentation of the thyroid gland was observed in males and females that received doses from 5,000 to 40,000 ppm. Thyroid pigmentation occurred in only one animal that received 80,000 ppm, presumably because most of these animals died within the first 2 weeks of the studies before sufficient time had elapsed for pigmentation to develop. Thyroid pigmentation appeared as a golden brown granular pigment within the cytoplasm of follicular epithelial cells. The severity of the pigmentation increased with dose; average severity was minimal in the 5,000 and 10,000 ppm dose groups, mild in the 20,000 ppm dose groups, and mild to moderate in the 40,000 ppm dose groups. Minimal pigmentation was characterized by scant, faintly visible amounts of pigment within the follicular epithelium, mild pigmentation was characterized by the presence of small but readily observable amounts of pigment, and moderate pigmentation was prominent and easily visible. The nature of the pigment was undetermined. Special histologic stains (Perl's stain and acid fast) showed the pigment was not hemosiderin or ceroid, and the periodic acid-Schiff method indicated the pigment was not colloid.

Mild to moderate depletion of lymphoid tissue and subsequent atrophy of the spleen and thymus were observed in the high-dose males and females; these findings were considered to be secondary to the decreased body weights in these groups. Minimal to mild uterine atrophy was observed in females in the 40,000 and 80,000 ppm dose groups and was characterized by thinner myometrium and endometrium with a decrease in the size and number of endometrial glands as compared with uteri from control females.

Dose Selection Rationale: A combination of deaths and decreased mean body weights relative to controls precluded the selection of doses above 10,000 ppm. Therefore, doses of 5,000 and 10,000 ppm were selected for mice in the 2-year studies.

TABLE 14
Incidences of Selected Treatment-Related Lesions in Mice in the 13-Week Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male						
Thyroid gland						
Pigmentation	0/10	10/10** (1.0)[b]	9/10** (1.2)	10/10** (1.6)	10/10** (2.0)	1/6 (1.0)
Spleen						
Lymphoid depletion/atrophy	0/10	–[c]	–	–	1/10 (2.0)	5/6** (3.0)
Thymus						
Lymphoid depletion/atrophy	0/10	–	–	–	1/10 (3.0)	3/6** (2.7)
Female						
Thyroid gland						
Pigmentation	0/10	4/10* (1.0)	5/10* (1.0)	10/10** (1.1)	7/9** (1.9)	0/5
Spleen						
Lymphoid depletion/atrophy	0/10	–	–	–	0/10	5/5** (3.0)
Thymus						
Lymphoid depletion/atrophy	0/10	–	–	–	1/10 (2.0)	4/5** (3.0)
Uterus						
Atrophy	0/10	–	–	–	5/10* (1.2)	4/5** (1.8)

* Significantly different ($P \leq 0.05$) from the control group by the Fisher exact test
** $P \leq 0.01$
[a] Incidences given as number of lesions/number of tissues examined
[b] Average severity grades for affected animals. Minimal = 1, Mild = 2, Moderate = 3
[c] Not examined at this dose level

2-Year Studies

6-Month Interim Evaluations

Although statistically significant changes in thyroid hormone levels were observed, the biological significance of these findings was uncertain (Table G3).

Fine golden brown granular pigmentation was observed within follicular epithelial cells in the thyroid glands of all mice in the 5,000 and 10,000 ppm dose groups. The severity of pigmentation increased with dose, and pigmentation was more severe in males than in females. The pigmentation severity was mild in males that received 5,000 ppm and moderate in males that received 10,000 ppm, while severity was minimal in females that received 5,000 ppm and mild in females that received 10,000 ppm. Pigmentation severity was graded using the criteria described for the 13-week studies.

15-Month Interim Evaluations

Statistically significant changes in absolute or relative organ weights were considered to be secondary to body weight decreases (Table F6). No biologically significant changes in hematology or clinical chemistry values occurred (Table G4).

Golden yellow to golden brown granular pigmentation was observed within follicular epithelial cells and within the colloid of the thyroid gland of all dosed male and female mice. Severity of the pigmentation increased with dose and was more severe in males than in females. The severity was mild in low-dose males, moderate in high-dose males, minimal in low-dose females, and mild in high-dose females. Pigmentation was more severe in the follicular epithelium than in the colloid. Pigmentation severity was graded using criteria described for the 13-week studies. In addition to the pigmentation, minimal follicular cell hyperplasia was seen in 5 of 10 high-dose male mice. The hyperplasia was characterized by scattered follicles lined by columnar cells which were often crowded together and sometimes protruded into the follicular lumen.

A few neoplasms were observed in control and dosed mice at 15 months (Table 15). Neoplasms in dosed mice were not attributed to chemical administration.

TABLE 15
Incidences of Neoplasms in Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm[b]	10,000 ppm
Male			
Liver			
Hepatocellular adenoma	2/10	3/10	0/10
Hepatocellular carcinoma	0/10	0/10	1/10
Lung			
Alveolar/bronchiolar adenoma	1/10	0/10	0/10
Female			
Lung			
Alveolar/bronchiolar adenoma	0/10	1/10	0/10
Lymphoma, undifferentiated	0/10	1/10	0/10
Pituitary gland, pars distalis			
Adenoma	1/8	0/10	0/8

[a] Incidences given as number of lesions/number of tissues examined
[b] Only gross lesions were examined microscopically. The denominator is the number of tissues examined grossly.

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of all dosed groups were generally lower than those of the controls throughout the studies (Tables 16 and 17 and Figure 3). The mean body weights of low-dose males and females were more than 10% lower than those of the controls after week 53. The mean body weights of high-dose mice were more than 10% lower than those of the controls after week 17 for males and week 14 for females. Feed consumption values for dosed groups were higher than those of the controls throughout the studies (Tables I3 and I4). The apparent increase in feed consumption by dosed animals was due to the scattering of feed by animals searching for unadulterated feed. No clinical findings were attributed to the administration of HC Yellow 4.

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Feed Study of HC Yellow 4

Weeks on Study	0 ppm		5,000 ppm			10,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	22.8	50	23.0	101	50	23.1	101	50
2	23.9	50	23.9	100	50	23.7	99	50
3	24.0	50	24.7	103	50	24.2	101	50
4	26.7	50	27.1	102	50	25.7	96	50
5	28.3	50	28.5	101	50	27.3	97	50
6	29.2	50	29.2	100	50	28.3	97	50
7	30.2	50	30.2	100	50	29.3	97	50
8	30.5	50	30.2	99	50	28.3	93	50
9	31.7	49	31.4	99	50	30.4	96	50
10	32.6	48	32.3	99	50	31.1	95	50
11	33.3	48	32.9	99	50	31.3	94	50
12	33.7	48	33.5	99	49	31.6	94	50
13	34.1	48	34.0	100	49	31.6	93	50
14	35.0	48	34.3	98	49	32.4	93	50
17	36.5	46	35.7	98	47	32.7	90	50
21	38.1	46	37.3	98	47	33.5	88	50
25	38.4	45	38.0	99	45	33.8	88	50
29	39.4	45	38.7	98	45	34.2	87	50
33	39.3	44	39.1	100	45	32.0	81	49
37	41.0	43	39.7	97	45	34.1	83	49
41	41.3	43	39.4	95	43	33.5	81	49
45	41.8	43	38.7	93	42	33.9	81	47
49	42.5	42	38.6	91	41	34.6	81	47
53	42.7	42	38.3	90	41	33.3	78	47
57	42.0	41	37.3	89	39	33.3	79	47
61	43.6	38	38.1	87	37	34.6	79	45
65	43.7	38	38.0	87	37	34.3	79	44
69	43.5	37	37.9	87	37	33.8	78	44
73	42.6	37	37.8	89	37	34.0	80	43
77	42.2	35	37.3	88	36	33.2	79	43
81	42.8	35	37.2	87	36	33.0	77	43
85	40.0	35	35.2	88	35	31.0	78	42
88	40.4	34	35.4	88	33	31.1	77	41
93	37.6	34	34.7	92	32	31.1	83	40
97	38.3	34	35.0	91	31	31.1	81	38
101	38.2	32	35.0	92	29	30.2	79	36
104	37.3	31	35.0	94	29	30.5	82	35
Terminal sacrifice		28			29			35
Mean for weeks								
1-13	29.3		29.3	100		28.1	96	
14-52	39.3		38.0	97		33.5	85	
53-104	41.1		36.6	89		32.5	79	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Feed Study of HC Yellow 4

Weeks on Study	0 ppm		5,000 ppm			10,000 ppm		
	Av. Wt. (g)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors	Av. Wt. (g)	Wt. (% of controls)	Number of Survivors
1	17.1	50	16.9	99	50	17.1	100	50
2	18.3	50	18.0	98	50	17.8	97	50
3	19.3	50	18.9	98	50	18.3	95	50
4	19.3	50	19.4	101	50	18.9	98	50
5	20.9	50	20.1	96	50	19.8	95	50
6	21.1	50	20.7	98	50	20.2	96	50
7	22.1	50	21.6	98	50	21.2	96	50
8	22.2	50	22.0	99	50	21.1	95	50
9	23.2	50	22.6	97	50	21.7	94	50
10	23.9	50	23.1	97	50	21.9	92	50
11	25.2	50	24.0	95	50	22.6	90	50
12	24.9	50	23.9	96	50	22.9	92	50
14	26.7	50	25.1	94	50	23.9	90	50
17	28.9	50	27.2	94	50	24.6	85	50
21	31.5	50	29.3	93	50	26.5	84	50
25	32.7	50	30.3	93	50	27.0	83	50
29	34.4	50	32.4	94	49	27.9	81	50
33	35.3	50	33.1	94	49	28.1	80	50
37	35.9	50	33.1	92	49	28.5	79	50
41	37.7	49	34.5	92	49	29.6	79	50
45	38.4	49	35.4	92	49	29.6	77	50
49	39.8	49	35.9	90	49	29.9	75	50
53	40.2	49	36.0	90	49	29.9	74	50
57	39.7	49	35.5	89	49	29.1	73	50
61	41.1	49	37.1	90	49	29.8	73	50
65	41.5	49	35.5	86	49	29.3	71	50
69	42.2	47	36.1	86	49	30.4	72	50
73	41.6	47	36.3	87	49	30.2	73	50
77	42.0	46	37.7	90	48	31.4	75	50
81	42.2	45	37.2	88	48	30.1	71	48
85	42.3	45	36.3	86	48	29.3	69	47
89	42.7	45	35.8	84	47	28.6	67	45
93	42.2	43	35.9	85	47	28.5	68	45
97	43.0	43	35.0	81	45	28.1	65	44
101	41.7	43	35.3	85	42	27.8	67	44
104	42.0	43	35.4	84	39	27.7	66	44
Terminal sacrifice		43			38			43
Mean for weeks								
1-13	21.5		20.9	97		20.3	94	
14-52	34.1		31.6	93		27.6	81	
53-104	41.7		36.1	87		29.3	70	

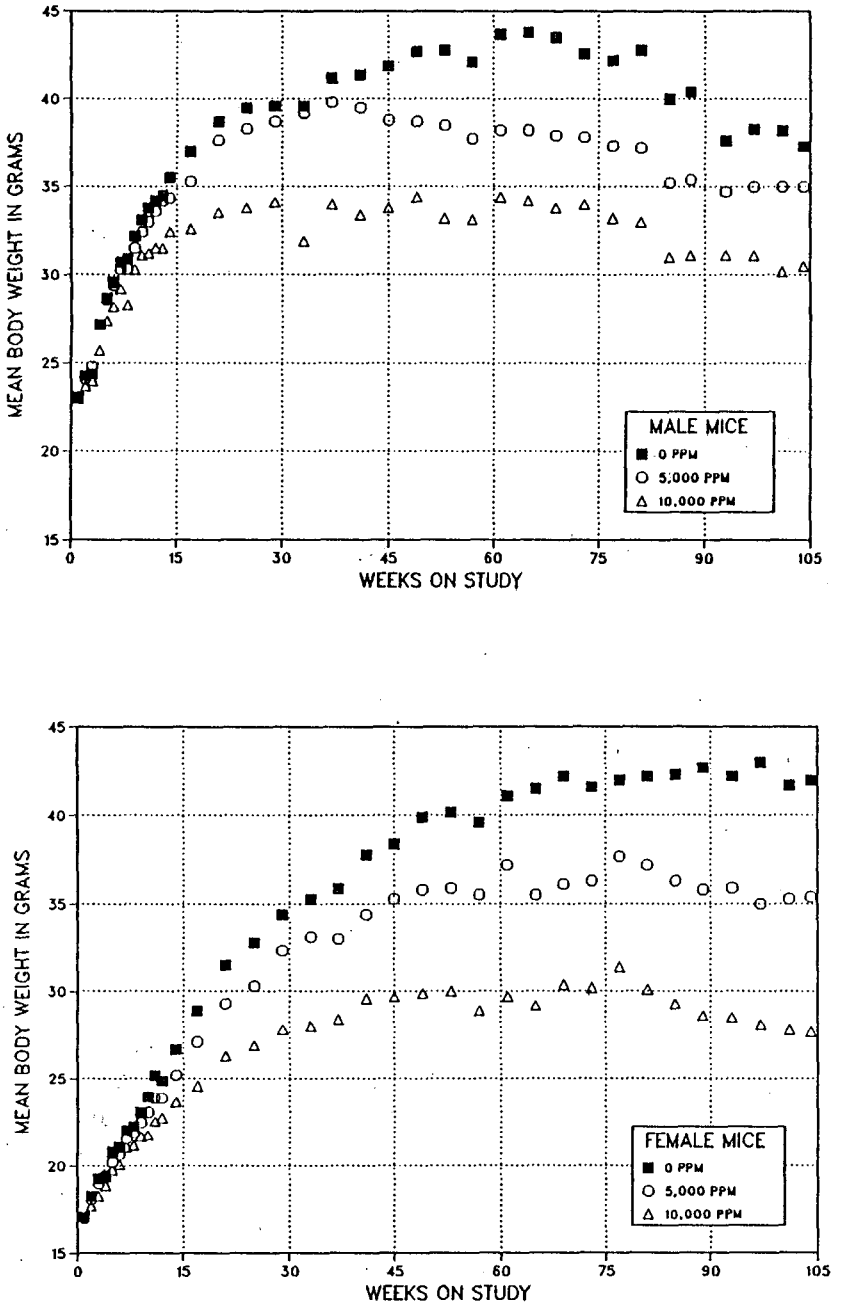


FIGURE 3
Growth Curves for Mice Administered HC Yellow 4 in Feed for 2 Years

Survival

Survival in dosed male and female mice was similar to that of the controls (Table 18 and Figure 4).

Pathology and Statistical Analyses of Results

This section describes the biologically noteworthy changes in the incidences of nonneoplastic lesions of the thyroid glands of mice. No neoplasms were attributed to the administration of HC Yellow 4. Summaries of the incidences of neoplasms and nonneoplastic lesions, individual animal tumor diagnoses, and statistical analyses of primary tumors that occurred with an incidence of at least 5% in at least one animal group are presented in Appendixes C for male mice and D for female mice.

TABLE 18
Survival of Mice in the 2-Year Feed Studies of HC Yellow 4

	0 ppm	5,000 ppm	10,000 ppm
Male			
Animals initially in study	70	70	70
6-month interim evaluation[a]	10	10	10
15-month interim evaluation[a]	10	10	10
Natural deaths	14	10	5
Moribund kills	8	10	9
Accidental deaths[a]	0	0	1
Missing[a]	0	1	0
Animals surviving to study termination	28	29	35
Percent survival at end of study[b]	60	63	73
Mean survival (days)[c]	516	512	568
Survival analyses[d]	P=0.119N	P=0.937N	P=0.133N
Female			
Animals initially in study	70	70	70
6-month interim evaluation[a]	10	10	10
15-month interim evaluation[a]	10	10	10
Natural deaths	4	2	2
Moribund kills	3	10	5
Animals surviving to study termination	43	38	43
Percent survival at end of study[b]	86	76	86
Mean survival (days)[c]	589	594	600
Survival analyses[d]	P=1.000N	P=0.375	P=1.000N

[a] Censored from survival analyses
[b] Kaplan-Meier determinations. Survival rates adjusted for interim evaluations.
[c] Mean of all deaths (uncensored, censored, terminal sacrifice)
[d] The result of the life table trend test (Tarone, 1975) is in the control column, and the results of the life table pairwise comparisons (Cox, 1972) with the controls are in the dosed columns. A negative trend or lower mortality in a dose group is indicated by N.

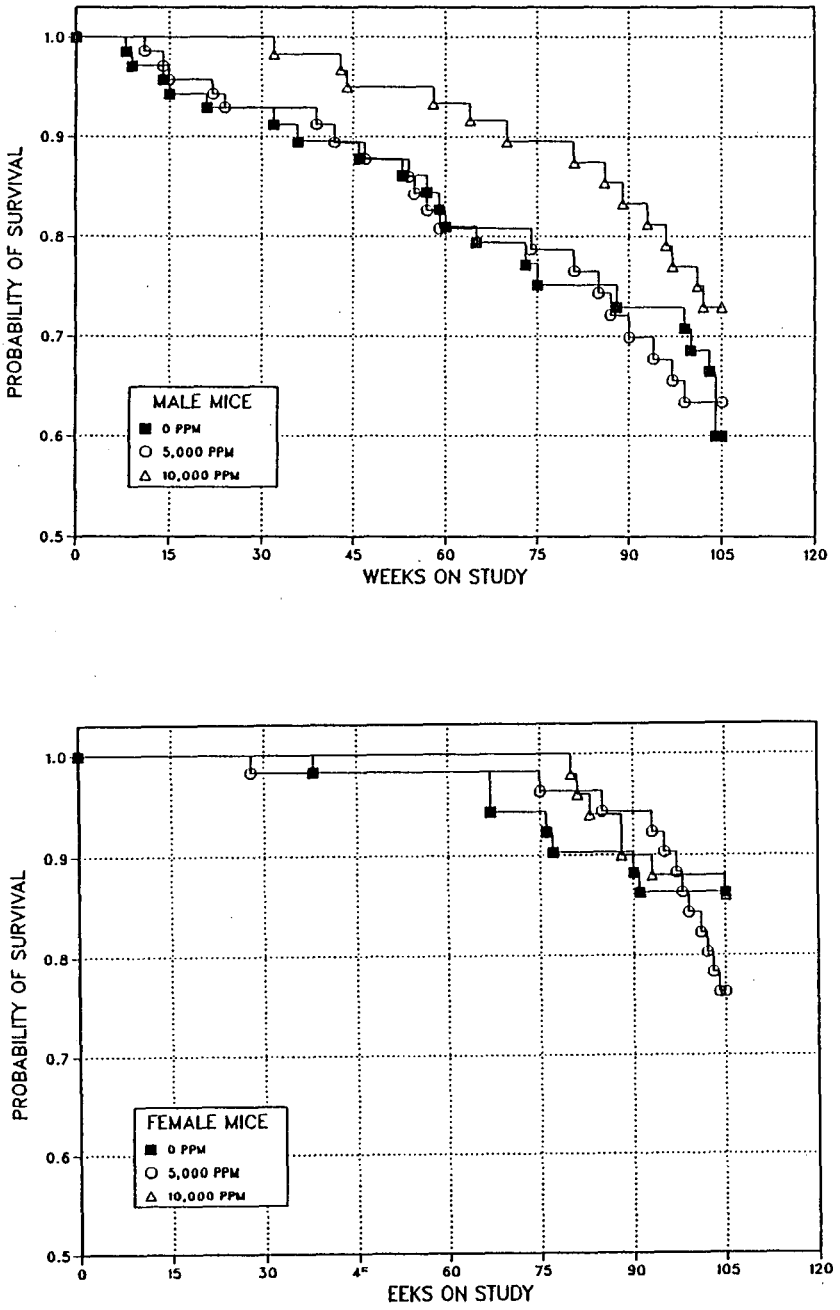


FIGURE 4
Kaplan-Meier Survival Curves for Mice Administered HC Yellow 4 in Feed for 2 Years

Thyroid Gland: The incidences of pigmentation and follicular cell hyperplasia were greatly increased in all dosed groups (Table 19). The pigment was gold-yellow to gold-brown and varied from fine granules to large aggregates. Pigment was present in the follicular cell cytoplasm (follicular cell, pigmentation), in the follicular lumens (follicle, pigmentation), and within macrophages in the interstitium between follicles (interstitium, pigmentation). Severity of pigmentation increased slightly with increasing dose and generally ranged from minimal to mild in the low-dose and mild to moderate in the high-dose groups (Plates 1 and 2). The severity was graded using the criteria described earlier in this report. The increase in hyperplasia was not accompanied by an increase in follicular cell neoplasms. Hyperplasia was of minimal to mild severity in all dosed groups. It involved multiple follicles lined by increased numbers of closely packed cells; as severity increased the follicular cells formed clusters that projected into the lumen. Chronic inflammation of minimal severity occurred in dosed males (Table 19). Inflammation, which consisted of scattered aggregates of small numbers of lymphocytes in the glandular interstitium, is not an uncommon finding in thyroid glands of older mice containing some degree of follicular cell hyperplasia.

TABLE 19
Incidences of Selected Thyroid Gland Lesions in Mice in the 2-Year Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Male			
Follicular cell adenoma[b]	1/47 (2%)	0/48 (0%)	2/49 (4%)
Follicular cell hyperplasia	0/47 (0%)	27/48 (56%)**	41/49 (84%)**
Follicular cell pigmentation	0/47 (0%)	44/48 (92%)**	49/49 (100%)**
Follicular pigmentation	0/47 (0%)	44/48 (92%)**	48/49 (98%)**
Interstitial pigmentation	0/47 (0%)	42/48 (88%)**	49/49 (100%)**
Chronic inflammation	0/47 (0%)	7/48 (15%)**	29/49 (59%)**
Female			
Follicular cell hyperplasia	0/48 (0%)	3/49 (6%)	13/50 (26%)**
Follicular cell pigmentation	0/48 (0%)	49/49 (100%)**	50/50 (100%)**
Follicular pigmentation	0/48 (0%)	48/49 (98%)**	50/50 (100%)**
Interstitial pigmentation	0/48 (0%)	46/49 (94%)**	50/50 (100%)**

** Significantly different ($P \leq 0.01$) from the control group by the logistic regression test
[a] Incidences given as number of lesion-bearing animals/number of animals examined at site
[b] Historical incidence for 2-year NTP feed studies of untreated control groups (mean ± standard deviation): 14/856 (1.6% ± 1.7%), range 0%–4%

GENETIC TOXICOLOGY

HC Yellow 4 (3 to 10,000 μg/plate) was tested for induction of gene mutations in four strains of *Salmonella typhimurium* in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; results were positive for strains TA100, TA1537, and TA98 with and without S9 (Table E1; Mortelmans *et al.*, 1986). An equivocal response was noted in strain TA1535 in the absence of S9 activation; results were negative with S9.

HC Yellow 4 induced sister chromatid exchanges in Chinese hamster ovary cells in the absence but not the presence of S9 activation (Table E2). In the two trials without S9, a significant increase in sister chromatid exchanges was observed only at the highest dose tested (167 or 200 μg/mL); the highest dose induced cell cycle delay and required an extended harvest to accumulate sufficient cells for analysis. With Aroclor 1254-induced male Sprague-Dawley rat liver S9, no significant increase was observed with concentrations up to 1,700 μg/mL HC Yellow 4; cell cycle delay was not noted with S9. When tested for induction of chromosomal aberrations in Chinese hamster ovary cells, HC Yellow 4 was negative with and without S9 (Table E3). In the one trial conducted without S9, a dose-related increase in aberrations was noted, but this increase was not statistically significant either by trend analysis ($P=0.027$) or peak response ($P>0.05$); a delayed harvest protocol was necessary to offset cell cycle delay caused by chemical administration. With S9, no cell cycle delay was observed in either trial and the weakly positive response observed at the highest nonlethal dose tested in the first trial (3,000 μg/mL) was not repeated in the second trial. A precipitate formed at the 2,500 μg/mL concentration in Trial 2 and no viable cells were present in the 3,000 μg/mL cultures.

HC Yellow 4 induced sex-linked recessive lethal mutations in germ cells of adult male *Drosophila melanogaster* when administered by injection at a dose of 10,000 ppm; results of the initial feeding test were negative (Table E4; Woodruff *et al.*, 1985). Following the positive result in the sex-linked recessive lethal assay, HC Yellow 4 (10,000 ppm by injection) was tested for induction of reciprocal translocations in germ cells of male *D. melanogaster*; results of this assay were negative (Table E5; Woodruff *et al.*, 1985).

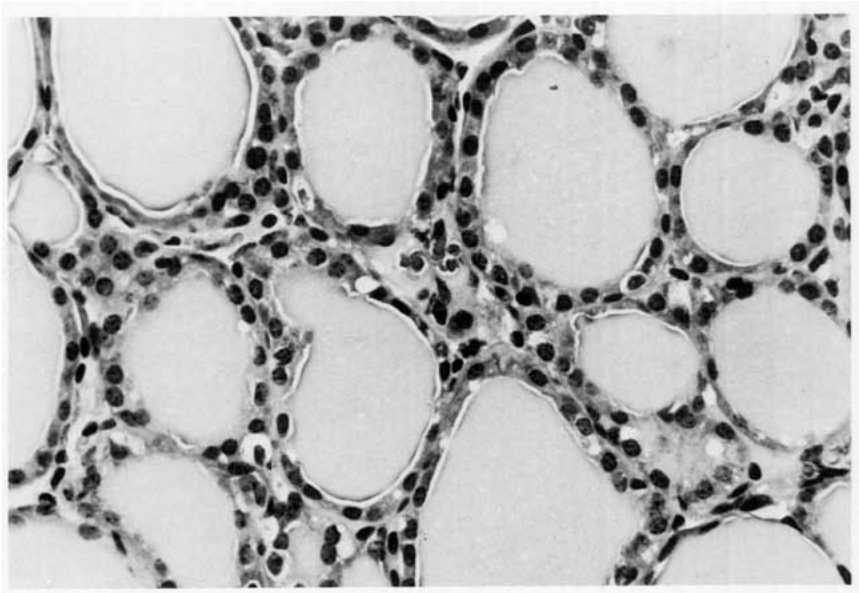


PLATE 1
Normal thyroid gland of a control male B6C3F$_1$ mouse in the 2-year feed study of HC Yellow 4. H&E, 300X

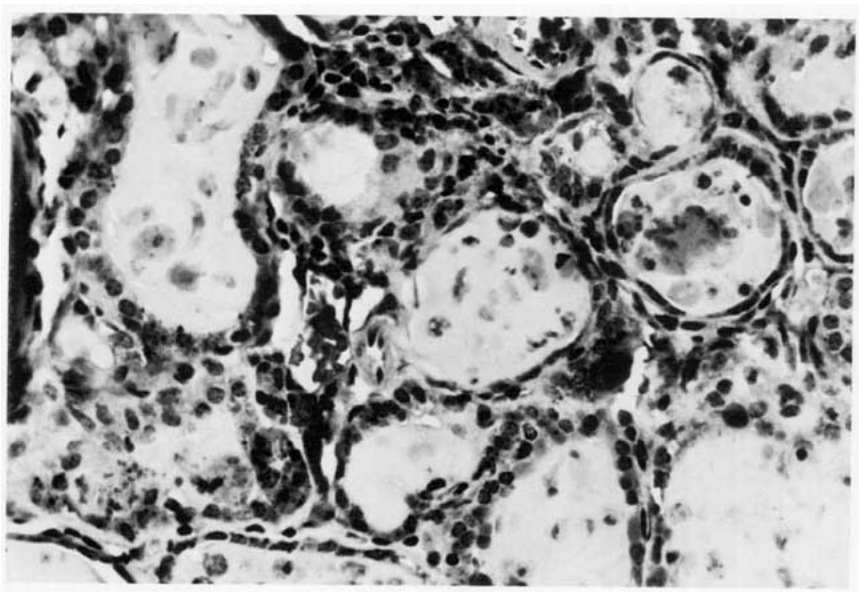


PLATE 2
Increased cellularity of the follicular epithelium in the thyroid gland of a male B6C3F$_1$ mouse receiving 10,000 ppm HC Yellow 4 in the 2-year feed study. Note the dark staining due to the presence of pigment. Compare with Plate 1. H&E, 300X

DISCUSSION AND CONCLUSIONS

Toxicity and carcinogenicity studies were conducted by administering HC Yellow 4 in feed to F344/N rats and B6C3F$_1$ mice. Although human exposure to HC Yellow 4 occurs primarily via the dermal route, the dosed feed route of administration was selected to ensure systemic exposure. In the 14-day rat feed studies, doses of 5,000 to 80,000 ppm (equivalent to 450 to 13,000 mg/kg body weight) caused decreases in body weight and feed intake. In the 14-day mouse feed studies, doses of 1,250 to 20,000 ppm (equivalent to 150 to 3,200 mg/kg) caused no toxic effects.

In the 13-week studies, doses of 2,500, 5,000, 10,000, 20,000, or 40,000 ppm were given to rats and doses of 5,000, 10,000, 20,000, 40,000, or 80,000 ppm were given to mice. All rats survived to the end of the studies. Final mean body weights of male rats that received doses of 10,000 ppm or greater and females that received doses of 20,000 ppm or greater were significantly lower than those of the controls. Histopathologic examination of the thyroid gland of rats revealed pigmentation in the follicular cells of 8 of 10 males and 2 of 10 females at the highest dose level. Slight mineralization of tubules in the renal papilla occurred in male rats that received 40,000 ppm. Uterine atrophy was seen in all female rats in the 20,000 and 40,000 ppm dose groups and may be related to the reduced body weights observed in these dose groups. Decreased body weight gain, when severe, is associated with uterine atrophy. Chemical-related deaths occurred in male mice that received 40,000 or 80,000 ppm and female mice that received 80,000 ppm. Final mean body weights were decreased in male and female mice that received doses above 5,000 ppm. Histopathologic examination of the thyroid gland revealed the presence of golden brown pigment in the follicular cells. Thyroid pigmentation was noted previously in the 13-week feed studies with the structurally related dyes HC Blue No. 1 (NTP, 1985a) and HC Blue No. 2 (NTP, 1985b). Lymphoid depletion and atrophy of the spleen and thymus were observed in mice in the 40,000 and 80,000 ppm dose groups and may have been associated with the decreases in mean body weight observed in these dose groups.

The doses selected for the 2-year studies were 0, 2,500, or 5,000 ppm for male rats and 0, 5,000, or 10,000 ppm for female rats and for male and female mice. The dose selection was based on mortality and decreased body weight.

In the 2-year studies, HC Yellow 4 caused a significant decrease in the mean body weight of high-dose female rats and all dosed mice. Mean body weights of dosed male and low-dose female rats were similar to those of the controls. The survival of dosed rats and mice was similar to that of the controls. Because HC Yellow 4 had no effect on body weight or survival of dosed male rats, it is possible that male rats could have tolerated higher doses, but probably not a doubling of the dose. In the 13-week studies, male rats were more sensitive to body weight depression than females. At doses of 10,000 ppm and above, males showed consistently higher percentages of body weight depression than females. Females receiving 10,000 ppm had no body weight depression at 13 weeks and 7% at the end of 2 years, as compared to 8% body weight depression at the end of 13 weeks in males receiving the same dose. Therefore, it is likely that if 10,000 ppm had been used for male rats in the 2-year study, they would have shown a considerable weight loss at the end of the study.

No chemical-related histopathologic lesions were observed in male or female rats evaluated at 15 months. In the 2-year studies, however, an increased incidence of pituitary gland adenomas of the pars distalis occurred in male rats (0 ppm, 17/45; 2,500 ppm, 20/49; 5,000 ppm, 28/49). The historical incidence for this tumor in untreated control rats is 230/785 (29.3%) with a range of 12% to 60%.

The only significant chemical-related effect observed in mice was a dose-related increased incidence in thyroid gland pigmentation and follicular cell hyperplasia. No chemical-related increase in the incidence of neoplastic lesions was observed in mice.

As in the 14-day and 13-week studies, the thyroid gland of mice was the organ primarily affected by

HC Yellow 4 in the 2-year studies. Dose-related increased incidences in follicular cell pigmentation and hyperplasia were observed in both sexes. The nature of the pigment was undetermined. Results of special histologic stains demonstrated that the pigment was not hemosiderin or ceroid, but were otherwise inconclusive. While it is possible to speculate that the pigment may represent HC Yellow or a metabolite, there is no definite proof of this. It is unclear if there was a relationship between the presence of pigment and follicular cell hyperplasia in these studies. In two other NTP studies of semipermanent hair dyes, C.I. Disperse Blue 1 (NTP, 1986a) and HC Red No. 3 (NTP, 1986b) caused increased thyroid gland pigmentation without a concomitant increase in hyperplasia. The presence of *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline as an impurity in HC Yellow 4 may have contributed to the increased incidence of thyroid follicular cell hyperplasia. Several aromatic amines, including aniline derivatives tested by NCI/NTP such as 4,4′-oxydianiline, 4,4′-methylenedianiline, and 4,4′-methylenebis (*N,N*-dimethyl)-benzamine, were found to increase the incidences of thyroid follicular cell adenoma and hyperplasia (Hayden *et al.*, 1978; Weisburger *et al.*, 1984; Hill *et al.*, 1989).

HC Yellow 4 bears a close structural resemblance to three of the five semipermanent hair dyes tested by the NTP: HC Blue No. 1, HC Blue No. 2, and HC Red No. 3 (Table 20). Of these structurally related dyes, the strongest evidence of carcinogenicity was obtained with HC Blue No. 1. This dye produced hepatocellular neoplasms in mice and, to a lesser degree, male rats. Dosed female rats had increased incidences of lung neoplasms. HC Red No. 3 caused a marginal increase in the incidence of liver tumors in male mice. No liver tumors were caused by HC Blue No. 2 or HC Yellow 4 in rats or mice. All of these dyes were mutagenic in *Salmonella typhimurium*. The *S. typhimurium* gene mutation assay has a high positive predictivity for carcinogenicity (89% of chemicals mutagenic in *S. typhimurium* are carcinogenic in rodents) (Tennant *et al.*, 1987). In the case of HC Yellow 4 and these two other noncarcinogenic dyes, positive results in the *in vitro S. typhimurium* gene mutation test are not reflected *in vivo*. The difference in the carcinogenic potential of these dyes may be due to differences in the metabolism or excretion of these dyes. The hydroxyethyl groups on the nitrogen in position 1 in HC Yellow 4 and positions 1 and 4 in HC Blue No. 2, as well as the hydroxyl group in position 2 in HC Yellow 4, may favor conjugation and excretion. The methyl group on the nitrogen in position 4 in HC Blue No. 1 may favor dealkylation and formation of an *N*-hydroxyl group. In HC Red No. 3, the primary amine may undergo *N*-acetylation or *N*-hydroxylation.

Although C.I. Acid Orange 3 (NTP, 1988) and C.I. Disperse Blue 1 do not bear a close structural resemblance to the other dyes, they too are mutagenic and have the potential to be metabolized to aromatic amines which are then *N*-hydroxylated to produce the final carcinogenic metabolite. Both dyes have been found to be carcinogenic; C.I. Acid Orange 3 induced kidney tumors in female rats, and C.I. Disperse Blue 1 induced urinary bladder neoplasms in male and female rats and marginally increased incidences of liver and lung neoplasms in male mice (Table 20).

Conclusions: Under the conditions of these 2-year feed studies, there was *equivocal evidence of carcinogenic activity** of HC Yellow 4 in male F344/N rats based on the increased incidence of pituitary gland adenomas and hyperplasia. The male rats may have been able to tolerate a slightly higher dose of the chemical. There was *no evidence of carcinogenic activity* of HC Yellow 4 in female F344/N rats given 5,000 or 10,000 ppm. There was *no evidence of carcinogenic activity* of HC Yellow 4 in male or female B6C3F$_1$ mice given 5,000 or 10,000 ppm.

There was a chemical-related increase in the incidence of thyroid gland pigmentation and follicular cell hyperplasia in mice.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 8. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 10.

TABLE 20
Comparison of Results of NTP Studies of Semipermanent Hair Dyes

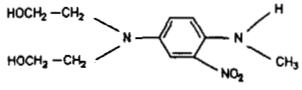
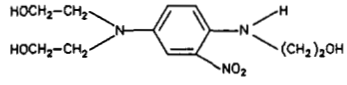
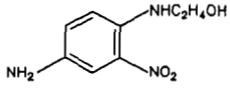
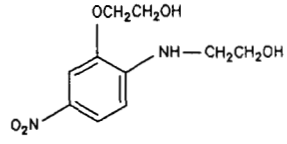
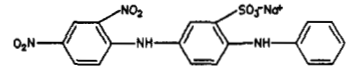
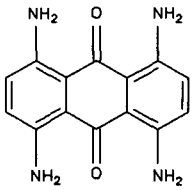
Chemical (route)/ Structure	Species/ Sex	Dose (mg/kg)	Level of Evidence[a]	Organ/Tumor
HC Blue No. 1 (feed) 	Rat			
	male	66 or 129	EE	Liver: neoplastic nodules, carcinoma
	female	74 or 154	SE	Lung: alveolar/bronchiolar neoplasms
	Mouse			
	male	309 or 650	CE	Liver: carcinoma; thyroid gland: adenoma
	female	778 or 1,634	CE	Liver: carcinoma
HC Blue No. 2 (feed) 	Rat			
	male	194 or 390	NE	None
	female	464 or 999	NE	None
	Mouse			
	male	1,319 or 2,239	NE	None
	female	2,331 or 5,603	NE	None
HC Red No. 3 (gavage) 	Rat			
	male	250 or 500	NE	None
	female	250 or 500	NE	None
	Mouse			
	male	125 or 250	EE	Liver: adenoma, carcinoma
	female	125 or 250	I	
HC Yellow 4 (current studies) (feed) 	Rat			
	male	140 or 230	EE	Pituitary gland: adenoma
	female	260 or 500	NE	None
	Mouse			
	male	1,380 or 2,500	NE	None
	female	1,080 or 2,800	NE	None
C.I. Acid Orange 3 (gavage) 	Rat			
	male	375 or 750	NE	None
	female	375 or 750	CE	Kidney: transitional cell carcinoma
	Mouse			
	male	125 or 250	NE	None
	female	250 or 500	NE	None

TABLE 20
Comparison of Results of NTP Studies of Semipermanent Hair Dyes (continued)

Chemical (route)/ Structure	Species/ Sex	Dose (mg/kg)	Level of Evidence	Organ/Tumor
C.I. Disperse Blue 1 (feed) 	Rat			
	male	45, 95, or 217	CE	Urinary bladder (males and females): transitional cell papilloma and carcinoma, leiomyoma and leiomysarcoma, squamous cell papilloma and carcinoma
	female	56, 111, or 240	CE	
	Mouse			
	male	112, 239, or 540	EE	Liver: adenoma and carcinoma; lung: alveolar/bronchiolar adenoma and carcinoma
	female	108, 235, or 520	NE	None

[a] Levels of evidence of carcinogenic activity: CE = clear evidence; SE = some evidence; EE = equivocal evidence; NE = no evidence; I = inadequate study

REFERENCES

Armitage, P. (1971). *Statistical Methods in Medical Research*, pp. 362-365. John Wiley and Sons, New York, NY.

Boorman, G.A., Montgomery, C.A., Jr., Eustis, S.L., Wolfe, M.J., McConnell, E.E., and Hardisty, J.F. (1985). Quality assurance in pathology for rodent carcinogenicity studies. In *Handbook of Carcinogen Testing* (H.A. Milman and E.K. Weisburger, Eds.), pp. 345-357. Noyes Publications, Park Ridge, NJ.

Burnett, C., Goldenthal, E.I., Harris, S.B., Wazeter, F.X., Strausburg, J., Kapp, R., and Voelker, R. (1976). Teratology and percutaneous toxicity studies of dyes. *J. Toxicol. Environ. Health* 1, 1027-1040.

Code of Federal Regulations (CFR). 21, Part 58.

Cosmetic, Toiletry and Fragrance Association (CTFA) (1977), 2nd ed. *Cosmetic Ingredient Dictionary.* Washington, DC.

Cosmetic, Toiletry and Fragrance Association (CTFA) (1982), 3rd ed. *Cosmetic Ingredient Dictionary.* (N.F. Estrin, P.A. Crosby, and C.R. Haynes, Eds.), p. 119. Washington DC.

Cox, D.R. (1972). Regression models and life tables. *J. R. Stat. Soc.* B34, 187-220.

Dinse, G.E., and Haseman, J.K. (1986). Logistic regression analysis of incidental-tumor data from animal carcinogenicity experiments. *Fundam. Appl. Toxicol.* 6, 44-52.

Dinse, G.E., and Lagakos, S.W. (1983). Regression analysis of tumor prevalence data. *Appl. Statist.* 32, 236-248.

Draize, J.H. (1959). *Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics.* pp. 49-51. Association of Food and Drug Officials of the United States. Austin, TX.

Dunn, O.J. (1964). Multiple comparisons using rank sums. *Technometrics* 6, 241-252.

Dunnett, W. (1955). A multiple comparison procedure for comparing several treatments with a control. *J. Am. Stat. Assoc.* 50, 1095-1121.

Galloway, S.M., Bloom, A.D., Resnick, M., Margolin, B.H., Nakamura, F., Archer, P., and Zeiger, E. (1985). Development of a standard protocol for *in vitro* cytogenetic testing with Chinese hamster ovary cells: Comparison of results for 22 compounds in two laboratories. *Environ. Mutagen.* 7, 1-51.

Galloway, S.M., Armstrong, M.J., Reuben, C., Colman, S., Brown, B., Cannon, C., Bloom, A.D., Nakamura, F., Ahmed, M., Duk, S., Rimpo, J., Margolin, B.H., Resnick, M.A., Anderson, B., and Zeiger, E. (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: Evaluations of 108 chemicals. *Environ. Mol. Mutagen.* 10 (Suppl. 10), 1-175.

Gart, J.J., Chu, K.C., and Tarone, R.E. (1979). Statistical issues in interpretation of chronic bioassay tests for carcinogenicity. *J. Natl. Cancer Inst.* 62, 957-974.

Haseman, J.K. (1984). Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. *Environ. Health Perspect.* 58, 385-392.

Haseman, J.K., Huff, J., and Boorman, G.A. (1984). Use of historical control data in carcinogenicity studies in rodents. *Toxicol. Pathol.* 12, 126-135.

Haseman, J.K., Huff, J.E., Rao, G.N., Arnold, J.E., Boorman, G.A., and McConnell, E.E. (1985). Neoplasms observed in untreated and corn oil gavage control groups of F344/N rats and (C57BL/6N x C3H/HeN)F_1 (B6C3F_1) mice. *JNCI* 75, 975-984.

Hayden, D.W., Wade, G.G., and Handler, A.H. (1978). Goitrogenic effect of 4,4′-oxydianiline in rats and mice. *Vet. Pathol.* **15**, 640-662.

HSDB [database online] (1990). Bethesda, MD: National Institute for Occupational Safety and Health. Available from: National Library of Medicine, Bethesda, MD.

Hill, R.N., Erdreich, L.S., Paynter, O.E., Roberts, P.A., Rosenthal, S.L., and Wilkinson, C.F. (1989). Thyroid follicular cell carcinogenesis. *Fundam. Appl. Toxicol.* **12**, 629-697.

Jonckheere, A. (1954). A distribution-free k-sample test against ordered alternatives. *Biometrika* **41**, 133-145.

Kaplan, E.L., and Meier, P. (1958). Nonparametric estimation of incomplete observations. *J. Am. Stat. Assoc.* **53**, 457-481.

Kastenbaum, M.A., and Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. *Mutat. Res.* **9**, 527-549.

Margolin, B.H., Collings, B.J., and Mason, J.M. (1983). Statistical analysis and sample-size determinations for mutagenicity experiments with binomial responses. *Environ. Mutagen.* **5**, 705-716.

Maronpot, R.R., and Boorman, G.A. (1982). Interpretation of rodent hepatocellular proliferative alterations and hepatocellular tumors in chemical safety assessment. *Toxicol. Pathol.* **10**, 71-80.

McConnell, E.E., Solleveld, H.A., Swenberg, J.A., and Boorman, G.A. (1986). Guidelines for combining neoplasms for evaluation of rodent carcinogenesis studies. *JNCI* **76**, 283-289.

Mortelmans, K., Haworth, S., Lawlor, T., Speck, W., Tainer, B., and Zeiger, E. (1986). *Salmonella* mutagenicity tests: II. Results from the testing of 270 chemicals. *Environ. Mutagen.* **8** (Suppl. 7), 1-119.

National Cancer Institute (NCI) (1976). Guidelines for Carcinogen Bioassay in Small Rodents. Technical Report Series No. 1. NIH Publication No. 76-801. National Institutes of Health, Bethesda, MD.

National Institutes of Health (NIH) (1978). Open Formula Rat and Mouse Ration (NIH-07). NIH Publication No. 11-1335. National Institutes of Health, Bethesda, MD.

National Institute for Occupational Safety and Health (NIOSH) (1990). National Occupational Health Survey (NOES) (1981-1983), unpublished provisional data as of July 1, 1990.

National Toxicology Program (NTP) (1985a). Toxicology and Carcinogenesis Studies of HC Blue No. 1 (CAS No. 2784-94-3) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). NTP TR No. 271. NIH Publication No. 85-2527. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

National Toxicology Program (NTP) (1985b). Toxicology and Carcinogenesis Studies of HC Blue No. 2 (CAS No. 33229-34-4) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). NTP TR No. 293. NIH Publication No. 85-2549. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

National Toxicology Program (NTP) (1986a). Toxicology and Carcinogenesis Studies of C.I. Disperse Blue 1 (CAS No. 2475-45-8) in F344/N Rats and B6C3F$_1$ Mice (Feed Studies). NTP TR No. 299. NIH Publication No. 86-2555. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

National Toxicology Program (NTP) (1986b). Toxicology and Carcinogenesis Studies of HC Red No. 3 (CAS No. 2871-01-4) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). NTP TR No. 281. NIH Publication No. 86-2537. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

National Toxicology Program (NTP) (1988). Toxicology and Carcinogenesis Studies of C.I. Acid Orange 3 (CAS No. 6373-74-6) in F344/N Rats and B6C3F$_1$ Mice (Gavage Studies). NTP TR No. 335. NIH Publication No. 89-2591. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health. Research Triangle Park, NC.

Sadtler Standard Spectra. Sadtler Research Laboratories, Philadelphia, PA.

Shirley, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics* 33, 386-389.

Tarone, R.E. (1975). Tests for trend in life table analysis. *Biometrika* 62, 679-682.

Tennant, R.W., Margolin, B.H., Shelby, M.D., Zeiger, E., Haseman, J.K., Spalding, J., Caspary, W., Resnick, M., Stasiewicz, S., Anderson, B., and Minor, R. (1987). Prediction of chemical carcinogenicity in rodents from *in vitro* genetic toxicity assays. *Science* 236, 933-941.

U.S. Food and Drug Administration (USFDA) (1975). Voluntary Cosmetic Regulatory Program, Cosmetics Registration File. U.S. FDA, Washington, DC.

U.S. Food and Drug Administration (USFDA) (1976). Voluntary Cosmetic Regulatory Program, Cosmetics Product Formulation Data. U.S. FDA, Washington, DC.

Weisburger, E.K., Murthy, A.S.K., Lilja, H.S., and Lamb, J.C. (1984). Neoplastic response of F344 rats and B6C3F$_1$ mice to the polymer and dyestuff intermediates 4,4′-methylenebis (*N,N*-dimethyl)-benzamine, 4,4′-oxydianiline, and 4,4′-methylenedianiline. *JNCI* 72 (Suppl. 6), 1457-1463.

Wernick, T., Lanman, B.M., and Fraux, J.L. (1975). Chronic toxicity, teratologic, and reproduction studies with hair dyes. *Toxicol. Appl. Pharmacol.* 32, 450-460.

Williams, D.A. (1971). A test for differences between treatment means when several dose levels are compared with a zero dose control. *Biometrics* 27, 103-117.

Williams, D.A. (1972). The comparison of several dose levels with a zero dose control. *Biometrics* 28, 519-531.

Woodruff, R.C., Mason, J.M., Valencia, R., and Zimmering, S. (1985). Chemical mutagenesis testing in *Drosophila*: V. Results of 53 coded compounds tested for the National Toxicology Program. *Environ. Mutagen.* 7, 677-702.

Zimmering, S., Mason, J.M., Valencia, R., and Woodruff, R.C. (1985). Chemical mutagenesis testing in *Drosophila*: II. Results of 20 coded compounds tested for the National Toxicology Program. *Environ. Mutagen.* 7, 87-100.

APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF HC YELLOW 4

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	9	10	10
Early deaths			
Natural deaths	6	4	3
Moribund kills	24	17	18
Survivors			
Terminal sacrifice	21	29	28
Missexed			1
Animals examined microscopically	50[b]	50	49
Alimentary System			
Esophagus	(48)	(48)	(49)
Intestine large, cecum	(47)	(48)	(46)
Intestine large, colon	(48)	(44)	(45)
Polyp adenomatous		1 (2%)	
Intestine small, duodenum	(47)	(48)	(47)
Intestine small, ileum	(45)	(48)	(46)
Intestine small, jejunum	(46)	(47)	(46)
Sarcoma			1 (2%)
Liver	(50)	(50)	(49)
Adenocarcinoma, metastatic, lung			1 (2%)
Fibrosarcoma, metastatic, skin	1 (2%)		
Hepatocellular adenoma	1 (2%)		
Mesentery	(3)	(5)	(1)
Pancreas	(50)	(48)	(48)
Acinus, adenoma	1 (2%)	3 (6%)	2 (4%)
Acinus, adenoma, multiple	1 (2%)	1 (2%)	
Pharynx	(1)		
Palate, papilloma squamous	1 (100%)		
Salivary glands	(49)	(50)	(48)
Sarcoma		1 (2%)	
Stomach, forestomach	(50)	(48)	(49)
Papilloma squamous	1 (2%)	1 (2%)	
Stomach, glandular	(49)	(48)	(48)
Tongue			(3)
Papilloma squamous			2 (67%)
Cardiovascular System			
Heart	(50)	(50)	(49)
Endocrine System			
Adrenal gland, cortex	(50)	(50)	(48)
Carcinoma	1 (2%)		
Adrenal gland, medulla	(50)	(49)	(48)
Pheochromocytoma malignant	2 (4%)	1 (2%)	
Pheochromocytoma benign	13 (26%)	11 (22%)	14 (29%)
Bilateral, pheochromocytoma benign	6 (12%)	6 (12%)	2 (4%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	2,500 ppm	5,000 ppm
Endocrine System (continued)			
Islets, pancreatic	(48)	(47)	(48)
Adenoma	1 (2%)	4 (9%)	3 (6%)
Parathyroid gland	(47)	(44)	(37)
Adenoma			2 (5%)
Pituitary gland	(45)	(49)	(49)
Pars distalis, adenoma	17 (38%)	18 (37%)	26 (53%)
Pars distalis, adenoma, multiple		2 (4%)	2 (4%)
Thyroid gland	(49)	(49)	(48)
C-cell, adenoma	6 (12%)	5 (10%)	6 (13%)
C-cell, adenoma, multiple	1 (2%)		
Follicular cell, adenoma			1 (2%)
General Body System			
None			
Genital System			
Epididymis	(49)	(50)	(49)
Preputial gland	(48)	(50)	(49)
Adenoma	8 (17%)	13 (26%)	7 (14%)
Carcinoma	1 (2%)		
Bilateral, adenoma	1 (2%)		1 (2%)
Prostate	(48)	(49)	(46)
Seminal vesicle	(48)	(50)	(48)
Testes	(50)	(50)	(49)
Bilateral, interstitial cell, adenoma	37 (74%)	40 (80%)	36 (73%)
Interstitial cell, adenoma	10 (20%)	3 (6%)	5 (10%)
Hematopoietic System			
Bone marrow	(49)	(50)	(48)
Lymph node	(50)	(50)	(49)
Lymph node, mandibular	(46)	(46)	(46)
Lymph node, mesenteric	(49)	(50)	(47)
Spleen	(50)	(50)	(48)
Sarcoma		1 (2%)	
Thymus	(41)	(43)	(47)
Thymoma benign	1 (2%)		
Integumentary System			
Mammary gland	(28)	(18)	(26)
Adenoma	1 (4%)		1 (4%)
Fibroadenoma	3 (11%)	1 (6%)	
Skin	(48)	(50)	(47)
Basal cell carcinoma	1 (2%)		
Keratoacanthoma		1 (2%)	1 (2%)
Papilloma squamous		1 (2%)	
Squamous cell carcinoma			1 (2%)
Subcutaneous tissue, fibroma		5 (10%)	
Subcutaneous tissue, fibrosarcoma	2 (4%)		1 (2%)
Subcutaneous tissue, lipoma		1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Musculoskeletal System			
Bone	(50)	(50)	(49)
Osteosarcoma		1 (2%)	
Skeletal muscle	(1)	(2)	
Nervous System			
Brain	(50)	(50)	(49)
Astrocytoma malignant			1 (2%)
Respiratory System			
Lung	(50)	(50)	(49)
Adenocarcinoma			1 (2%)
Alveolar/bronchiolar adenoma	2 (4%)	1 (2%)	3 (6%)
Carcinoma, metastatic, adrenal gland	1 (2%)		
Carcinoma, metastatic, preputial gland	1 (2%)		
Osteosarcoma, metastatic, bone		1 (2%)	
Osteosarcoma, metastatic, uncertain primary site	1 (2%)		
Pheochromocytoma malignant, metastatic, adrenal gland		1 (2%)	
Mediastinum, adenocarcinoma, metastatic, lung			1 (2%)
Mediastinum, sarcoma, metastatic, salivary glands		1 (2%)	
Nose	(47)	(50)	(49)
Papilloma squamous	1 (2%)		
Special Senses System			
Ear	(6)	(4)	(2)
Papilloma squamous	1 (17%)		
Squamous cell carcinoma		1 (25%)	
Harderian gland	(1)	(1)	(6)
Adenoma		1 (100%)	
Urinary System			
Kidney	(50)	(50)	(49)
Adenocarcinoma, metastatic, lung			1 (2%)
Renal tubule, adenoma	1 (2%)		
Urinary bladder	(48)	(48)	(47)
Systemic Lesions			
Multiple organs[c]	(50)	(50)	(49)
Leukemia mononuclear	19 (38%)	21 (42%)	16 (33%)
Lymphoma malignant undifferentiated cell		2 (4%)	1 (2%)
Mesothelioma malignant	2 (4%)	1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Tumor Summary			
Total animals with primary neoplasms[d]	49	50	48
Total primary neoplasms	143	148	137
Total animals with benign neoplasms	49	49	48
Total benign neoplasms	115	119	115
Total animals with malignant neoplasms	27	27	19
Total malignant neoplasms	28	29	22
Total animals with secondary neoplasms[e]	4	3	1
Total secondary neoplasms	4	3	3
Total animals with malignant neoplasms of uncertain primary site	1		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include one early death that occurred prior to the 15-month interim evaluation
[c] Number of animals with any tissue examined microscopically
[d] Primary tumors: all tumors except metastatic tumors
[e] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm

Number of Days on Study	2 8 8	4 8 5	5 0 8	5 8 7	5 8 9	6 0 8	6 1 0	6 1 9	6 2 5	6 3 7	6 3 7	6 3 9	6 5 1	6 5 5	6 5 8	6 6 2	6 6 5	6 6 6	6 7 3	6 8 9	6 9 4	7 0 7	7 1 0	7 1 0	7 1 3
Carcass ID Number	0 7 5	1 1 5	1 4 5	0 5 1	0 2 5	1 2 4	0 6 4	0 9 5	0 7 4	0 2 4	0 7 3	1 4 4	0 9 3	0 3 5	0 7 1	0 5 4	0 3 3	1 1 4	0 2 3	0 5 3	1 2 3	0 2 2	0 2 2	0 5 2	1 9 2	1 0 1
Alimentary System																									
Esophagus	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A
Intestine large, colon	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A
Intestine small	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A
Intestine small, duodenum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	A
Intestine small, ileum	+	+	+	A	+	+	+	+	+	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+	A
Intestine small, jejunum	+	+	+	A	+	+	+	+	+	+	+	+	+	M	+	A	+	+	+	+	+	+	+	+	A
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Fibrosarcoma, metastatic, skin																									
Hepatocellular adenoma																									
Mesentery													+					+	+						
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Acinus, adenoma, multiple																									
Pharynx									+																
Palate, papilloma squamous									X																
Salivary glands	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous							X																		
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Tooth						+																			
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma																									X
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant											X														
Pheochromocytoma benign											X	X	X	X					X				X	X	X
Bilateral, pheochromocytoma benign																						X			
Islets, pancreatic	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pituitary gland	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma					X		X			X		X					X						X	X	
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
C-cell, adenoma											X														
C-cell, adenoma, multiple																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 2 1	7 2 2	7 2 4	7 2 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 8 5	1 4 3	0 3 2	0 6 2	0 1 1	0 1 2	0 3 1	0 4 3	0 4 4	0 6 1	0 8 1	1 1 1	1 1 2	1 2 1	1 3 1	1 3 2	1 3 3	1 3 3	1 1 3	1 2 2	1 8 3	1 8 3	1 1 3	1 2 2	1 3 4	1 3 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Fibrosarcoma, metastatic, skin															X											1
Hepatocellular adenoma																					X					1
Mesentery																										3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma		X																								1
Acinus, adenoma, multiple																			X							1
Pharynx																										1
Palate, papilloma squamous																										1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Pheochromocytoma malignant										X																2
Pheochromocytoma benign										X			X			X			X			X				13
Bilateral, pheochromocytoma benign			X			X			X									X	X							6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	48
Adenoma								X																		1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma						X	X	X		X	X		X	X	X				X						X		17
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma														X	X	X					X		X			6
C-cell, adenoma, multiple									X																	1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	2 8 8	4 8 5	5 0 8	5 8 7	5 9 8	6 0 5	6 1 0	6 1 9	6 2 5	6 3 7	6 3 7	6 3 9	6 5 1	6 5 5	6 5 8	6 6 2	6 6 5	6 7 6	6 8 3	6 9 9	7 0 4	7 1 7	7 1 0	7 1 0	7 1 3
Carcass ID Number	0 7 5	1 1 5	1 4 5	0 5 1	0 2 5	1 2 4	0 6 4	0 9 5	0 7 4	0 2 4	0 7 3	0 4 4	1 9 3	0 3 5	0 7 1	0 5 4	0 3 3	1 1 4	0 2 3	0 5 3	1 2 3	0 2 2	0 5 2	0 9 2	1 0 1
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Adenoma												X													
Carcinoma			X																						
Bilateral, adenoma																									
Prostate	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X	X			X		X		X			X	X	X		X	X	X	X		X	X	X
Interstitial cell, adenoma					X	X		X		X			X				X					X			
Hematopoietic System																									
Blood					+																				
Bone marrow	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	M	+	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Thymoma benign																									
Integumentary System																									
Mammary gland	+	+	M	M	M	+	+	M	+	+	+	M	+	+	M	+	+	+	+	M	+	M	+	M	M
Adenoma																							X		
Fibroadenoma																									
Skin	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Basal cell carcinoma																									
Subcutaneous tissue, fibrosarcoma								X																	
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																		+							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 2 1	7 2 2	7 2 4	7 2 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 8 5	1 4 3	0 3 2	0 6 2	0 1 1	0 1 2	0 3 1	0 4 3	0 4 4	0 6 1	0 8 1	1 1 1	1 1 2	1 2 1	1 3 1	1 3 2	1 3 3	1 1 3	2 8 1	2 8 2	3 8 3	0 1 3	1 2 3	1 3 2	1 2 4	1 3 5	Total Tissues/ Tumors
General Body System																										
None																										
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma			X								X	X						X		X			X	X		8
Carcinoma																										1
Bilateral, adenoma									X																	1
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	37
Interstitial cell, adenoma			X					X																X		10
Hematopoietic System																										
Blood																										1
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	M	M	M	+	41
Thymoma benign																									X	1
Integumentary System																										
Mammary gland	+	+	M	+	M	+	+	+	+	+	+	+	M	M	+	M	+	M	+	M	M	M	M	M	M	28
Adenoma																										1
Fibroadenoma				X							X						X									3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Basal cell carcinoma			X																							1
Subcutaneous tissue, fibrosarcoma																X										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	2 4 5 5 5 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 7 7 7 7 8 8 0 8 9 0 1 1 2 3 3 3 5 5 5 6 6 6 7 8 9 0 1 1 8 5 8 7 8 5 0 9 5 7 7 9 1 5 8 2 5 6 6 3 9 4 7 0 0 3
Carcass ID Number	0 1 1 0 0 1 0 0 0 0 0 1 0 0 0 0 0 1 0 0 1 0 0 0 1 7 1 4 5 2 2 6 9 7 2 7 4 9 3 7 5 3 1 2 5 2 2 5 9 0 5 5 5 1 5 4 4 5 4 4 3 4 3 5 1 4 3 4 3 3 3 2 2 2 1
Respiratory System	
Lung	+ +
Alveolar/bronchiolar adenoma	X
Carcinoma, metastatic, adrenal gland	X
Carcinoma, metastatic, preputial gland	X
Osteosarcoma, metastatic, uncertain primary site	X
Nose	+ + M + + + + + + + M + + + + + + + + + + + + +
Papilloma squamous	
Trachea	+ +
Special Senses System	
Ear	+ + + + +
Papilloma squamous	
Eye	+ + + + +
Harderian gland	+
Urinary System	
Kidney	+ +
Renal tubule, adenoma	X
Urinary bladder	+ M + + + + + + + + + + + + + + + M + + + + + + +
Systemic Lesions	
Multiple organs	+ +
Leukemia mononuclear	X X X X X X X X X X X X X X X X X
Mesothelioma malignant	X

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 2 1	7 2 2	7 2 4	7 2 5	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	0 8 5	1 4 3	0 3 2	0 6 2	0 1 1	0 1 2	0 3 1	0 4 3	0 4 4	0 6 1	0 8 1	1 1 1	1 1 2	1 2 1	1 3 1	1 3 2	1 3 3	1 3 3	1 1 3	0 2 1	0 2 2	0 8 3	0 8 3	1 1 3	1 2 2	1 3 4	1 3 5	Total Tissues/ Tumors
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																								X			2
Carcinoma, metastatic, adrenal gland																											1
Carcinoma, metastatic, preputial gland																											1
Osteosarcoma, metastatic, uncertain primary site																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Papilloma squamous																		X									1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Ear													+											+			6
Papilloma squamous																								X			1
Eye		+											+														6
Harderian gland																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Renal tubule, adenoma																											1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X		X						X								X									19
Mesothelioma malignant						X																					2

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm

Number of Days on Study	3 6 7	3 9 6	4 6 7	4 8 1	5 8 8	6 1 9	6 2 0	6 2 3	6 2 6	6 2 6	6 4 6	6 5 3	6 5 8	6 6 8	6 7 2	6 7 3	7 1 8	7 2 3	7 2 3	7 2 8	7 2 8	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	2 8 5	1 7 5	2 2 4	1 8 5	1 8 4	1 5 5	1 5 4	1 9 5	1 7 3	1 8 3	1 9 4	2 1 4	1 9 3	2 0 5	1 6 5	2 8 2	1 6 2	2 4 5	2 6 4	1 8 1	2 0 4	1 5 3	1 6 1	1 7 1	1 7 2
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Intestine large	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Polyp adenomatous																									
Intestine large, rectum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	A	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery			+	+	+				+					+											
Pancreas	+	+	+	+	+	+	+	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																						X			
Acinus, adenoma, multiple																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																						X			
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous	X																								
Stomach, glandular	+	+	A	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign													X				X		X						
Bilateral, pheochromocytoma benign																				X		X		X	
Islets, pancreatic	+	+	+	+	+	+	+	A	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																		X						X	X
Parathyroid gland	+	+	+	M	+	+	+	M	+	M	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+
Pituitary gland	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma									X			X	X	X	X	X	X		X			X	X	X	
Pars distalis, adenoma, multiple																									
Thyroid gland	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma					X										X							X			
General Body System																									
Tissue NOS																									

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	1 8 2	1 9 2	2 0 1	2 0 2	2 1 1	2 1 3	2 2 1	2 2 3	2 3 2	2 3 5	2 4 2	2 4 3	2 4 4	2 5 1	2 5 2	2 5 3	2 6 2	2 6 3	2 6 1	2 7 2	2 7 3	2 7 4	2 8 1	2 8 3	2 8 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	M	+	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	44
Polyp adenomatous														X												1
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																										5
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Acinus, adenoma										X		X														3
Acinus, adenoma, multiple							X																			1
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma																										1
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	48
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																							X			1
Pheochromocytoma benign			X				X	X	X				X					X	X						X	11
Bilateral, pheochromocytoma benign		X								X														X		6
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Adenoma																X										4
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma				X									X	X				X	X	X		X				18
Pars distalis, adenoma, multiple							X										X									2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
C-cell, adenoma															X									X		5
General Body System																										
Tissue NOS																		+								1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm (continued)

Number of Days on Study	3 6 7	3 9 6	4 6 7	4 8 1	5 8 8	6 1 9	6 2 0	6 2 3	6 2 6	6 2 6	6 4 6	6 5 3	6 5 8	6 6 8	6 7 2	6 7 3	7 1 8	7 2 3	7 2 3	7 2 8	7 2 8	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	2 8 5	1 7 5	2 2 4	1 8 5	1 8 4	1 5 5	1 5 4	1 9 5	1 7 3	1 8 3	1 9 4	2 1 4	1 9 3	2 0 5	1 6 5	2 8 2	1 6 2	2 4 5	2 6 4	1 8 1	2 0 4	1 5 3	1 6 1	1 7 1	1 7 2
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X						X			X	X						X			X		X
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma			X			X		X	X	X	X		X		X		X	X	X		X	X	X	X	X
Interstitial cell, adenoma							X									X									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																									
Thymus	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	M	+	
Integumentary System																									
Mammary gland	M	+	+	+	+	M	+	+	+	M	M	M	M	M	M	M	M	+	M	+	+	+	M	M	M
Fibroadenoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Keratoacanthoma																									
Papilloma squamous																				X					
Subcutaneous tissue, fibroma															X					X					
Subcutaneous tissue, lipoma							X																		
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma						X																			
Skeletal muscle								+		+															
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	182	192	201	202	211	213	221	223	232	235	242	243	244	251	252	253	262	263	271	272	273	274	281	283	284	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X		X			X				X				X					X					13
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, interstitial cell, adenoma	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	40
Interstitial cell, adenoma														X												3
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	46
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma													X													1
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	M	+	+	43
Integumentary System																										
Mammary gland	M	M	M	M	M	M	+	+	M	M	M	M	M	M	M	M	M	+	M	M	M	+	+	+	+	18
Fibroadenoma							X																			1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Keratoacanthoma			X																							1
Papilloma squamous																										1
Subcutaneous tissue, fibroma			X														X					X				5
Subcutaneous tissue, lipoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma																										1
Skeletal muscle																										2
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm (continued)

Number of Days on Study	3 6 7	3 9 6	4 6 7	4 8 1	5 8 8	6 1 9	6 2 0	6 2 3	6 2 6	6 2 6	6 4 6	6 5 3	6 5 8	6 6 8	6 7 2	6 7 3	7 1 8	7 2 3	7 2 3	7 2 8	7 2 8	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	2 8 5	1 7 5	2 2 4	1 8 5	1 8 4	1 5 5	1 5 4	1 9 5	1 7 3	1 8 3	1 9 4	2 1 4	1 9 3	2 0 5	1 6 5	2 8 2	1 6 2	2 4 5	2 6 4	1 8 1	2 0 4	1 5 3	1 6 1	1 7 1	1 7 2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																									
Osteosarcoma, metastatic, bone						X																			
Pheochromocytoma malignant, metastatic, adrenal gland																									
Mediastinum, sarcoma, metastatic, salivary glands																						X			
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear		+																							
Squamous cell carcinoma		X																							
Eye							+																		
Harderian gland																									
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X				X	X	X	X	X	X					X	X	X		X				
Lymphoma malignant undifferentiated cell type	X				X																				
Mesothelioma malignant									X																

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 2,500 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	731	731	731	731	731	731	731	
Carcass ID Number	182	192	201	202	211	213	221	223	232	235	242	243	244	251	252	253	262	263	271	272	273	274	281	283	284	**Total Tissues/ Tumors**
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																								X		1
Osteosarcoma, metastatic, bone																										1
Pheochromocytoma malignant, metastatic, adrenal gland																							X			1
Mediastinum, sarcoma, metastatic, salivary glands																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear				+													+					+				4
Squamous cell carcinoma																										1
Eye				+					+								+	+				+				6
Harderian gland									+																	1
Adenoma									X																	1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X		X		X			X		X	X		X			X								X	X	21
Lymphoma malignant undifferentiated cell type																										2
Mesothelioma malignant																										1

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm

Number of Days on Study	3 6 7	4 3 0	4 3 6	5 5 3	5 5 7	5 7 0	5 8 8	6 0 5	6 5 4	6 5 8	6 6 8	6 7 6	6 8 0	6 9 4	6 9 4	6 9 4	6 9 6	7 0 2	7 0 4	7 1 4	7 1 5	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 8 5	3 9 5	3 9 4	3 9 2	4 1 4	4 1 3	3 6 5	4 0 4	3 1 4	3 8 4	3 0 5	3 3 4	4 2 5	3 1 3	3 4 3	3 5 3	4 1 2	3 8 2	3 2 4	3 4 1	3 6 4	2 9 1	2 9 3	2 9 4
Alimentary System																								
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	A	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma																								
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, lung									X															
Mesentery		M			+										M									
Pancreas	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma										X														
Salivary glands	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tongue			+																		+	+		
Papilloma squamous																					X	X		
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																								
Adrenal gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign						X					X	X			X					X			X	
Bilateral, pheochromocytoma benign														X										
Islets, pancreatic	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma						X													X					
Parathyroid gland	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	+
Adenoma																								
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X			X	X	X	X				X	X	X		X		X	X			X	X	X	X
Pars distalis, adenoma, multiple																X								
Thyroid gland	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma																						X		
Follicular cell, adenoma																								
General Body System																								
None																								

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	2 9 5	3 0 1	3 0 3	3 1 1	3 1 2	3 2 2	3 2 3	3 3 1	3 3 2	3 5 2	3 5 3	3 5 4	3 5 5	3 6 3	3 7 1	3 7 2	3 7 3	3 7 4	3 7 5	4 0 3	3 8 1	3 9 1	4 0 1	4 1 1	4 2 1	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large, colon	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Sarcoma	X																									1
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, metastatic, lung																										1
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Acinus, adenoma													X													2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Tongue																										3
Papilloma squamous																										2
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pheochromocytoma benign				X	X	X				X			X	X				X						X		14
Bilateral, pheochromocytoma benign									X																	2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																			X							3
Parathyroid gland	+	+	+	+	+	+	+	M	+	+	+	M	+	+	M	M	+	+	+	M	+	+		M	+	38
Adenoma		X																			X					2
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma		X	X		X	X	X		X	X				X				X	X						X	26
Pars distalis, adenoma, multiple																							X			2
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
C-cell, adenoma					X										X	X					X		X			6
Follicular cell, adenoma								X																		1
General Body System																										
None																										

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	3 6 7	4 3 0	4 3 6	5 5 3	5 5 7	5 7 0	5 8 8	6 0 5	6 5 4	6 5 8	6 6 8	6 7 6	6 8 0	6 9 4	6 9 4	6 9 4	6 9 6	7 0 2	7 0 4	7 1 4	7 1 5	7 2 9	7 2 9	7 2 9
Carcass ID Number	3 8 5	3 9 5	3 9 4	3 9 2	4 1 4	4 1 3	3 6 5	4 0 4	3 1 4	3 8 4	3 0 5	3 3 4	4 2 5	3 1 3	3 4 3	3 8 3	4 1 2	3 8 2	3 2 4	3 4 1	3 6 4	2 9 1	2 9 3	2 9 4
Genital System																								
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma				X			X										X							
Bilateral, adenoma																								
Prostate	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, interstitial cell, adenoma								X	X	X	X	X	X		X	X				X	X	X	X	X
Interstitial cell, adenoma			X	X	X		X																	
Hematopoietic System																								
Bone marrow	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Spleen	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																								
Mammary gland	+	+	+	+	+	M	M	M	M	+	M	+	+	+	M	+	+	+	+	M	M	+	+	+
Adenoma																								
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+
Keratoacanthoma																								
Squamous cell carcinoma			X																					
Subcutaneous tissue, fibrosarcoma												X												
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Astrocytoma malignant															X									
Spinal cord										+														
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma								X																
Alveolar/bronchiolar adenoma																							X	
Mediastinum, adenocarcinoma, metastatic, lung								X																
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
Carcass ID Number	29 5	30 1	30 3	31 1	31 2	32 2	32 3	33 1	33 2	35 2	35 3	35 4	35 5	36 3	37 1	37 2	37 3	37 4	37 5	40 3	38 1	39 1	40 1	41 1	42 1	Total Tissues/ Tumors
Genital System																										
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Preputial gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenoma													X							X		X	X			7
Bilateral, adenoma		X																								1
Prostate	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Bilateral, interstitial cell, adenoma	X	X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	36
Interstitial cell, adenoma					X																					5
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Lymph node, mesenteric	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	M	+	M	M	+	+	+	M	M	M	+	M	M	M	+	M	+	+	M	M	+	M	M	M	+	26
Adenoma																									X	1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	47
Keratoacanthoma			X																							1
Squamous cell carcinoma																										1
Subcutaneous tissue, fibrosarcoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Astrocytoma malignant																										1
Spinal cord																										1
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Alveolar/bronchiolar adenoma									X						X											3
Mediastinum, adenocarcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	3	4	4	5	5	5	5	6	6	6	6	6	6	6	6	6	7	7	7	7	7	7
	6	3	3	5	5	7	8	0	5	5	6	7	8	9	9	9	0	0	1	1	2	2
	7	0	6	3	7	0	8	5	4	8	8	6	0	4	4	4	6	2	4	4	5	9	9	9
Carcass ID Number	3	3	3	3	4	4	3	4	3	3	3	3	4	3	3	3	4	3	3	3	3	2	2	2
	8	9	9	9	1	1	6	0	1	8	0	3	2	1	4	8	1	8	2	4	6	9	9	9
	5	5	4	2	4	3	5	4	4	4	5	4	5	3	3	3	2	2	4	1	4	1	3	4
Special Senses System																								
Ear																		+	+					
Eye	+							+									+		+	+				
Harderian gland																	+		+					
Urinary System																								
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, lung									X															
Urinary bladder	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear						X	X	X			X		X	X	X	X		X			X	X		
Lymphoma malignant undifferentiated cell type							X																	

TABLE A2
Individual Animal Tumor Pathology of Male Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	729	730	730	730	730	730	
Carcass ID Number	29 5	30 1	30 3	31 1	31 2	32 2	32 3	33 1	33 2	35 2	35 3	35 4	35 5	36 3	37 1	37 2	37 3	37 4	37 5	40 3	38 1	39 1	40 1	41 1	42 1	Total Tissues/ Tumors
Special Senses System																										
Ear																										2
Eye			+		+		+						+									+		+	+	12
Harderian gland													+									+		+	+	6
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, metastatic, lung																										1
Urinary bladder	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Leukemia mononuclear	X					X		X	X										X							16
Lymphoma malignant undifferentiated cell type																										1

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4

	0 ppm	2,500 ppm	5,000 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	19/50 (38%)	17/49 (35%)	16/48 (33%)
Adjusted rates[b]	58.9%	51.1%	45.6%
Terminal rates[c]	9/21 (43%)	13/29 (45%)	10/28 (36%)
First incidence (days)	637	658	570
Life table tests[d]	P=0.108N	P=0.132N	P=0.143N
Logistic regression tests[d]	P=0.253N	P=0.321N	P=0.317N
Cochran-Armitage test[d]	P=0.352N		
Fisher exact test[d]		P=0.447N	P=0.393N
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	19/50 (38%)	18/49 (37%)	16/48 (33%)
Adjusted rates	58.9%	54.1%	45.6%
Terminal rates	9/21 (43%)	14/29 (48%)	10/28 (36%)
First incidence (days)	637	658	570
Life table tests	P=0.105N	P=0.171N	P=0.143N
Logistic regression tests	P=0.251N	P=0.398N	P=0.317N
Cochran-Armitage test	P=0.354N		
Fisher exact test		P=0.531N	P=0.393N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	2/50 (4%)	1/50 (2%)	3/49 (6%)
Adjusted rates	7.6%	3.4%	10.7%
Terminal rates	1/21 (5%)	1/29 (3%)	3/28 (11%)
First incidence (days)	666	729 (T)	729 (T)
Life table tests	P=0.501	P=0.412N	P=0.619
Logistic regression tests	P=0.429	P=0.481N	P=0.535
Cochran-Armitage test	P=0.391		
Fisher exact test		P=0.500N	P=0.490
Lung: Alveolar/bronchiolar Adenoma or Adenocarcinoma			
Overall rates	2/50 (4%)	1/50 (2%)	4/49 (8%)
Adjusted rates	7.6%	3.4%	12.9%
Terminal rates	1/21 (5%)	1/29 (3%)	3/28 (11%)
First incidence (days)	666	729 (T)	654
Life table tests	P=0.321	P=0.412N	P=0.451
Logistic regression tests	P=0.246	P=0.481N	P=0.347
Cochran-Armitage test	P=0.231		
Fisher exact test		P=0.500N	P=0.329
Mammary Gland: Fibroadenoma			
Overall rates	3/50 (6%)	1/50 (2%)	0/49 (0%)
Adjusted rates	13.6%	3.4%	0.0%
Terminal rates	2/21 (10%)	1/29 (3%)	0/28 (0%)
First incidence (days)	725	729 (T)	-[e]
Life table tests	P=0.034N	P=0.198N	P=0.079N
Logistic regression tests	P=0.039N	P=0.207N	P=0.090N
Cochran-Armitage test	P=0.062N		
Fisher exact test		P=0.309N	P=0.125N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	2,500 ppm	5,000 ppm
Mammary Gland: Adenoma or Fibroadenoma			
Overall rates	4/50 (8%)	1/50 (2%)	1/49 (2%)
Adjusted rates	16.7%	3.4%	3.6%
Terminal rates	2/21 (10%)	1/29 (3%)	1/28 (4%)
First incidence (days)	710	729 (T)	729 (T)
Life table tests	P=0.060N	P=0.104N	P=0.119N
Logistic regression tests	P=0.073N	P=0.124N	P=0.137N
Cochran-Armitage test	P=0.104N		
Fisher exact test		P=0.181N	P=0.187N
Pancreas: Adenoma			
Overall rates	2/50 (4%)	4/48 (8%)	2/48 (4%)
Adjusted rates	8.7%	13.8%	6.0%
Terminal rates	1/21 (5%)	4/29 (14%)	1/28 (4%)
First incidence (days)	722	729 (T)	658
Life table tests	P=0.478N	P=0.493	P=0.616N
Logistic regression tests	P=0.556N	P=0.464	P=0.689N
Cochran-Armitage test	P=0.569		
Fisher exact test		P=0.319	P=0.676
Pancreatic Islets: Adenoma			
Overall rates	1/48 (2%)	4/47 (9%)	3/48 (6%)
Adjusted rates	5.0%	13.4%	8.8%
Terminal rates	1/20 (5%)	3/28 (11%)	1/28 (4%)
First incidence (days)	729 (T)	723	570
Life table tests	P=0.339	P=0.295	P=0.368
Logistic regression tests	P=0.264	P=0.266	P=0.305
Cochran-Armitage test	P=0.253		
Fisher exact test		P=0.174	P=0.308
Parathyroid Gland: Adenoma			
Overall rates	0/47 (0%)	0/44 (0%)	2/37 (5%)
Adjusted rates	0.0%	0.0%	10.0%
Terminal rates	0/20 (0%)	0/27 (0%)	2/20 (10%)
First incidence (days)	–	–	729 (T)
Life table tests	P=0.083	–	P=0.237
Logistic regression tests	P=0.083	–	P=0.237
Cochran-Armitage test	P=0.072		
Fisher exact test		–	P=0.191
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	17/45 (38%)	20/49 (41%)	28/49 (57%)
Adjusted rates	59.1%	53.0%	67.4%
Terminal rates	10/20 (50%)	12/29 (41%)	15/28 (54%)
First incidence (days)	598	626	367
Life table tests	P=0.140	P=0.434N	P=0.176
Logistic regression tests	P=0.034	P=0.489	P=0.047
Cochran-Armitage test	P=0.036		
Fisher exact test		P=0.464	P=0.047

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	2,500 ppm	5,000 ppm
Preputial Gland: Adenoma			
Overall rates	9/48 (19%)	13/50 (26%)	8/49 (16%)
Adjusted rates	37.9%	36.6%	23.9%
Terminal rates	7/21 (33%)	8/29 (28%)	5/28 (18%)
First incidence (days)	639	481	553
Life table tests	P=0.261N	P=0.485	P=0.310N
Logistic regression tests	P=0.410N	P=0.295	P=0.451N
Cochran-Armitage test	P=0.430N		
Fisher exact test		P=0.269	P=0.481N
Preputial Gland: Adenoma or Carcinoma			
Overall rates	10/48 (21%)	13/50 (26%)	8/49 (16%)
Adjusted rates	39.2%	36.6%	23.9%
Terminal rates	7/21 (33%)	8/29 (28%)	5/28 (18%)
First incidence (days)	508	481	553
Life table tests	P=0.192N	P=0.576	P=0.232N
Logistic regression tests	P=0.325N	P=0.373	P=0.367N
Cochran-Armitage test	P=0.335N		
Fisher exact test		P=0.358	P=0.379N
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	0/50 (0%)	5/50 (10%)	0/49 (0%)
Adjusted rates	0.0%	15.6%	0.0%
Terminal rates	0/21 (0%)	3/29 (10%)	0/28 (0%)
First incidence (days)	-	672	-
Life table tests	P=0.520N	P=0.067	-
Logistic regression tests	P=0.585N	P=0.041	-
Cochran-Armitage test	P=0.603		
Fisher exact test		P=0.028	-
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	2/50 (4%)	5/50 (10%)	1/49 (2%)
Adjusted rates	7.0%	15.6%	2.6%
Terminal rates	1/21 (5%)	3/29 (10%)	0/28 (0%)
First incidence (days)	619	672	676
Life table tests	P=0.318N	P=0.336	P=0.449N
Logistic regression tests	P=0.408N	P=0.232	P=0.508N
Cochran-Armitage test	P=0.421N		
Fisher exact test		P=0.218	P=0.508N
Testes: Adenoma			
Overall rates	47/50 (94%)	43/50 (86%)	41/49 (84%)
Adjusted rates	100.0%	100.0%	97.5%
Terminal rates	21/21 (100%)	29/29 (100%)	27/28 (96%)
First incidence (days)	508	467	436
Life table tests	P=0.011N	P=0.018N	P=0.018N
Logistic regression tests	P=0.056N	P=0.140N	P=0.075N
Cochran-Armitage test	P=0.077N		
Fisher exact test		P=0.159N	P=0.094N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	2,500 ppm	5,000 ppm
Thyroid Gland (C-cell): Adenoma			
Overall rates	7/49 (14%)	5/49 (10%)	6/48 (13%)
Adjusted rates	30.3%	14.7%	21.4%
Terminal rates	6/21 (29%)	3/29 (10%)	6/28 (21%)
First incidence (days)	637	588	729 (T)
Life table tests	P=0.272N	P=0.220N	P=0.294N
Logistic regression tests	P=0.407N	P=0.344N	P=0.410N
Cochran-Armitage test	P=0.454N		
Fisher exact test		P=0.380N	P=0.516N
All Organs: Mononuclear Cell Leukemia			
Overall rates	19/50 (38%)	21/50 (42%)	16/49 (33%)
Adjusted rates	46.1%	51.1%	40.5%
Terminal rates	2/21 (10%)	10/29 (34%)	6/28 (21%)
First incidence (days)	485	467	570
Life table tests	P=0.192N	P=0.466N	P=0.238N
Logistic regression tests	P=0.332N	P=0.414	P=0.371N
Cochran-Armitage test	P=0.330N		
Fisher exact test		P=0.419	P=0.365N
All Organs: Benign Tumors			
Overall rates	49/50 (98%)	49/50 (98%)	48/49 (98%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	21/21 (100%)	29/29 (100%)	28/28 (100%)
First incidence (days)	485	367	367
Life table tests	P=0.085N	P=0.084N	P=0.104N
Logistic regression tests	P=0.694N	P=0.962	P=0.887N
Cochran-Armitage test	P=0.634N		
Fisher exact test		P=0.753N	P=0.747N
All Organs: Malignant Tumors			
Overall rates	27/50 (54%)	27/50 (54%)	19/49 (39%)
Adjusted rates	59.9%	59.8%	44.7%
Terminal rates	4/21 (19%)	12/29 (41%)	6/28 (21%)
First incidence (days)	485	367	436
Life table tests	P=0.054N	P=0.307N	P=0.070N
Logistic regression tests	P=0.085N	P=0.541	P=0.100N
Cochran-Armitage test	P=0.079N		
Fisher exact test		P=0.579N	P=0.094N
All Organs: Benign or Malignant Tumors			
Overall rates	49/50 (98%)	50/50 (100%)	48/49 (98%)
Adjusted rates	100.0%	100.0%	100.0%
Terminal rates	21/21 (100%)	29/29 (100%)	28/28 (100%)
First incidence (days)	485	367	367
Life table tests	P=0.087N	P=0.111N	P=0.104N
Logistic regression tests	P=0.608N	-[f]	P=0.887N
Cochran-Armitage test	P=0.665N		
Fisher exact test		P=0.500	P=0.747N

TABLE A3
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group
[f] Value of statistic cannot be computed

TABLE A4
Historical Incidence of Pituitary Gland Neoplasms in Untreated Male F344/N Rats[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at EG&G Mason Research Institute			
4-Hydroxyacetanilide	16/48	1/48	17/48
HC Yellow 4	17/45	0/45	17/45
Pentaerythritol tetranitrate	13/49	0/49	13/49
Quercetin	14/46	0/46	14/46
Total	60/188 (31.9%)	1/188 (0.5%)	6/188 (32.4%)
Standard deviation	4.7%	1.0%	4.9%
Range	27%–38%	0%–2%	27%–38%
Overall Historical Incidence			
Total	230/785 (29.3%)	3/785 (0.4%)	233/785 (29.7%)
Standard deviation	11.5%	0.8%	11.5%
Range	12%–60%	0%–2%	12%–60%

[a] Data as of 3 April 1991 for pars distalis or unspecified site

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	2,500 ppm	5,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	9	10	10
Early deaths			
Natural deaths	6	4	3
Moribund kills	24	17	18
Survivors			
Terminal sacrifice	21	29	28
Missexed			1
Animals examined microscopically	50[b]	50	49
Alimentary System			
Intestine large, cecum	(47)	(48)	(46)
Parasite	1 (2%)		
Intestine large, colon	(48)	(44)	(45)
Diverticulum	1 (2%)		
Parasite	4 (8%)		5 (11%)
Intestine large, rectum	(47)	(47)	(47)
Parasite	1 (2%)		2 (4%)
Thrombus	1 (2%)		
Liver	(50)	(50)	(49)
Angiectasis	4 (8%)	1 (2%)	4 (8%)
Angiectasis, focal	1 (2%)		1 (2%)
Basophilic focus	17 (34%)	21 (42%)	16 (33%)
Clear cell focus	6 (12%)	5 (10%)	10 (20%)
Cyst	1 (2%)		
Degeneration, cystic	2 (4%)	3 (6%)	4 (8%)
Eosinophilic focus	5 (10%)	2 (4%)	6 (12%)
Fatty change, diffuse	3 (6%)		1 (2%)
Fatty change, focal	9 (18%)	8 (16%)	3 (6%)
Hepatodiaphragmatic nodule	7 (14%)	4 (8%)	5 (10%)
Hyperplasia, focal		1 (2%)	1 (2%)
Mixed cell focus	1 (2%)	3 (6%)	1 (2%)
Necrosis	1 (2%)	3 (6%)	3 (6%)
Thrombus	3 (6%)	1 (2%)	
Bile duct, hyperplasia	34 (68%)	36 (72%)	41 (84%)
Mesentery	(3)	(5)	(1)
Fat, inflammation, chronic active			1 (100%)
Fat, necrosis	2 (67%)		1 (100%)
Pancreas	(50)	(48)	(48)
Acinus, atrophy	22 (44%)	21 (44%)	23 (48%)
Acinus, basophilic focus	5 (10%)	1 (2%)	3 (6%)
Acinus, hyperplasia	1 (2%)	3 (6%)	5 (10%)
Artery, fibrosis			4 (8%)
Artery, inflammation, chronic active			2 (4%)
Duct, hyperplasia	1 (2%)		
Stomach, forestomach	(50)	(48)	(49)
Acanthosis	2 (4%)	2 (4%)	
Hyperkeratosis	1 (2%)	1 (2%)	1 (2%)
Necrosis		3 (6%)	1 (2%)
Stomach, glandular	(49)	(48)	(48)
Hyperplasia		1 (2%)	
Mineralization	3 (6%)		1 (2%)
Necrosis	2 (4%)	1 (2%)	

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	2,500 ppm	5,000 ppm
Cardiovascular System			
Heart	(50)	(50)	(49)
Cardiomyopathy	33 (66%)	42 (84%)	34 (69%)
Thrombus		2 (4%)	2 (4%)
Endocrine System			
Adrenal gland, cortex	(50)	(50)	(48)
Hyperplasia			3 (6%)
Adrenal gland, medulla	(50)	(49)	(48)
Hyperplasia	12 (24%)	16 (33%)	14 (29%)
Islets, pancreatic	(48)	(47)	(48)
Hyperplasia		3 (6%)	
Parathyroid gland	(47)	(44)	(38)
Hyperplasia	2 (4%)	1 (2%)	1 (3%)
Pituitary gland	(45)	(49)	(49)
Pars distalis, angiectasis	8 (18%)	11 (22%)	10 (20%)
Pars distalis, cyst	1 (2%)	1 (2%)	3 (6%)
Pars distalis, hyperplasia	8 (18%)	13 (27%)	18 (37%)
Pars intermedia, cyst			1 (2%)
Thyroid gland	(49)	(49)	(48)
C-cell, hyperplasia	4 (8%)	9 (18%)	6 (13%)
Follicle, cyst	1 (2%)		
Follicular cell, hyperplasia		1 (2%)	1 (2%)
General Body System			
None			
Genital System			
Epididymis	(49)	(50)	(49)
Granuloma sperm			1 (2%)
Preputial gland	(48)	(50)	(49)
Inflammation, chronic active	11 (23%)	6 (12%)	5 (10%)
Prostate	(48)	(49)	(46)
Epithelium, hyperplasia	4 (8%)	1 (2%)	2 (4%)
Testes	(50)	(50)	(49)
Interstitial cell, hyperplasia	20 (40%)	27 (54%)	29 (59%)
Seminiferous tubule, atrophy	34 (68%)	31 (62%)	21 (43%)
Hematopoietic System			
Lymph node	(50)	(50)	(49)
Mediastinal, fibrosis			1 (2%)
Renal, pigmentation	1 (2%)	1 (2%)	
Lymph node, mesenteric	(49)	(50)	(47)
Fibrosis	1 (2%)		
Infiltration cellular, histiocyte	10 (20%)	3 (6%)	7 (15%)
Spleen	(50)	(50)	(48)
Cyst		1 (2%)	
Fibrosis	4 (8%)	2 (4%)	5 (10%)
Hematopoietic cell proliferation	20 (40%)	12 (24%)	25 (52%)
Thymus	(41)	(43)	(47)
Cyst			1 (2%)
Epithelial cell, hyperplasia	1 (2%)		

TABLE A5
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	2,500 ppm	5,000 ppm
Integumentary System			
Mammary gland	(28)	(18)	(26)
Galactocele	2 (7%)	2 (11%)	2 (8%)
Acinus, hyperplasia	7 (25%)	6 (33%)	5 (19%)
Skin	(48)	(50)	(47)
Acanthosis	1 (2%)		1 (2%)
Cyst epithelial inclusion	2 (4%)		3 (6%)
Hyperkeratosis	2 (4%)	2 (4%)	4 (9%)
Musculoskeletal System			
None			
Nervous System			
Brain	(50)	(50)	(49)
Hemorrhage	2 (4%)	2 (4%)	1 (2%)
Respiratory System			
Lung	(50)	(50)	(49)
Fibrosis	2 (4%)		2 (4%)
Infiltration cellular, histiocyte	6 (12%)	6 (12%)	5 (10%)
Inflammation, acute	2 (4%)		1 (2%)
Metaplasia, osseous		1 (2%)	1 (2%)
Alveolar epithelium, hyperplasia		4 (8%)	2 (4%)
Nose	(47)	(50)	(49)
Fungus	2 (4%)	1 (2%)	1 (2%)
Inflammation, acute	4 (9%)	4 (8%)	2 (4%)
Special Senses System			
Ear	(6)	(4)	(2)
Inflammation, acute	1 (17%)		
Eye	(6)	(6)	(12)
Hemorrhage	1 (17%)		
Lens, cataract	4 (67%)	4 (67%)	6 (50%)
Retina, atrophy	2 (33%)		3 (25%)
Urinary System			
Kidney	(50)	(50)	(49)
Cyst	3 (6%)	2 (4%)	4 (8%)
Hydronephrosis			2 (4%)
Nephropathy	49 (98%)	49 (98%)	47 (96%)
Urinary bladder	(48)	(48)	(47)
Calculus gross observation			1 (2%)
Calculus micro observation only	2 (4%)		1 (2%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Does not include one early death that occurred prior to the 15-month interim evaluation

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF HC YELLOW 4

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	2	4	1
Moribund kills	21	15	15
Survivors			
Terminal sacrifice	26	30	34
Moribund	1	1	
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(48)	(49)	(49)
Intestine large, colon	(48)	(47)	(47)
Intestine large, rectum	(48)	(46)	(49)
Intestine small, duodenum	(49)	(48)	(49)
Intestine small, ileum	(49)	(47)	(50)
Liver	(50)	(50)	(50)
Carcinoma, metastatic, ovary		1 (2%)	
Hepatocellular carcinoma	1 (2%)		
Hepatocellular adenoma		1 (2%)	
Mesentery	(3)	(4)	(6)
Carcinoma, metastatic, ovary		1 (25%)	
Pancreas	(49)	(50)	(49)
Acinus, adenoma	2 (4%)	2 (4%)	
Salivary glands	(50)	(50)	(50)
Stomach, forestomach	(50)	(48)	(50)
Papilloma squamous			1 (2%)
Stomach, glandular	(50)	(50)	(50)
Cardiovascular System			
Heart	(50)	(50)	(50)
Endocrine System			
Adrenal gland, cortex	(50)	(49)	(50)
Adenoma	1 (2%)		1 (2%)
Adrenal gland, medulla	(49)	(46)	(49)
Pheochromocytoma malignant	1 (2%)		
Pheochromocytoma benign	1 (2%)	3 (7%)	2 (4%)
Islets, pancreatic	(50)	(50)	(48)
Adenoma	1 (2%)		1 (2%)
Parathyroid gland	(46)	(39)	(45)
Adenoma	1 (2%)	1 (3%)	
Pituitary gland	(49)	(48)	(49)
Pars distalis, adenoma	31 (63%)	31 (65%)	29 (59%)
Pars distalis, adenoma, multiple	2 (4%)	4 (8%)	1 (2%)
Pars distalis, carcinoma	1 (2%)		
Thyroid gland	(50)	(50)	(50)
Bilateral, C-cell, adenoma			1 (2%)
C-cell, adenoma	9 (18%)	7 (14%)	7 (14%)
C-cell, carcinoma	1 (2%)		
Follicular cell, adenoma		1 (2%)	1 (2%)

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	5,000 ppm	10,000 ppm
General Body System			
None			
Genital System			
Clitoral gland	(47)	(47)	(44)
Adenoma	6 (13%)	5 (11%)	2 (5%)
Carcinoma			2 (5%)
Bilateral, adenoma	1 (2%)		
Ovary	(50)	(49)	(50)
Carcinoma		1 (2%)	
Granulosa cell tumor benign		1 (2%)	
Uterus	(48)	(50)	(50)
Polyp stromal	4 (8%)	7 (14%)	12 (24%)
Polyp stromal, multiple		1 (2%)	
Sarcoma stromal		1 (2%)	
Hematopoietic System			
Bone marrow	(50)	(49)	(49)
Lymph node	(50)	(50)	(50)
Mediastinal, carcinoma, metastatic, ovary		1 (2%)	
Mediastinal, carcinoma, metastatic, thyroid gland	1 (2%)		
Lymph node, mandibular	(49)	(45)	(48)
Adenocarcinoma, metastatic, mammary gland	1 (2%)		
Lymph node, mesenteric	(49)	(50)	(49)
Spleen	(50)	(49)	(50)
Thymus	(47)	(42)	(43)
Integumentary System			
Mammary gland	(48)	(37)	(47)
Adenocarcinoma	1 (2%)		
Adenoma	3 (6%)		2 (4%)
Fibroadenoma	19 (40%)	16 (43%)	16 (34%)
Fibroadenoma, multiple	9 (19%)	3 (8%)	2 (4%)
Skin	(48)	(50)	(49)
Basal cell carcinoma	1 (2%)		
Carcinoma adenosquamous		1 (2%)	
Keratoacanthoma		1 (2%)	
Subcutaneous tissue, fibroma	1 (2%)	3 (6%)	1 (2%)
Subcutaneous tissue, lipoma	1 (2%)		
Musculoskeletal System			
Skeletal muscle	(1)	(1)	(1)
Nervous System			
Brain	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland	1 (2%)		
Oligodendroglioma malignant	1 (2%)		
Spinal cord	(1)		(2)
Neoplasm NOS	1 (100%)		

TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Respiratory System			
Lung	(50)	(50)	(50)
Adenocarcinoma, metastatic, mammary gland	1 (2%)		
Alveolar/bronchiolar adenoma	1 (2%)	1 (2%)	2 (4%)
Alveolar/bronchiolar adenoma, multiple	1 (2%)		
Carcinoma adenosquamous, metastatic, skin		1 (2%)	
Squamous cell carcinoma, metastatic, nose	1 (2%)		
Nose	(50)	(49)	(48)
Squamous cell carcinoma	1 (2%)		
Trachea	(50)	(50)	(50)
Special Senses System			
Ear	(2)	(3)	(3)
Fibroma	1 (50%)		
Papilloma squamous			1 (33%)
Harderian gland	(9)	(4)	(15)
Adenoma	1 (11%)		1 (7%)
Zymbal's gland			(1)
Carcinoma			1 (100%)
Urinary System			
Kidney	(49)	(50)	(50)
Urinary bladder	(48)	(48)	(50)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Leukemia mononuclear	14 (28%)	15 (30%)	10 (20%)
Tumor Summary			
Total animals with primary neoplasms[c]	49	49	48
Total primary neoplasms	119	106	96
Total animals with benign neoplasms	43	48	43
Total benign neoplasms	96	88	83
Total animals with malignant neoplasms	19	18	13
Total malignant neoplasms	22	18	13
Total animals with secondary neoplasms[d]	4	2	
Total secondary neoplasms	5	4	
Total animals with neoplasms uncertain-benign or malignant	1		
Total uncertain neoplasms	1		

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm

Number of Days on Study	4 0 9	4 2 2	4 4 7	5 3 1	5 8 2	6 0 0	6 1 2	6 2 5	6 4 5	6 4 7	6 5 0	6 5 8	6 5 8	6 5 8	6 9 2	6 9 9	7 0 0	7 0 1	7 0 2	7 1 0	7 1 0	7 2 3	7 2 8	7 3 1	7 3 5
Carcass ID Number	5 5 5	4 9 5	4 8 5	4 7 5	5 3 5	5 4 5	5 0 5	4 7 4	4 7 2	4 4 4	5 3 4	4 9 3	5 4 4	5 6 4	1 5 4	8 4 4	5 1 4	4 3 5	5 3 3	4 5 4	5 0 4	5 0 3	4 5 3	4 6 4	4 3 1
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hepatocellular carcinoma																								X	
Mesentery			+																	+					
Pancreas	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																							X		
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Adrenal gland, medulla	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma malignant																									
Pheochromocytoma benign																									
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+
Adenoma																							X		
Pituitary gland	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma			X	X	X		X		X	X	X	X	X	X			X	X			X	X	X	X	X
Pars distalis, adenoma, multiple																									
Pars distalis, carcinoma																				X					
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma									X			X	X								X				X
C-cell, carcinoma						X																			
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	4 3 2	4 4 3	4 5 1	4 6 2	4 7 1	4 8 1	4 9 1	5 0 1	5 1 1	5 1 2	5 2 2	5 2 3	5 3 1	5 3 2	5 4 1	5 5 1	5 5 4	5 6 2	5 6 3	6 3 3	6 4 3	6 4 3	6 5 3	6 5 2	6 5 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Intestine large, rectum	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	48
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular carcinoma																										1
Mesentery																		+								3
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Acinus, adenoma																					X					2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma		X																								1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma malignant																		X								1
Pheochromocytoma benign																							X			1
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma			X																							1
Parathyroid gland	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	46
Adenoma																										1
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X				X	X	X			X	X		X	X	X			X	X	X	X				31
Pars distalis, adenoma, multiple			X	X																						2
Pars distalis, carcinoma																										1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma			X						X												X				X	9
C-cell, carcinoma																										1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm
(continued)

Number of Days on Study	4 0 9	4 2 2	4 4 7	5 3 1	5 8 2	6 0 0	6 1 2	6 2 5	6 4 5	6 4 7	6 5 0	6 5 8	6 5 8	6 5 8	6 9 2	6 9 9	7 0 0	7 0 1	7 0 2	7 1 0	7 1 0	7 2 3	7 2 8	7 3 1	7 3 5
Carcass ID Number	5 5 5	4 9 5	4 8 5	4 7 5	5 3 5	5 4 5	5 0 5	4 7 4	4 7 2	4 4 4	5 3 4	4 9 3	5 4 4	5 6 4	5 1 5	4 8 4	5 1 4	4 3 5	5 3 3	4 5 4	5 0 4	5 0 3	4 5 3	4 6 4	4 3 1
Genital System																									
Clitoral gland	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma								X	X			X													
Bilateral, adenoma																	X								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal								X						X								X	X		
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, carcinoma, metastatic, thyroid gland						X																			
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, mammary gland	X																								
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
Integumentary System																									
Mammary gland	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma	X																								
Adenoma																	X								
Fibroadenoma					X		X	X							X			X				X		X	
Fibroadenoma, multiple										X			X	X		X									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Basal cell carcinoma																									
Subcutaneous tissue, fibroma																					X				
Subcutaneous tissue, lipoma															X										
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, pituitary gland																				X					
Oligodendroglioma malignant		X																							
Spinal cord									+																
Neoplasm NOS									X																

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	4 3 2	4 4 3	4 5 1	4 6 2	4 7 1	4 8 1	4 9 1	5 0 1	5 1 1	5 1 2	5 2 2	5 2 3	5 3 1	5 3 2	5 4 1	5 5 1	5 5 4	5 6 2	6 6 3	4 3 3	4 4 3	4 8 3	5 0 2	5 2 5	5 4 3	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma								X									X		X							6
Bilateral, adenoma																										1
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	48
Polyp stromal																										4
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, carcinoma, metastatic, thyroid gland																										1
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma, metastatic, mammary gland																										1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Integumentary System																										
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenocarcinoma																										1
Adenoma							X							X												3
Fibroadenoma				X	X	X	X	X	X					X	X	X	X				X				X	19
Fibroadenoma, multiple	X		X										X					X				X				9
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Basal cell carcinoma				X																						1
Subcutaneous tissue, fibroma																										1
Subcutaneous tissue, lipoma																										1
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																		+								1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, pituitary gland																										1
Oligodendroglioma malignant																										1
Spinal cord																										1
Neoplasm NOS																										1

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm
(continued)

Number of Days on Study	4 0 9	4 2 2	4 4 7	5 3 1	5 8 2	6 0 0	6 1 2	6 2 5	6 4 5	6 4 7	6 5 0	6 5 8	6 5 8	6 5 8	6 9 2	6 9 9	7 0 0	7 0 1	7 0 2	7 1 0	7 1 0	7 2 3	7 2 8	7 3 1	7 3 5
Carcass ID Number	5 5 5	4 9 5	4 8 5	4 7 5	5 3 5	5 4 5	5 0 5	4 7 4	4 7 2	4 4 4	5 3 4	4 9 3	5 4 4	5 6 4	5 1 5	4 8 4	5 1 4	4 3 5	5 3 3	4 5 4	5 0 4	5 0 3	4 5 3	4 6 4	4 3 1
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, mammary gland	X																								
Alveolar/bronchiolar adenoma												X													
Alveolar/bronchiolar adenoma, multiple																X									
Squamous cell carcinoma, metastatic, nose																		X							
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Squamous cell carcinoma																		X							
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear																									
Fibroma																									
Eye							+			+		+	+			+		+			+				
Harderian gland										+		+				+					+				
Adenoma																					X				
Urinary System																									
Kidney	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	A	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear			X	X							X			X	X	X	X		X					X	

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 0 ppm
(continued)

Number of Days on Study	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	4 3 2	4 4 3	4 5 1	4 6 2	4 7 1	4 8 1	4 9 1	5 0 1	5 1 1	5 1 2	5 2 2	5 2 3	5 3 1	5 3 2	5 4 1	5 5 1	5 5 4	5 6 2	5 6 3	5 6 3	4 3 3	4 4 3	4 8 3	5 0 2	5 2 5	5 4 3	Total Tissues/ Tumors
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, mammary gland																											1
Alveolar/bronchiolar adenoma																											1
Alveolar/bronchiolar adenoma, multiple																											1
Squamous cell carcinoma, metastatic, nose																											1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Squamous cell carcinoma																											1
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																											
Ear													+										+				2
Fibroma													X														1
Eye						+								+		+							+	+			12
Harderian gland						+								+		+							+	+			9
Adenoma																											1
Urinary System																											
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																											
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear				X						X								X	X					X			14

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm

Number of Days on Study	4 7 8	5 4 4	5 4 5	6 1 1	6 1 2	6 1 9	6 2 8	6 6 4	6 6 6	6 6 8	6 7 2	6 8 5	6 8 6	7 1 0	7 1 0	7 1 0	7 1 0	7 1 3	7 1 3	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	6 6 5	5 7 5	6 5 5	5 9 5	6 9 5	6 7 5	6 0 4	6 4 5	6 9 4	5 8 4	6 3 5	6 5 4	6 0 3	6 1 4	6 1 5	6 2 5	6 8 4	5 7 4	6 9 2	6 7 4	5 7 1	5 7 2	5 7 3	5 8 2	5 8 3
Alimentary System																									
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	M	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma, metastatic, ovary									X																
Hepatocellular adenoma																							X		
Mesentery	+								+																
Carcinoma, metastatic, ovary									X																
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Acinus, adenoma																									
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	M	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	I	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																							X		
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	M	M	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+
Adenoma												X													
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma	X						X				X	X		X					X	X	X		X	X	
Pars distalis, adenoma, multiple		X				X		X	X																
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
C-cell, adenoma				X	X	X																			
Follicular cell, adenoma																									
General Body System																									
None																									

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm
(continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 9 2	6 0 2	6 1 3	6 2 4	6 7 1	6 7 3	6 8 1	6 8 2	6 9 1	7 0 1	7 0 2	7 0 4	6 1 1	6 1 2	6 2 1	6 3 1	6 3 4	6 4 1	6 4 4	6 5 1	6 5 2	6 5 3	6 6 1	6 6 2	6 6 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	46
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, duodenum	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	46
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma, metastatic, ovary																										1
Hepatocellular adenoma																										1
Mesentery								+			+															4
Carcinoma, metastatic, ovary																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Acinus, adenoma										X						X										2
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Pheochromocytoma benign																					X			X		3
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Parathyroid gland	+	+	+	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	M	M	+	39
Adenoma																										1
Pituitary gland	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Pars distalis, adenoma	X	X			X	X	X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	31
Pars distalis, adenoma, multiple																										4
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
C-cell, adenoma	X										X		X										X			7
Follicular cell, adenoma																				X						1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm
(continued)

Number of Days on Study	4 7 8	5 4 4	5 4 5	6 1 1	6 1 2	6 1 9	6 2 8	6 6 4	6 6 6	6 6 8	6 7 2	6 8 5	6 8 6	7 1 0	7 1 0	7 1 0	7 1 0	7 1 3	7 1 3	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	6 6 5	5 7 5	6 5 5	5 9 5	6 9 5	6 7 5	6 0 4	6 4 5	6 9 4	5 8 4	6 3 5	6 5 4	6 0 3	6 1 4	6 1 5	6 2 5	6 8 4	6 7 4	5 9 2	6 7 4	5 7 1	5 7 2	5 7 3	5 8 2	5 8 3
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																X	X								
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma									X																
Granulosa cell tumor benign																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal													X		X									X	
Polyp stromal, multiple																									
Sarcoma stromal																									
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mediastinal, carcinoma, metastatic, ovary									X																
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	M	M	+	+	+	+
Fibroadenoma					X	X			X		X		X				X	X				X			
Fibroadenoma, multiple																			X						
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Carcinoma adenosquamous			X																						
Keratoacanthoma																				X					
Subcutaneous tissue, fibroma		X								X		X													
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle																									
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 9 2	6 0 2	6 1 3	6 2 4	6 7 1	6 7 3	6 8 1	6 8 2	6 9 1	7 0 1	7 0 2	7 0 4	6 1 1	6 1 2	6 2 1	6 3 1	6 3 4	6 4 1	6 4 4	6 5 1	6 5 2	6 5 3	6 6 1	6 6 2	6 6 3	Total Tissues/ Tumors
Genital System																										
Clitoral gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	47
Adenoma	X			X																					X	5
Ovary	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Carcinoma																										1
Granulosa cell tumor benign	X																									1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal										X	X		X												X	7
Polyp stromal, multiple						X																				1
Sarcoma stromal							X																			1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mediastinal, carcinoma, metastatic, ovary																										1
Lymph node, mandibular	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	+	+	45
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	M	M	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	42
Integumentary System																										
Mammary gland	+	M	M	+	+	+	+	+	+	+	M	M	+	+	M	+	M	+	+	M	M	+	+	+	M	37
Fibroadenoma					X			X	X	X								X	X			X	X			16
Fibroadenoma, multiple							X									X										3
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Carcinoma adenosquamous																										1
Keratoacanthoma																										1
Subcutaneous tissue, fibroma																										3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																				+						1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm
(continued)

Number of Days on Study	4 7 8	5 4 4	5 4 5	6 1 1	6 1 2	6 1 9	6 2 8	6 6 4	6 6 6	6 6 8	6 7 2	6 8 5	6 8 6	7 1 0	7 1 0	7 1 0	7 1 0	7 1 3	7 1 3	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4
Carcass ID Number	6 6 5	5 7 5	6 5 5	5 9 5	6 9 5	6 7 5	6 0 4	6 4 5	6 9 4	5 8 4	6 3 5	6 5 4	6 0 3	6 1 4	6 1 5	6 2 5	6 8 4	6 7 4	5 9 2	5 7 4	5 7 1	5 7 2	5 7 3	5 8 2	5 8 3
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma			X																						
Carcinoma adenosquamous, metastatic, skin			X																						
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Special Senses System																									
Ear	+					+														+					
Eye				I	+								+					+		+			+	+	
Harderian gland													+					+					+	+	
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear		X		X	X		X			X	X		X	X	X	X			X					X	X

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm
(continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	
Carcass ID Number	5 9 2	6 0 2	6 1 3	6 2 4	6 7 1	6 7 3	6 8 1	6 8 2	6 9 1	7 0 1	7 0 2	7 0 4	6 1 1	6 1 2	6 2 1	6 3 1	6 3 4	6 4 1	6 4 4	6 5 1	6 5 2	6 5 3	6 6 1	6 6 2	6 6 3	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma																										1
Carcinoma adenosquamous, metastatic, skin																										1
Nose	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										3
Eye																				+					+	8
Harderian gland																										4
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear																							X	X		15

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm

Number of Days on Study	4 9 7	5 3 1	5 4 5	5 5 7	6 3 2	6 3 2	6 4 3	6 5 0	6 5 1	6 7 2	6 8 6	6 9 2	7 1 0	7 1 5	7 1 6	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	8 2 5	7 2 4	7 1 4	7 5 5	7 6 5	8 2 4	7 4 5	7 2 3	8 4 4	7 1 3	7 7 5	7 8 5	7 9 5	8 0 5	7 1 2	7 1 7	7 2 2	7 9 2	7 9 4	8 0 2	8 0 4	8 1 2	8 1 3	8 1 4	8 1 5	8 2 1
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mesentery			+		+																	+				+
Pancreas	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous														X												
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma					X																					
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Pheochromocytoma benign																										
Islets, pancreatic	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma											X															
Parathyroid gland	+	+	M	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pars distalis, adenoma				X		X	X	X	X		X	X	X	X	X				X			X			X	X
Pars distalis, adenoma, multiple																										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Bilateral, C-cell, adenoma																										
C-cell, adenoma		X								X	X										X					
Follicular cell, adenoma																										
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	8 2 3	8 3 1	8 3 5	7 9 1	8 0 1	7 1 1	7 4 1	7 4 2	7 4 4	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4	7 7 1	7 8 1	7 8 2	7 8 3	7 8 4	7 2 1	7 2 2	7 3 1	7 3 2	7 3 3	7 3 5	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Mesentery																+					+					6
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma																										1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pheochromocytoma benign																		X	X							2
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adenoma																										1
Parathyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	45
Pituitary gland	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Pars distalis, adenoma	X	X		X	X		X		X		X		X		X	X	X	X		X			X	X		29
Pars distalis, adenoma, multiple																						X				1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Bilateral, C-cell, adenoma							X																			1
C-cell, adenoma																	X	X					X			7
Follicular cell, adenoma	X																									1
General Body System																										
None																										

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm
(continued)

Number of Days on Study	4 9 7	5 3 1	5 4 5	5 5 7	6 3 2	6 3 2	6 4 3	6 5 0	6 5 1	6 7 2	6 8 6	6 9 2	7 1 0	7 1 5	7 1 6	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	8 2 5	7 2 4	7 1 4	7 5 5	7 6 5	8 2 4	7 4 5	7 2 3	8 4 4	7 1 3	7 7 5	7 8 5	7 9 5	8 0 5	7 1 2	7 7 2	7 9 2	7 9 4	8 0 2	8 0 4	8 1 2	8 1 3	8 1 4	8 1 5	8 2 1
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+
Adenoma																									
Carcinoma																								X	
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal					X	X	X												X		X				
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	+	+	M	+	M	+	+	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+
Integumentary System																									
Mammary gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+
Adenoma																									
Fibroadenoma								X		X								X	X						X
Fibroadenoma, multiple																				X					
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+
Subcutaneous tissue, fibroma					X																				
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+																						
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spinal cord		+											+												
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma														X											
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm
(continued)

Number of Days on Study	7 2 9	7 2 9	7 2 9	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	8 2 3	8 3 1	8 3 5	7 9 1	8 0 1	7 1 1	7 4 1	7 4 2	7 4 4	7 5 3	7 6 1	7 6 2	7 6 3	7 6 4	7 7 1	7 8 1	7 8 2	7 8 3	7 8 4	7 2 1	7 2 2	7 3 1	7 3 2	7 3 3	7 3 5	Total Tissues/ Tumors
Genital System																									
Clitoral gland	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M	+	+	+	M	+	M	+	44
Adenoma					X											X										2
Carcinoma																									X	2
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal		X		X		X			X								X			X	X					12
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	48
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	M	M	+	+	+	43
Integumentary System																									
Mammary gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Adenoma			X						X																	2
Fibroadenoma		X			X	X					X			X	X				X		X	X		X	X	16
Fibroadenoma, multiple								X																		2
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Subcutaneous tissue, fibroma																										1
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										1
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Spinal cord																										2
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Alveolar/bronchiolar adenoma		X																								2
Nose	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm
(continued)

Number of Days on Study	4 9 7	5 3 1	5 4 5	5 5 7	6 3 2	6 3 2	6 4 3	6 5 0	6 5 1	6 7 2	6 8 6	6 9 2	7 1 0	7 1 5	7 1 6	7 2 2	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9	7 2 9
Carcass ID Number	8 2 5	7 2 4	7 1 4	7 5 5	7 6 5	8 2 4	7 4 5	7 2 3	8 4 4	7 1 3	7 7 5	7 8 5	7 9 5	8 0 5	7 1 2	7 7 2	7 9 2	7 9 4	8 0 2	8 0 4	8 1 2	8 1 3	8 1 4	8 1 5	8 2 1
Special Senses System																									
Ear				+								+													
Papilloma squamous																									
Eye			I			+		+	+	+			+	+			+			+			+	+	+
Harderian gland								+	+	+			+	+			+			+			+	+	
Adenoma																									
Zymbal's gland												+													
Carcinoma												X													
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Leukemia mononuclear	X	X	X				X				X					X							X		

TABLE B2
Individual Animal Tumor Pathology of Female Rats in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm
(continued)

Number of Days on Study	729	729	729	730	700	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	734	734	734	734	734	
Carcass ID Number	82313	83315	83595	79011	80111	71111	74111	74412	74424	74435	76131	76232	76633	76644	76711	78112	78823	78834	78842	82121	82212	83321	83332	83345	83355	Total Tissues/ Tumors
Special Senses System																										
Ear								+																		3
Papilloma squamous								X																		1
Eye			+			+								+						+				+	+	17
Harderian gland			+			+								+						+				+	+	15
Adenoma			X																							1
Zymbal's gland																										1
Carcinoma																										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Leukemia mononuclear	X	X								X																10

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4

	0 ppm	5,000 ppm	10,000 ppm
Adrenal Medulla: Benign Pheochromocytoma			
Overall rates[a]	1/49 (2%)	3/46 (7%)	2/49 (4%)
Adjusted rates[b]	3.7%	10.0%	5.9%
Terminal rates[c]	1/27 (4%)	3/30 (10%)	2/34 (6%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests[d]	P=0.492	P=0.342	P=0.581
Logistic regression tests[d]	P=0.492	P=0.342	P=0.581
Cochran-Armitage test[d]	P=0.400		
Fisher exact test[d]		P=0.285	P=0.500
Adrenal Medulla: Benign or Malignant Pheochromocytoma			
Overall rates	2/49 (4%)	3/46 (7%)	2/49 (4%)
Adjusted rates	7.4%	10.0%	5.9%
Terminal rates	2/27 (7%)	3/30 (10%)	2/34 (6%)
First incidence (days)	729 (T)	729 (T)	729 (T)
Life table tests	P=0.493N	P=0.549	P=0.610N
Logistic regression tests	P=0.493N	P=0.549	P=0.610N
Cochran-Armitage test	P=0.593		
Fisher exact test		P=0.470	P=0.691N
Clitoral Gland: Adenoma			
Overall rates	7/47 (15%)	5/47 (11%)	2/44 (5%)
Adjusted rates	20.6%	15.2%	6.7%
Terminal rates	3/25 (12%)	3/29 (10%)	2/30 (7%)
First incidence (days)	625	710	729 (T)
Life table tests	P=0.039N	P=0.303N	P=0.059N
Logistic regression tests	P=0.063N	P=0.357N	P=0.091N
Cochran-Armitage test	P=0.073N		
Fisher exact test		P=0.379N	P=0.095N
Clitoral Gland: Carcinoma			
Overall rates	0/47 (0%)	0/47 (0%)	2/44 (5%)
Adjusted rates	0.0%	0.0%	6.7%
Terminal rates	0/25 (0%)	0/29 (0%)	2/30 (7%)
First incidence (days)	-[e]	-	729 (T)
Life table tests	P=0.112	-	P=0.279
Logistic regression tests	P=0.112	-	P=0.279
Cochran-Armitage test	P=0.088		
Fisher exact test		-	P=0.231
Clitoral Gland: Adenoma or Carcinoma			
Overall rates	7/47 (15%)	5/47 (11%)	4/44 (9%)
Adjusted rates	20.6%	15.2%	13.3%
Terminal rates	3/25 (12%)	3/29 (10%)	4/30 (13%)
First incidence (days)	625	710	729 (T)
Life table tests	P=0.144N	P=0.303N	P=0.193N
Logistic regression tests	P=0.212N	P=0.357N	P=0.277N
Cochran-Armitage test	P=0.240N		
Fisher exact test		P=0.379N	P=0.301N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Mammary Gland: Adenoma			
Overall rates	3/50 (6%)	0/50 (0%)	2/50 (4%)
Adjusted rates	10.1%	0.0%	5.9%
Terminal rates	2/27 (7%)	0/31 (0%)	2/34 (6%)
First incidence (days)	700	-	729 (T)
Life table tests	P=0.319N	P=0.102N	P=0.407N
Logistic regression tests	P=0.345N	P=0.107N	P=0.442N
Cochran-Armitage test	P=0.390N		
Fisher exact test		P=0.121N	P=0.500N
Mammary Gland: Fibroadenoma			
Overall rates	28/50 (56%)	19/50 (38%)	18/50 (36%)
Adjusted rates	74.5%	47.7%	49.6%
Terminal rates	18/27 (67%)	11/31 (35%)	16/34 (47%)
First incidence (days)	582	612	650
Life table tests	P=0.005N	P=0.030N	P=0.005N
Logistic regression tests	P=0.013N	P=0.034N	P=0.015N
Cochran-Armitage test	P=0.028N		
Fisher exact test		P=0.054N	P=0.035N
Mammary Gland: Adenoma or Fibroadenoma			
Overall rates	29/50 (58%)	19/50 (38%)	20/50 (40%)
Adjusted rates	75.3%	47.7%	55.2%
Terminal rates	18/27 (67%)	11/31 (35%)	18/34 (53%)
First incidence (days)	582	612	650
Life table tests	P=0.009N	P=0.021N	P=0.008N
Logistic regression tests	P=0.022N	P=0.021N	P=0.023N
Cochran-Armitage test	P=0.044N		
Fisher exact test		P=0.036N	P=0.055N
Mammary Gland: Adenoma, Fibroadenoma, or Adenocarcinoma			
Overall rates	30/50 (60%)	19/50 (38%)	20/50 (40%)
Adjusted rates	75.8%	47.7%	55.2%
Terminal rates	18/27 (67%)	11/31 (35%)	18/34 (53%)
First incidence (days)	409	612	650
Life table tests	P=0.006N	P=0.015N	P=0.005N
Logistic regression tests	P=0.016N	P=0.016N	P=0.019N
Cochran-Armitage test	P=0.028N		
Fisher exact test		P=0.022N	P=0.036N
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	33/49 (67%)	35/48 (73%)	30/49 (61%)
Adjusted rates	77.8%	89.2%	69.2%
Terminal rates	18/27 (67%)	25/29 (86%)	20/33 (61%)
First incidence (days)	447	478	557
Life table tests	P=0.091N	P=0.526N	P=0.131N
Logistic regression tests	P=0.248N	P=0.390	P=0.295N
Cochran-Armitage test	P=0.295N		
Fisher exact test		P=0.353	P=0.337N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	5,000 ppm	10,000 ppm
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma			
Overall rates	34/49 (69%)	35/48 (73%)	30/49 (61%)
Adjusted rates	78.5%	89.2%	69.2%
Terminal rates	18/27 (67%)	25/29 (86%)	20/33 (61%)
First incidence (days)	447	478	557
Life table tests	P=0.067N	P=0.453N	P=0.100N
Logistic regression tests	P=0.182N	P=0.479	P=0.222N
Cochran-Armitage test	P=0.225N		
Fisher exact test		P=0.437	P=0.262N
Skin (Subcutaneous Tissue): Fibroma			
Overall rates	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rates	3.2%	6.9%	2.2%
Terminal rates	0/27 (0%)	0/31 (0%)	0/34 (0%)
First incidence (days)	710	544	632
Life table tests	P=0.568N	P=0.349	P=0.727N
Logistic regression tests	P=0.533	P=0.250	P=0.749
Cochran-Armitage test	P=0.610		
Fisher exact test		P=0.309	P=0.753N
Thyroid Gland (C-cell): Adenoma			
Overall rates	9/50 (18%)	7/50 (14%)	8/50 (16%)
Adjusted rates	27.0%	18.5%	20.5%
Terminal rates	5/27 (19%)	4/31 (13%)	5/34 (15%)
First incidence (days)	645	611	531
Life table tests	P=0.317N	P=0.310N	P=0.356N
Logistic regression tests	P=0.454N	P=0.390N	P=0.485N
Cochran-Armitage test	P=0.446N		
Fisher exact test		P=0.393N	P=0.500N
Thyroid Gland (C-cell): Adenoma or Carcinoma			
Overall rates	10/50 (20%)	7/50 (14%)	8/50 (16%)
Adjusted rates	28.6%	18.5%	20.5%
Terminal rates	5/27 (19%)	4/31 (13%)	5/34 (15%)
First incidence (days)	600	611	531
Life table tests	P=0.234N	P=0.230N	P=0.271N
Logistic regression tests	P=0.363N	P=0.308N	P=0.399N
Cochran-Armitage test	P=0.344N		
Fisher exact test		P=0.298N	P=0.398N
Uterus: Stromal Polyp			
Overall rates	4/50 (8%)	8/50 (16%)	12/50 (24%)
Adjusted rates	11.4%	23.6%	31.3%
Terminal rates	0/27 (0%)	6/31 (19%)	9/34 (26%)
First incidence (days)	625	686	632
Life table tests	P=0.056	P=0.246	P=0.071
Logistic regression tests	P=0.025	P=0.201	P=0.031
Cochran-Armitage test	P=0.020		
Fisher exact test		P=0.178	P=0.027

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Uterus: Stromal Polyp or Stromal Sarcoma			
Overall rates	4/50 (8%)	9/50 (18%)	12/50 (24%)
Adjusted rates	11.4%	26.7%	31.3%
Terminal rates	0/27 (0%)	7/31 (23%)	9/34 (26%)
First incidence (days)	625	686	632
Life table tests	P=0.061	P=0.176	P=0.071
Logistic regression tests	P=0.028	P=0.137	P=0.031
Cochran-Armitage test	P=0.022		
Fisher exact test		P=0.117	P=0.027
All Organs: Mononuclear Cell Leukemia			
Overall rates	14/50 (28%)	15/50 (30%)	10/50 (20%)
Adjusted rates	37.1%	34.6%	23.2%
Terminal rates	6/27 (22%)	5/31 (16%)	4/34 (12%)
First incidence (days)	447	544	497
Life table tests	P=0.135N	P=0.534N	P=0.159N
Logistic regression tests	P=0.292N	P=0.443	P=0.321N
Cochran-Armitage test	P=0.212N		
Fisher exact test		P=0.500	P=0.241N
All Organs: Benign Tumors			
Overall rates	43/50 (86%)	48/50 (96%)	43/50 (86%)
Adjusted rates	95.4%	96.0%	91.4%
Terminal rates	25/27 (93%)	29/31 (94%)	30/34 (88%)
First incidence (days)	447	478	531
Life table tests	P=0.106N	P=0.540N	P=0.131N
Logistic regression tests	P=0.441N	P=0.122	P=0.470N
Cochran-Armitage test	P=0.564N		
Fisher exact test		P=0.080	P=0.613N
All Organs: Malignant Tumors			
Overall rates	19/50 (38%)	18/50 (36%)	13/50 (26%)
Adjusted rates	44.6%	39.9%	30.2%
Terminal rates	6/27 (22%)	6/31 (19%)	6/34 (18%)
First incidence (days)	409	544	497
Life table tests	P=0.078N	P=0.373N	P=0.093N
Logistic regression tests	P=0.205N	P=0.533	P=0.233N
Cochran-Armitage test	P=0.122N		
Fisher exact test		P=0.500N	P=0.142N
All Organs: Benign or Malignant Tumors			
Overall rates	49/50 (98%)	49/50 (98%)	48/50 (96%)
Adjusted rates	98.0%	98.0%	96.0%
Terminal rates	26/27 (96%)	30/31 (97%)	32/34 (94%)
First incidence (days)	409	478	497
Life table tests	P=0.074N	P=0.238N	P=0.095N
Logistic regression tests	P=0.451N	P=0.731	P=0.581N
Cochran-Armitage test	P=0.378N		
Fisher exact test		P=0.753N	P=0.500N

TABLE B3
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Feed Study of HC Yellow 4
(continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors in animal group

TABLE B4a
Historical Incidence of Uterine Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Stromal Polyps	Stromal Sarcoma	Stromal Polyp or Stromal Sarcoma
Historical Incidence at EG&G Mason Research Institute			
4-Hydroxyacetanilide	15/50	0/50	15/50
HC Yellow 4	4/50	0/50	4/50
Pentaerythritol tetranitrate	8/50	0/50	8/50
Quercetin	7/50	0/50	7/50
Total	34/200 (17.0%)		34/200 (17.0%)
Standard deviation	9.3%		9.3%
Range	8%–30%		8%–30%
Overall Historical Incidence			
Total	142/800 (17.8%)	8/800 (1.0%)	149/800 (18.6%)
Standard deviation	5.1%	1.8%	5.4%
Range	8%–30%	0%–6%	8%–30%

[a] Data as of 3 April 1991

TABLE B4b
Historical Incidence of Mammary Gland Neoplasms in Untreated Female F344/N Rats[a]

Study	Incidence in Controls		
	Fibroma	Fibroadenoma	Fibroma, Fibroadenoma, or Adenoma
Historical Incidence at EG&G Mason Research Institute			
4-Hydroxyacetanilide	0/50	19/50	19/50
HC Yellow 4	0/50	28/50	29/50
Pentaerythritol tetranitrate	0/50	27/50	27/50
Quercetin	0/50	29/50	29/50
Total		103/200 (51.5%)	104/200 (52.0%)
Standard deviation		9.2%	9.5%
Range		38%–58%	38%–58%
Overall Historical Incidence			
Total	0/800	314/800 (39.3%)	322/800 (40.3%)
Standard deviation		15.1%	15.2%
Range		8%–58%	8%–58%

[a] Data as of 3 April 1991

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	2	4	1
Moribund kills	21	15	15
Survivors			
Terminal sacrifice	26	30	34
Moribund	1	1	
Animals examined microscopically	50	50	50
Alimentary System			
Intestine large, cecum	(48)	(49)	(49)
Parasite	1 (2%)		
Intestine large, colon	(48)	(47)	(47)
Parasite	1 (2%)	1 (2%)	
Intestine large, rectum	(48)	(46)	(49)
Parasite	2 (4%)	1 (2%)	
Liver	(50)	(50)	(50)
Basophilic focus	37 (74%)	32 (64%)	41 (82%)
Clear cell focus	1 (2%)	3 (6%)	2 (4%)
Eosinophilic focus		1 (2%)	2 (4%)
Fatty change, diffuse	7 (14%)	7 (14%)	4 (8%)
Fatty change, focal	7 (14%)	2 (4%)	3 (6%)
Hepatodiaphragmatic nodule	4 (8%)	3 (6%)	9 (18%)
Hyperplasia	1 (2%)		
Inflammation, granulomatous	30 (60%)	22 (44%)	27 (54%)
Mixed cell focus	6 (12%)	5 (10%)	7 (14%)
Necrosis		3 (6%)	
Thrombus		1 (2%)	1 (2%)
Bile duct, hyperplasia	26 (52%)	24 (48%)	32 (64%)
Pancreas	(49)	(50)	(49)
Acinus, atrophy	1 (2%)		
Duct, hyperplasia		1 (2%)	
Stomach, forestomach	(50)	(48)	(50)
Acanthosis	1 (2%)	7 (15%)	4 (8%)
Hyperkeratosis	2 (4%)	4 (8%)	2 (4%)
Mineralization	1 (2%)		
Necrosis	1 (2%)		
Ulcer		2 (4%)	1 (2%)
Stomach, glandular	(50)	(50)	(50)
Hyperplasia		1 (2%)	
Cardiovascular System			
Heart	(50)	(50)	(50)
Cardiomyopathy	23 (46%)	28 (56%)	23 (46%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Endocrine System			
Adrenal gland, cortex	(50)	(49)	(50)
Hyperplasia	1 (2%)	2 (4%)	2 (4%)
Adrenal gland, medulla	(49)	(46)	(49)
Hyperplasia	6 (12%)	3 (7%)	4 (8%)
Pituitary gland	(49)	(48)	(49)
Hyperplasia			1 (2%)
Pars distalis, angiectasis	29 (59%)	28 (58%)	22 (45%)
Pars distalis, cyst	5 (10%)	5 (10%)	9 (18%)
Pars distalis, hyperplasia	11 (22%)	14 (29%)	11 (22%)
Pars intermedia, angiectasis	1 (2%)		
Pars intermedia, cyst			1 (2%)
Thyroid gland	(50)	(50)	(50)
Hyperplasia		1 (2%)	
C-cell, hyperplasia	10 (20%)	14 (28%)	11 (22%)
General Body System			
None			
Genital System			
Clitoral gland	(47)	(47)	(44)
Necrosis	1 (2%)	4 (9%)	
Ovary	(50)	(49)	(50)
Cyst	2 (4%)	5 (10%)	3 (6%)
Uterus	(48)	(50)	(50)
Endometrium, hyperplasia		1 (2%)	1 (2%)
Hematopoietic System			
Lymph node	(50)	(50)	(50)
Mediastinal, infiltration cellular, histiocyte	1 (2%)		2 (4%)
Lymph node, mesenteric	(49)	(50)	(49)
Degeneration, cystic		1 (2%)	1 (2%)
Infiltration cellular, histiocyte	2 (4%)	1 (2%)	1 (2%)
Spleen	(50)	(49)	(50)
Fibrosis	1 (2%)	2 (4%)	
Hematopoietic cell proliferation	30 (60%)	23 (47%)	31 (62%)
Infiltration cellular, histiocyte	3 (6%)	1 (2%)	1 (2%)
Pigmentation	28 (56%)	15 (31%)	31 (62%)
Thrombus		1 (2%)	
Thymus	(47)	(42)	(43)
Epithelial cell, hyperplasia	1 (2%)		
Integumentary System			
Mammary gland	(48)	(37)	(47)
Galactocele	14 (29%)	7 (19%)	9 (19%)
Acinus, hyperplasia	3 (6%)	6 (16%)	5 (11%)

TABLE B5
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Musculoskeletal System			
Bone	(50)	(50)	(50)
Hyperostosis			1 (2%)
Nervous System			
Brain	(50)	(50)	(50)
Hemorrhage	2 (4%)	1 (2%)	2 (4%)
Spinal cord	(1)		(2)
Hemorrhage			1 (50%)
Respiratory System			
Lung	(50)	(50)	(50)
Infiltration cellular, histiocyte	9 (18%)	16 (32%)	8 (16%)
Alveolar epithelium, hyperplasia			3 (6%)
Nose	(50)	(49)	(48)
Fungus		1 (2%)	
Inflammation, acute	2 (4%)	2 (4%)	4 (8%)
Special Senses System			
Eye	(12)	(8)	(17)
Hemorrhage	1 (8%)		1 (6%)
Inflammation, acute	2 (17%)	2 (25%)	
Lens, cataract	5 (42%)	7 (88%)	8 (47%)
Retina, atrophy	1 (8%)	1 (13%)	
Urinary System			
Kidney	(49)	(50)	(50)
Cyst	1 (2%)		
Nephropathy	37 (76%)	44 (88%)	41 (82%)
Urinary bladder	(48)	(48)	(50)
Inflammation, chronic active			1 (2%)
Transitional epithelium, hyperplasia			1 (2%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR FEED STUDY OF HC YELLOW 4

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	14	10	5
Moribund kills	8	10	9
Accidental death			1
Survivors			
Terminal sacrifice	28	29	35
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Gallbladder	(39)	(9)	(46)
Intestine large, colon	(47)	(14)	(47)
Intestine large, rectum	(40)	(14)	(49)
Adenocarcinoma			1 (2%)
Intestine small, ileum	(41)	(14)	(45)
Intestine small, jejunum	(42)	(14)	(45)
Liver	(49)	(22)	(48)
Hemangioma	1 (2%)		
Hemangiosarcoma	1 (2%)		
Hemangiosarcoma, multiple	1 (2%)		
Hepatocellular carcinoma	4 (8%)	5 (23%)	8 (17%)
Hepatocellular carcinoma, multiple	1 (2%)	2 (9%)	1 (2%)
Hepatocellular adenoma	8 (16%)	7 (32%)	4 (8%)
Lipoma	1 (2%)		
Mesentery	(1)		(1)
Sarcoma			1 (100%)
Pancreas	(45)	(16)	(49)
Salivary glands	(50)	(17)	(49)
Stomach, forestomach	(46)	(19)	(48)
Papilloma squamous	3 (7%)	2 (11%)	1 (2%)
Stomach, glandular	(46)	(16)	(47)
Cardiovascular System			
Heart	(50)	(17)	(49)
Sarcoma, metastatic, skeletal muscle		1 (6%)	
Endocrine System			
Adrenal gland, cortex	(50)	(17)	(48)
Adenoma	1 (2%)		
Adenoma, multiple	1 (2%)		
Thyroid gland	(47)	(48)	(49)
Follicular cell, adenoma	1 (2%)		2 (4%)
General Body System			
None			

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	5,000 ppm	10,000 ppm
Genital System			
Epididymis	(50)	(47)	(49)
Preputial gland	(9)	(11)	(6)
Fibrosarcoma	1 (11%)		
Prostate	(47)	(15)	(45)
Testes	(50)	(17)	(49)
Sertoli cell tumor benign	1 (2%)		
Hematopoietic System			
Bone marrow	(49)	(16)	(47)
Lymph node	(46)	(28)	(47)
Mediastinal, pancreatic, sarcoma, metastatic, skeletal muscle		1 (4%)	
Lymph node, mandibular	(42)	(9)	(40)
Lymph node, mesenteric	(38)	(24)	(45)
Spleen	(49)	(21)	(48)
Hemangiosarcoma	1 (2%)		
Thymus	(28)	(9)	(29)
Integumentary System			
Skin	(49)	(32)	(48)
Squamous cell carcinoma			1 (2%)
Subcutaneous tissue, fibroma		1 (3%)	
Subcutaneous tissue, fibrosarcoma	5 (10%)	2 (6%)	3 (6%)
Subcutaneous tissue, fibrosarcoma, multiple	1 (2%)		
Musculoskeletal System			
Skeletal muscle		(1)	
Sarcoma		1 (100%)	
Nervous System			
Brain	(50)	(18)	(49)
Meningioma benign		1 (6%)	
Respiratory System			
Lung	(50)	(29)	(49)
Alveolar/bronchiolar adenoma	7 (14%)	8 (28%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple			1 (2%)
Alveolar/bronchiolar carcinoma	2 (4%)	1 (3%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver		1 (3%)	2 (4%)
Sarcoma, metastatic, skeletal muscle		1 (3%)	
Special Senses System			
Harderian gland	(2)		(3)
Adenoma	1 (50%)		3 (100%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Urinary System			
Kidney	(49)	(22)	(49)
Adenocarcinoma			1 (2%)
Sarcoma, metastatic, skeletal muscle		1 (5%)	
Urinary bladder	(44)	(17)	(50)
Systemic Lesions			
Multiple organs[b]	(50)	(49)	(50)
Lymphoma malignant mixed	1 (2%)		
Lymphoma malignant undifferentiated cell	1 (2%)	1 (2%)	1 (2%)
Tumor Summary			
Total animals with primary neoplasms[c]	28	23	23
Total primary neoplasms	44	31	34
Total animals with benign neoplasms	19	17	13
Total benign neoplasms	25	19	16
Total animals with malignant neoplasms	16	12	16
Total malignant neoplasms	19	12	18
Total animals with secondary neoplasms[d]		2	2
Total secondary neoplasms		5	2

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm

Number of Days on Study	0 5 1	0 6 2	0 9 3	1 0 4	1 4 6	2 2 4	2 4 8	3 2 2	3 7 1	3 9 8	4 0 9	4 2 0	4 4 0	4 0 9	5 1 8	5 1 9	6 1 6	6 9 2	6 9 4	7 2 1	7 2 3	7 2 3	7 3 3	7 3 4	7 4 4	7 3 4
Carcass ID Number	1 0 1 5	1 3 5	0 1 5	1 1 5	1 2 5	1 2 3	1 3 4	0 3 5	0 5 4	0 2 4	0 3 4	0 3 3	0 6 5	0 5 1	0 4 1	0 3 3	1 0 4	0 8 4	1 2 1	0 7 2	0 6 4	0 8 1	0 9 5	1 1 1	1 1 2	0 1 3
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	A	M	+	+	+	A	M	A	M	+	M	+	+	M	+	+	+	+	+	+	+	+	+
Intestine large	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	M	M	+	+	+	+	A	+	M	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	M	+	+	+	M	+	A	+	+	M	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine small	+	+	M	A	+	+	A	+	A	A	+	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	+	+	M	A	+	+	A	+	A	A	+	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+
Intestine small, ileum	+	+	M	A	+	+	A	+	A	A	M	A	+	+	+	A	A	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	+	+	M	A	+	+	A	+	A	A	+	+	+	+	A	A	A	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Hemangioma																										
Hemangiosarcoma															X											
Hemangiosarcoma, multiple																			X							
Hepatocellular carcinoma																		X								
Hepatocellular carcinoma, multiple																				X						
Hepatocellular adenoma																										
Lipoma																										
Mesentery									A		M		+													
Pancreas	+	+	A	A	+	+	M	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	A	+	+	+	A	+	M	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Papilloma squamous																										
Stomach, glandular	+	+	A	+	+	+	A	+	A	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+
Tooth																	+									
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant undifferentiated cell type, minimal													X													
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenoma																										
Adenoma, multiple																										
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	+	+	A	+	+	+	M	+	+	M	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+
Parathyroid gland	M	M	+	+	M	+	M	M	+	+	M	+	+	+	M	+	M	I	M	+	+	M	+	+	+	M
Pituitary gland	M	+	I	+	+	+	+	M	+	+	M	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+
Thyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Follicular cell, adenoma														X												

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 1 4	0 1	0 2 2	0 2 3	0 3 1	0 4 3	0 4 4	0 6 1	0 6 2	0 6 3	0 8 3	0 9 1	0 9 2	0 9 3	0 9 4	1 0 2	1 0 3	1 1 1	1 1 2	1 1 3	1 1 4	1 3 2	1 4 1	1 4 2	1 4 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Gallbladder	+	+	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	39
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	40
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	43
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	41
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hemangioma																				X						1
Hemangiosarcoma																										1
Hemangiosarcoma, multiple																										1
Hepatocellular carcinoma	X			X				X																		4
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma			X								X						X				X	X	X	X	X	8
Lipoma																	X									1
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Papilloma squamous								X							X		X									3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Tooth										+	+					+		+	+				+	+	+	9
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant undifferentiated cell type, minimal																										1
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenoma														X												1
Adenoma, multiple																						X				1
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Parathyroid gland	+	I	M	+	M	+	+	+	+	M	+	+	+	+	+	M	+	M	M	M	M	M	+	+	M	27
Pituitary gland	M	+	+	M	+	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	M	38
Thyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	47
Follicular cell, adenoma																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	0	0	0	1	1	2	2	3	3	3	4	4	4	5	5	6	6	6	7	7	7	7	7	7	7
	5	6	9	0	4	2	4	2	7	9	0	2	4	0	1	1	9	9	2	2	2	2	3	3	3
	1	2	3	4	6	4	8	2	1	8	9	0	9	8	9	6	2	4	1	3	3	3	4	4	4
Carcass ID Number	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0
	0	3	1	1	2	2	3	3	5	2	3	3	6	5	4	3	8	2	7	6	8	9	1	1	1
	5	5	5	5	5	3	4	5	4	4	4	3	5	1	1	3	4	1	2	4	1	5	1	2	3
General Body System																									
None																									
Genital System																									
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis			M		+	+		+		+		+													
Preputial gland						+					+									+					
Fibrosarcoma																				X					
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sertoli cell tumor benign																									
Hematopoietic System																									
Bone marrow	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	M	+	+	+	+	+	+	M	A	M	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	M	M	M	+	+	+	M	+	+	M	+	+	+	M	M	M	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	A	+	+	M	M	M	+	A	+	+	+	+	M	A	M	+	+	+	+	+	+	+	+
Spleen	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangiosarcoma															X										
Thymus	+	+	A	A	+	M	M	M	+	M	A	+	+	+	M	M	I	M	+	+	M	M	+	M	+
Integumentary 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	M	M	M
Skin	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Subcutaneous tissue, fibrosarcoma														X							X	X			
Subcutaneous tissue, fibrosarcoma, multiple																				X					
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																									
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Alveolar/bronchiolar adenoma																			X	X			X		
Alveolar/bronchiolar carcinoma																									
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Trachea	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 1 4	0 2 1	0 2 2	0 2 3	0 3 1	0 4 3	0 4 4	0 6 1	0 6 2	0 6 3	0 8 3	0 9 1	0 9 2	0 9 3	0 9 4	1 0 2	1 0 3	1 1 1	1 1 2	1 1 3	1 1 4	1 3 2	1 4 1	1 4 2	1 4 3	Total Tissues/ Tumors	
General Body System																											
None																											
Genital System																											
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Penis																										5	
Preputial gland		+			+		+					+	+				+									9	
Fibrosarcoma																										1	
Prostate	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	47	
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Sertoli cell tumor benign			X																							1	
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46	
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42	
Lymph node, mesenteric	+	I	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	38	
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Hemangiosarcoma																										1	
Thymus	+	+	M	M	M	+	+	I	+	M	+	+	+	+	+	+	M	M	+	M	+	+	+	+	+	28	
Integumentary 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	M	M	1	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49	
Subcutaneous tissue, fibrosarcoma																			X				X			5	
Subcutaneous tissue, fibrosarcoma, multiple																										1	
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50	
Alveolar/bronchiolar adenoma												X	X	X						X						7	
Alveolar/bronchiolar carcinoma														X	X											2	
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	49	
Trachea	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	0	0	0	1	1	2	2	3	3	3	4	4	4	5	5	6	6	6	7	7	7	7	7	7	7	
	5	6	9	0	4	2	4	2	7	9	0	2	4	0	1	1	9	9	2	2	2	2	3	3	3	
	1	2	3	4	6	4	8	2	1	8	9	0	9	8	9	6	2	4	1	3	3	3	4	4	4	
Carcass ID Number	1	1	0	1	1	1	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	
	0	3	1	1	2	2	3	3	5	2	3	3	6	5	4	3	8	2	7	6	8	9	1	1	1	
	5	5	5	5	5	3	4	5	4	4	4	3	5	1	1	3	4	1	2	4	1	5	1	2	3	
Special Senses System																										
Harderian gland																										
Adenoma																										
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	
Ureter																+										
Urethra		+																								
Urinary bladder	+	+	A	A	+	+	A	+	A	+	A	+	+	+	+	+	A	+	+	+	+	+	+	+	+	
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
Lymphoma malignant mixed																										
Lymphoma malignant undifferentiated cell type													X													

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	7 3 4	
Carcass ID Number	0 1 4	0 2 1	0 2 2	0 2 3	0 3 1	0 4 3	0 4 4	0 6 1	0 6 2	0 6 3	0 8 3	0 9 1	0 9 2	0 9 3	0 9 4	1 0 2	1 0 3	1 1 1	1 1 2	1 1 3	1 1 4	1 3 2	1 4 1	1 4 2	1 4 3	Total Tissues/ Tumors
Special Senses System																										
Harderian gland									+							+										2
Adenoma																X										1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Ureter																										1
Urethra																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	44
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant mixed									X																	1
Lymphoma malignant undifferentiated cell type																										1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm

Number of Days on Study	0 7 3	0 9 4	1 0 0	1 5 2	1 6 8	2 7 1	2 9 1	3 2 8	3 7 7	3 8 4	3 9 9	4 0 9	5 1 2	5 6 7	5 9 2	6 0 7	6 2 6	6 5 8	6 7 5	6 8 7	7 3 1	7 3 4	7 3 4	7 3 4
Carcass ID Number	1 6 5	1 5 5	1 7 5	1 5 4	2 5 5	2 7 3	2 4 4	2 4 3	1 7 3	2 8 4	2 1 5	2 5 4	1 8 3	2 3 3	1 8 4	2 6 4	1 6 1	2 5 1	2 8 1	2 7 2	1 5 1	1 5 2	1 6 2	1 6 3
Alimentary System																								
Esophagus	+	+	+	+	A	+	+	+	+	+	+	+	+	+	M	+	+							
Gallbladder	M	M	+	A	A	A	I	+	+	+	+	M	M	+	+	+	+							
Intestine large	A	+	+	+	A	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, cecum	A	+	+	M	A	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, colon	A	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine large, rectum	A	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine small	+	+	+	A	A	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, duodenum	+	+	+	M	M	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, ileum	A	+	+	A	A	M	+	+	+	+	+	+	+	+	+	+	+							
Intestine small, jejunum	A	+	+	A	M	M	+	+	+	+	+	+	+	+	+	+	+							
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+			+		
Hepatocellular carcinoma															X	X			X			X		
Hepatocellular carcinoma, multiple													X	X										
Hepatocellular adenoma													X	X			X					X		
Mesentery											M													
Pancreas	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+							
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Papilloma squamous																								
Stomach, glandular	+	+	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+							
Tooth																+								
Cardiovascular System																								
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Sarcoma, metastatic, skeletal muscle																	X							
Endocrine System																								
Adrenal gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+							
Adrenal gland, cortex	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+							
Adrenal gland, medulla	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+							
Islets, pancreatic	+	+	+	+	M	M	+	+	+	+	+	+	+	M	+	+	+							
Parathyroid gland	M	M	+	+	+	M	+	M	M	+	+	+	M	M	M	M	+							
Pituitary gland	+	+	M	+	M	+	+	+	M	+	+	+	+	M	+	+	+							
Thyroid gland	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
General Body System																								
None																								
Genital System																								
Epididymis	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Penis				+		+		+				+												
Preputial gland		+										+	+				+							
Prostate	+	+	+	+	A	+	+	+	+	+	+	M	+	+	+	+	+							
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+					
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	1 7 1	1 8 1	1 9 4	1 8 2	1 9 1	1 9 2	1 9 3	2 0 1	2 1 1	2 1 2	2 1 3	2 2 1	2 2 2	2 2 3	2 3 1	2 3 4	2 4 1	2 4 2	2 5 2	2 6 1	2 6 2	2 6 5	2 7 1	2 8 2	2 8 3	Total Tissues/ Tumors
Alimentary System																									
Esophagus																									15
Gallbladder																									9
Intestine large																									14
Intestine large, cecum																									13
Intestine large, colon																									14
Intestine large, rectum																									14
Intestine small																					+				15
Intestine small, duodenum																					+				15
Intestine small, ileum																					+				14
Intestine small, jejunum																					+				14
Liver							+										+							+	22
Hepatocellular carcinoma							X																		5
Hepatocellular carcinoma, multiple																									2
Hepatocellular adenoma							X										X							X	7
Mesentery																									
Pancreas																					+				16
Salivary glands																									17
Stomach			+															+		+					20
Stomach, forestomach			+															+							19
Papilloma squamous			X															X							2
Stomach, glandular																				+					16
Tooth																									1
Cardiovascular System																									
Heart																									17
Sarcoma, metastatic, skeletal muscle																									1
Endocrine System																									
Adrenal gland																	+								17
Adrenal gland, cortex																	+								17
Adrenal gland, medulla																									16
Islets, pancreatic																					+				15
Parathyroid gland																									8
Pituitary gland																									13
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
General Body System																									
None																									
Genital System																									
Epididymis	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Penis				+	+		+																	+	8
Preputial gland	+		+					+		+	+								+		+				11
Prostate																									15
Seminal vesicle						+										+				+					21
Testes																									17

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	0 7 3	0 9 4	1 0 0	1 5 2	1 6 8	2 7 1	2 9 1	3 2 8	3 7 7	3 8 4	3 9 9	4 0 9	5 1 2	5 6 7	5 9 2	6 0 7	6 2 6	6 5 8	6 7 5	6 8 7	7 3 1	7 3. 4	7 3 4	7 3 4
Carcass ID Number	1 6 5	1 5 5	1 7 5	1 5 4	2 5 5	2 7 3	2 4 4	2 4 3	1 7 3	2 8 4	2 1 5	2 5 4	1 8 3	2 3 3	1 8 4	2 6 4	1 6 1	2 5 1	2 8 1	2 7 2	1 5 1	1 5 2	1 6 2	1 6 3
Hematopoietic System																								
Bone marrow	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+							
Lymph node	+	+	+	+	M	A	+	+	M	+	+	+	+	+	+	+	+			+				+
Mediastinal, pancreatic, sarcoma, metastatic, skeletal muscle																	X							
Lymph node, mandibular	M	+	M	M	M	A	+	+	M	+	M	+	+	+	+	+	M							
Lymph node, mesenteric	+	M	+	+	M	M	M	+	M	+	+	+	+	+	+	M	+			M				+
Spleen	+	+	+	+	M	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+				
Thymus	+	M	+	+	+	A	M	+	M	+	M	+	+	M	M	M	+							
Integumentary System																								
Mammary gland	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M	M							
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+
Subcutaneous tissue, fibroma																								
Subcutaneous tissue, fibrosarcoma							X			X														
Musculoskeletal System																								
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+	+	+		+
Skeletal muscle																	+							
Sarcoma																	X							
Nervous System																								
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+							
Meningioma benign																								
Respiratory System																								
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+			+				
Alveolar/bronchiolar adenoma																								
Alveolar/bronchiolar carcinoma																				X				
Hepatocellular carcinoma, metastatic, liver														X										
Sarcoma, metastatic, skeletal muscle																	X							
Nose	+	+	+	+	M	I	+	+	+	+	M	+	+	+	+	+	+							
Trachea	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+							
Special Senses System																								
None																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	1 7 1	1 8 1	1 9 4	1 8 2	1 9 1	1 9 2	1 9 3	2 0 1	2 1 1	2 1 2	2 1 3	2 2 1	2 2 2	2 2 3	2 3 1	2 3 4	2 4 1	2 4 2	2 5 2	2 6 1	2 6 2	2 6 5	2 7 1	2 8 2	2 8 3	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow																										16
Lymph node	+	+				+			+			+		+	+				+	+	+	+			+	28
Mediastinal, pancreatic, sarcoma, metastatic, skeletal muscle																										1
Lymph node, mandibular																										9
Lymph node, mesenteric	+	+				+			+			+		+	+				+	+	+	+			+	24
Spleen		+																+			+					21
Thymus																										9
Integumentary System																										
Mammary gland																										
Skin		+				+			+		+	+	+							+		+	+	M	+	32
Subcutaneous tissue, fibroma												X														1
Subcutaneous tissue, fibrosarcoma																										2
Musculoskeletal System																										
Bone	+	+	+	+	+	+		+	+			+	+	+	+				+	+	+		+	+	+	39
Skeletal muscle																										1
Sarcoma																										1
Nervous System																										
Brain					+																					18
Meningioma benign					X																					1
Respiratory System																										
Lung	+		+	+	+	+				+	+							+			+		+		+	29
Alveolar/bronchiolar adenoma	X		X		X					X	X										X		X		X	8
Alveolar/bronchiolar carcinoma																										1
Hepatocellular carcinoma, metastatic, liver																										1
Sarcoma, metastatic, skeletal muscle																										1
Nose																										14
Trachea																										16
Special Senses System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	0 7 3	0 9 4	1 0 0	1 5 2	1 6 8	2 7 1	2 9 1	3 2 8	3 7 7	3 8 4	3 9 9	4 0 9	4 1 2	5 6 7	5 9 2	5 0 7	6 2 6	6 5 8	6 7 5	6 8 7	7 3 1	7 3 4	7 3 4	7 3 4
Carcass ID Number	1 6 5	1 5 5	1 7 5	1 5 4	2 5 5	2 7 3	2 4 4	2 4 3	1 7 3	2 8 4	2 1 5	2 5 4	1 8 3	2 3 3	1 8 4	2 6 4	1 6 1	2 5 1	2 8 1	2 7 2	1 5 1	1 5 2	1 6 2	1 6 3
Urinary System																								
Kidney	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+			+		+		
Sarcoma, metastatic, skeletal muscle																	X							
Urethra					M			+																
Urinary bladder	+	+	+	+	A	A	+	+	A	+	M	+	+	+	+	+	+			+			+	+
Systemic Lesions																								
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant undifferentiated cell type																								

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 3 4	7 3 4	7 3 4	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 5	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	7 3 6	
Carcass ID Number	1 7 1	1 8 1	1 9 4	1 8 2	1 9 1	1 9 2	1 9 3	2 0 1	2 1 1	2 1 2	2 1 3	2 2 1	2 2 2	2 2 3	2 3 1	2 3 4	2 4 1	2 4 2	2 5 2	2 6 1	2 6 2	2 6 5	2 7 1	2 8 2	2 8 3	Total Tissues/ Tumors
Urinary System																									
Kidney																+	+				+		+		22
Sarcoma, metastatic, skeletal muscle																									1
Urethra																									1
Urinary bladder																	+								17
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymphoma malignant undifferentiated cell type																					X				1

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm

Number of Days on Study	2 2 3 3 4 4 4 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 2 9 0 9 0 4 8 6 0 1 4 6 7 0 0 3 3 3 3 3 3 3 3 3 3 0 7 8 4 5 8 4 7 2 7 5 8 9 1 8 0 0 0 0 0 0 0 0 0 0
Carcass ID Number	3 3 3 3 4 4 3 4 3 3 3 2 3 3 3 2 2 2 3 3 3 3 3 3 3 0 1 0 3 1 0 2 2 5 0 9 9 7 6 8 9 9 9 0 1 1 1 1 2 3 4 5 3 5 4 5 3 2 3 2 1 1 1 2 1 2 3 4 1 1 2 3 4 2 1
Alimentary System	
Esophagus	+ M + + + + + M + M + + + + + + + + + + + + + +
Gallbladder	+ M + + M + + + + + + + + + M + + + + M + + + + +
Intestine large	+ + + + + + + + + + + + + + A + + + + + + + + + +
Intestine large, cecum	+ + + + + + + + + + + + + + A + + + + + + + + + +
Intestine large, colon	+ + + + + + + + + + + + + + A + + + M + + + + + +
Intestine large, rectum	+ + + + + + + + + + + + + + A + + + + + + + + + +
Adenocarcinoma	
Intestine small	+ + A + + + + + + + + + + + A + + + + + + + + + +
Intestine small, duodenum	+ + A + + + + + + + + + + + A + + + + + + + + + +
Intestine small, ileum	+ M A + + + + + + + M + + + A + + + + + + + + + M
Intestine small, jejunum	+ M A + + + + + + + + + + + A + + + M + + + + + +
Liver	+ M + + + + + + + + + + + + A + + + + + + + + + +
Hepatocellular carcinoma	X X X X
Hepatocellular carcinoma, multiple	X
Hepatocellular adenoma	X X
Mesentery	M +
Sarcoma	X
Pancreas	+ + + + + + + + + + + + + + A + + + + + + + + + +
Salivary glands	+ M +
Stomach	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Stomach, forestomach	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Papilloma squamous	X
Stomach, glandular	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Tooth	+ +
Cardiovascular System	
Heart	+ M +
Endocrine System	
Adrenal gland	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Adrenal gland, cortex	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Adrenal gland, medulla	+ M + + + + + + + + + + + + + A + + + + + + + + + +
Islets, pancreatic	+ + + + + + + + + + + + + + + A + + + + + + + + + +
Parathyroid gland	M M M M + + + M M M + + M + A M + M M + M M M + M
Pituitary gland	M M + + M M + + + + M + + M A + + + + + + + M + +
Thyroid gland	+ M +
Follicular cell, adenoma	
General Body System	
None	

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 3 2	3 3 4	3 4 1	3 4 2	3 5 1	3 5 2	3 2 1	3 3 3	3 5 4	3 6 1	3 6 3	3 6 4	3 7 2	3 7 3	3 8 2	3 9 2	4 0 1	4 0 2	4 0 3	4 1 1	4 1 2	4 1 3	4 2 1	4 2 3	4 2 4	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	48
Intestine large, colon	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma												X														1
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Hepatocellular carcinoma		X					X		X			X														8
Hepatocellular carcinoma, multiple																										1
Hepatocellular adenoma														X									X			4
Mesentery																										1
Sarcoma																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Papilloma squamous																										1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	47
Tooth	+																			+			+		+	6
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	M	I	+	M	+	M	+	M	M	M	M	M	+	M	M	+	I	+	M	+	+	+	M	+	20
Pituitary gland	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I	+	+	+	+	+	40
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Follicular cell, adenoma									X			X														2
General Body System																										
None																										

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	2 2 3 3 4 4 4 5 6 6 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7
	2 9 0 9 0 4 8 6 0 1 4 6 7 0 0 3 3 3 3 3 3 3 3 3 3 3
	0 7 8 4 5 8 4 7 2 7 5 8 9 1 8 0 0 0 0 0 0 0 0 0 0 0
Carcass ID Number	3 3 3 3 4 4 3 4 3 3 3 2 3 3 3 2 2 2 3 3 3 3 3 3 3 3
	0 1 0 3 1 0 2 2 5 0 9 9 7 6 8 9 9 9 0 1 1 1 1 2 3
	4 5 3 5 4 5 3 2 3 2 1 1 1 2 1 2 3 4 1 1 2 3 4 2 1
Genital System	
Coagulating gland	+
Epididymis	M +
Penis	+ + + + + + +
Preputial gland	+ + + +
Prostate	+ + + + + + + + + + + + + + + + M + + + M + + + +
Seminal vesicle	+ + + + + + + + + + + + + + A + + + + + + + + + +
Testes	+ + + + + + + + + + + + + + A + + + + + + + + + +
Hematopoietic System	
Bone marrow	+ M + + + + + + + + + + + M A + + + + + + + + + +
Lymph node	+ M + + + + + + + + + + M + A + + + + + + + + + +
Lymph node, mandibular	+ M + + + M + M M + M + M + A + M + + + + + + + +
Lymph node, mesenteric	+ M + + + + + + + M + + M + M + + + + + + + + + +
Spleen	+ M + + + + + + + + + + + + M + + + + + + + + + +
Thymus	A M M M M + M M M + M M M + M M + I + + M + + + M
Integumentary 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 M M M
Skin	+ + + + + + + + + + + + + + + M + + + + + + + + +
Squamous cell carcinoma	X
Subcutaneous tissue, fibrosarcoma	X X X
Musculoskeletal System	
Bone	+ M + + + + + + + + + + + + M + + + + + + + + + +
Nervous System	
Brain	+ M +
Respiratory System	
Lung	+ M +
Alveolar/bronchiolar adenoma	X
Alveolar/bronchiolar adenoma, multiple	X
Alveolar/bronchiolar carcinoma	
Hepatocellular carcinoma, metastatic, liver	X X
Nose	+ M + + + + + + + + + + + + M + + + + + + + + + +
Trachea	+ M + + + + + + + + + + + + A + + + + + + + + + +

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 3 2	3 3 4	3 4 1	3 4 2	3 5 1	3 5 2	3 2 1	3 3 3	3 5 4	3 6 1	3 6 3	3 6 4	3 7 2	3 7 3	3 8 2	3 9 2	4 0 1	4 0 2	4 0 3	4 1 1	4 1 2	4 1 3	4 2 1	4 2 3	4 2 4	Total Tissues/ Tumors
Genital System																										
Coagulating gland																										1
Epididymis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Penis										+																4
Preputial gland							+														+		+			6
Prostate	+	+	+	+	+	+	+	+	I	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	+	45
Seminal vesicle	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Testes	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	+	+	40
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Thymus	+	M	+	+	+	+	+	+	M	+	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	29
Integumentary 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	M	M	M	
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	48
Squamous cell carcinoma																										1
Subcutaneous tissue, fibrosarcoma																										3
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Alveolar/bronchiolar adenoma			X				X												X					X		5
Alveolar/bronchiolar adenoma, multiple																										1
Alveolar/bronchiolar carcinoma					X																					1
Hepatocellular carcinoma, metastatic, liver																										2
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	2	2	3	3	4	4	4	5	6	6	6	6	6	7	7	7	7	7	7	7	7	7	7	7	7
	2	9	0	9	0	4	8	6	0	1	4	6	7	0	0	3	3	3	3	3	3	3	3	3	3
	0	7	8	4	5	8	4	7	2	7	5	8	9	1	8	0	0	0	0	0	0	0	0	0	0
Carcass ID Number	3	3	3	3	4	4	3	4	3	3	3	2	3	3	3	2	2	2	3	3	3	3	3	3	3
	0	1	0	3	1	0	2	2	5	0	9	9	7	6	8	9	9	9	0	1	1	1	1	2	3
	4	5	3	5	4	5	3	2	3	2	1	1	1	2	1	2	3	4	1	1	2	3	4	2	1
Special Senses System																									
Ear												+	+												
Eye																									
Harderian gland																					+				
Adenoma																					X				
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	A	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma			X																						
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant undifferentiated cell type																									

TABLE C2
Individual Animal Tumor Pathology of Male Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	3 3 2	3 4 4	3 1 1	3 4 2	3 5 1	3 5 2	3 2 1	3 3 3	3 5 4	3 6 1	3 6 3	3 6 4	3 7 2	3 7 3	3 8 2	3 9 2	4 0 1	4 0 2	4 0 3	4 1 1	4 1 2	4 1 3	4 2 1	4 2 3	4 2 4	Total Tissues/ Tumors
Special Senses System																										
Ear						+	+																			4
Eye																				+						1
Harderian gland			+																	+						3
Adenoma			X																	X						3
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Adenocarcinoma																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant undifferentiated cell type					X																					1

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4

	0 ppm	5,000 ppm	10,000 ppm
Harderian Gland: Adenoma			
Overall rates[a]	1/50 (2%)	0/49 (0%)	3/50 (6%)
Adjusted rates[b]	3.6%	0.0%	8.6%
Terminal rates[c]	1/28 (4%)	0/29 (0%)	3/35 (9%)
First incidence (days)	730 (T)	–[e]	730 (T)
Life table tests[d]	P=0.230	P=0.493N	P=0.387
Logistic regression tests[d]	P=0.230	P=0.493N	P=0.387
Cochran-Armitage test[d]	P=0.177		
Fisher exact test[d]		P=0.505N	P=0.309
Liver: Hepatocellular Adenoma			
Overall rates	8/49 (16%)	7/22 (32%)[f]	4/48 (8%)
Adjusted rates	28.6%		11.4%
Terminal rates	8/28 (29%)		4/35 (11%)
First incidence (days)	730 (T)		730 (T)
Life table tests			P=0.083N
Logistic regression tests			P=0.083N
Fisher exact test			P=0.188N
Liver: Hepatocellular Carcinoma			
Overall rates	5/49 (10%)	7/22 (32%)[f]	9/48 (19%)
Adjusted rates	16.2%		24.7%
Terminal rates	3/28 (11%)		8/35 (23%)
First incidence (days)	694		617
Life table tests			P=0.326
Logistic regression tests			P=0.279
Fisher exact test			P=0.182
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	13/49 (27%)	10/22 (45%)[f]	12/48 (25%)
Adjusted rates	43.0%		33.1%
Terminal rates	11/28 (39%)		11/35 (31%)
First incidence (days)	694		617
Life table tests			P=0.262N
Logistic regression tests			P=0.327N
Fisher exact test			P=0.524N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	7/50 (14%)	8/29 (28%)	6/49 (12%)
Adjusted rates	23.0%	72.7%	17.1%
Terminal rates	5/28 (18%)	8/11 (73%)	6/35 (17%)
First incidence (days)	721	730 (T)	730 (T)
Life table tests	P=0.259N	P=0.029	P=0.345N
Logistic regression tests	P=0.318N	P=0.005	P=0.395N
Cochran-Armitage test	P=0.464N		
Fisher exact test		P=0.119	P=0.516N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	8/50 (16%)	9/29 (31%)	7/49 (14%)
Adjusted rates	26.3%	73.6%	20.0%
Terminal rates	6/28 (21%)	8/11 (73%)	7/35 (20%)
First incidence (days)	721	687	730 (T)
Life table tests	P=0.251N	P=0.027	P=0.330N
Logistic regression tests	P=0.314N	P=0.003	P=0.385N
Cochran-Armitage test	P=0.468N		
Fisher exact test		P=0.101	P=0.517N
Skin (Subcutaneous Tissue): Fibrosarcoma			
Overall rates	6/50 (12%)	2/49 (4%)	3/50 (6%)
Adjusted rates	18.4%	4.8%	7.1%
Terminal rates	2/28 (7%)	0/29 (0%)	0/35 (0%)
First incidence (days)	508	291	484
Life table tests	P=0.129N	P=0.149N	P=0.179N
Logistic regression tests	P=0.182N	P=0.141N	P=0.217N
Cochran-Armitage test	P=0.170N		
Fisher exact test		P=0.141N	P=0.243N
Skin (Subcutaneous Tissue): Fibroma or Fibrosarcoma			
Overall rates	6/50 (12%)	3/49 (6%)	3/50 (6%)
Adjusted rates	18.4%	8.1%	7.1%
Terminal rates	2/28 (7%)	1/29 (3%)	0/35 (0%)
First incidence (days)	508	291	484
Life table tests	P=0.134N	P=0.256N	P=0.179N
Logistic regression tests	P=0.185N	P=0.252N	P=0.217N
Cochran-Armitage test	P=0.179N		
Fisher exact test		P=0.254N	P=0.243N
Stomach (Forestomach): Squamous Papilloma			
Overall rates	3/50 (6%)	2/49 (4%)	1/50 (2%)
Adjusted rates	10.7%	6.9%	2.9%
Terminal rates	3/28 (11%)	2/29 (7%)	1/35 (3%)
First incidence (days)	730 (T)	730 (T)	730 (T)
Life table tests	P=0.159N	P=0.484N	P=0.228N
Logistic regression tests	P=0.159N	P=0.484N	P=0.228N
Cochran-Armitage test	P=0.223N		
Fisher exact test		P=0.510N	P=0.309N
All Organs: Hemangioma or Hemangiosarcoma			
Overall rates	3/50 (6%)	0/49 (0%)	0/50 (0%)
Adjusted rates	9.2%	0.0%	0.0%
Terminal rates	1/28 (4%)	0/29 (0%)	0/35 (0%)
First incidence (days)	519	–	–
Life table tests	P=0.031N	P=0.128N	P=0.097N
Logistic regression tests	P=0.033N	P=0.124N	P=0.108N
Cochran-Armitage test	P=0.038N		
Fisher exact test		P=0.125N	P=0.121N

TABLE C3
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	5,000 ppm	10,000 ppm
All Organs: Benign Tumors			
Overall rates	19/50 (38%)	17/49 (35%)	13/50 (26%)
Adjusted rates	60.9%	52.6%	37.1%
Terminal rates	16/28 (57%)	14/29 (48%)	13/35 (37%)
First incidence (days)	508	512	730 (T)
Life table tests	P=0.021N	P=0.374N	P=0.026N
Logistic regression tests	P=0.034N	P=0.465N	P=0.044N
Cochran-Armitage test	P=0.121N		
Fisher exact test		P=0.447N	P=0.142N
All Organs: Malignant Tumors			
Overall rates	16/50 (32%)	12/49 (24%)	16/50 (32%)
Adjusted rates	44.2%	30.9%	37.3%
Terminal rates	8/28 (29%)	3/29 (10%)	9/35 (26%)
First incidence (days)	449	291	308
Life table tests	P=0.335N	P=0.288N	P=0.354N
Logistic regression tests	P=0.476N	P=0.270N	P=0.485N
Cochran-Armitage test	P=0.544		
Fisher exact test		P=0.272N	P=0.585N
All Organs: Benign or Malignant Tumors			
Overall rates	28/50 (56%)	23/49 (47%)	23/50 (46%)
Adjusted rates	77.7%	60.1%	54.1%
Terminal rates	20/28 (71%)	14/29 (48%)	16/35 (46%)
First incidence (days)	449	291	308
Life table tests	P=0.047N	P=0.222N	P=0.050N
Logistic regression tests	P=0.061N	P=0.222N	P=0.066N
Cochran-Armitage test	P=0.184N		
Fisher exact test		P=0.242N	P=0.212N

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Not applicable; no tumors were found at the site in this group
[f] Tissue was examined microscopically only when it was observed to be abnormal at necropsy.

TABLE C4
Historical Incidence of Thyroid Gland Follicular Cell Neoplasms in Untreated Male B6C3F$_1$ Mice[a]

Study	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence at EG&G Mason Research Institute			
4-Hydroxyacetanilide	2/49	1/49	3/49
HC Yellow 4	1/47	0/47	1/47
Pentaerythritol tetranitrate	1/46	1/46	2/46
Total	4/142 (2.8%)	2/142 (1.4%)	6/142 (4.2%)
Standard deviation	1.2%	1.2%	2.0%
Range	2%–4%	0%–2%	2%–6%
Overall Historical Incidence			
Total	14/856 (1.6%)	4/856 (0.5%)	18/856 (2.1%)
Standard deviation	1.7%	0.9%	1.8%
Range	0%–4%	0%–2%	0%–6%

[a] Data as of 3 April 1991

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	14	10	5
Moribund kills	8	10	9
Accidental death			1
Survivors			
Terminal sacrifice	28	29	35
Missing		1	
Animals examined microscopically	50	49	50
Alimentary System			
Gallbladder	(39)	(9)	(46)
Inflammation, chronic	3 (8%)		4 (9%)
Intestine small, ileum	(41)	(14)	(45)
Hyperplasia, lymphoid	1 (2%)		
Liver	(49)	(22)	(48)
Basophilic focus			1 (2%)
Clear cell focus	1 (2%)		
Eosinophilic focus			1 (2%)
Infarct		1 (5%)	
Necrosis	9 (18%)	4 (18%)	2 (4%)
Thrombus		1 (5%)	
Pancreas	(45)	(16)	(49)
Inflammation, chronic	16 (36%)	1 (6%)	11 (22%)
Vacuolization cytoplasmic	12 (27%)	2 (13%)	8 (16%)
Duct, dilatation			1 (2%)
Salivary glands	(50)	(17)	(49)
Inflammation, chronic active	33 (66%)	7 (41%)	21 (43%)
Stomach, forestomach	(46)	(19)	(48)
Acanthosis	1 (2%)		
Diverticulum	1 (2%)		
Hyperkeratosis	1 (2%)		
Stomach, glandular	(46)	(16)	(47)
Hyperplasia		1 (6%)	
Inflammation, chronic	9 (20%)	1 (6%)	8 (17%)
Mineralization	2 (4%)	1 (6%)	4 (9%)
Tooth	(9)	(1)	(6)
Dysplasia	9 (100%)	1 (100%)	5 (83%)
Cardiovascular System			
Heart	(50)	(17)	(49)
Abscess	1 (2%)		
Inflammation, chronic	8 (16%)		7 (14%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Endocrine System			
Adrenal gland, cortex	(50)	(17)	(48)
Hyperplasia	7 (14%)	1 (6%)	7 (15%)
Adrenal gland, medulla	(50)	(16)	(47)
Hyperplasia	1 (2%)		2 (4%)
Islets, pancreatic	(45)	(15)	(49)
Hyperplasia	8 (18%)		
Pituitary gland	(38)	(13)	(40)
Pars distalis, cyst	1 (3%)	1 (8%)	
Pars distalis, hyperplasia	10 (26%)	1 (8%)	6 (15%)
Thyroid gland	(47)	(48)	(49)
Inflammation, chronic		7 (15%)	29 (59%)
Follicle, cyst			2 (4%)
Follicle, inflammation, acute		1 (2%)	
Follicle, pigmentation		44 (92%)	48 (98%)
Follicular cell, hyperplasia		27 (56%)	41 (84%)
Follicular cell, pigmentation		44 (92%)	49 (100%)
Interstitium, pigmentation		42 (88%)	49 (100%)
General Body System			
None			
Genital System			
Epididymis	(50)	(47)	(49)
Granuloma sperm	1 (2%)		1 (2%)
Inflammation, chronic active	16 (32%)	13 (28%)	13 (27%)
Penis	(5)	(8)	(4)
Inflammation, chronic active	3 (60%)	4 (50%)	2 (50%)
Preputial gland	(9)	(11)	(6)
Abscess	3 (33%)	3 (27%)	1 (17%)
Inflammation, chronic active	5 (56%)	8 (73%)	4 (67%)
Duct, dilatation	1 (11%)		
Prostate	(47)	(15)	(45)
Inflammation, chronic active	34 (72%)	7 (47%)	20 (44%)
Seminal vesicle	(49)	(21)	(49)
Inflammation, chronic active	11 (22%)	4 (19%)	14 (29%)
Testes	(50)	(17)	(49)
Spermatogenic arrest		1 (6%)	1 (2%)
Germinal epithelium, giant cell		1 (6%)	1 (2%)
Hematopoietic System			
Spleen	(49)	(21)	(48)
Depletion lymphoid	11 (22%)	9 (43%)	5 (10%)
Hematopoietic cell proliferation	6 (12%)	6 (29%)	5 (10%)
Hyperplasia, lymphoid			1 (2%)
Thymus	(28)	(9)	(29)
Cyst	8 (29%)	1 (11%)	4 (14%)

TABLE C5
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Integumentary System			
Skin	(49)	(32)	(48)
Cyst epithelial inclusion	1 (2%)		
Inflammation, chronic active	8 (16%)	4 (13%)	5 (10%)
Musculoskeletal System			
Bone	(50)	(39)	(48)
Joint, tarsal, hyperostosis	17 (34%)	24 (62%)	17 (35%)
Nervous System			
Brain	(50)	(18)	(49)
Thalamus, mineralization	29 (58%)	6 (33%)	25 (51%)
Respiratory System			
Lung	(50)	(29)	(49)
Crystals		1 (3%)	
Alveolar epithelium, hyperplasia	1 (2%)		3 (6%)
Bronchiole, epithelium, hyperplasia			1 (2%)
Nose	(49)	(14)	(47)
Cyst	1 (2%)		
Inflammation, acute	6 (12%)		3 (6%)
Special Senses System			
None			
Urinary System			
Kidney	(49)	(22)	(49)
Abscess	2 (4%)		
Cyst	1 (2%)		3 (6%)
Hydronephrosis	1 (2%)		1 (2%)
Inflammation, chronic active	38 (78%)	15 (68%)	44 (90%)
Necrosis	1 (2%)		
Vacuolization cytoplasmic		1 (5%)	
Papilla, mineralization			1 (2%)
Renal tubule, mineralization	7 (14%)	2 (9%)	4 (8%)
Urethra	(1)	(1)	
Inflammation, chronic active	1 (100%)	1 (100%)	
Urinary bladder	(44)	(17)	(50)
Calculus micro observation only		1 (6%)	1 (2%)
Hemorrhage		2 (12%)	
Inflammation, chronic active	28 (64%)	5 (29%)	25 (50%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE IN THE 2-YEAR FEED STUDY OF HC YELLOW 4

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	4	2	2
Moribund kills	3	10	5
Survivors			
Terminal sacrifice	43	38	43
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(46)	(3)	(48)
Intestine large, cecum	(47)	(3)	(49)
Intestine large, rectum	(49)	(3)	(47)
Intestine small, duodenum	(46)	(9)	(50)
Intestine small, ileum	(45)	(11)	(48)
Intestine small, jejunum	(47)	(8)	(49)
Liver	(50)	(18)	(50)
Hemangioma	1 (2%)		
Hepatocellular carcinoma	1 (2%)		
Hepatocellular adenoma	4 (8%)	6 (33%)	4 (8%)
Hepatocellular adenoma, multiple	1 (2%)	2 (11%)	
Hepatocholangiocarcinoma	1 (2%)		
Mesentery	(2)	(1)	(1)
Pancreas	(48)	(3)	(49)
Salivary glands	(50)	(2)	(50)
Stomach, forestomach	(49)	(7)	(50)
Papilloma squamous	3 (6%)	2 (29%)	1 (2%)
Stomach, glandular	(49)	(6)	(50)
Tooth	(2)		(1)
Cardiovascular System			
Heart	(50)	(4)	(50)
Endocrine System			
Adrenal gland, cortex	(50)	(3)	(50)
Pituitary gland	(42)		(45)
Pars distalis, adenoma	5 (12%)		1 (2%)
Thyroid gland	(48)	(49)	(50)
General Body System			
None			

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4
(continued)

	0 ppm	5,000 ppm	10,000 ppm
Genital System			
Ovary	(50)	(47)	(50)
Cystadenoma	2 (4%)		3 (6%)
Granulosa cell tumor benign	1 (2%)		1 (2%)
Teratoma			1 (2%)
Uterus	(50)	(24)	(50)
Polyp stromal	2 (4%)	1 (4%)	
Sarcoma stromal			1 (2%)
Hematopoietic System			
Bone marrow	(50)	(3)	(50)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Lymph node	(49)	(15)	(49)
Lymph node, mandibular	(45)	(5)	(45)
Adenocarcinoma, metastatic, harderian gland	1 (2%)		
Lymph node, mesenteric	(45)	(10)	(44)
Spleen	(49)	(17)	(50)
Hemangiosarcoma, metastatic, skeletal muscle		1 (6%)	
Thymus	(43)	(6)	(41)
Integumentary System			
Mammary gland	(31)	(2)	(37)
Adenocarcinoma	1 (3%)	1 (50%)	1 (3%)
Skin	(50)	(40)	(50)
Musculoskeletal System			
Skeletal muscle	(2)	(1)	(2)
Hemangiosarcoma		1 (100%)	
Osteosarcoma, metastatic, uncertain primary site			1 (50%)
Sarcoma	1 (50%)		
Nervous System			
Brain	(50)	(5)	(50)
Respiratory System			
Lung	(50)	(8)	(50)
Adenocarcinoma, metastatic, harderian gland	1 (2%)		
Adenocarcinoma, metastatic, mammary gland			1 (2%)
Alveolar/bronchiolar adenoma	1 (2%)	2 (25%)	1 (2%)
Alveolar/bronchiolar adenoma, multiple	2 (4%)		
Alveolar/bronchiolar carcinoma	1 (2%)		
Alveolar/bronchiolar carcinoma, multiple			1 (2%)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung			1 (2%)
Trachea	(49)	(3)	(50)

TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Special Senses System			
Harderian gland	(2)	(1)	(2)
Adenocarcinoma	1 (50%)		
Adenoma	1 (50%)	1 (100%)	1 (50%)
Urinary System			
Kidney	(50)	(4)	(50)
Osteosarcoma, metastatic, uncertain primary site			1 (2%)
Urinary bladder	(48)	(4)	(49)
Systemic Lesions			
Multiple organs[b]	(50)	(50)	(50)
Lymphoma malignant histiocytic	1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant lymphocytic	1 (2%)	3 (6%)	
Lymphoma malignant mixed	6 (12%)	4 (8%)	5 (10%)
Lymphoma malignant undifferentiated cell	2 (4%)	6 (12%)	2 (4%)
Tumor Summary			
Total animals with primary neoplasms[c]	30	24	20
Total primary neoplasms	39	30	24
Total animals with benign neoplasms	21	13	11
Total benign neoplasms	23	14	13
Total animals with malignant neoplasms	14	15	11
Total malignant neoplasms	16	16	11
Total animals with secondary neoplasms[d]	1	1	3
Total secondary neoplasms	2	1	6
Total animals with malignant neoplasms of uncertain primary site			1

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.
[b] Number of animals with any tissue examined microscopically
[c] Primary tumors: all tumors except metastatic tumors
[d] Secondary tumors: metastatic tumors or tumors invasive to an adjacent organ

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm

Number of Days on Study	2 6 3	4 6 3	4 6 6	5 3 2	5 3 3	6 2 5	6 3 4	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	4 5 4	5 2 4	5 4 4	5 1 2	4 9 4	4 4 5	5 6 2	4 3 1	4 3 2	4 3 3	4 3 4	4 4 1	4 4 2	4 4 3	4 5 2	4 5 3	4 6 1	4 6 2	4 4 4	4 5 1	4 6 3	4 7 1	4 7 2	4 7 3	4 8 1
Alimentary System																									
Esophagus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Gallbladder	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+
Intestine large	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, cecum	+	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine large, colon	+	+	A	A	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Intestine large, rectum	+	+	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, duodenum	A	+	A	A	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Intestine small, ileum	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+
Intestine small, jejunum	A	+	A	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Hemangioma							X																		
Hepatocellular carcinoma																			X						
Hepatocellular adenoma										X											X		X		
Hepatocellular adenoma, multiple																	X								
Hepatocholangiocarcinoma																									
Mesentery	M	M							M																
Pancreas	M	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Stomach, forestomach	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Papilloma squamous													X												
Stomach, glandular	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Tooth			+	+																					
Cardiovascular System																									
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Endocrine System																									
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adrenal gland, medulla	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Islets, pancreatic	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Parathyroid gland	+	+	+	+	M	M	+	+	M	+	+	M	+	+	M	+	+	+	I	+	M	M	+	M	+
Pituitary gland	+	+		+	+	+	+	+	M	M	+	+	M	+	M	+	+	+	+	+	M	+	M	+	+
Pars distalis, adenoma		X																							
Thyroid gland	+	+	+	+	M	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+
General Body System																									
None																									

+: Tissue examined microscopically
A: Autolysis precludes examination
M: Missing tissue
I: Insufficient tissue
X: Lesion present
Blank: Not examined

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 5	7 3 5	7 3 5	
Carcass ID Number	4 8 2	4 8 3	4 8 4	4 9 1	4 9 2	4 9 3	5 0 1	5 0 2	5 0 3	5 0 4	5 1 1	5 1 1	5 2 1	5 2 2	5 2 3	5 3 1	5 3 2	5 3 3	5 4 2	5 4 3	5 5 3	5 5 4	5 6 1	4 1 1	5 5 1	5 5 2	Total Tissues/ Tumors
Alimentary System																											
Esophagus	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, cecum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine large, colon	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine large, rectum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hemangioma																											1
Hepatocellular carcinoma																											1
Hepatocellular adenoma																				X							4
Hepatocellular adenoma, multiple																											1
Hepatocholangiocarcinoma							X																				1
Mesentery						+											+										2
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Papilloma squamous															X	X											3
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Tooth																											2
Cardiovascular System																											
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																											
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Parathyroid gland	+	+	M	M	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	37
Pituitary gland	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	42
Pars distalis, adenoma								X		X											X	X					5
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
General Body System																											
None																											

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	2 6 3	4 6 3	4 6 6	5 3 2	5 3 3	6 2 5	6 3 4	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2
Carcass ID Number	4 5 4	5 2 4	5 4 4	5 1 2	4 9 4	4 4 5	5 6 2	4 3 1	4 3 2	4 3 3	4 3 4	4 4 1	4 4 2	4 4 3	4 5 2	4 5 3	4 6 1	4 6 2	4 4 4	4 5 1	4 6 3	4 7 1	4 7 2	4 7 3	4 8 1
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																										X
Granulosa cell tumor benign																							X			
Oviduct	A																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Polyp stromal																			X							
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	I
Lymph node, mandibular	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	I
Adenocarcinoma, metastatic, harderian gland				X																						
Lymph node, mesenteric	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	M
Spleen	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	M	M	+	M	M	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+
Integumentary System																										
Mammary gland	+	+	+	+	M	+	M	M	+	M	M	M	+	+	+	M	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma						X																				
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle			+		+																					
Sarcoma					X																					
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Adenocarcinoma, metastatic, harderian gland				X																						
Alveolar/bronchiolar adenoma																										
Alveolar/bronchiolar adenoma, multiple																										
Alveolar/bronchiolar carcinoma																										
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	M	+	+	+	+	
Trachea	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	4 8 2	4 8 3	4 8 4	4 9 1	4 9 2	4 9 3	5 0 1	5 0 2	5 0 3	5 0 4	5 1 1	5 1 2	5 2 1	5 2 2	5 2 3	5 3 1	5 3 2	5 3 3	5 4 2	5 4 3	5 5 3	5 5 4	5 6 1	5 4 1	5 5 1	5 5 2	Total Tissues/ Tumors
Genital System																											
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma						X																					2
Granulosa cell tumor benign																											1
Oviduct																											
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Polyp stromal	X																										2
Hematopoietic System																											
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	45
Adenocarcinoma, metastatic, harderian gland																											1
Lymph node, mesenteric	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Thymus	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	43
Integumentary System																											
Mammary gland	M	+	M	+	+	M	+	+	M	+	M	M	M	M	M	+	+	+	+	+	+	+	M	M	M		31
Adenocarcinoma																											1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																											
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																											2
Sarcoma																											1
Nervous System																											
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Respiratory System																											
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, harderian gland																											1
Alveolar/bronchiolar adenoma																							X				1
Alveolar/bronchiolar adenoma, multiple									X											X							2
Alveolar/bronchiolar carcinoma								X																			1
Nose	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	47
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	2	4	4	5	5	6	6	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7	7
	6	6	6	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	3	3	6	2	3	5	4	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2	2	2
Carcass ID Number	4	5	5	5	4	4	5	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4	4
	5	2	4	1	9	4	6	3	3	3	3	4	4	4	5	5	6	6	4	5	6	7	7	7	8
	4	4	4	2	4	5	2	1	2	3	4	1	2	3	2	3	1	2	4	1	3	1	2	3	1
Special Senses System																									
Eye				+																					
Harderian gland				+																					
Adenocarcinoma				X																					
Adenoma																									
Urinary System																									
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Urinary bladder	A	+	A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic				X																					
Lymphoma malignant lymphocytic			X																						
Lymphoma malignant mixed							X									X									
Lymphoma malignant undifferentiated cell type			X															X							

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 0 ppm (continued)

Number of Days on Study	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	7 3 2	
Carcass ID Number	4 8 2	4 8 3	4 8 4	4 9 1	4 9 2	4 9 3	5 0 1	5 0 2	5 0 3	5 0 4	5 1 1	5 2 1	5 2 2	5 2 3	5 3 1	5 3 2	5 3 3	5 4 2	5 4 3	5 5 3	5 5 4	5 6 1	5 4 1	5 5 1	5 5 2	Total Tissues/ Tumors
Special Senses System																										
Eye																										1
Harderian gland																							+			2
Adenocarcinoma																										1
Adenoma																							X			1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic																										1
Lymphoma malignant mixed	X											X						X			X					6
Lymphoma malignant undifferentiated cell type																										2

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm

Number of Days on Study	1 9 2	5 1 9	5 9 2	6 4 7	6 6 4	6 7 6	6 8 2	6 8 8	7 0 4	7 1 1	7 1 8	7 2 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	6 9 5	7 0 5	5 7 5	6 2 2	6 0 3	5 9 4	6 8 4	6 3 2	5 8 4	7 0 4	5 9 3	7 0 3	5 7 1	5 7 2	5 7 3	5 7 4	5 8 1	5 8 2	5 8 3	5 9 1	5 9 2	6 0 1	6 0 2	6 1 2	6 1 3
Alimentary System																									
Esophagus	M	+	+																						
Gallbladder	+	+	+																						
Intestine large	+	+	+																						
Intestine large, cecum	+	+	+																						
Intestine large, colon	+	+	+																						
Intestine large, rectum	+	+	+																						
Intestine small	+	+	+				+		+				+		+							+			
Intestine small, duodenum	+	+	+				+		+				+												
Intestine small, ileum	+	+	+				+		+				+		+							+			
Intestine small, jejunum	M	+	+				+		+				+												
Liver	+	+	+		+		+	+	+	+									+					+	+
Hepatocellular adenoma																								X	X
Hepatocellular adenoma, multiple																			X						
Mesentery		+																							
Pancreas	+	+	+																						
Salivary glands	M	+	+																						
Stomach	+	+	+			+				+													+		
Stomach, forestomach	+	+	+			+				+													+		
Papilloma squamous										X															
Stomach, glandular	+	+	+			+																	+		
Cardiovascular System																									
Heart	+	+	+		+																				
Endocrine System																									
Adrenal gland	+	+	+																						
Adrenal gland, cortex	+	+	+																						
Adrenal gland, medulla	+	+	+																						
Islets, pancreatic	+	+	+																						
Parathyroid gland	+	+	M																						
Pituitary gland	M	M	M																						
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+
General Body System																									
None																									
Genital System																									
Ovary	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Uterus	+	+	+		+	+	+			+			+	+	+						+		+		+
Polyp stromal																					X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	730	730	730	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	731	
Carcass ID Number	62111	62311	62313	63111	63411	64412	64511	65512	65513	65514	66111	66612	66613	66614	67111	67713	67714	68111	68812	68813	69111	69912	69913	70112	70212	Total Tissues/ Tumors
Alimentary System																										
Esophagus																										2
Gallbladder																										3
Intestine large																										3
Intestine large, cecum																										3
Intestine large, colon																										3
Intestine large, rectum																										3
Intestine small			+																+			+				11
Intestine small, duodenum			+																+			+				9
Intestine small, ileum			+																+			+				11
Intestine small, jejunum			+																+			+				8
Liver	+	+		+			+							+					+	+						18
Hepatocellular adenoma	X	X																	X	X						6
Hepatocellular adenoma, multiple							X																			2
Mesentery																										1
Pancreas																										3
Salivary glands																										2
Stomach																				+						7
Stomach, forestomach																				+						7
Papilloma squamous																				X						2
Stomach, glandular																				+						6
Cardiovascular System																										
Heart																										4
Endocrine System																										
Adrenal gland																										3
Adrenal gland, cortex																										3
Adrenal gland, medulla																										3
Islets, pancreatic																										3
Parathyroid gland																										2
Pituitary gland																										
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
General Body System																										
None																										
Genital System																										
Ovary	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	47
Uterus	+	+	+	+			+		+					+	+						+			+	+	24
Polyp stromal																										1

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	1 9 2	5 1 9	5 9 2	6 4 7	6 6 4	6 7 6	6 8 2	6 8 8	7 0 4	7 1 1	7 1 8	7 2 2	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0
Carcass ID Number	6 9 5	7 0 5	5 7 5	6 2 2	6 0 3	5 9 4	6 8 4	6 3 2	5 8 4	7 0 4	5 9 3	7 0 3	5 7 1	5 7 2	5 7 3	5 7 4	5 8 1	5 8 2	5 8 3	5 9 1	5 9 2	6 0 1	6 0 2	6 1 2	6 1 3
Hematopoietic System																									
Bone marrow	+	+	+																						
Lymph node	+	+	+	+	+			+	+	+	+		+		+		+	M			+	+			
Lymph node, mandibular	+	M	+	+	+					+											M				
Lymph node, mesenteric	M	+	M	+				+	+	+			+		+						+	+			
Spleen	M	+	+	+		+		+	+	+	+				+	+	+	+			+	+			
Hemangiosarcoma, metastatic, skeletal muscle			X																						
Thymus	M	+	M	+					+					+		+									
Integumentary System																									
Mammary gland	M	M	+					+																	
Adenocarcinoma								X																	
Skin	M	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+		+	+	+
Musculoskeletal System																									
Bone	+	+	+													+									
Skeletal muscle			+																						
Hemangiosarcoma			X																						
Nervous System																									
Brain	+	+	+		+						+														
Respiratory System																									
Lung	+	+	+				+				+	+											+		
Alveolar/bronchiolar adenoma							X																X		
Nose	+	+	+																						
Trachea	+	+	+																						
Special Senses System																									
Harderian gland																				+					
Adenoma																				X					
Urinary System																									
Kidney	+	+	+						+																
Urinary bladder	+	+	+		+																				
Systemic Lesions																									
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymphoma malignant histiocytic					X																				
Lymphoma malignant lymphocytic				X							X														
Lymphoma malignant mixed										X							X					X		
Lymphoma malignant undifferentiated cell type		X						X	X												X				

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 5,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	7 3 1	
Carcass ID Number	6 2 1	6 3 1	6 3 3	6 1 1	6 4 1	6 4 2	6 5 1	6 5 2	6 5 3	6 5 4	6 6 1	6 6 2	6 6 3	6 6 4	6 7 1	6 7 3	6 7 4	6 8 1	6 8 2	6 8 3	6 9 1	6 9 2	6 9 3	7 0 1	7 0 2	Total Tissues/ Tumors
Hematopoietic System																										
Bone marrow																										3
Lymph node			+																							15
Lymph node, mandibular			M																							5
Lymph node, mesenteric			+																							10
Spleen			+						+										+							17
Hemangiosarcoma, metastatic, skeletal muscle																										1
Thymus							+																M			6
Integumentary System																										
Mammary gland																										2
Adenocarcinoma																										1
Skin	+	M		+	+	+	+	+	+		+	+	+	+				+	+	+	+	+	+	+		40
Musculoskeletal System																										
Bone																										4
Skeletal muscle																										1
Hemangiosarcoma																										1
Nervous System																										
Brain																										5
Respiratory System																										
Lung																								+		8
Alveolar/bronchiolar adenoma																										2
Nose																										3
Trachea																										3
Special Senses System																										
Harderian gland																										1
Adenoma																										1
Urinary System																										
Kidney																										4
Urinary bladder																										4
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant lymphocytic							X																			3
Lymphoma malignant mixed																		X								4
Lymphoma malignant undifferentiated cell type			X																			X				6

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm

Number of Days on Study	5 5 5 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	5 6 7 1 1 4 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
	4 1 8 0 3 7 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Carcass ID Number	7 8 8 8 8 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	5 2 4 0 2 3 8 1 1 1 2 2 3 3 3 3 4 4 4 4 4 5 5 5 6
	4 5 4 3 4 4 2 1 2 3 1 2 1 2 3 4 1 2 3 4 5 1 2 3 1
Alimentary System	
Esophagus	M +
Gallbladder	+ + + + + + + M + + + + + + + + + + + + + + + +
Intestine large	+ +
Intestine large, cecum	+ +
Intestine large, colon	+ +
Intestine large, rectum	+ + + + + + + + + + + M + + + + + + + + + + + +
Intestine small	+ +
Intestine small, duodenum	+ +
Intestine small, ileum	+ + + M + + + M + + + + + + + + + + + + + + + +
Intestine small, jejunum	+ A +
Liver	+ +
Hepatocellular adenoma	X X
Mesentery	+
Pancreas	+ +
Salivary glands	+ +
Stomach	+ +
Stomach, forestomach	+ +
Papilloma squamous	
Stomach, glandular	+ +
Tooth	+
Cardiovascular System	
Heart	+ +
Endocrine System	
Adrenal gland	+ +
Adrenal gland, cortex	+ +
Adrenal gland, medulla	+ + + + + + + + + + + + + + + + + M + + + + + +
Islets, pancreatic	+ +
Parathyroid gland	+ M M + + + M + + M + + M + + M + + M + + M + + +
Pituitary gland	+ + + + + + + + + + + + M + + + + + + + + + + + M
Pars distalis, adenoma	
Thyroid gland	+ +
General Body System	
None	

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	7 6 2	7 6 3	7 7 1	7 7 2	7 7 3	7 7 4	7 8 1	7 9 1	7 9 2	8 0 1	8 0 2	8 1 1	8 1 2	8 1 3	8 1 4	8 1 5	8 2 1	8 2 2	8 2 3	8 3 1	8 3 2	8 3 3	8 4 1	8 4 2	8 4 3	Total Tissues/ Tumors
Alimentary System																										
Esophagus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Gallbladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	48
Intestine large	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine large, cecum	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Intestine large, colon	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Intestine large, rectum	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	47
Intestine small	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, duodenum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Intestine small, ileum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	48
Intestine small, jejunum	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Liver	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Hepatocellular adenoma																			X				X			4
Mesentery																										1
Pancreas	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Salivary glands	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Stomach, forestomach	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Papilloma squamous		X																								1
Stomach, glandular	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Tooth																										1
Cardiovascular System																										
Heart	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Endocrine System																										
Adrenal gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, cortex	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adrenal gland, medulla	M	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	47
Islets, pancreatic	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	49
Parathyroid gland	+	+	+	+	I	+	I	+	I	+	M	M	+	M	I	+	M	+	+	M	+	M	+	M	+	31
Pituitary gland	I	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Pars distalis, adenoma																									X	1
Thyroid gland	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
General Body System																										
None																										

Table D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	554	561	578	610	613	647	729	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730
Carcass ID Number	754	825	844	803	824	834	782	711	712	713	721	722	731	732	733	734	741	742	743	744	745	751	752	753	761
Genital System																									
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Cystadenoma																						X	X		
Granulosa cell tumor benign																									
Teratoma																									
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Sarcoma stromal																			X						
Hematopoietic System																									
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Osteosarcoma, metastatic, uncertain primary site		X																							
Lymph node	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mandibular	+	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lymph node, mesenteric	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Thymus	+	+	+	M	M	+	+	M	+	+	+	+	+	+	+	M	M	+	+	+	+	+	+	+	+
Integumentary System																									
Mammary gland	M	+	+	+	M	M	+	M	M	M	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+
Adenocarcinoma																									
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Musculoskeletal System																									
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Skeletal muscle	+	+																							
Osteosarcoma, metastatic, uncertain primary site		X																							
Nervous System																									
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	7 3 0	
Carcass ID Number	7 6 2	7 6 3	7 7 1	7 7 2	7 7 3	7 7 4	7 8 1	7 9 1	7 9 2	8 0 1	8 0 2	8 1 1	8 1 2	8 1 3	8 1 4	8 1 5	8 2 1	8 2 2	8 2 3	8 3 1	8 3 2	8 3 3	8 4 1	8 4 2	8 4 3	Total Tissues/ Tumors
Genital System																										
Ovary	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Cystadenoma							X																			3
Granulosa cell tumor benign													X													1
Teratoma														X												1
Uterus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Sarcoma stromal																										1
Hematopoietic System																										
Bone marrow	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, uncertain primary site																										1
Lymph node	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	49
Lymph node, mandibular	M	+	M	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	45
Lymph node, mesenteric	+	+	+	+	+	I	+	M	+	M	+	I	+	+	+	+	+	+	+	M	+	+	+	+	+	44
Spleen	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Thymus	+	+	+	+	+	+	M	+	+	+	+	M	+	+	+	+	M	+	+	+	+	+	M	+	+	41
Integumentary System																										
Mammary gland	M	+	+	+	M	M	+	+	+	+	+	M	+	+	+	+	+	+	+	M	+	+	+	+	+	37
Adenocarcinoma		X																								1
Skin	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Musculoskeletal System																										
Bone	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Skeletal muscle																										2
Osteosarcoma, metastatic, uncertain primary site																										1
Nervous System																										
Brain	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	5 5 5 6 6 6 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	5 6 7 1 1 4 2 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3 3
	4 1 8 0 3 7 9 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
Carcass ID Number	7 8 8 8 8 8 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	5 2 4 0 2 3 8 1 1 1 2 2 3 3 3 3 4 4 4 4 4 5 5 5 6
	4 5 4 3 4 4 2 1 2 3 1 2 1 2 3 4 1 2 3 4 5 1 2 3 1
Respiratory System	
Lung	+ +
Adenocarcinoma, metastatic, mammary gland	
Alveolar/bronchiolar adenoma	
Alveolar/bronchiolar carcinoma, multiple	X
Osteosarcoma, metastatic, uncertain primary site	X
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung	X
Nose	+ M M +
Trachea	+ +
Special Senses System	
Ear	+
Harderian gland	+
Adenoma	
Urinary System	
Kidney	+ +
Osteosarcoma, metastatic, uncertain primary site	X
Urinary bladder	+ +
Systemic Lesions	
Multiple organs	+ +
Lymphoma malignant histiocytic	X
Lymphoma malignant mixed	X X X X
Lymphoma malignant undifferentiated cell type	X

TABLE D2
Individual Animal Tumor Pathology of Female Mice in the 2-Year Feed Study of HC Yellow 4: 10,000 ppm (continued)

Number of Days on Study	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	730	
Carcass ID Number	76 2	76 3	77 1	77 2	77 3	77 4	78 1	79 1	79 2	80 1	80 2	81 1	81 2	81 3	81 4	81 5	82 1	82 2	82 3	83 1	83 2	83 3	84 1	84 2	84 3	Total Tissues/ Tumors
Respiratory System																										
Lung	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Adenocarcinoma, metastatic, mammary gland		X																								1
Alveolar/bronchiolar adenoma																			X							1
Alveolar/bronchiolar carcinoma, multiple																										1
Osteosarcoma, metastatic, uncertain primary site																										1
Mediastinum, alveolar/bronchiolar carcinoma, metastatic, lung																										1
Nose	+	+	+	+	+	+	M	+	+	M	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	46
Trachea	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Special Senses System																										
Ear																										1
Harderian gland													+													2
Adenoma													X													1
Urinary System																										
Kidney	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Osteosarcoma, metastatic, uncertain primary site																										1
Urinary bladder	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	M	+	+	+	+	+	+	+	+	+	49
Systemic Lesions																										
Multiple organs	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	50
Lymphoma malignant histiocytic																										1
Lymphoma malignant mixed	X																									5
Lymphoma malignant undifferentiated cell type						X																				2

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4

	0 ppm	5,000 ppm	10,000 ppm
Liver: Hepatocellular Adenoma			
Overall rates[a]	5/50 (10%)	8/18 (44%)[e]	4/50 (8%)
Adjusted rates[b]	11.6%		9.3%
Terminal rates[c]	5/43 (12%)		4/43 (9%)
First incidence (days)	730 (T)		730 (T)
Life table tests[d]			P=0.500N
Logistic regression tests[d]			P=0.500N
Fisher exact test[d]			P=0.500N
Liver: Hepatocellular Adenoma or Carcinoma			
Overall rates	6/50 (12%)	8/18 (44%)[e]	4/50 (8%)
Adjusted rates	14.0%		9.3%
Terminal rates	6/43 (14%)		4/43 (9%)
First incidence (days)	730 (T)		730 (T)
Life table tests			P=0.369N
Logistic regression tests			P=0.369N
Fisher exact test			P=0.370N
Lung: Alveolar/bronchiolar Adenoma			
Overall rates	3/50 (6%)	2/8 (25%)[e]	1/50 (2%)
Adjusted rates	7.0%		2.3%
Terminal rates	3/43 (7%)		1/43 (2%)
First incidence (days)	730 (T)		730 (T)
Life table tests			P=0.305N
Logistic regression tests			P=0.305N
Fisher exact test			P=0.309N
Lung: Alveolar/bronchiolar Adenoma or Carcinoma			
Overall rates	4/50 (8%)	2/8 (25%)[e]	2/50 (4%)
Adjusted rates	9.3%		4.4%
Terminal rates	4/43 (9%)		1/43 (2%)
First incidence (days)	730 (T)		610
Life table tests			P=0.335N
Logistic regression tests			P=0.331N
Fisher exact test			P=0.339N
Ovary: Cystadenoma			
Overall rates	2/50 (4%)	0/47 (0%)	3/50 (6%)
Adjusted rates	4.7%	0.0%	7.0%
Terminal rates	2/43 (5%)	0/36 (0%)	3/43 (7%)
First incidence (days)	730 (T)	–[f]	730 (T)
Life table tests	P=0.393	P=0.278N	P=0.500
Logistic regression tests	P=0.393	P=0.278N	P=0.500
Cochran-Armitage test[d]	P=0.391		
Fisher exact test		P=0.263N	P=0.500
Pituitary Gland (Pars Distalis): Adenoma			
Overall rates	5/42 (12%)	0/0[e]	1/45 (2%)
Adjusted rates	12.9%		2.6%
Terminal rates	4/36 (11%)		1/38 (3%)
First incidence (days)	463		730 (T)
Life table tests			P=0.094N
Logistic regression tests			P=0.163N
Fisher exact test			P=0.086N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Stomach (Forestomach): Squamous Papilloma			
Overall rates	3/50 (6%)	2/50 (4%)	1/50 (2%)
Adjusted rates	7.0%	5.1%	2.3%
Terminal rates	3/43 (7%)	1/38 (3%)	1/43 (2%)
First incidence (days)	730 (T)	718	730 (T)
Life table tests	P=0.227N	P=0.551N	P=0.305N
Logistic regression tests	P=0.214N	P=0.505N	P=0.305N
Cochran-Armitage test	P=0.222N		
Fisher exact test		P=0.500N	P=0.309N
All Organs: Malignant Lymphoma (Histiocytic, Lymphocytic, Mixed, or Undifferentiated Cell Type)			
Overall rates	9/50 (18%)	14/50 (28%)	8/50 (16%)
Adjusted rates	19.4%	30.6%	16.6%
Terminal rates	6/43 (14%)	7/38 (18%)	3/43 (7%)
First incidence (days)	466	519	554
Life table tests	P=0.437N	P=0.151	P=0.481N
Logistic regression tests	P=0.527N	P=0.160	P=0.594
Cochran-Armitage test	P=0.451N		
Fisher exact test		P=0.171	P=0.500N
All Organs: Benign Tumors			
Overall rates	21/50 (42%)	13/50 (26%)	11/50 (22%)
Adjusted rates	46.6%	32.3%	25.6%
Terminal rates	19/43 (44%)	11/38 (29%)	11/43 (26%)
First incidence (days)	463	682	730 (T)
Life table tests	P=0.021N	P=0.141N	P=0.027N
Logistic regression tests	P=0.014N	P=0.060N	P=0.020N
Cochran-Armitage test	P=0.019N		
Fisher exact test		P=0.069N	P=0.026N
All Organs: Malignant Tumors			
Overall rates	14/50 (28%)	15/50 (30%)	12/50 (24%)
Adjusted rates	29.2%	32.0%	24.0%
Terminal rates	9/43 (21%)	7/38 (18%)	5/43 (12%)
First incidence (days)	466	519	554
Life table tests	P=0.362N	P=0.435	P=0.395N
Logistic regression tests	P=0.473N	P=0.471	P=0.544N
Cochran-Armitage test	P=0.368N		
Fisher exact test		P=0.500	P=0.410N
All Organs: Benign or Malignant Tumors			
Overall rates	30/50 (60%)	24/50 (48%)	21/50 (42%)
Adjusted rates	61.2%	50.7%	42.0%
Terminal rates	24/43 (56%)	15/38 (39%)	14/43 (33%)
First incidence (days)	463	519	554
Life table tests	P=0.071N	P=0.321N	P=0.078N
Logistic regression tests	P=0.057N	P=0.163N	P=0.072N
Cochran-Armitage test	P=0.045N		
Fisher exact test		P=0.158N	P=0.055N

TABLE D3
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Feed Study of HC Yellow 4
(continued)

(T)Terminal sacrifice
[a] Number of tumor-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, bone marrow, brain, clitoral gland, epididymis, gallbladder (mouse), heart, kidney, larynx, liver, lung, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, spleen, testes, thyroid gland, and urinary bladder; for other tissues, denominator is number of animals necropsied.
[b] Kaplan-Meier estimated tumor incidence at the end of the study after adjustment for intercurrent mortality
[c] Observed 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 the controls and that dosed group. The life table analysis regards tumors in animals dying prior to terminal kill as being (directly or indirectly) the cause of death. The logistic regression tests regard these lesions as nonfatal. The Cochran-Armitage and Fisher exact tests compare directly the overall incidence rates. For all tests, a negative trend or a lower incidence in a dose group is indicated by N.
[e] Tissue was examined microscopically only when it was observed to be abnormal at necropsy.
[f] Not applicable; no tumors were found at the site in this group

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Disposition Summary			
Animals initially in study	70	70	70
6-month interim evaluation	10	10	10
15-month interim evaluation	10	10	10
Early deaths			
Natural deaths	4	2	2
Moribund kills	3	10	5
Survivors			
Terminal sacrifice	43	38	43
Animals examined microscopically	50	50	50
Alimentary System			
Gallbladder	(46)	(3)	(48)
Inflammation, chronic	10 (22%)		4 (8%)
Liver	(50)	(18)	(50)
Basophilic focus			1 (2%)
Clear cell focus	2 (4%)		
Fatty change	2 (4%)		
Inflammation, chronic active	17 (34%)		23 (46%)
Necrosis	4 (8%)	1 (6%)	13 (26%)
Mesentery	(2)	(1)	(1)
Fibrosis	1 (50%)		
Inflammation, chronic active	1 (50%)		
Necrosis	1 (50%)		
Pancreas	(48)	(3)	(49)
Inflammation, chronic	25 (52%)		22 (45%)
Salivary glands	(50)	(2)	(50)
Inflammation, chronic	4 (8%)		
Inflammation, chronic active	33 (66%)	1 (50%)	32 (64%)
Stomach, forestomach	(49)	(7)	(50)
Acanthosis	1 (2%)		3 (6%)
Hyperkeratosis	1 (2%)		2 (4%)
Hyperplasia, basal cell			2 (4%)
Hyperplasia, pseudoepitheliomatous			1 (2%)
Inflammation, chronic active	3 (6%)	1 (14%)	4 (8%)
Ulcer			1 (2%)
Cardiovascular System			
None			
Endocrine System			
Adrenal gland, cortex	(50)	(3)	(50)
Hyperplasia	3 (6%)		
Adrenal gland, medulla	(49)	(3)	(47)
Hyperplasia	1 (2%)		1 (2%)
Islets, pancreatic	(49)	(3)	(49)
Hyperplasia	1 (2%)		
Pituitary gland	(42)		(45)
Pars distalis, hyperplasia	9 (21%)		8 (18%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Endocrine System (continued)			
Thyroid gland	(48)	(49)	(50)
Inflammation, acute			2 (4%)
Inflammation, chronic	1 (2%)	6 (12%)	6 (12%)
Inflammation, chronic active			2 (4%)
C-cell, hyperplasia			1 (2%)
Follicle, cyst	1 (2%)	1 (2%)	1 (2%)
Follicle, pigmentation		48 (98%)	50 (100%)
Follicular cell, hyperplasia		3 (6%)	13 (26%)
Follicular cell, pigmentation		49 (100%)	50 (100%)
Interstitium, pigmentation		46 (94%)	50 (100%)
General Body System			
None			
Genital System			
Ovary	(50)	(47)	(50)
Angiectasis		2 (4%)	4 (8%)
Cyst	14 (28%)	17 (36%)	23 (46%)
Cyst, multiple	4 (8%)	4 (9%)	1 (2%)
Hemorrhage		9 (19%)	11 (22%)
Inflammation, chronic	16 (32%)	4 (9%)	2 (4%)
Mineralization		1 (2%)	2 (4%)
Thrombus			1 (2%)
Uterus	(50)	(24)	(50)
Endometriosis	1 (2%)		
Endometrium, hydrometra			10 (20%)
Endometrium, hyperplasia	41 (82%)	12 (50%)	36 (72%)
Endometrium, metaplasia, squamous			1 (2%)
Endometrium, thrombus	1 (2%)		
Hematopoietic System			
Bone marrow	(50)	(3)	(50)
Myelofibrosis	36 (72%)		42 (84%)
Lymph node	(49)	(15)	(49)
Renal, hyperplasia, lymphoid		1 (7%)	
Lymph node, mesenteric	(45)	(10)	(44)
Infiltration cellular, histiocyte	26 (58%)	2 (20%)	23 (52%)
Spleen	(49)	(17)	(50)
Hematopoietic cell proliferation	6 (12%)	3 (18%)	2 (4%)
Hyperplasia, lymphoid	11 (22%)	4 (24%)	3 (6%)
Pigmentation			1 (2%)
Thymus	(43)	(6)	(41)
Cyst	4 (9%)		1 (2%)
Integumentary System			
None			

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Feed Study of HC Yellow 4 (continued)

	0 ppm	5,000 ppm	10,000 ppm
Musculoskeletal System			
Bone	(50)	(4)	(50)
Joint, tarsal, hyperostosis		1 (25%)	
Nervous System			
Brain	(50)	(5)	(50)
Gliosis		1 (20%)	
Thalamus, mineralization	34 (68%)	2 (40%)	19 (38%)
Respiratory System			
Lung	(50)	(8)	(50)
Hemorrhage	3 (6%)		5 (10%)
Alveolar epithelium, hyperplasia	1 (2%)		1 (2%)
Nose	(47)	(3)	(46)
Inflammation, acute	9 (19%)	1 (33%)	2 (4%)
Special Senses System			
None			
Urinary System			
Kidney	(50)	(4)	(50)
Inflammation, chronic	45 (90%)	3 (75%)	48 (96%)
Metaplasia, osseous			1 (2%)
Urinary bladder	(48)	(4)	(49)
Inflammation, chronic	43 (90%)	2 (50%)	39 (80%)

[a] Incidences are expressed as the ratio of animals with lesions to the number of animals examined microscopically at the site.

APPENDIX E
GENETIC TOXICOLOGY

GENETIC TOXICOLOGY

SALMONELLA Protocol

Testing was performed as reported by Mortelmans *et al.* (1986). HC Yellow 4 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the *Salmonella typhimurium* tester strains (TA98, TA100, TA1535, TA1537) either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37° C prior to the addition of soft agar supplemented with *l*-histidine and *d*-biotin, and subsequent plating on minimal glucose agar plates. Incubation continued for an additional 48 hours.

Each trial consisted of triplicate plates of concurrent positive and negative controls and of at least five doses of HC Yellow 4. High dose was limited to 10,000 μg per plate. All assays were repeated.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants which was not dose-related, not reproducible, or of insufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment.

CHINESE HAMSTER OVARY CELL CYTOGENETICS ASSAYS

Testing was performed as reported by Galloway *et al.* (1985, 1987) and as presented briefly below. HC Yellow 4 was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was tested in cultured Chinese hamster ovary (CHO) cells for induction of sister chromatid exchanges (SCEs) and chromosomal aberrations (Abs), both in the presence and absence of Aroclor 1254-induced male Sprague-Dawley rat liver S9 and cofactor mix. Cultures were handled under gold lights to prevent photolysis of bromodeoxyuridine-substituted DNA. Each trial consisted of concurrent solvent and positive controls and of at least three doses of HC Yellow 4; the high dose was limited by toxicity.

In the SCE test without S9, CHO cells were incubated for 26 hours with HC Yellow 4 in McCoy's 5A medium supplemented with 10% fetal bovine serum, *l*-glutamine (2mM), and antibiotics. Bromodeoxyuridine (BrdU) was added 2 hours after culture initiation. After 26 hours, the medium containing HC Yellow 4 was removed and replaced with fresh medium plus BrdU and Colcemid, and incubation was continued for 2 hours. Cells were then harvested by mitotic shake-off, fixed, and stained with Hoechst 33258 and Giemsa. In the SCE test with S9, cells were incubated with HC Yellow 4, serum-free medium, and S9 for 2 hours. The medium was then removed and replaced with medium containing BrdU and no HC Yellow 4 and incubation proceeded for an additional 26 hours, with Colcemid present for the final 2 to 3 hours. Harvesting and staining procedures were the same as for cells treated without S9. For the SCE test, significant chemical-induced cell cycle delay was seen in the absence of S9; therefore, incubation time was lengthened in several of the cultures to ensure a sufficient number of scorable cells.

In the Abs test without S9, a delayed harvest protocol was used, based on the information obtained in the SCE tests. Cells were incubated in McCoy's 5A medium with HC Yellow 4 for 16.5 hours; Colcemid was added and incubation was continued for 2 hours. The cells were then harvested by mitotic shake-off, fixed, and stained with Giemsa. For the Abs test with S9, cells were treated with HC Yellow 4 and S9 for 2 hours, after which the treatment medium was removed and the cells incubated for 10.5 hours in fresh medium, with Colcemid present for the final 2 hours. Cells were harvested in the the same manner as for the treatment without S9.

Cells were selected for scoring on the basis of good morphology and completeness of karyotype (21 ± 2 chromosomes). All slides were scored blind and those from a single test were read by the same person. For the SCE test, 50 second-division metaphase cells were scored for frequency of SCE per cell from each dose level; 100 first-division metaphase cells were scored at each dose level for the Abs test. Classes of aberrations included simple (breaks and terminal deletions), complex (rearrangements and translocations), and other (pulverized cells, despiralized chromosomes, and cells containing 10 or more aberrations).

Statistical analyses were conducted on both the slopes of the dose-response curves and the individual dose points. An SCE frequency 20% above the concurrent solvent control value was chosen as a statistically conservative positive response. The probability of this level of difference occurring by chance at one dose point is less than 0.01; the probability for such a chance occurrence at two dose points is less than 0.001. Abs data are presented as percentage of cells with aberrations. As with SCE data, both the dose-response curve and individual dose points were statistically analyzed. For a single trial, a statistically significant ($P \leq 0.05$) difference for one dose point and a significant trend ($P \leq 0.015$) was considered weak evidence for a positive response (+w); significant differences for two or more doses indicated the trial was positive (+) (Galloway *et al.*, 1987).

Drosophila Protocol

The assays for induction of mutations and chromosomal translocations were performed as described in Zimmering *et al.* (1985). HC Yellow 4 was supplied as a coded aliquot from Radian Corporation (Austin, TX). Initially, HC Yellow 4 was assayed in the sex-linked recessive lethal (SLRL) test by feeding for 3 days to adult Canton-S wild-type males no more than 24 hours old at the beginning of treatment. Because no response was obtained, the chemical was retested by injection into adult males. Because treatment by injection produced a positive result, the chemical was assayed for induction of reciprocal translocations (RT) using this same method of exposure.

To administer a chemical by injection, a glass Pasteur pipette was drawn out in a flame to a microfine filament and the tip was broken off to allow delivery of the test solution. Injection was performed either manually, by attaching a rubber bulb to the other end of the pipette and forcing through sufficient solution (0.2 to 0.3 μL) to slightly distend the abdomen of the fly, or by attaching the pipette to a microinjector which automatically delivered a calibrated volume. Flies were anesthetized with ether and immobilized on a strip of double stick tape; the chemical was injected into the thorax under the wing with the aid of a dissecting microscope.

Toxicity tests were performed to set concentrations of HC Yellow 4 at a level which would induce 30% mortality after 72 hours of feeding or 24 hours after injection, while keeping induced sterility at an acceptable level. For the SLRL test, oral exposure was achieved by allowing Canton-S males (10 to 20 flies per vial) to feed for 72 hours on a solution of HC Yellow 4 dissolved in 40% ethanol and diluted with 5% sucrose. In the injection experiments, 24- to 72-hour-old Canton-S males were treated with a solution of HC Yellow 4 dissolved in 40% ethanol diluted with 0.7% saline, and were allowed to recover for 24 hours. Exposed males were mated to three *Basc* females for 3 days and given fresh females at 2-day intervals to produce three matings of 3, 2, and 2 days; sample sperm from successive matings were treated at successively earlier post-meiotic stages. F_1 heterozygous females were allowed to mate with their siblings and were then placed in individual vials. F_1 daughters from the same parental male were kept together to identify clusters. (A cluster occurs when a number of mutants from a given male result from a single spontaneous premeiotic mutation event, and is identified when the number of mutants from that male exceeds the number predicted by a Poisson distribution.) If a cluster was identified, all data from the male in question were discarded. Presumptive lethal mutations were identified as occurring in vials containing no wild-type males after 17 days; these were retested. The feeding and injection experiments combined resulted in the testing of approximately 5,000 treated and 5,000 control chromosomes. The only exceptions occurred when the results of the first experiment were clearly positive (induced frequency of recessive lethal mutations equal to or greater than 1%); then the second trial was not run.

Recessive lethal data were analyzed by the normal approximation to the binomial test (Margolin *et al.*, 1983). A test result was considered to be positive if the P value was less than 0.01 and the mutation frequency in the tested group was greater than 0.10%, or if the P value was less than 0.05 and the frequency in the treatment group was greater than 0.15%. A test was considered to be inconclusive if (a) the P value was between 0.01 and 0.05 but the frequency in the treatment group was between 0.10% and 0.15%, or (b) the P value was between 0.05 and 0.10 but the frequency in the treatment group was greater than 0.10%. A result was considered to be negative if the P value was greater than 0.10 or if the frequency in the treatment group was less than 0.10%.

For the RT test, the exposure regimen was the same as that for the SLRL test except that small mass matings were used (10 males and 20 females). Exposed males were mated to three X.Y,y; bw; st females for 3 days and discarded. The females were transferred to fresh medium every 3 to 4 days for a period of about 3 weeks to produce a total of six broods. The results of the SLRL test were used to narrow the germ cell stage most likely to be affected by the chemical; for example, if earlier germ cell stages seemed to exhibit increased sensitivity, mating of the males was continued and translocation tests carried out from the offspring derived from these earlier germ cell stages. F_1 males were mated individually to X.Y,y; bw; st females and the progeny were examined for missing classes, which indicate the induction of a translocation in a germ cell of the parental male. The translocation data were analyzed according to the conditional binomial test (Kastenbaum and Bowman, 1970).

RESULTS

HC Yellow 4 (3 to 10,000 μg/plate) was tested for induction of gene mutations in four strains of *Salmonella typhimurium* in a preincubation protocol with and without Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver S9; results were positive for strains TA100, TA1537, and TA98 with and without S9. An equivocal response was noted in TA1535 in the absence of S9 activation; with S9 from either species, results were negative (Table E1; Mortelmans *et al.*, 1986).

HC Yellow 4 induced SCE in CHO cells in the absence, but not in the presence, of S9 activation (Table E2). In the two trials without S9, a significant increase in SCE was observed only at the highest dose tested (167 or 200 μg/mL); these doses induced cell cycle delay and required an extended harvest to accumulate sufficient cells for analysis. With Aroclor 1254-induced male Sprague-Dawley rat liver S9, no significant increase in SCE was observed with concentrations of up to 1,700 μg/mL HC Yellow 4; cell cycle delay was not noted with S9. When tested for induction of Abs in CHO cells, HC Yellow 4 was negative with and without S9 (Table E3). In the trial conducted without S9, a dose-related increase in aberrations was noted, but this increase was not significant either by trend analysis (P=0.027) or peak response (P>0.05); a delayed harvest protocol was necessary to offset chemical-induced cell cycle delay. With S9, no cell cycle delay was observed in either trial, and the response observed at the highest nonlethal dose tested in the first trial (3,000 μg/mL) was not repeated in the second trial. A precipitate formed at the 2,500 μg/mL concentration in trial 2 and no viable cells were present in the 3,000 μg/mL cultures.

HC Yellow 4 induced SLRL mutations in germ cells of adult male *Drosophila melanogaster* when administered by injection at a dose of 10,000 ppm; results of the initial feeding test were negative (Table E4; Woodruff *et al.*, 1985). Following the positive result in the SLRL assay, HC Yellow 4 (10,000 ppm by injection) was tested for induction of RT in germ cells of male *D. melanogaster*; results of this assay were negative (Table E5; Woodruff *et al.*, 1985).

TABLE E1
Mutagenicity of HC Yellow 4 in *Salmonella typhimurium*[a]

Strain	Dose (μg/plate)	Revertants/plate[b] -S9 Trial 1	-S9 Trial 2	+10% hamster S9 Trial 1	+10% hamster S9 Trial 2	+10% rat S9 Trial 1	+10% rat S9 Trial 2
TA100	0	166 ± 7.0	154 ± 12.6	138 ± 10.6	147 ± 13.0	156 ± 12.0	139 ± 4.8
	3				199 ± 11.7		
	10				262 ± 9.4[c]		
	33				723 ± 10.7[c]		
	100	133 ± 2.8	157 ± 7.0	1,180 ± 27.8	1,103 ± 7.6[c]	153 ± 14.7	143 ± 9.0
	333	181 ± 7.8	186 ± 33.8[c]	1,096 ± 136.5	1,121 ± 145.4[c]	169 ± 2.3	152 ± 3.3[c]
	1,000	307 ± 8.7	257 ± 28.4[c]	918 ± 32.7		175 ± 6.1	189 ± 3.2[c]
	3,333	617 ± 38.5	528 ± 37.9[c]	583 ± 97.5		313 ± 20.6	302 ± 4.2[c]
	10,000	283 ± 21.7[d]	324 ± 22.2[c]	210 ± 31.7		369 ± 25.4	469 ± 94.0[c]
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control[e]		482 ± 13.4	421 ± 4.7	1,978 ± 31.5	1,307 ± 20.1	1,703 ± 202.1	764 ± 16.7
TA1535	0	39 ± 3.4	34 ± 3.5	16 ± 0.6	11 ± 2.1	16 ± 0.9	13 ± 2.6
	100	33 ± 1.7	26 ± 2.7	20 ± 2.7	15 ± 2.8	10 ± 3.2	12 ± 1.7
	333	31 ± 1.2	26 ± 0.9[c]	19 ± 1.0	20 ± 3.2[c]	13 ± 2.1	4 ± 1.3[c]
	1,000	39 ± 2.4	30 ± 4.4[c]	24 ± 4.3	20 ± 3.2[c]	14 ± 3.2	4 ± 1.7[c]
	3,333	64 ± 2.0	46 ± 5.0[c]	25 ± 2.2	22 ± 5.0[c]	14 ± 4.1	9 ± 1.5[c]
	10,000	87 ± 11.3	58 ± 16.4[c]	31 ± 4.9	30 ± 6.9[c]	41 ± 9.7[d]	23 ± 2.4[c]
Trial summary		Positive	Equivocal	Negative	Equivocal	Equivocal	Negative
Positive control		452 ± 25.5	394 ± 2.3	606 ± 23.6	486 ± 14.9	528 ± 24.8	307 ± 5.5
TA1537	0	13 ± 0.3	9 ± 1.5	16 ± 2.1	8 ± 0.6	12 ± 3.2	9 ± 1.5
	10		10 ± 2.0				
	33		13 ± 2.8[c]				
	100	36 ± 2.8	19 ± 0.9[c]	19 ± 4.4	13 ± 2.9	12 ± 2.7	5 ± 1.2
	333	82 ± 1.2	79 ± 6.7[c]	35 ± 4.2	29 ± 6.7[c]	18 ± 4.7	17 ± 4.9[c]
	1,000	237 ± 9.0	167 ± 10.7[c]	66 ± 6.8	62 ± 9.2[c]	38 ± 4.2	27 ± 1.5[c]
	3,333	388 ± 48.7		236 ± 19.9	181 ± 27.0[c]	124 ± 10.9	114 ± 11.7[c]
	10,000	172 ± 24.0[d]		229 ± 27.8[d]	248 ± 55.5[c]	283 ± 27.7	239 ± 11.8[c]
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control		382 ± 35.8	242 ± 23.5	367 ± 4.4	424 ± 22.5	308 ± 36.0	304 ± 2.9
TA98	0	28 ± 2.2	21 ± 2.3	29 ± 0.7	38 ± 3.5	36 ± 3.2	35 ± 1.3
	10		25 ± 4.1		38 ± 3.6		
	33		30 ± 3.5[c]		48 ± 7.4[c]		
	100	60 ± 4.7	40 ± 10.9[c]	96 ± 9.5	75 ± 6.4[c]	30 ± 1.8	37 ± 0.9
	333	145 ± 4.7	124 ± 10.4[c]	111 ± 9.7	105 ± 7.1[c]	41 ± 0.7	47 ± 4.7[c]
	1,000	298 ± 26.7	252 ± 6.9[c]	138 ± 12.9	154 ± 8.5[c]	65 ± 3.5	84 ± 5.9[c]
	3,333	556 ± 22.0		310 ± 49.1		192 ± 15.0	142 ± 7.5[c]
	10,000	473 ± 62.7[d]		323 ± 38.5[d]		386 ± 64.0	276 ± 35.4[d]
Trial summary		Positive	Positive	Positive	Positive	Positive	Positive
Positive control		767 ± 21.9	687 ± 40.0	1,503 ± 69.9	1,219 ± 34.6	1,080 ± 15.6	571 ± 22.3

[a] Study performed at SRI, International. The detailed protocol and these data are presented in Mortelmans *et al.* (1986). Cells and HC Yellow 4 or solvent (dimethylsulfoxide) were incubated in the absence of exogenous metabolic activation (-S9) or with Aroclor 1254-induced S9 from male Syrian hamster liver or male Sprague-Dawley rat liver. High dose was limited to 10,000 μg/plate; 0 μg/plate dose is the solvent control.

[b] Revertants are presented as mean ± standard error from three plates.

[c] Precipitate on plate

[d] Slight toxicity

[e] 2-aminoanthracene was used on all strains in the presence of S9. In the absence of metabolic activation, 4-nitro-*o*-phenylenediamine was tested on TA98, sodium azide was tested on TA100 and TA1535, and 9-aminoacridine was tested on TA1537.

TABLE E2
Induction of Sister Chromatid Exchanges in Chinese Hamster Ovary Cells by HC Yellow 4[a]

Compound	Dose (μg/mL)	Total Cells	No. of Chromosomes	No. of SCEs	SCEs/Chromosomes	SCEs/Cell	Hrs in BrdU	Relative SCEs/Chromosome (%)[b]
-S9								
Trial 1								
Summary: Weak positive								
Dimethylsulfoxide		50	1,034	450	0.43	9.0	26.0	
Mitomycin-C	0.0010	50	1,035	574	0.55	11.5	26.0	27.43
	0.0100	5	105	241	2.29	48.2	26.0	427.40
HC Yellow 4	16.7	50	1,032	427	0.41	8.5	26.0	-4.93
	50.0	50	1,028	463	0.45	9.3	33.0	3.49
	167.0	50	1,030	575	0.55	11.5	33.0	28.27*
								P=0.000[d]
Trial 2								
Summary: Positive								
Dimethylsulfoxide		50	1,031	467	0.45	9.3	26.0	
Mitomycin-C	0.0010	50	1,034	631	0.61	12.6	26.0	34.73
	0.0100	5	105	226	2.15	45.2	26.0	375.19
HC Yellow 4	50.0	50	1,037	509	0.49	10.2	26.0	8.36
	100.0	50	1,033	559	0.54	11.2	32.5[c]	19.47
	200.0	50	1,026	609	0.59	12.2	32.5[c]	31.04*
								P=0.000
+S9								
Trial 1								
Summary: Negative								
Dimethylsulfoxide		50	1,033	440	0.42	8.8	26.0	
Cyclophosphamide	0.4	50	1,035	601	0.58	12.0	26.0	36.33
	2.0	5	104	135	1.29	27.0	26.0	204.75
HC Yellow 4								
	167.0	50	1,037	395	0.38	7.9	26.0	-10.58
	500.0	50	1,029	401	0.38	8.0	26.0	-8.51
	1,700.0	50	1,033	421	0.40	8.4	26.0	-4.32
								P=0.696

* Positive (≥20% increase over solvent control)
[a] Study performed at Litton Bionetics, Inc. SCE = sister chromatid exchange; BrdU = bromodeoxyuridine. A detailed description of the SCE protocol is presented by Galloway *et al.* (1985, 1987).
[b] Percent increase in SCEs/chromosome of culture exposed to HC Yellow 4 relative to those of culture exposed to solvent.
[c] Because HC Yellow 4 induced significant cell cylce delay, incubation time was lengthened to ensure a sufficient number of scorable (second-division metaphase) cells.
[d] Significance of relative SCEs/chromosome tested by the linear regression trend test vs. log of the dose

TABLE E3
Induction of Chromosomal Aberrations in Chinese Hamster Ovary Cells by HC Yellow 4[a]

-S9					+S9				
Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs	Dose (μg/mL)	Total Cells	No. of Abs	Abs/ Cell	Percent Cells with Abs
Trial 1 – Harvest time: 18.5 hours Summary: Negative					Trial 1 – Harvest time: 12.5 hours Summary: Weak positive				
Dimethylsulfoxide					Dimethylsulfoxide				
	100	1	0.01	1.0		100	1	0.01	1.0
Mitomycin-C					Cyclophosphamide				
0.0400	100	24	0.24	16.0	7.5	100	9	0.09	5.0
0.0625	25	15	0.60	36.0	37.5	25	20	0.80	32.0
HC Yellow 4					HC Yellow 4				
400	100	1	0.01	1.0	1,000	100	4	0.04	4.0
500	100	2	0.02	2.0	2,000	100	10	0.10	5.0
600	100	5	0.05	5.0	3,000	100	14	0.14	12.0°
				P=0.027[c]					P=0.001
					Trial 2 – Harvest time: 12.5 hours Summary: Negative				
					Dimethylsulfoxide				
						100	3	0.03	3.0
					Cyclophosphamide				
					7.5	100	17	0.17	14.0
					37.5	25	15	0.60	36.0
					HC Yellow 4				
					1,500	100	5	0.05	5.0
					2,000	100	2	0.02	2.0
					2,500[d]	100	3	0.03	3.0
					3,000	0			
									P=0.660

° Positive ($P \leq 0.05$)

[a] Study performed at Litton Bionetics, Incorporated. Abs = aberrations. A detailed presentation of the technique for detecting chromosomal aberrations is found in Galloway *et al.* (1985, 1987).

[b] Because HC Yellow 4 induced significant cell cycle delay, incubation time was lengthened to ensure a sufficient number of scorable (first-division metaphase) cells.

[c] Significance of percent cells with aberrations tested by the linear regression trend test vs. log of the dose

[d] Precipitate formed at this concentration.

TABLE E4
Induction of Sex-Linked Recessive Lethal Mutations in *Drosophila melanogaster* by HC Yellow 4[a]

Route of Exposure	Dose (ppm)	Incidence of Deaths (%)	Incidence of Sterility (%)	No. of Lethals/No. of X Chromosomes Tested Mating 1	Mating 2	Mating 3	Total[b]
Feeding	10,000	2	0	0/2,182	2/2,128	1/2,087	3/6,397 (0.05%)
	0			1/2,353	2/1,959	1/1,846	4/6,158 (0.06%)
Injection	10,000	0	0	4/2,075	3/1,995	3/1,834	10/5,904 (0.17%)*
	0			0/1,880	0/1,863	1/1,561	1/5,304 (0.02%)

* Results were significant at the 5% level (Margolin *et al.*, 1983).
[a] Study performed at Bowling Green State University. A detailed protocol of the sex-linked recessive lethal assay and these data are presented in Woodruff *et al.* (1985). Results of the feeding experiment were not significant at the 5% level (Margolin *et al.*, 1983).
[b] Combined total number of lethal mutations/number of X chromosomes tested for three mating trials.

TABLE E5
Induction of Reciprocal Translocations in *Drosophila melanogaster* by HC Yellow 4[a]

Route of Exposure	Dose (ppm)	Transfers Translocations/Total F_1 Tested 1	2	3	4	5	6	No. of Tests	Total No. of Translocations	Total Translocations (%)
Injection	10,000	0/914	0/959	0/1,075	0/1,045	0/927	0/0	4,920	0	0.00
Concurrent control								23,686	1	0.00
Historical control								116,163	2	0.00

[a] Study performed at Bowling Green State University. A detailed protocol of the reciprocal translocation assay and these data are presented in Woodruff *et al.* (1985). Results were not significant at the 5% level (Kastenbaum and Bowman, 1970).

APPENDIX F
ORGAN WEIGHTS
AND ORGAN-WEIGHT-TO-BODY-WEIGHT RATIOS

TABLE F1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 14-Day Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	183 ± 8	175 ± 6	170 ± 5	150 ± 6**	107 ± 3	74 ± 3**
Brain						
Absolute	1.69 ± 0.04	1.72 ± 0.02	1.74 ± 0.03	1.63 ± 0.01	1.62 ± 0.02	1.54 ± 0.04**
Relative	9.27 ± 0.20	9.83 ± 0.30	10.25 ± 0.17	10.95 ± 0.41**	15.15 ± 0.32**	20.85 ± 0.58**
Heart						
Absolute	0.66 ± 0.04	0.62 ± 0.02	0.61 ± 0.03	0.55 ± 0.04	0.38 ± 0.02	0.59 ± 0.33
Relative	3.61 ± 0.08	3.55 ± 0.08	3.58 ± 0.12	3.63 ± 0.11	3.61 ± 0.23	8.08 ± 4.50
R. Kidney						
Absolute	0.83 ± 0.06	0.84 ± 0.04	0.86 ± 0.03	0.73 ± 0.04	0.56 ± 0.02**	0.44 ± 0.02**
Relative	4.53 ± 0.21	4.80 ± 0.05	5.06 ± 0.03*	4.82 ± 0.11*	5.27 ± 0.06**	5.98 ± 0.13**
Liver						
Absolute	9.93 ± 0.69	9.69 ± 0.37	10.32 ± 0.64	8.79 ± 0.50	6.31 ± 0.17**	4.51 ± 0.21**
Relative	54.1 ± 2.1	55.4 ± 2.0	60.5 ± 2.2	58.4 ± 1.4	59.1 ± 2.4	60.8 ± 1.0*
Lungs						
Absolute	0.94 ± 0.07	1.17 ± 0.13	1.25 ± 0.11	1.22 ± 0.11	0.97 ± 0.12	0.89 ± 0.17
Relative	5.13 ± 0.31	6.69 ± 0.68	7.37 ± 0.59	8.06 ± 0.46*	9.01 ± 1.07**	11.78 ± 1.70**
R. Testis						
Absolute	1.08 ± 0.04	1.10 ± 0.04	1.05 ± 0.04	1.05 ± 0.03	0.77 ± 0.06**	0.34 ± 0.03**
Relative	5.91 ± 0.12	6.27 ± 0.21	6.17 ± 0.25	7.02 ± 0.26	7.20 ± 0.51*	4.65 ± 0.39*
Thymus						
Absolute	0.38 ± 0.06	0.40 ± 0.04	0.36 ± 0.05	0.29 ± 0.01	0.18 ± 0.02**	0.02 ± 0.00**
Relative	2.12 ± 0.35	2.29 ± 0.19	2.07 ± 0.27	1.91 ± 0.06	1.71 ± 0.15	0.31 ± 0.03**
Female						
n	5	5	5	5	5	4
Necropsy body wt	148 ± 2	140 ± 5	132 ± 2**	131 ± 3**	101 ± 3**	77 ± 5**
Brain						
Absolute	1.42 ± 0.24	1.70 ± 0.03	1.49 ± 0.13	1.58 ± 0.04	1.56 ± 0.01	1.51 ± 0.02
Relative	9.59 ± 1.64	12.13 ± 0.31	11.26 ± 0.93	12.07 ± 0.26	15.48 ± 0.39**	20.02 ± 1.40**
Heart						
Absolute	0.59 ± 0.01	0.52 ± 0.03**	0.50 ± 0.02**	0.49 ± 0.01**	0.38 ± 0.01**	0.29 ± 0.02**
Relative	4.01 ± 0.13	3.68 ± 0.11	3.75 ± 0.09	3.71 ± 0.07	3.78 ± 0.11	3.73 ± 0.13
R. Kidney						
Absolute	0.69 ± 0.01	0.64 ± 0.03	0.62 ± 0.02*	0.63 ± 0.02*	0.51 ± 0.02**	0.42 ± 0.02**
Relative	4.66 ± 0.10	4.58 ± 0.11	4.68 ± 0.10	4.77 ± 0.07	5.06 ± 0.11**	5.47 ± 0.06**
Liver						
Absolute	7.21 ± 0.34	7.27 ± 0.69	6.23 ± 0.26	6.70 ± 0.26	5.47 ± 0.19*	4.30 ± 0.83**
Relative	48.6 ± 2.3	51.8 ± 4.5	47.1 ± 1.4	50.9 ± 1.1	54.2 ± 1.8	54.6 ± 8.4
Lungs						
Absolute	1.11 ± 0.07	0.94 ± 0.09	0.91 ± 0.05	1.24 ± 0.10	0.88 ± 0.04	0.89 ± 0.15
Relative	7.51 ± 0.47	6.71 ± 0.59	6.90 ± 0.36	9.44 ± 0.64	8.65 ± 0.31	11.41 ± 1.37**
Thymus						
Absolute	0.38 ± 0.04	0.39 ± 0.15	0.34 ± 0.04	0.29 ± 0.05	0.27 ± 0.06	0.08 ± 0.02*
Relative	2.56 ± 0.27	2.70 ± 0.93	2.60 ± 0.29	2.21 ± 0.39	2.70 ± 0.64	1.09 ± 0.27

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 13-Week Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male						
n	10	10	10	9	10	10
Necropsy body wt	348 ± 7	356 ± 6	341 ± 6	316 ± 6**	273 ± 9**	244 ± 5**
Brain						
Absolute	1.94 ± 0.01	1.95 ± 0.02	1.92 ± 0.02	1.92 ± 0.02	1.89 ± 0.01*	1.80 ± 0.01**
Relative	5.61 ± 0.13	5.48 ± 0.08	5.65 ± 0.08	6.10 ± 0.08*	6.99 ± 0.27**	7.42 ± 0.11**
Heart						
Absolute	0.93 ± 0.02	0.97 ± 0.03[b]	0.94 ± 0.02	0.95 ± 0.03	0.91 ± 0.03	0.78 ± 0.02**
Relative	2.67 ± 0.04	2.75 ± 0.07[b]	2.75 ± 0.06	2.99 ± 0.11*	3.36 ± 0.18**	3.20 ± 0.07**
R. Kidney						
Absolute	1.14 ± 0.03	1.28 ± 0.02	1.23 ± 0.04	1.14 ± 0.03	1.10 ± 0.02	1.09 ± 0.03
Relative	3.28 ± 0.12	3.61 ± 0.07*	3.59 ± 0.08*	3.61 ± 0.07*	4.05 ± 0.12**	4.48 ± 0.05**
Liver						
Absolute	13.83 ± 0.25	15.35 ± 0.58	14.91 ± 0.52	13.78 ± 0.36	12.93 ± 0.40	13.82 ± 0.36
Relative	39.8 ± 0.9	43.1 ± 1.3	43.7 ± 1.3*	43.9 ± 0.7*	47.6 ± 2.0**	56.6 ± 0.9**
Lung						
Absolute	1.45 ± 0.06	1.67 ± 0.07	1.51 ± 0.04[b]	1.62 ± 0.12	1.54 ± 0.07	1.52 ± 0.08
Relative	4.21 ± 0.23	4.70 ± 0.19	4.47 ± 0.16[b]	5.05 ± 0.38*	5.65 ± 0.21**	6.20 ± 0.27**
R. Testis						
Absolute	1.47 ± 0.02[b]	1.37 ± 0.08	1.52 ± 0.02	1.49 ± 0.03	1.47 ± 0.03[b]	1.44 ± 0.02
Relative	4.20 ± 0.10[b]	3.87 ± 0.25	4.45 ± 0.06	4.72 ± 0.11*	5.43 ± 0.23**[b]	5.93 ± 0.09**
Thymus						
Absolute	0.26 ± 0.02	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.30 ± 0.02	0.23 ± 0.01
Relative	0.74 ± 0.04	0.69 ± 0.02	0.70 ± 0.03	0.79 ± 0.04	1.09 ± 0.05**	0.96 ± 0.03**
Female						
n	10	10	10	10	10	10
Necropsy body wt	200 ± 4	209 ± 3	195 ± 3	197 ± 3	188 ± 2**	177 ± 2**
Brain						
Absolute	1.79 ± 0.02[b]	1.78 ± 0.03	1.76 ± 0.01	1.79 ± 0.02[b]	1.76 ± 0.02	1.70 ± 0.02**
Relative	8.86 ± 0.12[b]	8.54 ± 0.15	9.02 ± 0.10	9.00 ± 0.09[b]	9.37 ± 0.14**	9.61 ± 0.14**
Heart						
Absolute	0.62 ± 0.01	0.63 ± 0.02[b]	0.63 ± 0.01	0.65 ± 0.01[b]	0.57 ± 0.01*[b]	0.57 ± 0.01*
Relative	3.11 ± 0.07	3.03 ± 0.09[b]	3.25 ± 0.09	3.27 ± 0.06[b]	3.03 ± 0.06[b]	3.23 ± 0.06
R. Kidney						
Absolute	0.70 ± 0.03	0.75 ± 0.01[b]	0.73 ± 0.01	0.73 ± 0.01	0.70 ± 0.01	0.72 ± 0.02
Relative	3.50 ± 0.13	3.60 ± 0.05[b]	3.73 ± 0.05	3.71 ± 0.06	3.72 ± 0.05	4.06 ± 0.09**
Liver						
Absolute	7.84 ± 0.28	7.93 ± 0.36	6.77 ± 0.11**	6.93 ± 0.11*	6.96 ± 0.09*	7.99 ± 0.22
Relative	39.1 ± 1.1	37.9 ± 1.5	34.8 ± 0.8	35.2 ± 0.6	37.1 ± 0.6	45.0 ± 1.0**
Lungs						
Absolute	1.10 ± 0.05	1.37 ± 0.05**	1.20 ± 0.03	1.32 ± 0.08*	1.24 ± 0.06	1.32 ± 0.06*
Relative	5.48 ± 0.22	6.57 ± 0.21*	6.17 ± 0.16*	6.67 ± 0.34**	6.64 ± 0.29**	7.46 ± 0.35**
Thymus						
Absolute	0.18 ± 0.01	0.20 ± 0.01	0.23 ± 0.01**	0.22 ± 0.01*	0.21 ± 0.01	0.20 ± 0.01
Relative	0.91 ± 0.03	0.97 ± 0.05	1.16 ± 0.05**	1.11 ± 0.05**	1.12 ± 0.05**	1.14 ± 0.03**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test

** $P \leq 0.01$

[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

[b]

TABLE F3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

	0 ppm	2,500 ppm	5,000 ppm
Male			
n	9	10	10
Necropsy body wt	463 ± 8	473 ± 7	452 ± 8
Brain			
Absolute	2.11 ± 0.02	2.06 ± 0.02	2.03 ± 0.03
Relative	4.56 ± 0.10	4.37 ± 0.08	4.50 ± 0.08
R. Kidney			
Absolute	1.52 ± 0.02	1.43 ± 0.03	1.42 ± 0.04
Relative	3.30 ± 0.08	3.04 ± 0.08	3.15 ± 0.07
Liver			
Absolute	15.81 ± 0.34	15.61 ± 0.33	15.13 ± 0.42
Relative	34.2 ± 0.8	33.0 ± 0.6	33.5 ± 0.6

	0 ppm	5,000 ppm	10,000 ppm
Female			
n	10	10	10
Necropsy body wt	328 ± 6	314 ± 8	297 ± 6**
Brain			
Absolute	1.85 ± 0.02	1.85 ± 0.02	1.87 ± 0.01
Relative	5.65 ± 0.11	5.92 ± 0.13	6.33 ± 0.14**
R. Kidney			
Absolute	0.888 ± 0.024	0.812 ± 0.016**	0.805 ± 0.015**
Relative	2.70 ± 0.05	2.59 ± 0.05	2.72 ± 0.03
Liver			
Absolute	9.30 ± 0.27	9.57 ± 0.33	9.76 ± 0.19
Relative	28.3 ± 0.4	30.5 ± 0.8*	33.0 ± 0.6**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

TABLE F4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 14-Day Feed Studies of HC Yellow 4[a]

	0 ppm	1,250 ppm	2,500 ppm	5,000 ppm	10,000 ppm	20,000 ppm
Male						
n	5	5	5	5	5	5
Necropsy body wt	26.2 ± 0.6	28.2 ± 0.3	28.4 ± 0.3	28.3 ± 0.2	27.0 ± 0.3	22.9 ± 0.9**
Brain						
Absolute	0.460 ± 0.004	0.451 ± 0.009	0.475 ± 0.007	0.475 ± 0.008	0.471 ± 0.010	0.462 ± 0.005
Relative	17.6 ± 0.3	16.0 ± 0.4	16.7 ± 0.3	16.8 ± 0.4	17.4 ± 0.3	20.3 ± 0.7**
Heart						
Absolute	0.133 ± 0.006	0.150 ± 0.008	0.166 ± 0.009*	0.166 ± 0.012*	0.153 ± 0.003	0.134 ± 0.005
Relative	5.08 ± 0.18	5.33 ± 0.25	5.85 ± 0.33	5.88 ± 0.43	5.66 ± 0.13	5.85 ± 0.22
R. Kidney						
Absolute	0.278 ± 0.010	0.300 ± 0.013	0.327 ± 0.006**	0.323 ± 0.008*	0.292 ± 0.009	0.243 ± 0.009
Relative	10.6 ± 0.2	10.6 ± 0.4	11.5 ± 0.2	11.4 ± 0.3	10.8 ± 0.3	10.6 ± 0.3
Liver						
Absolute	1.34 ± 0.02	1.57 ± 0.04**	1.78 ± 0.05**	1.83 ± 0.04**	1.66 ± 0.07**	1.34 ± 0.04
Relative	51.3 ± 0.2	55.6 ± 1.5*	62.5 ± 1.2**	64.8 ± 0.9**	61.4 ± 2.2**	58.5 ± 0.8**
Lungs						
Absolute	0.174 ± 0.007	0.280 ± 0.041**	0.228 ± 0.007	0.231 ± 0.005	0.208 ± 0.009	0.223 ± 0.029
Relative	6.64 ± 0.19	9.93 ± 1.48	8.02 ± 0.20	8.17 ± 0.23	7.70 ± 0.33	9.95 ± 1.72
R. Testis						
Absolute	0.108 ± 0.006	0.108 ± 0.003	0.119 ± 0.002	0.115 ± 0.004	0.115 ± 0.004	0.112 ± 0.002
Relative	4.12 ± 0.23	3.83 ± 0.07	4.18 ± 0.05	4.08 ± 0.18	4.26 ± 0.13	4.92 ± 0.18**
Thymus						
Absolute	0.039 ± 0.006	0.050 ± 0.002	0.056 ± 0.003	0.046 ± 0.005	0.049 ± 0.006	0.035 ± 0.004
Relative	1.5 ± 0.2	1.8 ± 0.1	2.0 ± 0.1	1.6 ± 0.2	1.8 ± 0.2	1.6 ± 0.2
Female						
n	5	5	5	5	5	5
Necropsy body wt	20.6 ± 0.4	21.7 ± 0.6	20.1 ± 0.4	20.8 ± 0.1	20.0 ± 0.3	20.8 ± 0.5
Brain						
Absolute	0.507 ± 0.013	0.487 ± 0.010	0.484 ± 0.008	0.502 ± 0.007	0.500 ± 0.008	0.517 ± 0.026
Relative	24.5 ± 0.6	22.5 ± 0.7	24.2 ± 0.7	24.2 ± 0.3	25.1 ± 0.4	24.8 ± 0.7
Heart						
Absolute	0.134 ± 0.011	0.127 ± 0.010	0.136 ± 0.010	0.131 ± 0.004	0.125 ± 0.003	0.147 ± 0.016
Relative	6.48 ± 0.45	5.91 ± 0.51	6.79 ± 0.59	6.28 ± 0.21	6.26 ± 0.14	7.03 ± 0.62
R. Kidney						
Absolute	0.196 ± 0.009	0.199 ± 0.005	0.200 ± 0.011	0.209 ± 0.006	0.187 ± 0.005	0.202 ± 0.015
Relative	9.51 ± 0.47	9.19 ± 0.34	9.96 ± 0.60	10.05 ± 0.25	9.35 ± 0.13	9.72 ± 0.61
Liver						
Absolute	1.17 ± 0.05	1.32 ± 0.04	1.30 ± 0.01	1.35 ± 0.05	1.25 ± 0.05	1.44 ± 0.08**
Relative	56.4 ± 1.8	61.0 ± 1.3	65.0 ± 1.2**	64.8 ± 2.1**	62.8 ± 2.0**	69.4 ± 2.2**
Lungs						
Absolute	0.231 ± 0.018	0.221 ± 0.011	0.249 ± 0.016	0.221 ± 0.010	0.219 ± 0.010	0.231 ± 0.022
Relative	11.2 ± 0.9	10.3 ± 0.8	12.4 ± 0.9	10.6 ± 0.4	11.0 ± 0.6	11.1 ± 0.8
Thymus						
Absolute	0.084 ± 0.007	0.063 ± 0.016	0.083 ± 0.003	0.077 ± 0.006	0.073 ± 0.008	0.093 ± 0.014
Relative	4.08 ± 0.30	2.94 ± 0.78	4.11 ± 0.14	3.68 ± 0.29	3.69 ± 0.43	4.45 ± 0.64

* Significantly different ($P \leq 0.05$) from the control group by Wiliams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

TABLE F5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 13-Week Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm	20,000 ppm	40,000 ppm	80,000 ppm
Male						
n	10	10	10	10	9	2
Necropsy body wt	28.2 ± 0.5	29.7 ± 0.7	27.6 ± 0.6	27.9 ± 0.5	26.4 ± 0.5	21.3 ± 1.1
Brain						
Absolute	0.463 ± 0.005	0.455 ± 0.004	0.453 ± 0.011	0.446 ± 0.008[b]	0.439 ± 0.007	0.502 ± 0.060
Relative	16.5 ± 0.3	15.4 ± 0.3	16.5 ± 0.5	16.1 ± 0.4[b]	16.7 ± 0.4	23.5 ± 1.6**
Heart						
Absolute	0.144 ± 0.005	0.155 ± 0.007[b]	0.149 ± 0.007	0.133 ± 0.006	0.121 ± 0.004*	0.124 ± 0.026
Relative	5.10 ± 0.14	5.23 ± 0.19[b]	5.40 ± 0.24	4.75 ± 0.21	4.61 ± 0.19	5.76 ± 0.92
R. Kidney						
Absolute	0.251 ± 0.011	0.283 ± 0.005	0.258 ± 0.006[b]	0.230 ± 0.006	0.210 ± 0.008**	0.162 ± 0.005**
Relative	8.87 ± 0.29	9.61 ± 0.31	9.49 ± 0.20[b]	8.21 ± 0.15	7.96 ± 0.28*	7.59 ± 0.16*
Liver						
Absolute	1.23 ± 0.06	1.52 ± 0.05**	1.42 ± 0.04*	1.30 ± 0.04[b]	1.35 ± 0.05	1.03 ± 0.08
Relative	43.5 ± 1.6	51.4 ± 1.5**	51.4 ± 1.2**	46.6 ± 0.7[b]	51.1 ± 1.5**	48.5 ± 1.1
Lungs						
Absolute	0.211 ± 0.011	0.249 ± 0.017	0.251 ± 0.015	0.230 ± 0.009	0.231 ± 0.012	0.309 ± 0.058*
Relative	7.47 ± 0.33	8.41 ± 0.56	9.11 ± 0.50	8.13 ± 0.32	8.84 ± 0.55	14.40 ± 1.98**
R. Testis						
Absolute	0.117 ± 0.002	0.112 ± 0.002	0.114 ± 0.004	0.111 ± 0.004	0.113 ± 0.004[c]	0.095 ± 0.012*
Relative	4.15 ± 0.06	3.78 ± 0.07*	4.15 ± 0.13	3.98 ± 0.09	4.28 ± 0.07[c]	4.42 ± 0.35
Thymus						
Absolute	0.033 ± 0.002	0.041 ± 0.002*	0.041 ± 0.003*	0.043 ± 0.002**	0.042 ± 0.003**	0.043 ± 0.010
Relative	1.16 ± 0.07	1.38 ± 0.07	1.48 ± 0.10*	1.56 ± 0.08**	1.61 ± 0.10**	2.02 ± 0.37**
Female						
n	10	10	10	9	9	3
Necropsy body wt	24.8 ± 0.6	23.8 ± 0.3	22.6 ± 0.2**	21.0 ± 0.3**	18.7 ± 0.2**	17.2 ± 0.8**
Brain						
Absolute	0.479 ± 0.004	0.471 ± 0.006	0.443 ± 0.008**	0.470 ± 0.006*	0.433 ± 0.006**	0.431 ± 0.011**
Relative	19.4 ± 0.5	19.8 ± 0.2	19.6 ± 0.4	22.3 ± 0.4**	23.2 ± 0.3**	25.1 ± 0.6**
Heart						
Absolute	0.114 ± 0.002[b]	0.121 ± 0.005	0.115 ± 0.003	0.116 ± 0.006	0.096 ± 0.003**	0.092 ± 0.009*
Relative	4.63 ± 0.10[b]	5.06 ± 0.16	5.08 ± 0.15	5.51 ± 0.27**	5.16 ± 0.15**	5.34 ± 0.28
R. Kidney						
Absolute	0.181 ± 0.006	0.185 ± 0.004	0.170 ± 0.004[b]	0.158 ± 0.003**	0.136 ± 0.003**	0.136 ± 0.010**
Relative	7.31 ± 0.22	7.78 ± 0.13	7.50 ± 0.17[b]	7.46 ± 0.11	7.25 ± 0.18	7.87 ± 0.25
Liver						
Absolute	1.245 ± 0.043	1.205 ± 0.049	1.136 ± 0.027	1.115 ± 0.089	0.810 ± 0.021**	0.883 ± 0.080**
Relative	50.2 ± 1.0	50.5 ± 1.7	50.2 ± 0.9	52.7 ± 4.1	43.3 ± 1.0	51.3 ± 3.7
Lungs						
Absolute	0.233 ± 0.017	0.255± 0.014	0.244 ± 0.015	0.258 ± 0.024	0.192 ± 0.022	0.176 ± 0.005
Relative	9.43 ± 0.69	10.66 ± 0.51	10.79 ± 0.64	12.14 ± 1.05	10.23 ± 1.16	10.30 ± 0.64
Thymus						
Absolute	0.043 ± 0.003	0.044 ± 0.003	0.036 ± 0.002	0.041 ± 0.001	0.047 ± 0.003	0.048 ± 0.006
Relative	1.74 ± 0.11	1.83 ± 0.10	1.60 ± 0.08	1.93 ± 0.04	2.52 ± 0.13**	2.80 ± 0.31**

* Significantly different (P≤0.05) from the control group by Williams' or Dunnett's test
** P≤0.01
[a] Organ weights and body weights are given in grams; organ-weight-to-body weight ratios are given as mg organ weight/g body weight (mean ± standard error).
[b] n=9
[c] n=8

TABLE F6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

	0 ppm	5,000 ppm	10,000 ppm
Male			
n	10	10	10
Necropsy body wt	37.4 ± 1.7	33.7 ± 1.6	30.8 ± 0.8**
Brain			
Absolute	0.466 ± 0.005	0.467 ± 0.008	0.459 ± 0.006
Relative	12.7 ± 0.6	14.1 ± 0.6	15.0 ± 0.3**
R. Kidney			
Absolute	0.316 ± 0.010	0.292 ± 0.007	0.256 ± 0.009**
Relative	8.52 ± 0.29	8.77 ± 0.32	8.30 ± 0.17
Liver			
Absolute	1.51 ± 0.07	1.42 ± 0.07	1.28 ± 0.04*
Relative	40.3 ± 1.0	42.2 ± 1.2	41.7 ± 0.8
Female			
n	10	10	10
Necropsy body wt	39.2 ± 1.0	33.8 ± 1.1**	27.7 ± 1.0**
Brain			
Absolute	0.484 ± 0.005	0.476 ± 0.007	0.464 ± 0.006*
Relative	12.4 ± 0.3	14.2 ± 0.4**	16.9 ± 0.5**
R. Kidney			
Absolute	0.206 ± 0.005	0.198 ± 0.004	0.176 ± 0.008**
Relative	5.26 ± 0.14	5.90 ± 0.24*	6.32 ± 0.11**
Liver			
Absolute	1.39 ± 0.03	1.32 ± 0.03	1.17 ± 0.03**
Relative	35.7 ± 0.9	39.4 ± 1.1**	42.3 ± 0.7**

* Significantly different ($P \leq 0.05$) from the control group by Williams' or Dunnett's test
** $P \leq 0.01$
[a] Organ weights and body weights are given in grams; organ-weight-to-body-weight ratios are given as mg organ weight/g body weight (mean ± standard error).

APPENDIX G
HEMATOLOGY AND CLINICAL CHEMISTRY RESULTS

TABLE G1
Clinical Chemistry Data for Rats at the 6-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

Analysis	0 ppm	5,000 ppm	10,000 ppm
Male			
n	10	9	
Thyroid-stimulating hormone (ng/mL)	395 ± 27	452 ± 46[b]	
Triiodothyronine (ng/dL)	74 ± 4	69 ± 6	
Thyroxine (μg/dL)	2 ± 0	3 ± 0	
Female			
n	10	10	10
Thyroid-stimulating hormone (ng/mL)	399 ± 18	–[c]	355 ± 14*
Triiodothyronine (ng/dL)	79 ± 6	–	62 ± 4
Thyroxine (μg/dL)	2 ± 0	–	2 ± 0

* Significantly different (P≤0.05) from the control group by Dunn's or Shirley's test
** P≤0.01
[a] Mean ± standard error. No male rats received doses of 10,000 ppm.
[b] n=10
[c] No measurements were taken for this dose group.

TABLE G2
Hematology and Clinical Chemistry Data for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

Analysis	0 ppm	2,500 ppm	5,000 ppm
Male			
n	10	10	10
Hematology			
Hematocrit (%)	44.0 ± 2.0	46.0 ± 1.1	44.3 ± 1.1
Hemoglobin (g/dL)	15.9 ± 0.8	16.9 ± 0.5	16.1 ± 0.5
Erythrocytes ($10^6/\mu L$)	9.03 ± 0.33	9.55 ± 0.27	9.42 ± 0.07
Mean cell volume (fL)	48.6 ± 0.7	48.2 ± 0.3	46.9 ± 1.0*
Mean cell hemoglobin (pg)	17.5 ± 0.4	17.7 ± 0.1	17.0 ± 0.5
Mean cell hemoglobin concentration (g/dL)	36.1 ± 0.4	36.8 ± 0.3	36.2 ± 0.3
Leukocytes ($10^3/\mu L$)	5.94 ± 0.58[b]	5.74 ± 0.29	6.85 ± 0.45
Segmented neutrophils ($10^3/\mu L$)	2.52 ± 0.52[b]	2.13 ± 0.21	3.14 ± 0.43
Lymphocytes ($10^3/\mu L$)	3.00 ± 0.10[b]	3.24 ± 0.17	3.26 ± 0.16
Monocytes ($10^3/\mu L$)	0.19 ± 0.06[b]	0.16 ± 0.04	0.22 ± 0.04
Eosinophils ($10^3/\mu L$)	0.07 ± 0.02[b]	0.08 ± 0.02	0.06 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.07 ± 0.04[b]	0.09 ± 0.04	0.05 ± 0.04
n	9	9	10
Clinical chemistry			
Blood urea nitrogen (mg/dL)	15.4 ± 1.3	15.4 ± 1.7	30.2 ± 6.1*
Alkaline phosphatase (IU/L)	146 ± 5	144 ± 9[c]	155 ± 7
Alanine aminotransferase (IU/L)	104 ± 15	102 ± 6	101 ± 8
Aspartate aminotransferase (IU/L)	180 ± 21	154 ± 7	146 ± 7
Sorbitol dehydrogenase (SU/mL)	948 ± 103	625 ± 26*	856 ± 86

TABLE G2
Hematology and Clinical Chemistry Data for Rats at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4 (continued)

Analysis	0 ppm	5,000 ppm	10,000 ppm
Female			
n	10	10	10
Hematology			
Hematocrit (%)	44.4 ± 0.2	44.3 ± 0.3	43.9 ± 0.3
Hemoglobin (g/dL)	16.1 ± 0.1	16.1 ± 0.1	15.8 ± 0.1*
Erythrocytes ($10^6/\mu L$)	8.28 ± 0.05	8.32 ± 0.08	8.24 ± 0.08
Mean cell volume (fL)	53.7 ± 0.2	53.2 ± 0.3	53.3 ± 0.2
Mean cell hemoglobin (pg)	19.5 ± 0.1	19.3 ± 0.1	19.1 ± 0.1*
Mean cell hemoglobin concentration (g/dL)	36.3 ± 0.2	36.2 ± 0.2	36.0 ± 0.1
Leukocytes ($10^3/\mu L$)	3.41 ± 0.19	3.58 ± 0.11	4.08 ± 0.26
Segmented neutrophils ($10^3/\mu L$)	1.13 ± 0.08	1.23 ± 0.09	1.59 ± 0.22
Lymphocytes ($10^3/\mu L$)	2.07 ± 0.15	2.11 ± 0.09	2.25 ± 0.10
Monocytes ($10^3/\mu L$)	0.11 ± 0.02	0.16 ± 0.01*	0.18 ± 0.02*
Eosinophils ($10^3/\mu L$)	0.06 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.04 ± 0.02	0.04 ± 0.02	0.01 ± 0.01
Clinical chemistry			
Blood urea nitrogen (mg/dL)	13.7 ± 0.5	17.0 ± 1.0*	15.9 ± 0.6*
Alkaline phosphatase (IU/L)	145 ± 6	148 ± 7	148 ± 6
Alanine aminotransferase (IU/L)	58 ± 4	57 ± 4	75 ± 13
Aspartate aminotransferase (IU/L)	94 ± 6	96 ± 7	115 ± 17
Sorbitol dehydrogenase (SU/mL)	666 ± 42	745 ± 68	872 ± 152

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error.
[b] n=8
[c] n=10

TABLE G3
Clinical Chemistry Data for Mice at the 6-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

Analysis	0 ppm	5,000 ppm	10,000 ppm
Male			
n	10	10	10
Triiodothyronine (ng/dL)	105 ± 5	99 ± 3	78 ± 6**
Thyroxine (μg/dL)	4.09 ± 0.23	6.09 ± 0.25**	6.32 ± 0.28**
Female			
n	10	10	10
Triiodothyronine (ng/dL)	83 ± 5[b]	94 ± 2	70 ± 4[b]
Thyroxine (μg/dL)	4.98 ± 0.18	6.34 ± 0.30**	6.51 ± 0.55**

** Significantly different (P≤0.01) from the control group by Dunn's or Shirley's test
[a] Mean ± standard error.
[b] n=9

TABLE G4
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4[a]

Analysis	0 ppm	5,000 ppm	10,000 ppm
Male			
n	10	10	10
Hematology			
Hematocrit (%)	42.2 ± 0.9	39.9 ± 0.9	43.2 ± 0.3
Hemoglobin (g/dL)	15.3 ± 0.3	14.6 ± 0.3	15.8 ± 0.2
Erythrocytes ($10^6/\mu L$)	8.83 ± 0.19	8.27 ± 0.23	9.02 ± 0.11
Mean cell volume (fL)	47.8 ± 0.2	48.2 ± 0.7	47.9 ± 0.5
Mean cell hemoglobin (pg)	17.3 ± 0.2	17.7 ± 0.2	17.5 ± 0.2
Mean cell hemoglobin concentration (g/dL)	36.3 ± 0.4	36.7 ± 0.3	36.5 ± 0.4
Leukocytes ($10^3/\mu L$)	5.23 ± 0.56	5.33 ± 0.70	4.46 ± 0.58
Segmented neutrophils ($10^3/\mu L$)	2.81 ± 0.50	1.55 ± 0.18	1.05 ± 0.22**
Lymphocytes ($10^3/\mu L$)	1.98 ± 0.17	3.41 ± 0.59*	3.05 ± 0.44*
Monocytes ($10^3/\mu L$)	0.07 ± 0.02	0.09 ± 0.03	0.06 ± 0.02
Eosinophils ($10^3/\mu L$)	0.07 ± 0.03	0.11 ± 0.03	0.09 ± 0.02
Nucleated erythrocytes ($10^3/\mu L$)	0.02 ± 0.01	0.10 ± 0.04*	0.12 ± 0.03**
n	10	9	10
Clinical chemistry			
Blood urea nitrogen (mg/dL)	22.8 ± 1.4	24.4 ± 3.2	24.4 ± 1.4
Alkaline phosphatase (IU/L)	48 ± 5	46 ± 2	52 ± 2
Alanine aminotransferase (IU/L)	34 ± 3[b]	38 ± 5	42 ± 3[b]
Aspartate aminotransferase (IU/L)	108 ± 14[b]	125 ± 10	183 ± 22**
Sorbitol dehydrogenase (SU/mL)	1,858 ± 73	1,845 ± 143	1,651 ± 103

TABLE G4
Hematology and Clinical Chemistry Data for Mice at the 15-Month Interim Evaluations in the 2-Year Feed Studies of HC Yellow 4 (continued)

Analysis	0 ppm	5,000 ppm	10,000 ppm
Female			
n	10	10	10
Hematology			
Hematocrit (%)	43.4 ± 0.6	43.8 ± 0.4	43.6 ± 0.7
Hemoglobin (g/dL)	15.3 ± 0.2	15.1 ± 0.1	15.4 ± 0.2
Erythrocytes ($10^6/\mu L$)	9.29 ± 0.12	9.44 ± 0.10	9.57 ± 0.19
Mean cell volume (fL)	46.5 ± 0.2	46.4 ± 0.2	45.6 ± 0.3*
Mean cell hemoglobin (pg)	16.5 ± 0.1	15.9 ± 0.1**	16.1 ± 0.2*
Mean cell hemoglobin concentration (g/dL)	35.3 ± 0.3	34.4 ± 0.2*	35.2 ± 0.2
Leukocytes ($10^3/\mu L$)	2.49 ± 0.29	3.46 ± 0.37	3.65 ± 0.40*
Segmented neutrophils ($10^3/\mu L$)	0.60 ± 0.13	0.88 ± 0.07*	0.92 ± 0.14*
Lymphocytes ($10^3/\mu L$)	1.66 ± 0.18	2.34 ± 0.33	2.53 ± 0.25*
Monocytes ($10^3/\mu L$)	0.08 ± 0.02	0.09 ± 0.01	0.09 ± 0.03
Eosinophils ($10^3/\mu L$)	0.07 ± 0.02	0.04 ± 0.01	0.04 ± 0.01
Nucleated erythrocytes ($10^3/\mu L$)	0.04 ± 0.01	0.05 ± 0.02	0.02 ± 0.01
Clinical chemistry			
Blood urea nitrogen (mg/dL)	13.8 ± 1.0[b]	18.0 ± 2.3[c]	20.2 ± 1.9*[b]
Alkaline phosphatase (IU/L)	81 ± 6[b]	83 ± 3[b]	99 ± 7*
Alanine aminotransferase (IU/L)	34 ± 4	34 ± 3[b]	45 ± 3*
Aspartate aminotransferase (IU/L)	100 ± 9	133 ± 18	194 ± 24**[b]
Sorbitol dehydrogenase (SU/mL)	941 ± 74	947 ± 119	1,004 ± 57

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error.
[b] n=9
[c] n=8

APPENDIX H
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

HC Yellow 4 was obtained from the Southland Corporation, Grant Meadow, New Jersey (lots 0-218 and 3-074), and from Prochemie International, Incorporated (lot 81031). Lot 0-218 was used in the 14-day, 13-week, and the first 11 months of the 2-year studies. Lot 3-074 was used in the next 7 months of the 2-year study, and lot 81031 was used the final 6 months of the 2-year studies. Identity, purity, and stability analyses were conducted by the analytical chemistry laboratory, Midwest Research Institute (Kansas City, MO). MRI reports on analyses performed in support of the HC Yellow 4 studies are on file at the National Institute of Environmental Health Sciences.

The three lots of dye, a fluffy, yellow powder, were identified as HC Yellow 4 by infrared, ultraviolet/visible, and nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with those expected for the structure and with the literature spectra of HC Yellow 4, as shown in Figures H1 and H2 (*Sadtler Standard Spectra*).

The purity of the three lots was determined by elemental analysis, Karl Fischer water analysis, weight loss on drying, titration, ultraviolet/visible spectrophotometry (lot 0-218), thin-layer chromatography (TLC), and high-performance liquid chromatography (HPLC). Titration was performed by dissolving the sample in 50% aqueous ethanol containing 7.5% sodium citrate, followed by reduction of the nitro group with 0.5 N titanous chloride. TLC was performed on silica gel 60 F-254 plates with two solvent systems: A) methylene chloride:acetone:glacial acetic acid (70:26:4), and B) methanol:toluene (75:25). Visualization was accomplished with visible light, short (254 nm) and long (366 nm) wavelength ultraviolet light, a 2,6-dibromoquinonechloroimide spray (lot 0-218), and a spray of 0.4% methanolic solution of 2,6-dichloroquinonechlorimide followed by a spray of 10% aqueous sodium carbonate solution (lots 3-074 and 81031). HPLC was performed with a μBondapak C_{18} column in a mixture of two solvents: A) 0.005 M heptanesulfonic acid in water, with pH adjusted to approximately 2.00 with concentrated phosphoric acid and B) 0.005 M heptanesulfonic acid in methanol, with an equal volume of phosphoric acid added as solvent A, with a ratio of 80:20 A:B (lot 0-218), 67:33 A:B (lot 3-074), or 85:15 A:B (lot 81031), at a flow rate of 1 mL/minute. Visible detection was at 405 nm for all lots, and ultraviolet detection was at 254 nm for lots 3-074 and 81031.

For lot 0-218, elemental analyses for carbon, hydrogen, and nitrogen were in agreement with theoretical values. Karl Fischer water analysis indicated 0.84 ± 0.02% water. Weight loss on drying indicated 0.38 ± 0.01% water. Titration by reduction of the nitro group indicated a purity of 105.6 ± 1.2%. TLC indicated one major spot, one trace impurity, and one slight impurity by solvent system A, and one homogeneous spot by solvent system B. HPLC indicated one major peak and three impurities; the area of the largest impurity was 6.5% relative to the major peak. The two remaining impurities had a combined area of 0.40% relative to the major peak. The identity of the major impurity was tentatively identified by mass spectroscopy and synthesis data as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline, with a concentration of 7% to 8% of the total peak area estimated from HPLC data. A comparison of the chromatographic profiles of lot 0-218 and the manufacturer's pure standard of HC Yellow 4 indicated the relative purity of lot 0-218 was 93.4 ± 0.7% and the concentration of the major impurity was 1/25th as large in the standard. Based upon the above data, the purity of Lot 0-218 was estimated at greater than 93%.

For lot 3-074, elemental analyses for carbon, hydrogen, and nitrogen were in agreement with theoretical values. Karl Fischer water analysis indicated 0.33 ± 0.06% water. Weight loss on drying indicated 0.15 ± 0.02% water. Titration by reduction of the nitro group indicated a purity of 103.4 ± 0.7%.

TLC indicated one major spot, one minor impurity, and one trace impurity by solvent system A, and one major spot and a slight trace impurity by solvent system B. HPLC indicated one major peak and four impurities with a combined area of 2.9% at 254 nm and 3.1% at 405 nm relative to the major peak. The largest of the impurities (approximately 2.5%) was tentatively identified as *N*-(2-hydroxyethyl)-2-hydroxy-4-nitroaniline. Major peak comparison of lots 0-218 and 3-074 indicated a purity of 105.1 ± 0.4% for lot 3-074 relative to lot 0-218. Based upon the above data, the purity of lot 3-074 was estimated at greater than 97%.

For lot 81031, elemental analyses for carbon, hydrogen, and nitrogen were in agreement with theoretical values. Karl Fischer water analysis indicated less than 0.05% water. Weight loss on drying indicated 0.04 ± 0.01% water. Titration by reduction of the nitro group, with concomitant analyses of lots 0-218 and 81031, indicated a purity of 101.1 ± 0.3% for lot 0-218 and 100.6 ± 0.8% for lot 81031. TLC indicated one major spot and three trace impurities by solvent system A, and one major spot and a minor impurity by solvent system B. HPLC indicated one major peak and six impurities with a combined area of 1.2% at 254 nm and one major peak and four impurities with a relative combined area of 0.7% at 405 nm. The largest impurity peak was 0.3% of the major peak. Major peak comparison of lots 0-218 and 81031 indicated a purity of 105.1% for lot 81031 relative to lot 0-218. Based upon the above data, the purity of lot 81031 was estimated at greater than 98%.

All three lots were analyzed for the possible presence of nitrosamines by HPLC equipped with a thermal energy analyzer (Thermo Electron Corp., Waltham, MA). Two or three nonpolar nitrosamines with combined concentrations of less than 0.5 ppm were found in each lot. A polar nitrosamine present at approximately 1.1 ppm was found in lot 81031. Another peak present at approximately 100 ppm could not be confirmed as a polar nitrosamine.

Stability studies performed by HPLC with the system described for analysis of the purity of lot 0-218 but with a ratio of 10:90 A:B and a flow rate of 2.5 mL/minute, with acetophenone added as an internal standard, indicated that HC Yellow 4, when stored protected from light, was stable as a bulk chemical for 2 weeks at temperatures up to 60° C. During the 2-year studies, the stability of the bulk chemical was monitored by the study laboratory using HPLC, titration of the nitro group, and infrared spectroscopy; no degradation of HC Yellow 4 was seen throughout the studies.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared by mixing HC Yellow 4 with feed in a Patterson-Kelly twin-shell blender (Table H1). Dose formulations were prepared weekly.

Homogeneity and stability analyses of the dosed feed preparations were conducted by the analytical chemistry laboratory. For the homogeneity analyses, the formulations were extracted with 100 mL acetonitrile and centrifuged, then further diluted with acetonitrile. The absorbance of the samples was measured versus acetonitrile by ultraviolet spectroscopy at 398 nm. For the stability studies, feed samples were extracted with 100 mL of methanol:hydrochloric acid (99:1 v/v) and centrifuged; the extracts were then diluted with water:methanol (80:20), and were injected into an HPLC system equipped with a μBondapak C_{18} column and a 365 nm detector. The mobile phase was a mixture of two solvents: A) 0.005 M heptanesulfonic acid, sodium salt, in water, with pH adjusted to 2.0 with phosphoric acid and B) 0.005 M heptanesulfonic acid, sodium salt, in methanol, with an equal volume of phosphoric acid added as solvent A, with a ratio of 80:20 A:B. Homogeneity of these formulations was confirmed; stability of the formulation was established for at least 2 weeks when stored in the dark at temperatures up to 25° C.

Periodic analyses of the dose formulations of HC Yellow 4 were conducted at the study laboratory and at the analytical chemistry laboratory using spectroscopy at 398 nm. Dose formulations were analyzed once during the 14-day studies. For the 13-week studies, dose formulations were analyzed at the

beginning of the studies, after the third mix, midway through the studies, and at the end of the studies. During the 14-day and 13-week studies, all dose formulations for rats and mice were within 10% of target concentrations (Table H2, H3). During the 2-year studies, the first and one of every eight sets of the dose formulations were analyzed; all dose formulations for rats and mice were within 10% of the target concentrations. Results of the dose formulation analyses studies for the 2-year studies are presented in Table H4. Results of periodic referee analysis performed by the analytical chemistry laboratory indicated good agreement with the results obtained by the study laboratory (Table H5).

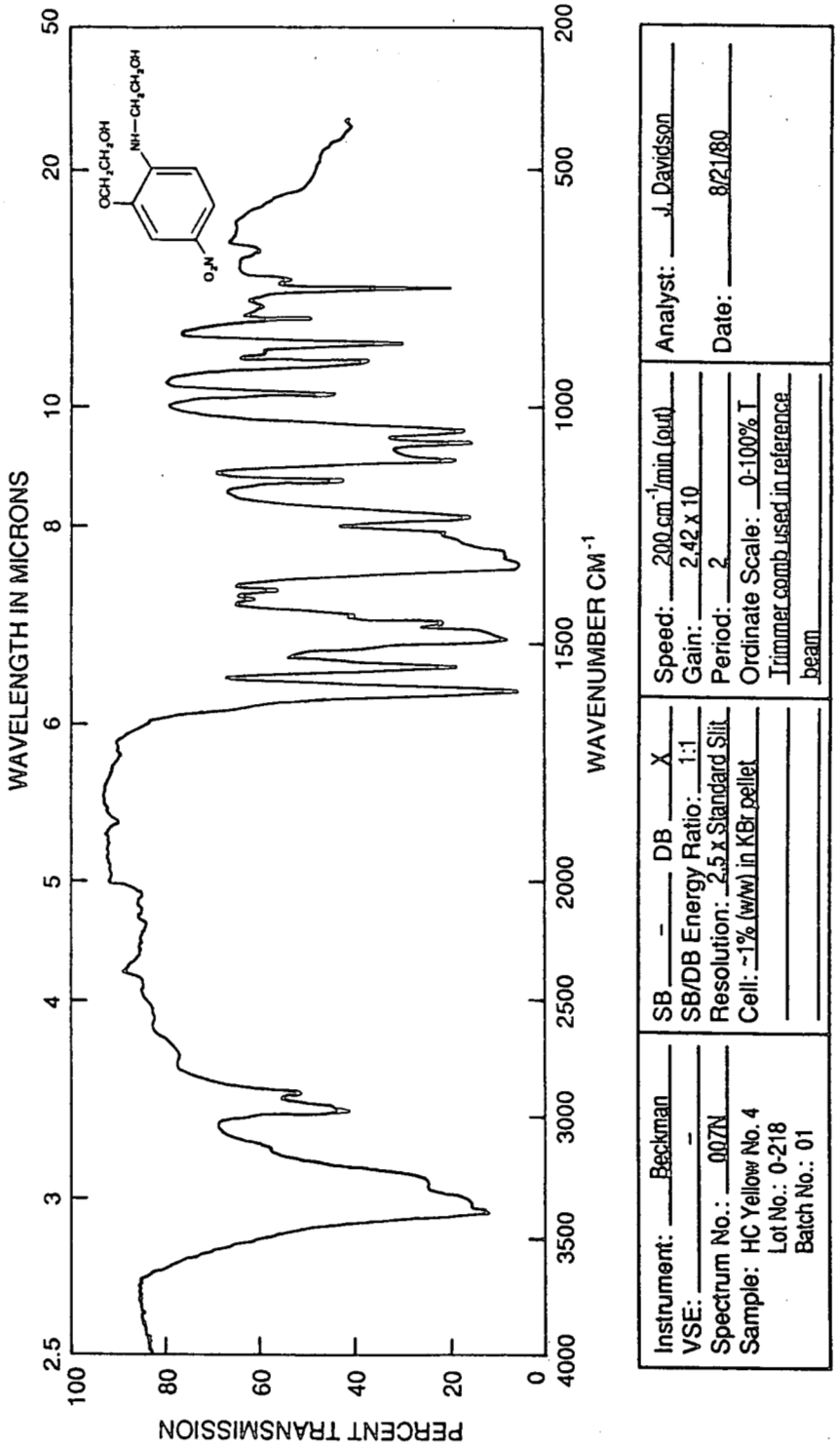


FIGURE H1
Infrared Absorption Spectrum of HC Yellow 4

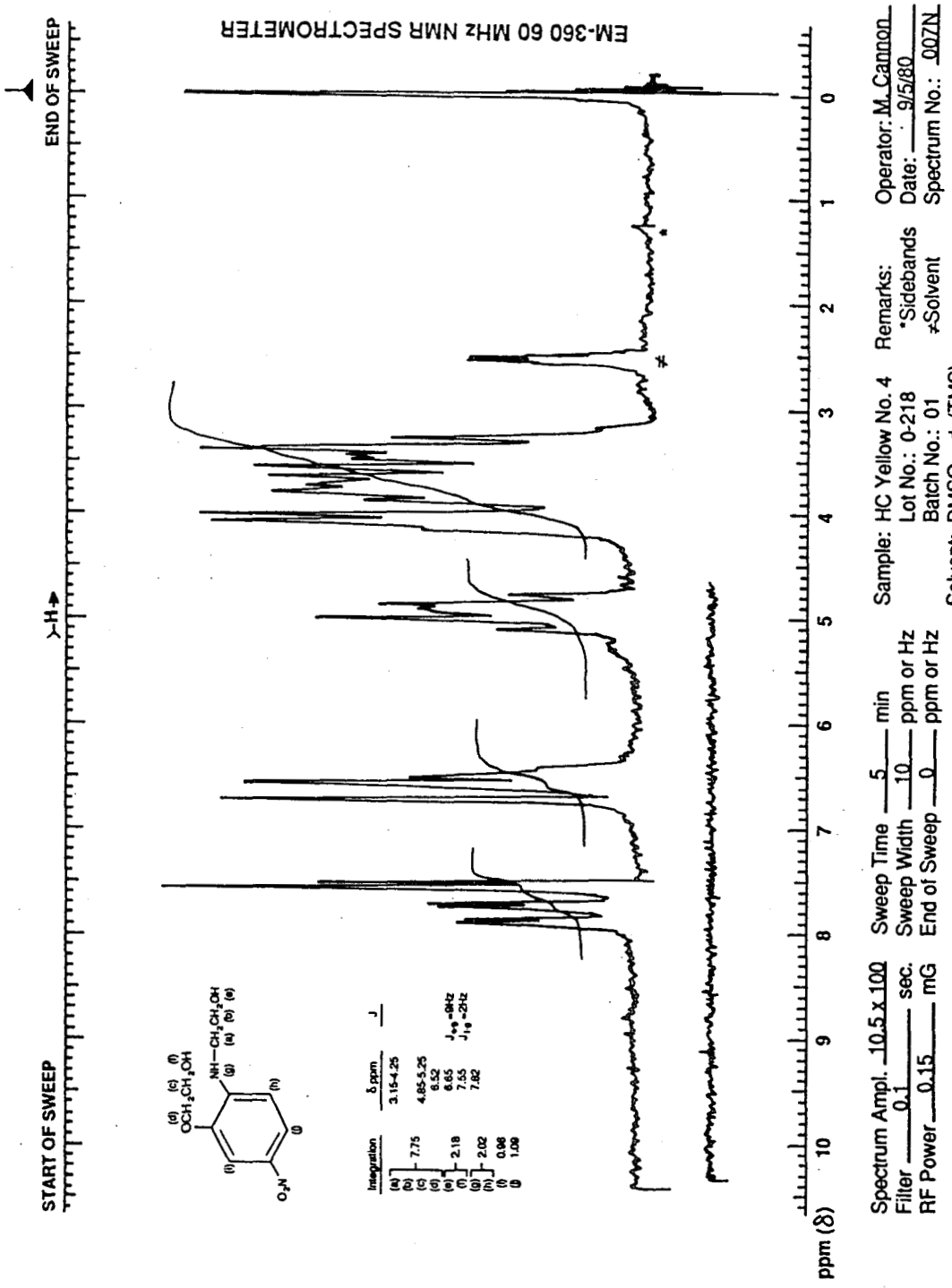


FIGURE H2
Nuclear Magnetic Resonance Spectrum of HC Yellow 4

TABLE H1
Preparation and Storage of Dose Formulations in the Feed Studies of HC Yellow 4

14-Day Studies	13-Week Studies	2-Year Studies
Preparation		
A premix with HC Yellow 4 and feed (wt:vol) was prepared using a mortar and pestle; premix and remainder of feed was layered into a blender with an intensifier bar and mixed for 15 min. Dose formulations were prepared weekly.	Same as 14-day studies.	Same as 14-day studies.
Chemical Lot Number		
0-218	0-218	0-218 3-074 81031
Maximum Storage Time		
14 days from date of preparation	14 days from date of preparation	14 days from date of preparation
Storage Conditions		
In double plastic bags, in the dark, at 0 ± 5° C	In double, clear plastic bags, at approximately 4° C	In double plastic bags (inner bag opaque) at 0 ± 5° C
Study Laboratory		
EG&G Mason Research Institute, Worcester, MA	Same as 14-day studies.	Same as 14-day studies.
Referee Laboratory		
Midwest Research Institute, Kansas City, MO	Same as 14-day studies.	Same as 14-day studies.

TABLE H2
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 14-Day Feed Studies of HC Yellow 4

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
8 July 1981	9 July 1981	1,250	1,140	-9
		2,500	2,330	-7
		5,000	5,060	+1
	10 July 1981	10,000	9,800	-2
		20,000	18,000	-10
		40,000	39,400	-2
		80,000	73,000	-9

[a] Results of duplicate analyses

TABLE H3
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 13-Week Feed Studies of HC Yellow 4

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
10 February 1982	11 February 1982	2,500	2,330	−7
		5,000	4,760	−5
		10,000	10,300	+3
	12 February 1982	20,000	19,200	−4
		40,000	39,200	−2
		80,000	76,500	−4
26 February 1982	3 March 1982	2,500	2,400	−4[b]
		2,500	2,370	−5[c]
		2,500	2,250	−10[d]
		80,000	79,300	−1[b]
		80,000	79,800	0[c]
		80,000	79,500	−1[d]
13 April 1982	15 April 1982	2,500	2,360	−6
		5,000	5,320	+6
		10,000	10,400	+4
		20,000	21,000	+5
		40,000	39,200	−2
		80,000	76,900	−4
18 May 1982	19 May 1982	2,500	2,380	−5
		5,000	4,690	−6
		10,000	9,540	−5
		20,000	19,600	−2
		40,000	38,600	−3
		80,000	78,000	−3

[a] Results of duplicate analyses
[b] Sample selection from top left of twin-shell blender
[c] Sample selection from top right of twin-shell blender
[d] Sample selection from bottom of twin-shell blender

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of HC Yellow 4

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration[a] (ppm)	% Difference from Target
9 March 1983	14 March 1983	2,500	2,260	-10[b]
		2,500	2,300	-8[c]
		2,500	2,440	-3[d]
		5,000	4,790	-4
		10,000	9,580	-4[b]
		10,000	9,520	-5[c]
		10,000	10,000	0[d]
5 May 1983	6 May 1983	2,500	2,540	+2
		5,000	4,840	-3
		10,000	9,700	-3
7 July 1983	8 July 1983	2,500	2,380	-5[b]
		2,500	2,520	+1[c]
		2,500	2,340	-6[d]
		5,000	4,870	-3
		10,000	10,100	+1[b]
		10,000	10,000	0[c]
		10,000	9,580	-4[d]
22 September 1983	23 September 1983	2,500	2,290	-8
		5,000	4,860	-3
		10,000	9,890	-1
15 December 1983	20 December 1983	2,500	2,540	+2
		5,000	4,840	-3
		10,000	9,840	-2
9 February 1984	9 February 1984	2,500	2,370	-5
		5,000	4,710	-6
		10,000	9,980	0
5 April 1984	6 April 1984	2,500	2,290	-8
		5,000	4,730	-5
		10,000	9,800	-2
24 May 1984	25 May 1984	2,500	2,440	-3
		5,000	5,470	+9[e]
		10,000	10,040	0
	30 May 1984[f]	5,000	4,790	-4
28 June 1984	29 June 1984	2,500	2,560	+3
		5,000	5,080	+2
		10,000	10,000	0
7 August 1984	9 August 1984	2,500	2,500	0
		5,000	4,880	-2
		10,000	10,200	+2

TABLE H4
Results of Analysis of Dose Formulations Administered to Rats and Mice in the 2-Year Feed Studies of HC Yellow 4 (continued)

Date Prepared	Date Analyzed	Target Concentration (ppm)	Determined Concentration (ppm)	% Difference from Target
16 October 1984	18 October 1984	2,500	2,440	−2
		5,000	4,780	−4
		10,000	10,200	+2
18 December 1984	20 December 1984	2,500	2,300	−8
		5,000	4,780	−4
		10,000	9,700	−3
12 February 1985	13 February 1985	2,500	2,550	+2
		5,000	5,020	+1
		10,000	10,000	0
26 March 1985	2 April 1985	2,500	2,440	−2
		5,000	5,020	0
		10,000	9,820	−2

[a] Results of duplicate analyses
[b] Sample selection from top left of twin-shell blender
[c] Sample selection from top right of twin-shell blender
[d] Sample selection from bottom of twin-shell blender
[e] Variation between duplicate samples was >10%, and samples contained relatively large aggregates of HC Yellow 4. Samples remixed.
[f] Analysis results of remix

TABLE H5
Results of Referee Analysis of Dose Formulations in the 13-Week and 2-Year Feed Studies of HC Yellow 4

Date Prepared	Target Concentration (ppm)	Determined Concentration (ppm)	
		Study Laboratory[a]	Referee Laboratory[b]
13-Week Studies			
16 February 1982	2,500	2,330	2,460 ± 60
2-Year Studies			
9 March 1983	2,250	2,330	2,490 ± 20
22 September 1983	10,000	9,890	9,990 ± 0
9 February 1984	5,000	4,710	4,780 ± 50
7 August 1984	2,500	2,500	2,500 ± 30
12 February 1985	10,000	10,000	9,890 ± 100

[a] Results of duplicate analysis
[b] Results of triplicate analysis. Mean ± standard deviation

APPENDIX I
FEED AND COMPOUND CONSUMPTION IN THE 2-YEAR FEED STUDIES

TABLE I1
Feed and Compound Consumption by Male Rats in the 2-Year Feed Study of HC Yellow 4

Week	0 ppm		2,500 ppm			5,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	17.1	107	16.5	103	400	16.7	104	808
2	16.8	159	16.0	153	262	16.0	152	527
4	19.2	222	17.7	216	205	18.0	211	427
5	20.2	246	19.6	242	202	18.9	239	395
8	20.4	291	20.2	286	177	19.2	283	338
9	21.0	308	19.5	305	160	20.3	301	337
13	20.5	343	19.7	342	144	18.9	338	279
17	24.8	367	21.8	367	149	20.3	363	280
21	22.8	388	21.2	384	138	20.4	380	268
25	24.2	406	21.8	410	133	21.0	405	259
29	26.6	420	22.3	426	131	21.5	421	256
33	22.1	433	20.2	434	116	20.2	431	235
37	23.4	439	19.8	439	113	19.4	431	225
41	23.7	449	19.8	449	110	20.3	446	227
45	22.4	461	19.2	458	105	20.5	454	226
49	24.4	465	20.4	467	109	21.1	463	228
53	19.2	465	16.1	465	87	16.1	465	173
57	16.5	472	15.8	473	84	16.3	473	172
61	15.8	478	15.9	482	82	15.9	478	166
65	15.8	473	16.2	481	84	16.5	479	172
69	14.8	472	14.6	480	76	15.2	488	156
73	14.9	473	15.5	487	80	15.5	489	159
77	15.1	466	15.4	479	80	15.3	481	159
81	14.7	464	15.4	477	81	15.6	481	162
85	14.9	456	15.4	472	81	16.2	483	167
89	15.4	445	14.9	454	82	16.1	473	170
93	13.9	435	13.1	441	74	14.6	463	158
97	15.0	434	14.7	439	84	13.6	445	153
101	15.0	416	14.5	430	84	13.5	439	153
104	15.4	413	15.0	417	90	15.5	440	176
Weeks 1-13:								
Mean	19.3	239	18.4	235	221	18.3	233	444
SD[c]	1.7		1.7		87	1.5		179
CV[d]	8.9		9.3		39.5	8.0		40.2
Weeks 14-52:								
Mean	23.8	425	20.7	426	123	20.5	421	245
SD	1.4		1.1		15	0.6		21
CV	5.7		5.3		12.6	3.0		8.6
Weeks 53-104:								
Mean	15.5	454	15.2	463	82	15.4	470	164
SD	1.2		0.8		4	0.9		8
CV	8.0		5.4		4.8	6.1		4.8

[a] Grams of feed consumed per animal per day
[b] Milligrams of HC Yellow 4 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I2
Feed and Compound Consumption by Female Rats in the 2-Year Feed Study of HC Yellow 4

Week	0 ppm		5,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
1	15.0	99	13.6	99	687	12.6	98	1,284
2	14.0	131	14.3	134	533	14.3	128	1,114
4	14.6	154	14.3	162	441	13.7	156	878
5	16.3	165	13.7	171	401	13.5	165	817
8	15.0	190	14.5	191	380	14.3	184	775
9	15.8	196	13.9	197	354	13.3	189	706
12	15.3	206	15.5	208	374	14.8	200	740
13	15.8	209	15.2	212	357	13.6	204	667
17	15.9	221	15.7	225	350	15.2	217	701
21	15.2	228	14.3	229	311	13.6	222	613
25	15.7	237	14.8	238	310	14.0	230	611
29	15.8	247	13.9	247	281	14.4	239	605
33	17.4	254	14.8	250	297	14.2	239	594
37	15.5	263	14.1	258	273	13.5	245	551
41	17.2	276	14.9	266	281	13.9	250	555
45	15.5	277	13.6	271	251	12.9	255	504
49	18.1	292	16.1	279	289	15.5	262	594
53	10.8	300	12.6	288	218	12.3	272	451
57	12.2	315	12.6	299	211	11.9	281	425
61	10.9	317	10.8	300	181	10.4	280	372
65	11.9	334	11.8	318	186	11.5	297	386
69	12.4	336	11.9	321	185	11.5	298	385
73	12.7	349	12.5	335	187	10.6	309	343
77	14.2	355	13.3	342	194	12.4	314	396
81	13.1	360	14.4	352	204	13.6	322	424
85	12.2	360	11.3	348	163	11.9	321	370
89	12.7	358	12.7	351	181	12.0	326	369
93	12.8	356	13.0	356	182	12.2	324	376
97	12.5	356	11.8	351	169	11.9	327	364
101	12.2	355	13.2	351	189	13.0	330	394
104	13.1	354	13.3	353	189	12.2	330	369
Weeks 1-13:								
Mean	15.2	169	14.4	172	441	13.8	166	873
SD[c]	0.7		0.7		116	0.7		217
CV[d]	4.9		4.7		26.2	4.9		24.8
Weeks 14-52:								
Mean	16.2	255	14.7	251	294	14.1	240	592
SD	1.0		0.8		28	0.8		54
CV	6.3		5.7		9.6	5.9		9.2
Weeks 53-104:								
Mean	12.4	343	12.5	333	189	12.0	309	388
SD	0.9		0.9		15	0.8		29
CV	6.9		7.3		7.9	7.0		7.4

[a] Grams of feed consumed per animal per day
[b] Milligrams of HC Yellow 4 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I3
Feed and Compound Consumption by Male Mice in the 2-Year Feed Study of HC Yellow 4

Week	0 ppm		5,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	4.9	23.9	5.1	23.9	1,075	6.0	23.7	2,527
5	5.8	28.3	6.0	28.5	1,052	7.8	27.3	2,849
9	5.7	31.7	6.2	31.4	980	7.1	30.4	2,337
13	5.3	34.1	5.9	34.0	874	7.2	31.6	2,268
17	5.9	36.5	6.0	35.7	834	6.9	32.7	2,102
21	5.6	38.1	5.6	37.3	745	7.2	33.5	2,145
25	5.7	38.4	5.9	38.0	771	7.9	33.8	2,343
29	5.9	39.4	5.7	38.7	736	8.0	34.2	2,335
33	6.4	39.3	7.4	39.1	947	8.0	32.0	2,498
37	5.8	41.0	5.7	39.7	721	7.6	34.1	2,239
41	5.8	41.3	6.1	39.4	769	8.1	33.5	2,422
45	5.6	41.8	5.9	38.7	763	8.2	33.9	2,404
49	4.9	42.5	5.4	38.6	706	7.1	34.6	2,059
53	5.9	42.7	6.3	38.3	828	7.5	33.3	2,258
57	5.3	42.0	5.7	37.3	763	7.7	33.3	2,304
61	5.3	43.6	6.1	38.1	798	7.6	34.6	2,204
65	5.8	43.7	5.8	38.0	760	8.0	34.3	2,318
69	6.2	43.5	6.5	37.9	860	8.5	33.8	2,502
77	11.2	42.2	14.1	37.3	1,889	17.5	33.2	5,276
81	10.1	42.8	15.0	37.2	2,010	18.6	33.0	5,621
85			5.5	35.2	774	6.0	31.0	1,923
88	5.2	40.4	5.4	35.4	767	7.4	31.1	2,392
93	4.8	37.6	4.1	34.7	586	5.1	31.1	1,654
97	4.8	38.3	4.5	35.0	650	6.3	31.1	2,012
101	5.2	38.2	5.0	35.0	713	6.8	30.2	2,246
104	5.3	37.3	5.1	35.0	724	6.3	30.5	2,054
Weeks 1-13:								
Mean	5.4	29.5	5.8	29.5	995	7.0	28.3	2,495
SD[c]	0.4		0.5		91	0.7		260
CV[d]	7.9		7.8		9.1	10.6		10.4
Weeks 14-52:								
Mean	5.7	39.8	6.0	38.4	777	7.7	33.6	2,283
SD	0.4		0.6		74	0.5		154
CV	6.7		9.7		9.5	6.3		6.8
Weeks 53-104:								
Mean	6.3	41.0	6.8	36.5	932	8.7	32.3	2,674
SD	2.1		3.5		458	4.2		1,253
CV	33.8		50.8		49.1	48.8		46.9

[a] Grams of feed consumed per animal per day
[b] Milligrams of HC Yellow 4 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

TABLE I4
Feed and Compound Consumption by Female Mice in the 2-Year Feed Study of HC Yellow 4

Week	0 ppm		5,000 ppm			10,000 ppm		
	Feed (g/day)[a]	Body Weight (g)	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)[b]	Feed (g/day)	Body Weight (g)	Dose/ Day (mg/kg/day)
2	5.2	18.3	5.5	18.0	1,528	6.2	17.8	3,498
5	6.1	20.9	6.5	20.1	1,618	7.4	19.8	3,759
9	5.9	23.2	6.1	22.6	1,344	6.6	21.7	3,028
17	5.5	28.9	6.3	27.2	1,166	6.8	24.6	2,768
21	5.7	31.5	6.0	29.3	1,021	6.8	26.5	2,553
25	6.2	32.7	6.9	30.3	1,142	7.8	27.0	2,901
29	7.6	34.4	7.8	32.4	1,207	8.1	27.9	2,907
33	6.8	35.3	7.6	33.1	1,147	8.2	28.1	2,919
37	6.8	35.9	7.1	33.1	1,069	7.2	28.5	2,529
41	7.0	37.7	7.1	34.5	1,036	8.2	29.6	2,779
45	5.1	38.4	6.3	35.4	895	6.0	29.6	2,011
49	6.1	39.8	7.1	35.9	992	8.1	29.9	2,718
53	5.4	40.2	6.6	36.0	914	6.3	29.9	2,108
57	6.2	39.7	7.3	35.5	1,023	7.6	29.1	2,621
61	6.4	41.1	6.6	37.1	889	6.9	29.8	2,307
65	7.0	41.5	5.8	35.5	820	6.2	29.3	2,120
69	8.2	42.2	7.3	36.1	1,010	7.7	30.4	2,542
73	8.3	41.6	8.0	36.3	1,095	8.7	30.2	2,875
77	7.9	42.0	8.2	37.7	1,085	8.7	31.4	2,784
81	5.5	42.2	6.5	37.2	878	7.6	30.1	2,533
85	8.8	42.3	7.5	36.3	1,029	9.1	29.3	3,095
89	7.9	42.7	6.9	35.8	962	7.2	28.6	2,526
93	7.1	42.2	6.9	35.9	955	7.4	28.5	2,594
97	6.3	43.0	6.4	35.0	909	9.5	28.1	3,371
101	7.4	41.7	6.6	35.3	936	8.4	27.8	3,009
104	8.9	42.0	9.0	35.4	1,266	10.6	27.7	3,825
Weeks 1-13:								
Mean	5.7	20.8	6.0	20.2	1,497	6.7	19.8	3,428
SD[c]	0.5		0.5		139	0.6		370
CV[d]	8.9		8.3		9.3	9.3		10.8
Weeks 14-52:								
Mean	6.3	35.0	6.9	32.4	1,075	7.5	28.0	2,676
SD	0.8		0.6		99	0.8		288
CV	13.0		8.7		9.8	11.0		10.8
Weeks 53-104:								
Mean	7.2	41.7	7.1	36.1	984	8.0	29.3	2,736
SD	1.1		0.8		114	1.2		475
CV	15.8		11.7		11.5	15.5		17.3

[a] Grams of feed consumed per animal per day
[b] Milligrams of HC Yellow 4 consumed per day per kilogram body weight
[c] Standard deviation of weekly means
[d] Coefficient of variation = (standard deviation/mean) x 100

APPENDIX J
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN NIH-07 RAT AND MOUSE RATION

TABLE J1
Ingredients of NIH-07 Rat and Mouse Ration[a]

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

[a] NCI, 1976; NIH, 1978
[b] Ingredients ground to pass through a U.S. Standard Screen No. 16 before being mixed

TABLE J2
Vitamins and Minerals in NIH-07 Rat and Mouse Ration[a]

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

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

TABLE J3
Nutrient Composition of NIH-07 Rat and Mouse Ration

Nutrients	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	22.31 ± 0.86	21.00–24.30	23
Crude fat (% by weight)	5.30 ± 0.66	4.20–6.40	23
Crude fiber (% by weight)	3.59 ± 0.33	2.90–4.50	23
Ash (% by weight)	6.65 ± 0.28	5.96–7.27	23
Amino Acids (% of total diet)			
Arginine	1.308 ± 0.606	1.210–1.390	8
Cystine	0.306 ± 0.084	0.181–0.400	8
Glycine	1.150 ± 0.047	1.060–1.210	8
Histidine	0.576 ± 0.024	0.531–0.607	8
Isoleucine	0.917 ± 0.029	0.881–0.944	8
Leucine	1.946 ± 0.055	1.850–2.040	8
Lysine	1.270 ± 0.058	1.200–1.370	8
Methionine	0.448 ± 0.128	0.306–0.699	8
Phenylalanine	0.987 ± 0.140	0.665–1.110	8
Threonine	0.877 ± 0.042	0.824–0.940	8
Tryptophan	0.236 ± 0.176	0.107–0.671	8
Tyrosine	0.676 ± 0.105	0.564–0.794	8
Valine	1.103 ± 0.040	1.050–1.170	8
Essential Fatty Acids (% of total diet)			
Linoleic	2.393 ± 0.258	1.830–2.570	7
Linolenic	0.280 ± 0.040	0.210–0.320	7
Vitamins			
Vitamin A (IU/kg)	11,491 ± 4,854	4,200–22,000	23
Vitamin D (IU/kg)	4,450 ± 1,382	3,000–6,300	4
α–Tocopherol (ppm)	37.95 ± 9.41	22.50–48.90	8
Thiamine (ppm)	20.00 ± 5.29	12.0–37.0	23
Riboflavin (ppm)	7.92 ± 0.87	6.10–9.00	8
Niacin (ppm)	103.38 ± 26.59	65.0–150.0	8
Pantothenic acid (ppm)	29.54 ± 3.60	23.0–34.0	8
Pyridoxine (ppm)	9.55 ± 3.48	5.60–14.0	8
Folic acid (ppm)	2.25 ± 0.73	1.80–3.70	8
Biotin (ppm)	0.254 ± 0.042	0.19–0.32	8
Vitamin B_{12} (ppb)	38.45 ± 22.01	10.6–65.0	8
Choline (ppm)	3,089 ± 329	2,400–3,430	8
Minerals			
Calcium (%)	1.21 ± 0.14	0.91–1.43	23
Phosphorus (%)	0.95 ± 0.06	0.84–1.10	23
Potassium (%)	0.883 ± 0.078	0.772–0.971	6
Chloride (%)	0.526 ± 0.092	0.380–0.635	8
Sodium (%)	0.313 ± 0.390	0.258–0.371	8
Magnesium (%)	0.168 ± 0.010	0.151–0.181	8
Sulfur (%)	0.280 ± 0.064	0.208–0.420	8
Iron (ppm)	361 ± 100	255.0–523.0	8
Manganese (ppm)	91.97 ± 6.01	81.70–99.40	8
Zinc (ppm)	54.72 ± 5.67	46.10–64.50	8
Copper (ppm)	11.06 ± 2.50	8.090–15.39	8
Iodine (ppm)	3.37 ± 0.92	1.52–4.13	6
Chromium (ppm)	1.79 ± 0.36	1.04–2.09	8
Cobalt (ppm)	0.68 ± 0.14	0.490–0.780	4

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration

Contaminants	Mean ± Standard Deviation[a]	Range	Number of Samples
Arsenic (ppm)	0.56 ± 0.18	0.18–0.80	23
Cadmium (ppm)[b]	0.11 ± 0.03	<0.10–0.20	23
Lead (ppm)	0.54 ± 0.20	0.24–1.00	23
Mercury (ppm)	<0.05		23
Selenium (ppm)	0.33 ± 0.05	0.23–0.45	23
Aflatoxins (ppb)	<5.0		23
Nitrate nitrogen (ppm)[c]	10.55 ± 5.40	2.50–22.0	23
Nitrite nitrogen (ppm)[c]	0.84 ± 1.41	<0.10–6.10	23
BHA (ppm)[d]	<2.00		23
BHT (ppm)[d]	2.26 ± 1.05	<1.00–4.00	23
Aerobic plate count (CFU/g)[e]	140,291 ± 151,986	6,200–420,000	23
Coliform (MPN/g)[f]	313 ± 555	<3.00–2,400	23
E. coli (MPN/g)	9.39 ± 30.64	<3.00–150	23
Total nitrosoamines (ppb)[g]	6.16 ± 6.15	0.80–30.30	23
N-Nitrosodimethylamine (ppb)[g]	5.46 ± 6.19	0.50–30.00	23
N-Nitrosopyrrolidine (ppb)[g]	0.70 ± 0.73	0.30–2.70	23
Pesticides (ppm)			
α-BHC[h]	<0.01		23
β-BHC	<0.02		23
γ-BHC	<0.01		23
δ-BHC	<0.01		23
Heptachlor	<0.01		23
Aldrin	<0.01		23
Heptachlor epoxide	<0.01		23
DDE	<0.01		23
DDD	<0.01		23
DDT	<0.01		23
HCB	<0.01		23
Mirex	<0.01		23
Methoxychlor	<0.05		23
Dieldrin	<0.01		23
Endrin	<0.01		23
Telodrin	<0.01		23
Chlordane	<0.05		23
Toxaphene	<0.1		23
Estimated PCBs	<0.2		23
Ronnel	<0.01		23
Ethion	<0.02		23
Trithion	<0.05		23
Diazinon	<0.1		23
Methyl parathion	<0.02		23
Ethyl parathion	<0.02		23
Malathion[i]	0.17 ± 0.21	0.05–0.81	23
Endosulfan I	<0.01		23
Endosulfan II	<0.01		23
Endosulfan sulfate	<0.03		23

TABLE J4
Contaminant Levels in NIH-07 Rat and Mouse Ration (continued)

[a] For values less than the limit of detection, the detection limit is given for the mean.
[b] Three batches (milled on 22 February 1984, 14 March 1984, and 9 May 1984) contained 0.20 ppm; all others contained <0.10 ppm.
[c] Sources of contamination: alfalfa, grains, and fish meal
[d] Sources of contamination: soy oil and fish meal
[e] CFU = colony-forming unit
[f] MPN = most probable number
[g] All values were corrected for percent recovery.
[h] BHC = hexachlorocyclohexane or benzene hexachloride
[i] Twelve lots contained more than 0.05 ppm.

APPENDIX K
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

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 study 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 serology on sera from extra (sentinel) animals in the study rooms. These animals are untreated, and these animals and the study animals are 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.

Rats

During the 13-week studies, samples for viral screening were collected from five diet control animals of each sex. At the termination of the 13-week studies, the animals were bled. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM (pneumonia virus of mice)	Study termination
Sendai	Study termination
KRV (Kilham rat virus)	Study termination
H-1 (Toolan's H-1 virus)	Study termination
Complement Fixation	
RCV (rat corona virus)	Study termination

During the 2-year studies, 15 F344/N rats of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6 and 12 months
Sendai	6 and 12 months
KRV	6, 12, 18, and 24 months
H-1	6, 12, 18, and 24 months
ELISA	
RCV/SDA (sialodacryoadenitis virus)	6, 12, 18, and 24 months
Mycoplasma pulmonis	18 and 24 months
Mycoplasma arthritidis	18 and 24 months
PVM	18 and 24 months
Sendai	18 and 24 months

Mice

During the 13-week studies, samples for viral screening were collected from five diet control animals of each sex. At the termination of the 13-week studies, the animals were bled. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	Study termination
Reovirus 3	Study termination
GDVII (mouse encephalomyelitis virus)	Study termination
Polyoma virus	Study termination
MVM (minute virus of mice)	Study termination
Ectromelia virus (mouse pox)	Study termination
Complement Fixation	
Sendai	Study termination
Mouse adenoma virus	Study termination
LCM (lymphocytic choriomeningitis virus)	Study termination
ELISA	
MHV (mouse hepatitis virus)	Study termination

During the 2-year studies, 15 $B6C3F_1$ mice of each sex were selected at the time of randomization and allocation of the animals to the various study groups. Five animals of each designated sentinel group were killed at 6, 12, and 18 months on study. Samples for viral screening at 24 months were collected from five diet control animals of each sex. Blood collected from each animal was allowed to clot, and the serum was separated. The serum was cooled on ice and shipped to Microbiological Associates, Inc. (Bethesda, MD) for determination of the antibody titers. The following tests were performed:

Method of Analysis	Time of Analysis
Hemagglutination Inhibition	
PVM	6 and 12 months
Reovirus 3	6 and 12 months
GDVII	6 and 12 months
Polyoma virus	6, 12, 18, and 24 months
Sendai	6 and 12 months
MVM	6, 12, 18, and 24 months
Ectromelia virus	6 and 12 months
K (papovavirus)	24 months
Complement Fixation	
Mouse adenoma virus	6 and 12 months
LCM	6, 12, 18, and 24 months

Method of Analysis (continued)	Time of Analysis
ELISA	
PVM	18 and 24 months
Reovirus 3	18 and 24 months
GDVII	18 and 24 months
Sendai	18 and 24 months
Ectromelia virus	18 and 24 months
Mouse adenoma virus	18 and 24 months
Mycoplasma pulmonis	18 and 24 months
Mycoplasma arthritidis	18 and 24 months
MHV	6, 12, 18, and 24 months
Immunofluorescent Antibody	
EDIM (epizootic diarrhea of infant mice)	24 months

TABLE K1
Murine Virus Antibody Determinations for Rats and Mice in the 13-Week and 2-Year Feed Studies of HC Yellow 4

	Interval	Incidence of Antibody in Sentinel Animals	Positive Serologic Reaction for
13-Week Studies			
Rats	13 weeks	4/10	PVM
Mice	13 weeks	0/10	None positive
2-Year Studies			
Rats	6 months	0/10	None positive
	12 months	0/10	None positive
	18 months	0/9	None positive
	24 months	2/10	KRV
Mice	6 months	0/10	None positive
	12 months	0/10	None positive
	18 months	0/9	None positive
	24 months	3/10	*M. arthritidis*[a]

[a] Possible *Mycoplasma arthritidis*

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TR No.	CHEMICAL
201	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Dermal)
206	1,2-Dibromo-3-chloropropane
207	Cytembena
208	FD & C Yellow No. 6
209	2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (Gavage)
210	1,2-Dibromoethane
211	C.I. Acid Orange 10
212	Di(2-ethylhexyl)adipate
213	Butyl Benzyl Phthalate
214	Caprolactam
215	Bisphenol A
216	11-Aminoundecanoic Acid
217	Di(2-ethylhexyl)phthalate
219	2,6-Dichloro-p-phenylenediamine
220	C.I. Acid Red 14
221	Locust Bean Gum
222	C.I. Disperse Yellow 3
223	Eugenol
224	Tara Gum
225	D & C Red No. 9
226	C.I. Solvent Yellow 14
227	Gum Arabic
228	Vinylidene Chloride
229	Guar Gum
230	Agar
231	Stannous Chloride
232	Pentachloroethane
233	2-Biphenylamine Hydrochloride
234	Allyl Isothiocyanate
235	Zearalenone
236	*D*-Mannitol
237	1,1,1,2-Tetrachloroethane
238	Ziram
239	Bis(2-chloro-1-methylethyl)ether
240	Propyl Gallate
242	Diallyl Phthalate (Mice)
243	Trichloroethylene (Rats and Mice)
244	Polybrominated Biphenyl Mixture
245	Melamine
246	Chrysotile Asbestos (Hamsters)
247	L-Ascorbic Acid
248	4,4'-Methylenedianiline Dihydrochloride
249	Amosite Asbestos (Hamsters)
250	Benzyl Acetate
251	2,4- & 2,6-Toluene Diisocyanate
252	Geranyl Acetate
253	Allyl Isovalerate
254	Dichloromethane (Methylene Chloride)
255	1,2-Dichlorobenzene
257	Diglycidyl Resorcinol Ether
259	Ethyl Acrylate
261	Chlorobenzene
263	1,2-Dichloropropane
266	Monuron
267	1,2-Propylene Oxide
269	Telone II® (1,3-Dichloropropene)
271	HC Blue No. 1
272	Propylene
273	Trichloroethylene (Four Rat Strains)
274	Tris(2-ethylhexyl)phosphate
275	2-Chloroethanol
276	8-Hydroxyquinoline
277	Tremolite
278	2,6-Xylidine
279	Amosite Asbestos
280	Crocidolite Asbestos
281	HC Red No. 3
282	Chlorodibromomethane
284	Diallylphthalate (Rats)
285	C.I. Basic Red 9 Monohydrochloride
287	Dimethyl Hydrogen Phosphite
288	1,3-Butadiene
289	Benzene
291	Isophorone
293	HC Blue No. 2
294	Chlorinated Trisodium Phosphate
295	Chrysotile Asbestos (Rats)
296	Tetrakis(hydroxymethyl) phosphonium Sulfate & Tetrakis(hydroxymethyl) phosphonium Chloride
298	Dimethyl Morpholinophosphoramidate
299	C.I. Disperse Blue 1
300	3-Chloro-2-methylpropene
301	*o*-Phenylphenol
303	4-Vinylcyclohexene
304	Chlorendic Acid
305	Chlorinated Paraffins (C_{23}, 43% chlorine)
306	Dichloromethane (Methylene Chloride)
307	Ephedrine Sulfate
308	Chlorinated Paraffins (C_{12}, 60% chlorine)
309	Decabromodiphenyl Oxide
310	Marine Diesel Fuel and JP-5 Navy Fuel
311	Tetrachloroethylene (Inhalation)
312	*n*-Butyl Chloride
313	Mirex
314	Methyl Methacrylate
315	Oxytetracycline Hydrochloride
316	1-Chloro-2-methylpropene
317	Chlorpheniramine Maleate
318	Ampicillin Trihydrate
319	1,4-Dichlorobenzene
320	Rotenone
321	Bromodichloromethane
322	Phenylephrine Hydrochloride
323	Dimethyl Methylphosphonate
324	Boric Acid
325	Pentachloronitrobenzene
326	Ethylene Oxide
327	Xylenes (Mixed)
328	Methyl Carbamate
329	1,2-Epoxybutane
330	4-Hexylresorcinol
331	Malonaldehyde, Sodium Salt
332	2-Mercaptobenzothiazole
333	*N*-Phenyl-2-naphthylamine
334	2-Amino-5-nitrophenol
335	C.I. Acid Orange 3
336	Penicillin VK
337	Nitrofurazone

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TR No.	CHEMICAL
338	Erythromycin Stearate
339	2-Amino-4-nitrophenol
340	Iodinated Glycerol
341	Nitrofurantoin
342	Dichlorvos
343	Benzyl Alcohol
344	Tetracycline Hydrochloride
345	Rotenone
346	Chloroethane
347	D-Limonene
348	*a*-Methyldopa Sesquihydrate
349	Pentachlorophenol
350	Tribromomethane
351	*p*-Chloroaniline Hydrochloride
352	N-Methylolacrylamide
353	2,4-Dichlorophenol
354	Dimethoxane
355	Diphenhydramine Hydrochloride
356	Furosemide
357	Hydrochlorothiazide
358	Ochratoxin A
359	8-Methoxypsoralen
360	N,N-Dimethylaniline
361	Hexachloroethane
362	4-Vinyl-1-Cyclohexene Diepoxide
363	Bromoethane (Ethyl Bromide)
364	Rhodamine 6G (C.I. Basic Red 1)
365	Pentaerythritol Tetranitrate
366	Hydroquinone
367	Phenylbutazone
368	Nalidixic Acid
369	Alpha-Methylbenzyl Alcohol
370	Benzofuran
371	Toluene
372	3,3'-Dimethoxybenzidine Dihydrochloride
373	Succinic Anhydride
374	Glycidol
375	Vinyl Toluene
376	Allyl Glycidyl Ether
377	*o*-Chlorobenzalmalononitrile
378	Benzaldehyde
379	2-Chloroacetophenone
380	Epinephrine Hydrochloride
381	*d*-Carvone
382	Furfural
385	Methyl Bromide
386	Tetranitromethane
387	Amphetamine Sulfate
388	Ethylene Thiourea
389	Sodium Azide
390	3,3'-Dimethylbenzidine Dihydrochloride
391	Tris(2-chloroethyl) Phosphate
392	Chlorinated Water and Chloraminated Water
393	Sodium Fluoride
395	Probenecid
396	Monochloroacetic Acid
399	Titanocene Dichloride
401	2,4-Diaminophenol Dihydrochloride
405	C.I. Acid Red 114
406	γ-Butyrolactone
407	C.I. Pigment Red 3
410	Naphthalene
415	Polysorbate 80

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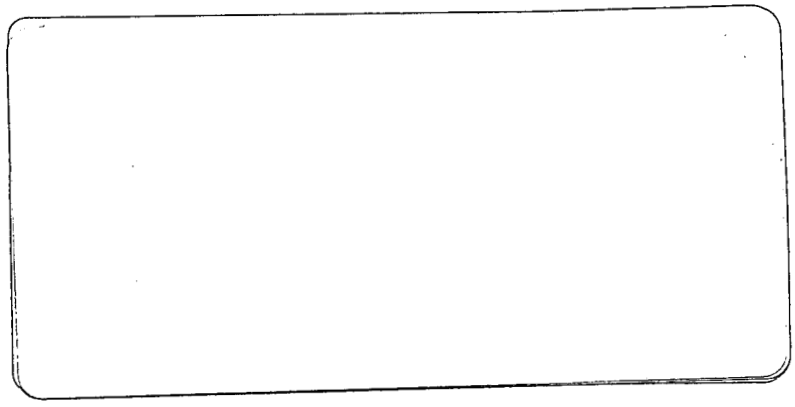

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