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U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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FOREWORD: This report presents the results of the bioassay of C.I. vat yellow 4 conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a greater incidence of cancer than control animals, do not necessarily mean that the test chemical is not a carcinogen, inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical is a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic to animals requires a wider analysis.

CONTRIBUTORS: This bioassay of C. I. vat yellow 4 was conducted at the NCI Frederick Cancer Research Center (FCRC) (1), Frederick, Maryland, operated for NCI (2) by Litton Bionetics, Inc. (3), Kensington, Maryland.

The manager of the bioassay at FCRC was Dr. B. Ulland, the toxicologist was Dr. E. Gordon, and Drs. R. Cardy and D. Creasia compiled the data. Ms. S. Toms was responsible for management of data, Mr. D. Cameron for management of histopathology, Mr. L. Callahan for management of the computer branch, and Mr. R. Cypher for management of the facilities. Mr. A. Butler performed computer services. Histopathologic evaluations were performed by Dr. C. E. Gilmore (4), and the diagnoses included in this report represent his interpretation.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were performed by Dr. J. R. Joiner (6) and Ms. P. L. Yong (6), using methods selected for the bioassay program by Dr. J. J. Gart (7). The chemicals used in this bioassay were analyzed at FCRC by Dr.

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This report was prepared at Tracor Jitco (6) under the direction of NCI. Those responsible for the report at Tracor Jitco were Dr. L. A. Campbell, Director of the Bioassay Program; Dr. S. S. Olin, Deputy Director for Science; Dr. J. F. Robens, toxicologist; Dr. R. L. Schueler, pathologist; Dr. G. L. Miller, Ms. M. S. King, and Mr. W. D. Reichardt, bioscience writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley and Ms. P. J. Graboske.

The following scientists at NCI (2) were responsible for evaluating the bioassay, interpreting the results, and reporting the findings: Dr. Kenneth C. Chu, Dr. Cipriano Cueto, Jr., Dr. J. Fielding Douglas, Dr. Thomas E. Hamm, Dr. William V. Hartwell, Dr. Richard A. Griesemer, Dr. Morton H. Levitt, Dr. Harry A. Milman, Dr. Thomas W. Orme, Dr. Sherman F. Stinson, Dr. Jerrold M. Ward, and Dr. Carrie E. Whitmire.

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SUMMARY

A bioassay of C. I. vat yellow 4, a commercial formulation containing dibenzo(b,def)chrysene-7,14-dione, for possible carcinogenicity was conducted by administering the test chemical in feed to Fischer 344 rats and B6C3F1 mice.

Groups of 50 rats of each sex and 50 mice of each sex were administered C. I. vat yellow 4 in the diet at one of two doses, either 3,500 or 7,000 ppm for the rats, either 25,000 or 50,000 ppm for the male mice, and either 12,500 or 25,000 ppm for the female mice. The rats were administered the test chemical for 104 weeks; the mice, for 106 weeks. Matched controls consisted of 20 untreated rats and 20 untreated mice of each sex. All surviving animals were killed at the end of the period of administration of the test chemical.

Mean body weights of the dosed rats were lower than those of corresponding controls throughout the bioassay, but the differences in weights were slight for the males. Mean body weights of the dosed mice were not affected by the test chemical. Survival of the rats and mice was not affected adversely by the chemical, and sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

In the male and female rats and the female mice, no tumors occurred at incidences that were significantly higher in dosed groups than in control groups.

In the male mice, lymphomas occurred at incidences that were dose related ($P = 0.002$), and, in a direct comparison, the incidence of the tumor in the high-dose group was significantly higher ($P = 0.019$) than that in the control group (controls 3/20, or 15%; low-dose 7/47, or 15%; high-dose 22/50, or 44%). The incidence of lymphomas and leukemias in historical-control male B6C3F1 mice at this laboratory was 38/323 (12%).

It is concluded that under the conditions of this bioassay, the formulated product containing C. I. vat yellow 4 was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice, but was carcinogenic for male B6C3F1 mice, causing an increased incidence of lymphomas.

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I. INTRODUCTION

C. I. vat yellow 4 (CAS 128-66-5; NCI C03565) is the common name adopted in the Colour Index (Society of Dyers and Colourists, 1971) for a commercial formulation that consists in part of dibenzo(b,def)chrysene-7,14-dione, the color-imparting component (Owens and Ward, 1974). This chemical has an anthraquinoid structure. In the dye process, the keto groups are reduced to the hydroxyl level of oxidation, which allows the dye to penetrate the fiber of the material being dyed. The dye is then fixed by oxidation back to the keto state, reforming the original insoluble dyestuff. C. I. vat yellow 4 is used on cellulose fibers, on some cellulose synthetics such as cellulose acetate and cellulose polyester, and on wool and silk. It is also used as a pigment for paper.

C. I. vat yellow 4 is used by the armed services as a smokescreen and as a signaling agent (Owens and Ward, 1974). Smoke dyes were effectively used to mask the movement of troops in World War II and in Korea, where in some instances men were exposed to chemical smokescreens for several months (Puro, 1964). Although there are no production data on C. I. vat yellow 4, the vat dyes

as a class achieved a production volume of 1,254,000 pounds in 1976 (USITC, 1977).

This chemical was selected for study in the Carcinogenesis Testing Program because these uses indicated that instances of significant long-term human exposure may exist.

II. MATERIALS AND METHODS

A. Chemical

The commercial formulated material used in the bioassay was obtained in a single batch from the American Aniline Products, Inc., Philadelphia, Pennsylvania. This material was manufactured by the American Hoechst Corporation in Somerville, New Jersey, as a formulated product. The specifications from the manufacturer required 18.2% of the color-imparting component, 30.8% sorbitol, 5.5% dispersant (Lomar TWC), 2.7% glycerine, and 42.8% water.

The test material was stored at $5^{o}C$ until used.

B. Dietary Preparation

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

third additions of feed, and final mixing was performed with the remaining quantity of feed for a minimum of 15 minutes in a Patterson-Kelly twin-shell blender. The diets were routinely stored at 7°C until used.

No homogeneity or stability studies of the C.I. vat yellow 4 feed mixtures were performed.

C. Animals

Male and female Fischer 344 rats and B6C3F1 mice were obtained through a National Cancer Institute contract from the Frederick Cancer Research Center Animal Farm (Frederick, Md.) as 4-week-old weanlings, all within 3 days of the same age. The animals were housed within the test facility for 2 weeks and then were assigned four rats to a cage and five mice to a cage by a procedure that gave the same average weights per cage for each cage of animals of a given species and sex. For use in the chronic study, the male rats were required to weigh 90 to 105 g, averaging at least 100 g; the female rats, 80 to 95 g, averaging at least 90 g; the male mice, 18 to 22 g, averaging at least 19.5 g; and the female mice, 17 to 21 g, averaging at least 18.5 g. Individual animals were identified by ear punch.

D. Animal Maintenance

The animals were housed in polycarbonate cages (Lab Products, Inc., Garfield, N. J.), 19 x 10-1/2 x 8 inches for the rats and 11-1/2 x 7-1/2 x 5 inches for the mice. The cages were suspended from aluminum racks (Scientific Cages, Inc., Bryan, Tex.) and were covered by nonwoven polyester-fiber 12-mil-thick filter paper (Hoeltge, Inc., Cincinnati, Ohio). The bedding used was Absorb-dri® hardwood chips (Northeastern Products, Inc., Warrenburg, N. Y.). The feed supplied was Wayne® Sterilizable Lab Meal, which was steam autoclaved for 20 minutes before mixing with the test compound. The feed was provided *ad libitum* in suspended stainless steel hoppers and replenished at least three times per week. Water, acidified to pH 2.5, was supplied *ad libitum* from glass bottles. Sipper tubes (Lab Products, Inc.) were suspended through the tops of the cages.

The contaminated bedding was disposed of through an enclosed vacuum line that led to a holding tank from which the bedding was fed periodically into an incinerator. The cages were sanitized twice per week and the feed hoppers twice per month at 82 to 88°C in a tunnel-type cagewasher (Industrial Washing Machine Corp., Mataway, N. J.), using the detergents Clout® (Pharmacal Research Laboratories, Greenwich, Conn.) or Oxford D'Chlor

(Oxford Chemicals, Atlanta, Ga.). The water bottles were sanitized at 82 to 88°C in a tunnel-type bottle washer (Consolidated Equipment Supply Co., Mercersburg, Pa.) three times per week, using a Calgen Commercial Division detergent (St. Louis, Mo.). The racks for the cages were sanitized at or above 82°C in a rack washer (Consolidated Equipment Supply Co.) once per month, using the Calgen Commercial Division detergent, and the filter paper was changed at the same time.

The air in the animal rooms was maintained at a temperature of 22 to 24°C and a relative humidity of 45 to 55%. Nonrecirculated air was passed through a filter of 65% efficiency and a bag filter of 95% efficiency at the intake and through "Z"-type roughing filter of 30% efficiency and a bag system of 90 to 95% efficiency at the exhaust (American Air Filters, Louisville, Ky.; Mine Safety Appliances, Pittsburgh, Pa.). The rate of movement allowed 15 changes of room air per hour. The air pressure was maintained negative to a clean hallway and positive to a return hallway. Fluorescent lighting was provided on an automatic 12-hour-per-day cycle.

Both control and dosed rats were housed in the same room as rats on feeding studies of the following chemicals:

(CAS 298-00-0) methyl parathion
(CAS 148-18-5) sodium diethyldithiocarbamate

Both control and dosed mice were housed in the same room as mice on feeding studies of the following chemicals:

(CAS 103-33-3) azobenzene
(CAS 20941-65-5) ethyl tellurac
(CAS 298-00-0) methyl parathion
(CAS 88-06-2) 2,4,6-trichlorophenol
(CAS 72-56-0) p,p'-ethyl-DDD
(CAS 85-44-9) phthalic anhydride
(CAS 51-03-6) piperonyl butoxide

E. Subchronic Studies

Subchronic feeding studies were conducted to estimate the maximum tolerated doses (MTD's) of C. I. vat yellow 4, on the basis of which two concentrations (hereinafter referred to as "low" and "high" doses) were selected for administration in the chronic studies. Groups of five rats of each sex and five mice of each sex were administered feed containing C. I. vat yellow 4 at one of several doses, and groups of five control animals of each species and sex were administered basal diet only. The period of administration of the test chemical was 7 weeks, followed by 1 week of additional observation. Each animal was weighed twice per week. Table 1 shows the survival of animals in each dosed group at the end of the course of administration and the week on

study when the last animal died; the table also shows the mean body weights of dosed animals at week 7, expressed as percentages of mean body weights of controls.

At the end of the subchronic studies, all animals were killed using CO_2 and necropsied. On clinical and histopathologic examination of the rats, slight to moderate centrilobular cytoplasmic vacuolation was noted in the liver of one male, and trace amounts of the vacuolation were noted in four females at 50,000 ppm. Tissues examined in rats at 6,200 ppm were essentially normal in both males and females. In mice, tissues examined in males at 50,000 and in females at 25,000 ppm were essentially normal.

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

Table 1. C. I. Vat Yellow 4 Subchronic Feeding Studies in Rats and Mice

	Male			Female		
Dose (ppm)	Survival (a)	Week on Study when Last Animal Died	Mean Weight at Week 7 as % of Control	Survival (a)	Week on Study when Last Animal Died	Mean Weight at Week 7 as % of Control
Rats						
6,200	5/5		84	5/5		91
12,500	5/5		87	5/5		85
25,000	5/5		88	5/5		87
50,000	5/5		77	5/5		83
Mice						
6,200	5/5		115	5/5		105
12,500	5/5		116	5/5		111
25,000	5/5		114	5/5		104
50,000	5/5		110	3/5	7	120

(a) Number surviving/number in group.

were used to estimate the doses required to induce 10% depression in weight.

Based on the data thus obtained, the low and high doses for chronic studies using rats were set at 3,500 and 7,000 ppm. The low dose for the chronic studies using male mice was set at 25,000 ppm, and the high dose was set at 50,000 ppm, the maximum amount recommended in Guidelines for Carcinogen Bioassay in Small Rodents (Sontag et al., 1976) in the Carcinogenesis Testing Program. The low and high doses for the chronic studies using female mice were set at 12,500 and 25,000 ppm.

F. Chronic Studies

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

G. Clinical and Pathological Examinations

All animals were observed twice daily for deaths. Observations on sick, tumor-bearing, or moribund animals were recorded daily. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least once per month.

Table 2. C. I. Vat Yellow 4 Chronic Studies in Rats

Sex and Test Group	Initial No. of Animals (a)	C. I. Vat Yellow 4 (b) in Diet (c) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	104
Low-Dose	50	3,500	104
High-Dose	50	7,000	104
Female			
Matched-Control	20	0	104
Low-Dose	50	3,500	104
High-Dose	50	7,000	104

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

(b) Commercially formulated product (see "Introduction" and "Chemical" sections, above).

(c) Test and control diets were provided *ad libitum*.

Table 3. C. I. Vat Yellow 4 Chronic Studies in Mice

Sex and Test Group	Initial No. of Animals (a)	C. I. Vat Yellow 4 (b) in Diet (c) (ppm)	Time on Study (weeks)
Male			
Matched-Control	20	0	106
Low-Dose	50	25,000	106
High-Dose	50	50,000	106
Female			
Matched-Control	20	0	106
Low-Dose	50	12,500	106
High-Dose	50	25,000	106

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

(b) Commercially formulated product (see "Introduction" and "Chemical" sections, above).

(c) Test and control diets were provided *ad libitum*.

Moribund animals and animals that survived to the end of the bioassay were killed using CO_2 and necropsied. Necropsies were also performed on all animals found dead, unless precluded by autolysis or severe cannibalization.

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

A few tissues from some animals were not examined, particularly from those animals that may have died early, been missing, or been in advanced states of cannibalization or autolysis. Thus, the number of animals from which particular organs or tissues

were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

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

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

Probabilities of survival were estimated by the product-limit

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

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

The purpose of the statistical analyses of tumor incidence is to

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

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

A time-adjusted analysis was applied when numerous early deaths

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

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

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95% of a large number of identical experiments, the true ratio of the risk in a dosed group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (P less than 0.025 one-tailed test when the control incidence is not zero, P less than 0.050 when the control incidence is zero) has occurred. When the lower limit is less

than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Mean body weights of dosed male rats were only slightly depressed throughout the bioassay when compared with those of matched controls; mean body weights of females were more depressed throughout the bioassay than those of the males (figure 1). No differences were observed in the effects of the high and low doses. Some fluctuation in the growth curves may be due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Clinical signs including wasting, arched back, rough hair coats, and alopecia occurred at low incidences in dosed groups and may have been related to administration of the test chemical. Corneal opacity and tissue masses occurred at comparable incidences in dosed and control groups.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered C. I. vat yellow 4

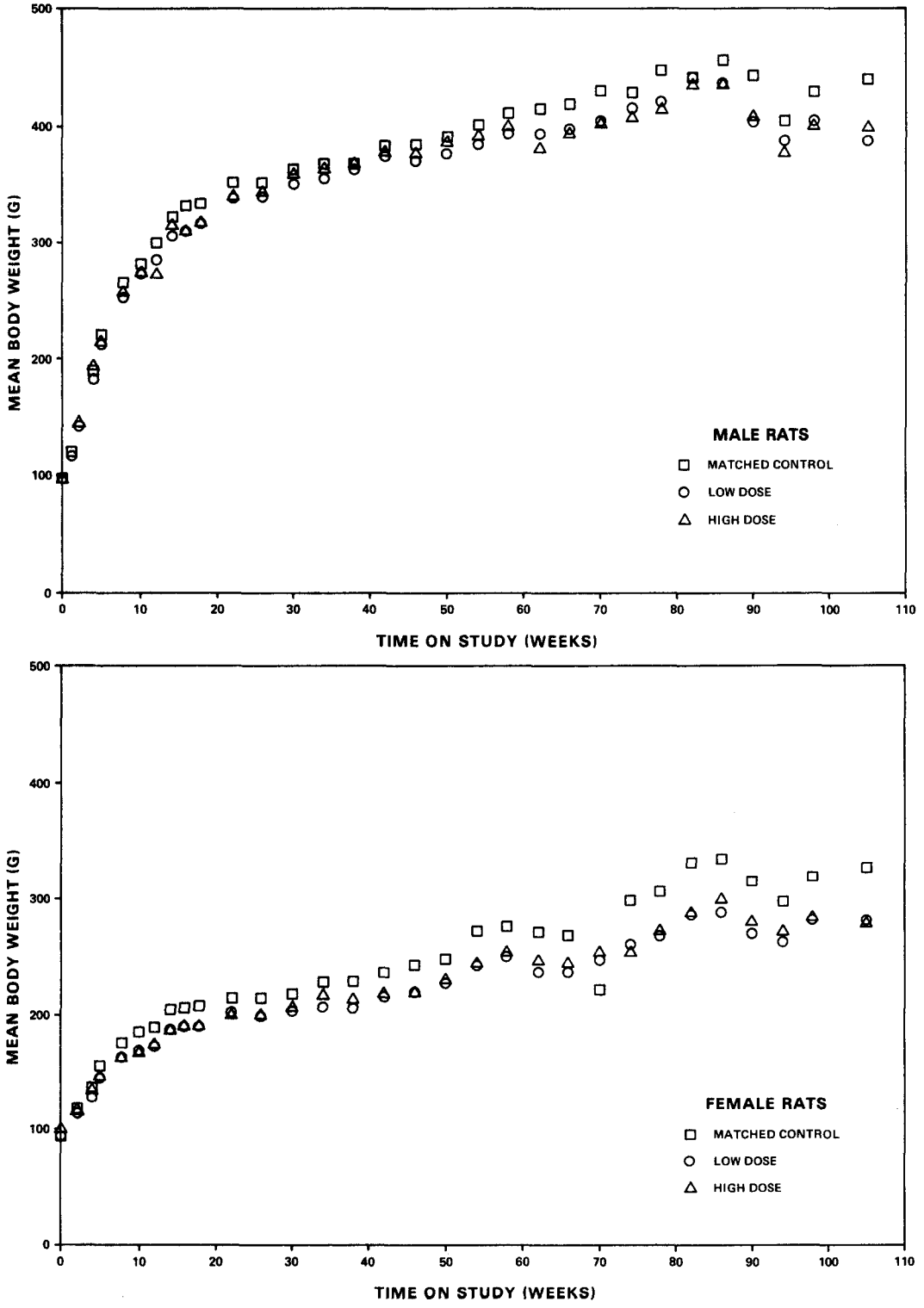

Figure 1. Growth Curves for Rats Administered C.I. Vat Yellow 4 in the Diet

in the diet at the doses of this bioassay, together with those of the matched controls, are shown in figure 2. In male rats, the result of the Tarone test for dose-related trend in mortality is not significant. In females, the result of the Tarone test is significant (P = 0.039), but in the negative direction, because the dosed groups lived longer than the control animals.

In male rats, 39/50 (78%) of the high-dose group, 37/50 (74%) of the low-dose group, and 19/20 (95%) of the control group lived to the end of the study. In females, 45/50 (90%) of the high-dose group, 40/50 (80%) of the low-dose group, and 14/20 (70%) of the control group lived to the end of the study.

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

C. Pathology (Rats)

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

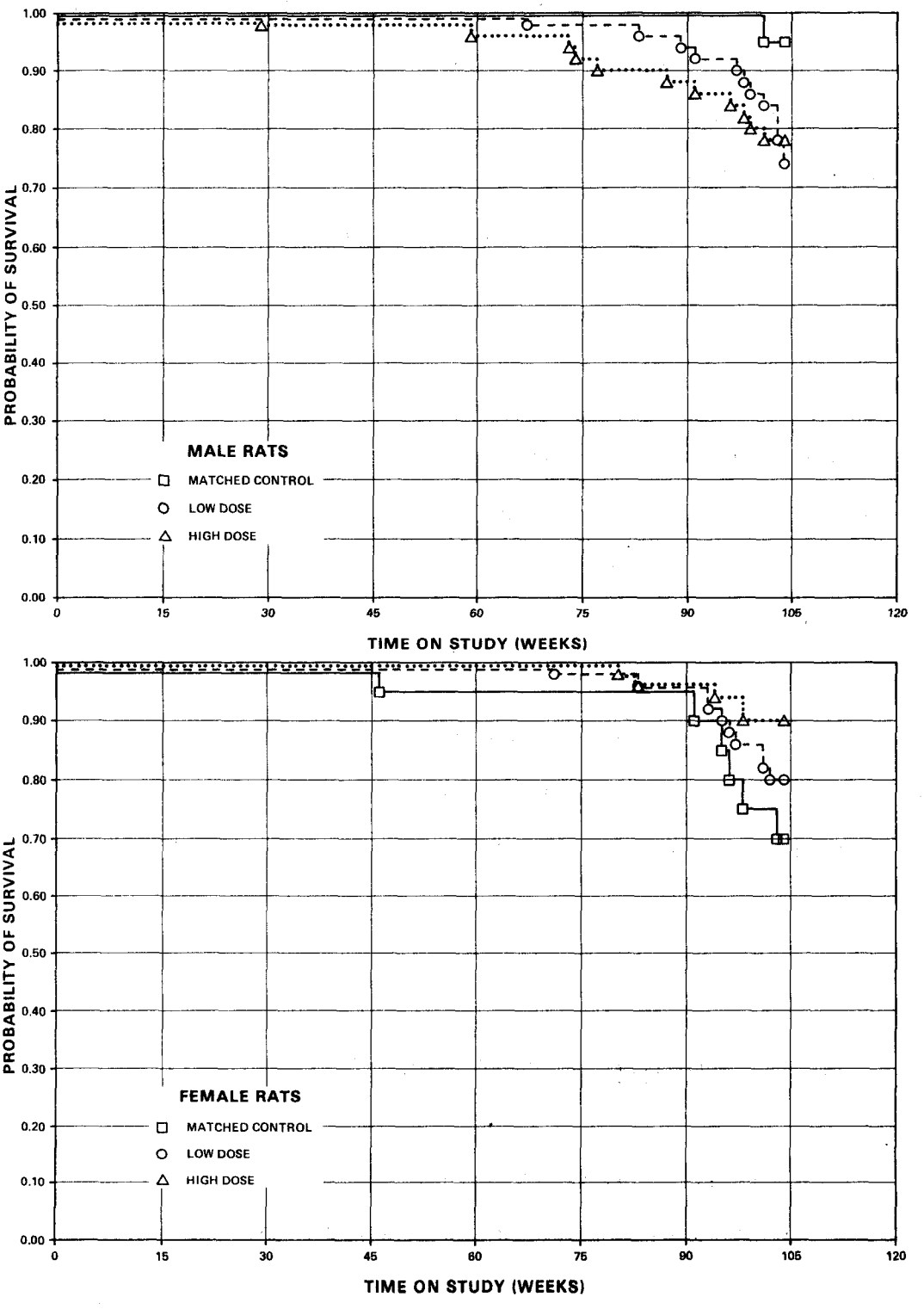

Figure 2. Survival Curves for Rats Administered C.I. Vat Yellow 4 in the Diet

The incidence of proliferative lesions, with the exception of interstitial-cell tumors of the testes in male rats, was low and approximately equal in control and dosed groups of animals. As expected from findings in previous studies using the Fischer 344 strain of rat, most males had interstitial-cell tumors involving one or both testicles.

Occasionally, there were inflammatory and degenerative lesions such as chronic nephritis (especially in males), pneumonia, and cysts of the pituitary and ovaries. Incidences of these changes also appeared to be approximately equal in dosed and control animals.

Based on the pathologic examination, there is no evidence that C. I. vat yellow 4 was carcinogenic or contributed to the incidence of nonneoplastic lesions in Fischer 344 rats under the conditions of this bioassay.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that occurred in at

least two animals of one group and at an incidence of at least 5% in one or more than one group.

In each sex, the results of the Cochran-Armitage test for positive dose-related trend in proportions and those of the Fisher exact test comparing the incidences in the dosed groups with those in the control group in the positive direction are not significant.

Significant results in the negative direction are observed in the incidences of pheochromocytomas of the adrenal and C-cell tumors of the thyroid in male rats and in the incidence of hematopoietic tumors in female rats where the incidences in the control group exceed those in the dosed groups.

In each of the 95% confidence intervals of relative risk, shown in the tables, the value of one or less than one is included; this indicates the absence of significant positive results. It should also be noted that each of the intervals (except that for the incidences of adrenal pheochromocytoma in low-dose male rats and hematopoietic tumors in high-dose female rats) has an upper limit greater than one, indicating the theoretical possibility of the induction of tumors by C. I. vat yellow 4, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of dosed male mice were unaffected by administration of the test chemical; mean body weights of the dosed females also were unaffected, except for a dose-related depression during the last 16 weeks of weight measurement (figure 3). Fluctuation in the growth curves may have been due to mortality; as the size of a group diminishes, the mean body weight may be subject to variation. Clinical signs included tissue masses, which occurred at comparable incidences in dosed and control groups; corneal opacity occurred in two low-dose females. Wasting, arched back, and paralysis each occurred in one or two mice, but none of the clinical signs could be clearly related to administration of the test chemical.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered C. I. vat yellow 4 in the diet at the doses of this bioassay, together with those of

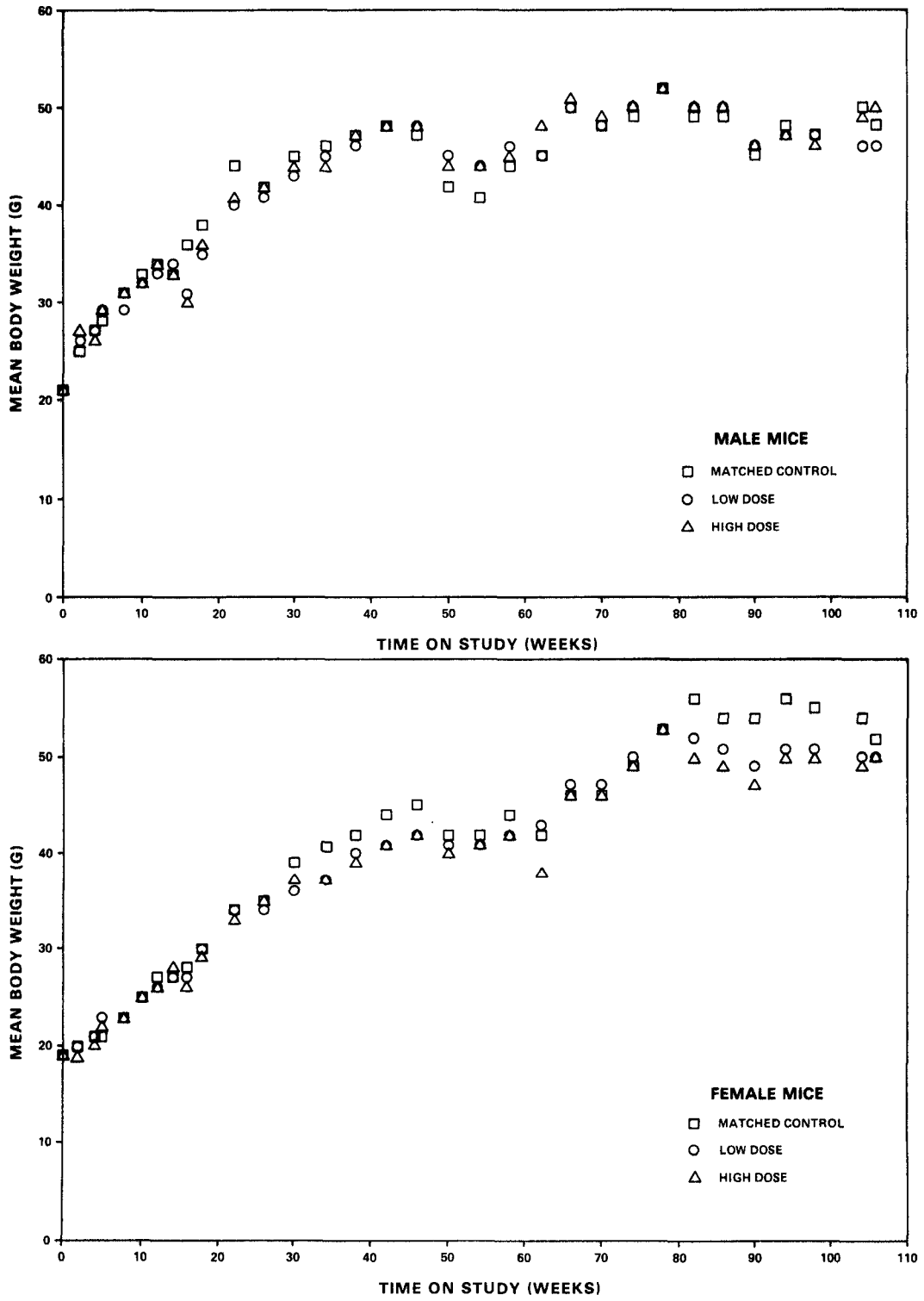

Figure 3. Growth Curves for Mice Administered C.I. Vat Yellow 4 in the Diet

the matched controls, are shown in figure 4. In each sex, the result of the Tarone test for dose-related trend in mortality is not significant.

In male mice, 32/50 (64%) of each dosed group and 16/20 (80%) of the control group were still alive at the end of the bioassay. In females, 39/50 (78%) of the high-dose group, 35/50 (72%) of the low-dose group, and 14/20 (70%) of the control group were alive at the end of the bioassay.

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

C. Pathology (Mice)

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

There was increased incidence of hepatocellular carcinomas in the dosed male and female mice when compared with respective controls. The incidences of these neoplasms were as follows:

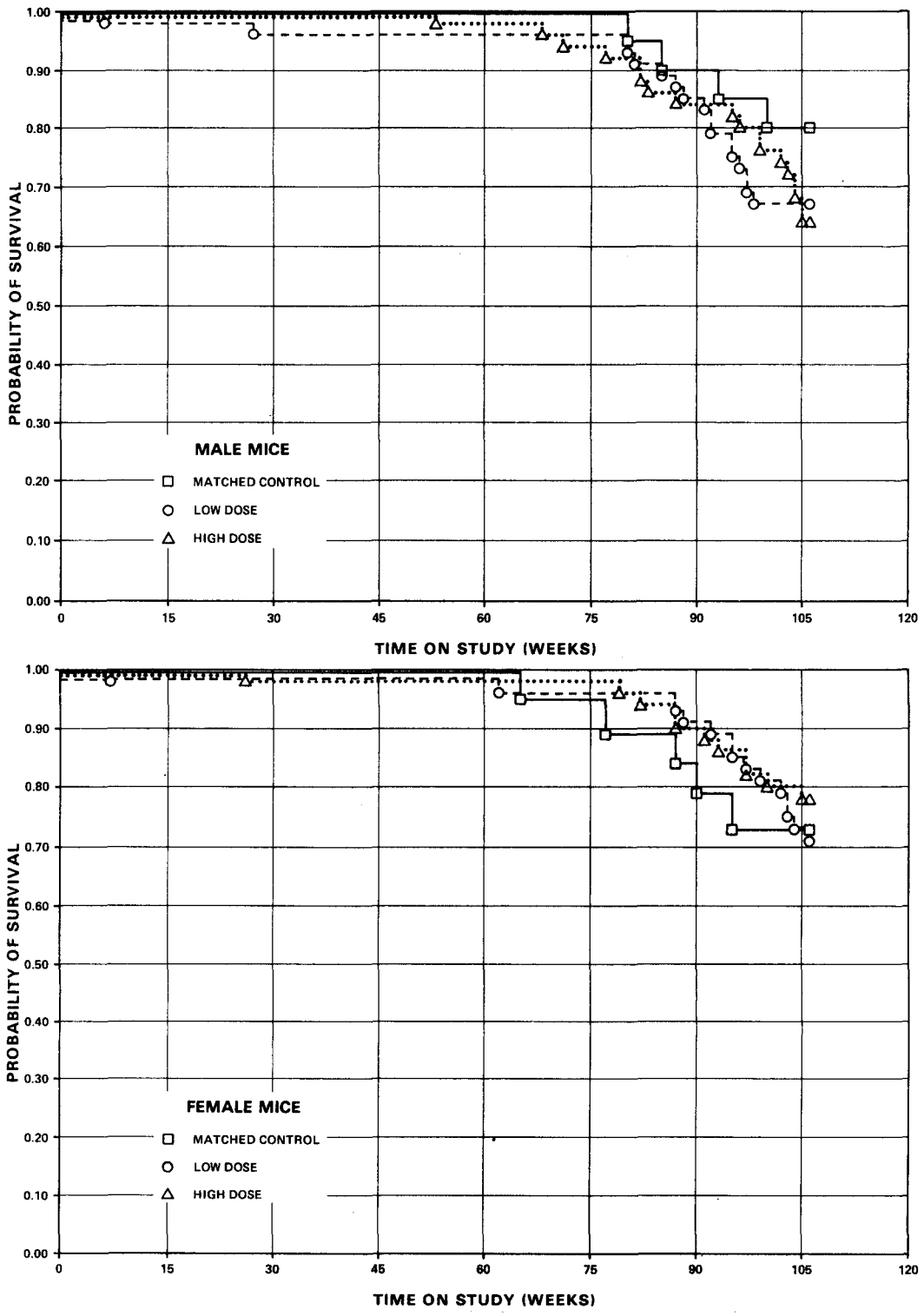

Figure 4. Survival Curves for Mice Administered C.I. Vat Yellow 4 in the Diet

	Controls	Low-Dose	High-Dose
Males	3/20 (15%)	22/47 (47%)	21/50 (42%)
Females	2/19 (11%)	6/48 (13%)	9/50 (18%)

Metastases of the tumor were found in one control male, three low-dose males, and two high-dose males.

The hepatocellular carcinomas had a wide morphologic spectrum, varying from well to very poorly differentiated. Some were small expanding and invasive nodules usually with increased basophilic staining; others were large masses involving large portions of the liver. These varied from a trabecular pattern to solid masses of large anaplastic cells. All had increased mitoses.

Although the incidence of the hepatocellular carcinomas is higher in dosed than in control male mice, the tumor is known to occur frequently in this strain of mouse. In addition, there is no evidence of a dose relationship in the incidence of the tumor in this study.

There was an increased incidence of malignant lymphoma, which frequently occurred in multiple sites in high-dose male mice. The incidences of lymphomas in the mice were as follows:

	Controls	Low-Dose	High-Dose
Males	3/20 (15%)	7/47 (15%)	22/50 (44%)
Females	6/19 (32%)	12/48 (25%)	17/50 (34%)

Other proliferative or neoplastic lesions were of a type, incidence, and distribution commonly found in aged B6C3F1 mice and therefore were believed to be unrelated to administration of the test compound.

In addition to the proliferative lesions, there were inflammatory and degenerative changes in some mice in each of the groups. These included focal mineralization of the brain, focal hepatitis, focal hepatic necrosis, testicular atrophy, and cystic uterine endometrium. The incidence of these changes appeared to be approximately equal in control and dosed groups.

Based on the pathologic examination, administration of C.I. vat yellow 4 may be associated with the increased incidence of lymphomas in male B6C3F1 mice.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses

of the incidences of those primary tumors that occurred in at least two animals of one group and at an incidence of at least 5% in one or more than one group.

In male mice, the result of the Cochran-Armitage test for positive dose-related trend in the incidence of lymphomas is significant (P = 0.002) and the Fisher exact test shows that the incidence in the high-dose group is significantly higher (P = 0.019) than that in the control group. When the life-table method is used on the incidence of lymphoma, as shown in figure 5, the result of the Tarone test shows a P value of 0.004. The statistical evaluation suggests that the incidence of lymphomas in male mice is associated with the administration of C. I. vat yellow 4.

Although the result of the Cochran-Armitage test for the incidence of hepatocellular carcinomas in male mice is not significant at the 0.05 level (the trend statistic has P = 0.07 with the departure statistic of P = 0.056), the Fisher exact comparisons of the incidences in the low- and high-dose groups with that in the control group show P values of 0.012 and 0.027, respectively. The latter is above the 0.025 level for significance when the Bonferroni inequality criterion is used for multiple comparison. Historical records at this laboratory

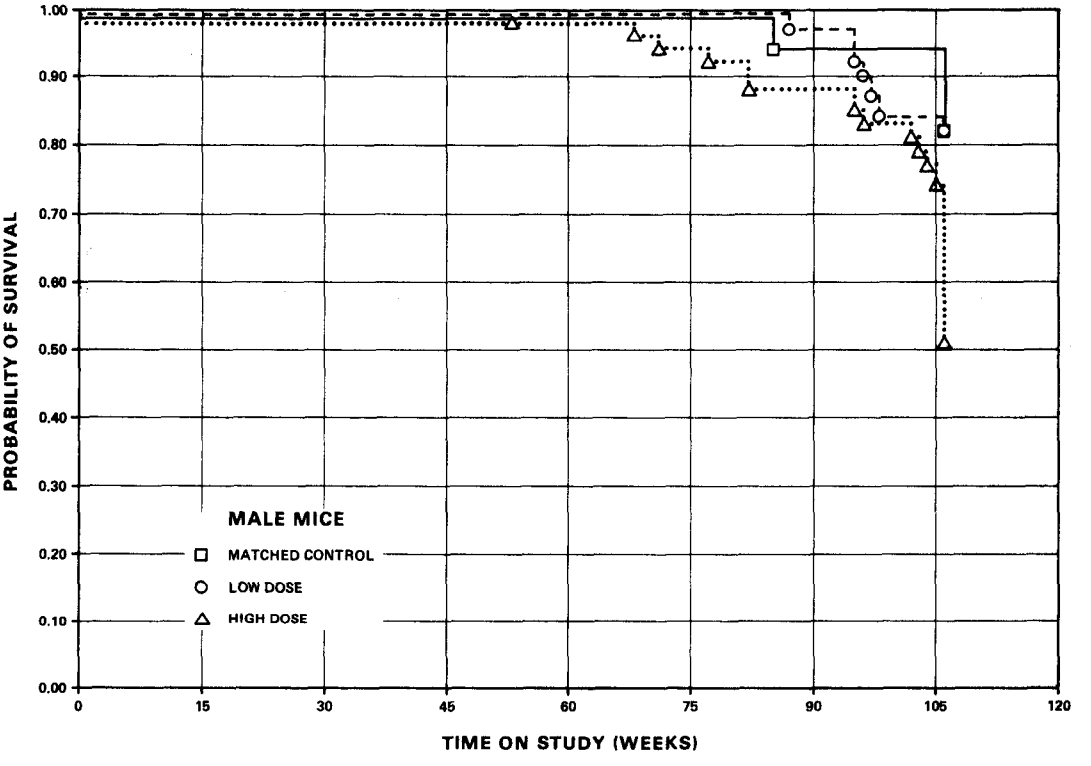

Figure 5. Life Table for Male Mice Administered C.I. Vat Yellow 4 in the Diet: Incidence of Lymphoma

indicate an overall incidence of hepatocellular carcinomas in control male B6C3F1 mice of 93/323 (29%); two separate control groups out of 11 groups had incidences of these tumors as high as 10/20 (50%) and 8/17 (47%) as compared with 22/47 (47%) in the low-dose group and 21/50 (42%) in the high-dose group of the present study. In view of these historical incidences, the occurrences of hepatocellular carcinoma in the dosed male mice of the present test do not convincingly establish a dose association.

The results of the Cochran-Armitage test and of the Fisher exact test are not significant for the incidences of any tumors in the female mice.

DISCUSSION

Depressions in mean body weight occurred throughout the bioassay in the rats administered C. I. vat yellow 4, a commercial formulation of the color-imparting chemical, dibenzo(b,def)-chrysene-7,14-dione. However, the depression was slight for the dosed male rats, and the mean body weights of the mice were not appreciably affected by the test chemical except for the last 16 weeks of the bioassay in the female mice. Clinical signs observed at low incidences in the dosed rats were wasting, arched back, rough hair coats, and alopecia; these signs may have been related to the chemical. Survival of the rats and mice was not affected by the C. I. vat yellow 4 except for an increased survival in the dosed female rats compared with the controls; in every group 82% or more of the animals were alive at week 90 of the bioassay. Thus, sufficient numbers of dosed and control rats and mice of each sex were at risk for the development of late-appearing tumors.

No tumors occurred in male or female rats or female mice at incidences that were significantly higher in dosed groups than in control groups.

Hepatocellular carcinomas occurred in the low-dose male mice at an incidence that was higher (P = 0.012) than that in the control group (controls 3/20, low-dose 22/47, high-dose 21/50). However, the incidence of this tumor in the high-dose group was not significant when the Bonferroni criterion was applied; the incidences in the dosed groups were not significantly dose related; and the incidence in the control group (3/20, or 15%) was lower than that observed for hepatocellular carcinomas or adenomas in historical-control male B6C3F1 mice at this laboratory (93/323, or 29%). Thus, the occurrence of hepatocellular carcinomas in the dosed male B6C3F1 mice of the present bioassay cannot be clearly related to administration of the test chemical.

Lymphomas occurred in the male mice at incidences that were dose related (P = 0.002), and, in a direct comparison, the incidence of the tumor in the high-dose group was significantly higher (P = 0.019) than that in the control group (controls 3/20, or 15%; low-dose 7/47, or 15%; high-dose 22/50, or 44%). When the life-table method is used on the incidence of lymphoma, a P value of 0.004 is obtained with the Tarone test. The incidence of lymphomas and leukemias in historical-control male B6C3F1 mice at this laboratory was 38/323 (12%).

No oral LD_{50} values have been reported for C. I. vat yellow 4 in rats or mice, and no chronic feeding studies with the test chemical have previously been carried out. No tumors appeared in strain P. D. mice in 12-month studies when 1.2 ml of a 0.5% solution of the chemical was injected subcutaneously or when a 0.4% solution in benzene was administered 115 times to the skin (Kleinenberg, 1939). Dibenzo(b,def)chrysene, the parent hydrocarbon analog of the color-imparting component of vat yellow 4, is a potent carcinogen (Haddow and Kon., 1947; Dipple, 1976) and may occur in small amounts as a contaminant in vat yellow 4 (Owens and Ward, 1974). No evidence has been reported for the metabolic reduction of dibenzo(b,def)chrysene-7,14-dione to dibenzo(b,def)chrysene.

It is concluded that under the conditions of this bioassay, the formulated product containing C. I. vat yellow 4 was not carcinogenic for male or female Fischer 344 rats or for female B6C3F1 mice, but was carcinogenic for male B6C3F1 mice, causing an increased incidence of lymphomas.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		2 (4%)	
LIPOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA		1 (2%)	1 (2%)
LIPOMA		2 (4%)	1 (2%)
HEMANGIOSARCOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		5 (10%)	
HEMANGIOSARCOMA			3 (6%)
OSTEOSARCOMA		1 (2%)	
OSTEOSARCOMA, METASTATIC			1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		3 (6%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA	1 (5%)	1 (2%)	1 (2%)
#SPLEEN	(20)	(50)	(50)
UNDIFFERENTIATED LEUKEMIA	2 (10%)	2 (4%)	1 (2%)
#THYMUS	(12)	(18)	(24)
MALIGNANT LYMPHOMA, NOS			1 (4%)
CIRCULATORY SYSTEM			
NONE			

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
HEPATOCELLULAR ADENOMA		1 (2%)	3 (6%)
#COLON	(18)	(48)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
#CECUM	(18)	(48)	(50)
ADENOCARCINOMA, NOS, INVASIVE		1 (2%)	
LIPOMA		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(45)	(49)
ADENOMA, NOS	3 (16%)	4 (9%)	6 (12%)
ADENOCARCINOMA, NOS			2 (4%)
#ADRENAL	(20)	(50)	(50)
CORTICAL CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA	4 (20%)	1 (2%)	5 (10%)
#THYROID	(19)	(50)	(50)
FOLLICULAR-CELL ADENOMA		1 (2%)	
FOLLICULAR-CELL CARCINOMA		2 (4%)	1 (2%)
C-CELL ADENOMA	3 (16%)	2 (4%)	1 (2%)
C-CELL CARCINOMA	1 (5%)		1 (2%)
#PANCREATIC ISLETS	(19)	(49)	(50)
ISLET-CELL ADENOMA		3 (6%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYSTADENOMA, NOS		1 (2%)	
FIBROADENOMA	1 (5%)		
#PROSTATE	(19)	(49)	(49)
CARCINOMA, NOS			1 (2%)

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	16 (80%)	44 (88%)	39 (78%)
*EPIDIDYMIS	(20)	(50)	(50)
LIPOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*BONE/LOWER EXTREMITY	(20)	(50)	(50)
OSTEOSARCOMA			1 (2%)
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	10	9
MORIBUND SACRIFICE		3	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	37	39
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	18	50	47
TOTAL PRIMARY TUMORS	33	82	75
TOTAL ANIMALS WITH BENIGN TUMORS	18	46	43
TOTAL BENIGN TUMORS	28	64	58
TOTAL ANIMALS WITH MALIGNANT TUMORS	4	17	13
TOTAL MALIGNANT TUMORS	5	18	16
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	1
TOTAL SECONDARY TUMORS		1	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA			1 (2%)
KERATOACANTHOMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
SEBACEOUS ADENOCARCINOMA		1 (2%)	
FIBROSARCOMA		2 (4%)	1 (2%)
LIPOMA	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
UNDIFFERENTIATED LEUKEMIA	1 (5%)	3 (6%)	
#SPLEEN	(20)	(48)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		
UNDIFFERENTIATED LEUKEMIA	1 (5%)	2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(50)	(50)
ADENOMA, NOS	2 (11%)	12 (24%)	6 (12%)
ADENOCARCINOMA, NOS	1 (5%)		
#ADRENAL	(20)	(49)	(50)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(50)	(50)
C-CELL ADENOMA	2 (10%)	1 (2%)	1 (2%)
C-CELL CARCINOMA		1 (2%)	3 (6%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOMA, NOS		1 (2%)	
CYSTADENOMA, NOS	1 (5%)	2 (4%)	1 (2%)
FIBROADENOMA	1 (5%)	8 (16%)	6 (12%)
#UTERUS/ENDOMETRIUM	(20)	(49)	(50)
ADENOCARCINOMA, NOS	1 (5%)		
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
GLIOMA, NOS		1 (2%)	1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	7	3
MORIBUND SACRIFICE	2	3	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	40	45
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	9	26	23
TOTAL PRIMARY TUMORS	14	34	27
TOTAL ANIMALS WITH BENIGN TUMORS	7	22	16
TOTAL BENIGN TUMORS	8	24	20
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	8	7
TOTAL MALIGNANT TUMORS	6	10	7
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN

MICE ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(47)	(50)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(20)	(47)	(50)
ADENOCARCINOMA, NOS			1 (2%)
KERATOACANTHOMA	1 (5%)		
FIBROMA		1 (2%)	
FIBROSARCOMA			2 (4%)
LIPOMA	2 (10%)	1 (2%)	4 (8%)
HEMANGIOMA		1 (2%)	
HEMANGIOSARCOMA			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(47)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)	3 (6%)	1 (2%)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	4 (8%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	4 (20%)	12 (26%)	11 (22%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(47)	(50)
MALIGNANT LYMPHOMA, NOS	2 (10%)	4 (9%)	16 (32%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			2 (4%)
*SUBCUT TISSUE	(20)	(47)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#SPLEEN	(19)	(45)	(50)
SARCOMA, NOS			1 (2%)
HEMANGIOSARCOMA		1 (2%)	1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(18)	(46)	(49)
MALIGNANT LYMPHOMA, NOS	1 (6%)	1 (2%)	1 (2%)
#LIVER	(20)	(47)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(50)
HEPATOCELLULAR CARCINOMA	3 (15%)	22 (47%)	21 (42%)
HEMANGIOSARCOMA			2 (4%)
#SMALL INTESTINE	(19)	(46)	(49)
ADENOCARCINOMA, NOS		3 (7%)	1 (2%)
SARCOMA, NOS			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (5%)		
TUBULAR-CELL ADENOMA	1 (5%)		
ENDOCRINE SYSTEM			
#ADRENAL	(19)	(46)	(46)
CORTICAL CARCINOMA	1 (5%)		
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(20)	(45)	(48)
ADENOCARCINOMA, NOS		1 (2%)	
FOLLICULAR-CELL CARCINOMA			1 (2%)
#PANCREATIC ISLETS	(19)	(46)	(47)
ISLET-CELL ADENOMA	1 (5%)	1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(20)	(47)	(50)
GLIOMA, NOS			1 (2%)
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(20)	(47)	(50)
ADENOMA, NOS		2 (4%)	1 (2%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(47)	(50)
LIPOMA			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	16	17
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	16	32	32
ANIMAL MISSING		2	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	42	48
TOTAL PRIMARY TUMORS	16	55	78
TOTAL ANIMALS WITH BENIGN TUMORS	4	7	10
TOTAL BENIGN TUMORS	5	8	11
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	38	44
TOTAL MALIGNANT TUMORS	11	47	67
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	3	1
TOTAL SECONDARY TUMORS	2	3	1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	19	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	48	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(48)	(50)
FIBROSARCOMA		1 (2%)	2 (4%)
LIPOMA	1 (5%)	1 (2%)	1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(19)	(48)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	2 (11%)	3 (6%)	2 (4%)
FIBROSARCOMA			1 (2%)
OSTEOMA	1 (5%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(19)	(48)	(50)
MALIGNANT LYMPHOMA, NOS	3 (16%)	8 (17%)	9 (18%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)	2 (4%)	2 (4%)
*PERITONEAL CAVITY	(19)	(48)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
*SUBCUT TISSUE	(19)	(48)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
#SPLEEN	(19)	(47)	(50)
HEMANGIOSARCOMA		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	2 (4%)
#MESENTERIC L. NODE	(19)	(48)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
#LIVER	(19)	(48)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#SMALL INTESTINE	(19)	(45)	(42)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (5%)		
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(18)	(47)	(50)
HEMANGIOMA			1 (2%)
#LIVER	(19)	(48)	(50)
HEPATOCELLULAR CARCINOMA	2 (11%)	6 (13%)	9 (18%)
HEMANGIOSARCOMA		1 (2%)	
#SMALL INTESTINE	(19)	(45)	(42)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(43)	(48)
ADENOMA, NOS	1 (5%)	1 (2%)	1 (2%)
#ADRENAL	(19)	(48)	(49)
CORTICAL ADENOMA		1 (2%)	
#THYROID	(19)	(47)	(49)
FOLLICULAR-CELL ADENOMA		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(48)	(48)
LEIOMYOSARCOMA			1 (2%)
NERVOUS SYSTEM			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(19)	(48)	(50)
ADENOMA, NOS	1 (5%)	2 (4%)	2 (4%)
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	4	14	11
MORIBUND SACRIFICE	1		
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	14	35	39
ANIMAL MISSING	1	1	

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	13	29	29
TOTAL PRIMARY TUMORS	14	33	39
TOTAL ANIMALS WITH BENIGN TUMORS	3	8	5
TOTAL BENIGN TUMORS	4	8	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	21	28
TOTAL MALIGNANT TUMORS	10	25	32
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
CYST, NOS	1 (5%)		
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
ATELECTASIS	2 (10%)		4 (8%)
INFLAMMATION, NOS			1 (2%)
ALVEOLAR MACROPHAGES	1 (5%)		
HEMATOPOIETIC SYSTEM			
#SPLEEN	(20)	(50)	(50)
FIBROSIS		1 (2%)	1 (2%)
HEMATOPOIESIS	1 (5%)	3 (6%)	3 (6%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL	1 (5%)	3 (6%)	
INFLAMMATION, CHRONIC	1 (5%)		
FIBROSIS	2 (10%)		1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(50)
GRANULOMA, NOS	1 (5%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HEPATOCYTOMEGALY		2 (4%)	2 (4%)
HYPERPLASIA, NODULAR		1 (2%)	
REGENERATIVE NODULE		1 (2%)	
#LIVER/HEPATOCYTES	(20)	(50)	(50)
CYTOPLASMIC VACUOLIZATION		6 (12%)	4 (8%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	3 (15%)	4 (8%)	4 (8%)
#PANCREAS	(19)	(49)	(50)
INFLAMMATION, NOS			1 (2%)
PERIARTERITIS		3 (6%)	1 (2%)
ATROPHY, NOS	2 (11%)	6 (12%)	13 (26%)
#STOMACH	(20)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
#COLON	(18)	(48)	(50)
POLYPOID HYPERPLASIA			1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, CHRONIC	17 (85%)	44 (88%)	43 (86%)
INFLAMMATION, GRANULOMATOUS			1 (2%)
INFARCT, HEALED	1 (5%)		
#KIDNEY/CORTEX	(20)	(50)	(50)
CYST, NOS		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(45)	(49)
CYST, NOS	2 (11%)	3 (7%)	3 (6%)
ANGIECTASIS	2 (11%)	4 (9%)	1 (2%)
#ADRENAL	(20)	(50)	(50)
ANGIECTASIS		3 (6%)	1 (2%)
#ADRENAL MEDULLA	(20)	(50)	(50)
ANGIECTASIS		1 (2%)	
#THYROID	(19)	(50)	(50)
CYSTIC FOLLICLES		1 (2%)	2 (4%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
CYST, NOS			1 (2%)
#PROSTATE	(19)	(49)	(49)
INFLAMMATION, NOS	2 (11%)	5 (10%)	3 (6%)
HYPERPLASIA, CYSTIC		1 (2%)	
#TESTIS	(20)	(50)	(50)
ATROPHY, NOS		2 (4%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(50)
INFLAMMATION, FOCAL		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
AUTO/NECROPSY/HISTO PERF	1		1

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(50)
INFLAMMATION, SUPPURATIVE		1 (2%)	
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(20)	(50)	(50)
HYPOPLASIA, HEMATOPOIETIC	1 (5%)		
#SPLEEN	(20)	(48)	(50)
PIGMENTATION, NOS	1 (5%)	2 (4%)	
HYPERPLASIA, LYMPHOID			1 (2%)
HEMATOPOIESIS	7 (35%)	19 (40%)	16 (32%)
CIRCULATORY SYSTEM			
#HEART	(20)	(50)	(50)
THROMBUS, MURAL			2 (4%)
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL		2 (4%)	1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(20)	(50)	(49)
INFLAMMATION, SUPPURATIVE	1 (5%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
#LIVER	(20)	(49)	(50)
NECROSIS, FOCAL			1 (2%)
HEPATOCYTOMEGALY	1 (5%)		
HYPERPLASIA, NODULAR		1 (2%)	
#LIVER/HEPATOCYTES	(20)	(49)	(50)
CYTOPLASMIC VACUOLIZATION	4 (20%)	3 (6%)	5 (10%)
*BILE DUCT	(20)	(50)	(50)
HYPERPLASIA, NOS	1 (5%)		
#PANCREAS	(19)	(48)	(50)
ECTOPIA			1 (2%)
ATROPHY, NOS		6 (13%)	13 (26%)
#STOMACH	(20)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL	1 (5%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERKERATOSIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(50)
INFLAMMATION, CHRONIC	4 (20%)	6 (12%)	10 (20%)
#KIDNEY/CORTEX	(20)	(49)	(50)
CYST, NOS			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(19)	(50)	(50)
CYST, NOS	2 (11%)	3 (6%)	5 (10%)
ANGIECTASIS	5 (26%)	2 (4%)	14 (28%)
#ADRENAL	(20)	(49)	(50)
ANGIECTASIS	1 (5%)	2 (4%)	2 (4%)
#THYROID	(20)	(50)	(50)
CYSTIC FOLLICLES			1 (2%)
HYPERPLASIA, C-CELL	3 (15%)	6 (12%)	

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
HYPERPLASIA, CYSTIC	2 (10%)	1 (2%)	2 (4%)
#UTERUS	(20)	(49)	(50)
POLYPOID HYPERPLASIA	4 (20%)	8 (16%)	4 (8%)
#UTERUS/ENDOMETRIUM	(20)	(49)	(50)
CYST, NOS	2 (10%)	1 (2%)	1 (2%)
#OVARY	(20)	(49)	(50)
CYST, NOS	1 (5%)	3 (6%)	6 (12%)
NERVOUS SYSTEM			
#BRAIN	(20)	(50)	(50)
HEMORRHAGE	1 (5%)	1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	1	3	6

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
AUTO/NECROPSY/HISTO PERF	1	2	

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING		2	
ANIMALS NECROPSIED	20	47	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	20	47	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(47)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(45)	(50)
HYPERPLASIA, LYMPHOID	1 (5%)	5 (11%)	5 (10%)
HEMATOPOIESIS	2 (11%)	10 (22%)	3 (6%)
#MESENTERIC L. NODE	(18)	(46)	(49)
HYPERPLASIA, LYMPHOID			3 (6%)
CIRCULATORY SYSTEM			
#HEART	(20)	(47)	(50)
MINERALIZATION			1 (2%)
#MYOCARDIUM	(20)	(47)	(50)
INFLAMMATION, FOCAL	1 (5%)		
DIGESTIVE SYSTEM			
#LIVER	(20)	(47)	(50)
INFLAMMATION, FOCAL			1 (2%)
NECROSIS, FOCAL		3 (6%)	1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
HYPERPLASIA, NODULAR	2 (10%)	4 (9%)	4 (8%)
ANGIECTASIS			3 (6%)
#LIVER/HEPATOCYTES	(20)	(47)	(50)
CYTOPLASMIC VACUOLIZATION	1 (5%)		
#PANCREAS	(19)	(46)	(47)
CYSTIC DUCTS		1 (2%)	
#STOMACH	(19)	(47)	(50)
INFLAMMATION, FOCAL		2 (4%)	2 (4%)
#COLON	(19)	(46)	(46)
PARASITISM		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(20)	(47)	(50)
INFLAMMATION, INTERSTITIAL	1 (5%)	1 (2%)	
INFLAMMATION, CHRONIC	1 (5%)		
INFARCT, HEALED			1 (2%)
LYMPHOCYTOSIS	1 (5%)	1 (2%)	
#KIDNEY/CORTEX	(20)	(47)	(50)
CYST, NOS			1 (2%)
ENDOCRINE SYSTEM			
#THYROID	(20)	(45)	(48)
ATROPHY, NOS	1 (5%)	1 (2%)	
#PANCREATIC ISLETS	(19)	(46)	(47)
HYPERPLASIA, NOS	3 (16%)	5 (11%)	1 (2%)
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(47)	(50)
CAST, NOS		1 (2%)	
#TESTIS	(19)	(46)	(50)
ATROPHY, NOS		3 (7%)	3 (6%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(20)	(47)	(50)
MINERALIZATION	5 (25%)	12 (26%)	7 (14%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	1	2
ANIMAL MISSING/NO NECROPSY		2	
AUTOLYSIS/NO NECROPSY		1	

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE ADMINISTERED C.I. VAT YELLOW 4 IN THE DIET

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING	1	1	
ANIMALS NECROPSIED	19	48	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	19	48	50
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(19)	(48)	(50)
CYST, NOS		2 (4%)	
RESPIRATORY SYSTEM			
#LUNG	(19)	(48)	(50)
HEMORRHAGE	1 (5%)	1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(19)	(47)	(50)
HYPERPLASIA, LYMPHOID		3 (6%)	2 (4%)
HEMATOPOIESIS	7 (37%)	17 (36%)	12 (24%)
#MANDIBULAR L. NODE	(19)	(48)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#MESENTERIC L. NODE	(19)	(48)	(50)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, LYMPHOID			1 (2%)
#THYMUS	(11)	(23)	(37)
HYPERPLASIA, LYMPHOID			1 (3%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(48)	(50)
INFLAMMATION, FOCAL	1 (5%)		
DIGESTIVE SYSTEM			
#LIVER	(19)	(48)	(50)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, GRANULOMATOUS		1 (2%)	
HYPERPLASIA, NODULAR	1 (5%)	2 (4%)	5 (10%)
LYMPHOCYTOSIS	1 (5%)		
#PANCREAS	(19)	(43)	(45)
CYSTIC DUCTS	1 (5%)		3 (7%)
#STOMACH	(19)	(48)	(49)
INFLAMMATION, FOCAL		2 (4%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(19)	(48)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
INFARCT, NOS			1 (2%)
METAPLASIA, OSSEOUS		1 (2%)	
LYMPHOCYTOSIS	1 (5%)		3 (6%)
#URINARY BLADDER	(17)	(46)	(44)
LYMPHOCYTOSIS			2 (5%)
ENDOCRINE SYSTEM			
#THYROID	(19)	(47)	(49)
CYSTIC FOLLICLES		1 (2%)	1 (2%)
REPRODUCTIVE SYSTEM			
#UTERUS	(19)	(48)	(48)
POLYPOID HYPERPLASIA			1 (2%)
#UTERUS/ENDOMETRIUM	(19)	(48)	(48)
CYST, NOS	7 (37%)	14 (29%)	7 (15%)
#OVARY	(17)	(48)	(46)
CYST, NOS	1 (6%)	10 (21%)	7 (15%)
NERVOUS SYSTEM			
#BRAIN	(19)	(48)	(50)
MINERALIZATION	2 (11%)	7 (15%)	10 (20%)
SPECIAL SENSE ORGANS			
NONE			

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	MATCHED CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(19)	(48)	(50)
FIBROSIS		1 (2%)	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		4	5
ANIMAL MISSING/NO NECROPSY	1	1	
AUTOLYSIS/NO NECROPSY		1	

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

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	0/20 (0)	5/50 (10)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.010		
Relative Risk (f)		Infinite	--
Lower Limit		0.525	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	104	--
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	1/20 (5)	8/50 (16)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Departure from Linear Trend (e)	P = 0.019		
Relative Risk (f)		3.200	0.400
Lower Limit		0.482	0.005
Upper Limit		138.771	30.802
Weeks to First Observed Tumor	104	101	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	3/20 (15)	6/50 (12)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.800	0.533
Lower Limit		0.195	0.102
Upper Limit		4.615	3.410
Weeks to First Observed Tumor	104	89	59
All Sites: Hemangiosarcoma (b)	1/20 (5)	0/50 (0)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.200
Lower Limit		0.000	0.106
Upper Limit		7.475	61.724
Weeks to First Observed Tumor	104	--	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Adenoma (b)	0/20 (0)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	101
Pituitary: Adenoma, NOS (b)	3/19 (16)	4/45 (9)	6/49 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.563	0.776
Lower Limit		0.108	0.191
Upper Limit		3.578	4.463
Weeks to First Observed Tumor	104	91	96

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma or Adenocarcinoma, NOS (b)	3/19 (16)	4/45 (9)	8/49 (16)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.563	1.034
Lower Limit		0.108	0.288
Upper Limit		3.578	5.620
Weeks to First Observed Tumor	104	91	96
Adrenal: Pheochromocytoma (b)	4/20 (20)	1/50 (2)	5/50 (10)
P Values (c,d)	N.S.	P = 0.021 (N)	N.S.
Departure from Linear Trend (e)	P = 0.015		
Relative Risk (f)		0.100	0.500
Lower Limit		0.002	0.124
Upper Limit		0.944	2.322
Weeks to First Observed Tumor	104	104	91

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: Follicular-cell Adenoma or Carcinoma (b)	0/19 (0)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.238	0.021
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	104	104
Thyroid: C-cell Adenoma or Carcinoma (b)	4/19 (21)	2/50 (4)	2/50 (4)
P Values (c,d)	P = 0.034 (N)	P = 0.045 (N)	P = 0.045 (N)
Relative Risk (f)		0.190	0.190
Lower Limit		0.019	0.019
Upper Limit		1.230	1.230
Weeks to First Observed Tumor	104	104	104

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pancreatic Islets: Islet-cell Adenoma (b)	0/19 (0)	3/49 (6)	0/50 (0)
P Values (c,d)	N.S.	N.S.	--
Departure from Linear Trend (e)	P = 0.047		
Relative Risk (f)		Infinite	--
Lower Limit		0.243	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	104	--
Testis: Interstitial-cell Tumor (b)	16/20 (80)	44/50 (88)	39/50 (78)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.100	0.975
Lower Limit		0.884	0.775
Upper Limit		1.458	1.382
Weeks to First Observed Tumor	104	83	91

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

(a) Dosed groups received 3,500 or 7,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P less than 0.05; otherwise, not signficant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered C. I. Vat Yellow 4 in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	1/20 (5)	0/50 (0)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.000	1.600
Lower Limit		0.000	0.175
Upper Limit		7.475	77.169
Weeks to First Observed Tumor	104	--	104
Hematopoietic System: Lymphoma or Leukemia (b)	4/20 (20)	5/50 (10)	1/50 (2)
P Values(c, d)	P = 0.011 (N)	N.S.	P = 0.021 (N)
Relative Risk (f)		0.500	0.100
Lower Limit		0.124	0.002
Upper Limit		2.322	0.944
Weeks to First Observed Tumor	95	93	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Pituitary: Adenoma, NOS (b)	2/19 (11)	12/50 (24)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.280	1.140
Lower Limit		0.587	0.232
Upper Limit		19.837	10.985
Weeks to First Observed Tumor	103	96	98
Pituitary: Adenoma or Adenocarcinoma, NOS (b)	3/19 (16)	12/50 (24)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.520	0.760
Lower Limit		0.481	0.187
Upper Limit		7.762	4.377
Weeks to First Observed Tumor	91	96	98

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Thyroid: C-cell Carcinoma (b)	0/20 (0)	1/50 (2)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.022	0.250
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	101	104
Thyroid: C-cell Carcinoma or Adenoma (b)	2/20 (10)	2/50 (4)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.400	0.800
Lower Limit		0.032	0.128
Upper Limit		5.277	8.436
Weeks to First Observed Tumor	91	101	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Mammary Gland: Adenoma or Cystadenoma, NOS (b)	1/20 (5)	3/50 (6)	1/50 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.200	0.400
Lower Limit		0.106	0.005
Upper Limit		61.724	30.802
Weeks to First Observed Tumor	91	102	104
Mammary Gland: Fibroadenoma (b)	1/20 (5)	8/50 (16)	6/50 (12)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		3.200	2.400
Lower Limit		0.482	0.325
Upper Limit		138.771	108.021
Weeks to First Observed Tumor	104	83	104

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

(a) Dosed groups received 3,500 or 7,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P less than 0.05; otherwise, not signficant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TURMORS IN MICE ADMINISTERED C. I. VAT YELLOW 4 IN THE DIET

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered C. I. Vat Yellow 4 in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Integumentary System: Lipoma of the the Subcutaneous Tissue (b)	2/20 (10)	1/47 (2)	4/50 (8)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.213	0.800
Lower Limit		0.004	0.128
Upper Limit		3.909	8.436
Weeks to First Observed Tumor	106	97	105
Lung: Alveolar/Bronchiolar Carcinoma (b)	4/20 (20)	12/47 (26)	11/50 (22)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.277	1.100
Lower Limit		0.457	0.384
Upper Limit		4.926	4.321
Weeks to First Observed Tumor	100	85	87

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	4/20 (20)	14/47 (30)	15/50 (30)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.489	1.500
Lower Limit		0.554	0.566
Upper Limit		5.616	5.627
Weeks to First Observed Tumor	100	85	82
Hematopoietic System: Lymphoma (b)	3/20 (15)	7/47 (15)	22/50 (44)
P Values (c,d)	P = 0.002	N.S.	P = 0.019
Relative Risk (f)		0.993	2.933
Lower Limit		0.261	1.040
Upper Limit		5.532	13.947
Weeks to First Observed Tumor	85	87	53

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	3/20 (15)	22/47 (47)	21/50 (42)
P Values (c,d)	N.S.	P = 0.012	P = 0.027
Relative Risk (f)		3.121	2.800
Lower Limit		1.110	0.986
Upper Limit		14.730	13.384
Weeks to First Observed Tumor	80	27	83
Small Intestine: Adenocarcinoma, NOS (b)	0/19 (0)	3/46 (7)	1/49 (2)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		Infinite	Infinite
Lower Limit		0.259	0.021
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	106	106

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

(a) Dosed groups received 25,000 or 50,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P less than 0.05; otherwise, not signficant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered C. I. Vat Yellow 4 in the Diet (a)

Topography: Morphology	Matched Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Carcinoma (b)	2/19 (11)	3/48 (6)	2/50 (4)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.594	0.380
Lower Limit		0.076	0.030
Upper Limit		6.774	5.009
Weeks to First Observed Tumor	106	106	106
Lung: Alveolar/Bronchiolar Carcinoma or Adenoma (b)	2/19 (11)	5/48 (10)	3/50 (6)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.990	0.570
Lower Limit		0.184	0.073
Upper Limit		9.880	6.511
Weeks to First Observed Tumor	106	106	106

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

Topography: Morphology	Matched Control	Low Dose	High Dose
Hematopoietic System:			
Lymphoma (b)	6/19 (32)	12/48 (25)	17/50 (34)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		0.792	1.077
Lower Limit		0.336	0.499
Upper Limit		2.267	2.920
Weeks to First Observed Tumor	77	62	26
Liver: Hepatocellular Carcinoma (b)	2/19 (11)	6/48 (13)	9/50 (18)
P Values (c,d)	N.S.	N.S.	N.S.
Relative Risk (f)		1.188	1.710
Lower Limit		0.242	0.407
Upper Limit		11.426	15.426
Weeks to First Observed Tumor	106	87	93

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Administered C. I. Vat Yellow 4 in the Diet (a)

(continued)

(a) Dosed groups received 12,500 or 25,000 ppm.

(b) Number of tumor-bearing animals/number of animals examined at site (percent).

(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P less than 0.05; otherwise, not signficant (N.S.) is indicated.

(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.

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

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

Review of the Bioassay of C.I. Vat Yellow 4* for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

August 31, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of C.I. Vat Yellow 4 for carcinogenicity.

Although the primary reviewer noted the increased incidence of lymphomas and hepatomas among treated high dose male mice, he said that the evidence was insufficient to designate C.I. Vat Yellow 4 to be a carcinogen. He commented on the large disparity between the dose levels administered to rats (3,500 and 7,000 ppm) compared to mice (male: 25,000 and 50,000 ppm; female: 12,500 and 25,000 ppm). Except for a question regarding the disparity in dosages, the primary reviewer said that the study was adequate. He said that the lack of a dose-response relationship for hepatomas may be due to the fact that both dose levels are on the upper slope of the curve. He opined that the absence of a response among treated female mice may be a result of the lower dose levels administered. The primary reviewer suggested that the report over emphasized statistics and that not enough attention was given to pharmacological considerations. He recommended that both the increased incidence of hepatomas and lymphomas, among treated male mice, recieve mention in the report's summary conclusion. He proposed that the conclusion be reworded as follows, "Increased incidences of lymphomas and of hepatomas were observed in male mice receiving the higher dose, suggesting carcinogenic activity and requiring consideration for further tests." He concluded by stating that the human risk posed by C.I. Vat Yellow 4, if any, is very low.

A program staff member said that the staff had rejected the significance of the liver tumors in the high dose male mice based on the Bonferroni criterion. In addition, the historical control data did not support the significance of the liver tumors. In regard to the lymphomas, he said that their significance was sustained by both the Bonferroni criterion and historical control data.

The primary reviewer said that the basic disagreement was not with the interpretation of the data but rather with how it should be stated. A vote on a proposal that the conclusion be reworded, as suggested, was defeated. Subsequently, a recommendation was approved unanimously that the report on the bioassay of C.I. Vat Yellow 4 be accepted as written.

Members present were:

Arnold Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center

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

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