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CARCINOGENESIS BIOASSAY OF FD & C YELLOW NO. 6

(CAS NO. 2783-94-0)

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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NTP Technical Report

on the

CARCINOGENESIS BIOASSAY

of

FD & C YELLOW NO. 6

(CAS No. 2783-94-0)

National Cancer Institute
NATIONAL TOXICOLOGY PROGRAM
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Comments and questions about the National Toxicology Program Technical Reports on Carcinogenesis Bioassays should be directed to Ms. Joan Chase, Technical Information Section, Room A-306, Landow Building, Bethesda, MD 20014 (301-496-1152).

CARCNOGENESIS BIOASSAY

OF

FD & C YELLOW NO. 6

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FOREWORD

This report presents the results of the bioassay of FD & C Yellow No. 6 conducted for the Carcinogenesis Testing Program, National Cancer Institute (NCI)/National Toxicology Program (NTP). 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 a test chemical is not a carcinogen inasmuch as the experiments are conducted under a limited set of circumstances. Positive results demonstrate that a test chemical is carcinogenic for animals under the conditions of the test and indicate that exposure to the chemical may pose a potential risk to man. The actual determination of the risk to man from chemicals found to be carcinogenic in animals requires a wider analysis which extends beyond the purview of this study.

CONTRIBUTORS

This bioassay was conducted December 1976 - January 1979 at Battelle Columbus Laboratories, Columbus, Ohio, under a subcontract to Tracor Jitco, Inc., Rockville, Maryland, prime contractor for the NCI Carcinogenesis Testing Program. Dr. A Peters (1) was the principal investigator for this study. Doses of the test chemical were selected by Dr. A. Peters and J. Robens (2,3). Drs. A. Peters, H. Harroff (1), and P. Stromberg (1) were in charge of animal care.

Necropsies were directed by Drs. G. D. Dill (1), R. Persing (1), R. Everett (1,4), and D. Thake (1). Histopathologic evaluations were performed by Drs. S. G. Dill (rats) and R. Persing (mice) (1). The pathology report and selected slides were evaluated by the NCI Pathology Working Group as described by Ward et al. (1978). The diagnoses represent a consensus of contracting pathologists and the NCI Pathology Working Group with final approval by the NCI Pathology Working Group.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute (5). Statistical analyses were performed by Dr. J. R. Joiner (2) and Ms. S. Vatsan (2) using methods selected for the bioassay program by Dr. J. J. Gart (6). Chemical analyses were conducted at Midwest Research Institute (7). Dosage analysis was supervised by Drs. R. Freudenthal (1), P. Leber (1,9), and D. Emmerling (1).

This report was prepared at Tracor Jitco (2) under the direction of Dr. L. A. Campbell, Acting Director of the Bioassay Program; Dr. S. S. Olin, Associate Director; Dr. R. L. Schueler, pathologist; Dr. D. J. Beach, reports manager; Dr. A. C. Jacobs, bioscience writer; and Dr. W. D. Theriault and Ms. M. W. Glasser, technical editors.

The following scientists at NCI/NTP (8) were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. Michael P. Dieter (chemical manager), Dr. J. Fielding Douglas, Dr. Richard A. Griesemer, Dr. Charles Grieshaber, Dr. Larry Hart, Dr. William V. Hartwell, Dr. Joseph Haseman, Dr. James E. Huff, Dr. C. W. Jameson, Dr. Ernest E. McConnell, Dr. John A. Moore, Dr. Sherman F. Stinson, Dr. Raymond Tennant, and Dr. Jerrold M. Ward.

On June 27, 1980, this report underwent peer review by the National Toxicology Program Board of Scientific Counselors' Technical Reports Review Subcommittee and associated Panel of Experts. The review meeting began at 9 a.m. in Room 1331, Switzer Building, 330 C Street, S.W., Washington, D.C.

Members of the Subcommittee are: Drs. Margaret Hitchcock (Chairperson), Curtis Harper, Thomas Shepard, and Alice Whittemore. Members of the Panel are: Drs. Norman Breslow, Joseph Highland, Charles Irving, Frank Mirer, Sheldon Murphy, Svend Nielsen, Bernard Schwetz, Roy Shore, James Swenberg, and Gary Williams. Drs. Highland, Schwetz, and Swenberg were unable to attend the review.

Dr. Shepard, the primary reviewer for the report on the bioassay of FD & C Yellow No. 6, agreed with the conclusion in the report that FD & C Yellow No. 6 was not carcinogenic under the conditions of the bioassay.

The secondary reviewer, Dr. Nielsen, also agreed with the conclusions. He suggested that a peer review of the hepatocellular tumors would be desirable in view of their high incidence in the low-dose male mice. Dr. Williams questioned whether the high dose was at a maximum tolerated dose level. The minimal effects of the compound on weight gain suggest this might be the case. He said that one cannot conclude there is a lack of carcinogenic effect unless one is testing at the maximum tolerated dose.

Dr. Shepard moved that the report on the bioassay of FD & C Yellow No. 6 be accepted under the condition that certain parts of the summary be reworded to reflect the Panel's consensus. Dr. Nielsen seconded the motion, and it was approved unanimously.

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SUMMARY

A carcinogenesis bioassay was conducted using groups of 50 male and 50 female F344 rats and B6C3F1 mice which were fed diets containing 12,500 or 25,000 ppm FD & C Yellow No. 6, a widely used food colorant, for 103 weeks. Groups of 90 male and 90 female rats and 50 male and 50 female mice served as undosed controls.

Throughout the study, mean body weights of high-dose female rats and all low-dose groups were comparable with those of the controls, but mean body weights of high-dose male rats and high-dose male and female mice were slightly lower (10% or less) than those of the controls.

No compound-related neoplastic or nonneoplastic lesions were observed in the rats.

The incidence of hepatocellular carcinomas in low-dose male mice was significantly higher than that in the controls, but the lack of a significant increase in high-dose males and the variability of liver tumors in B6C3F1 male mice precluded clearly relating the occurrence of these tumors to the administration of FD & C Yellow No. 6.

Under the conditions of this bioassay, there was no clear evidence of the carcinogenicity of FD & C Yellow No. 6 in F344 rats or B6C3F1 mice of either sex.

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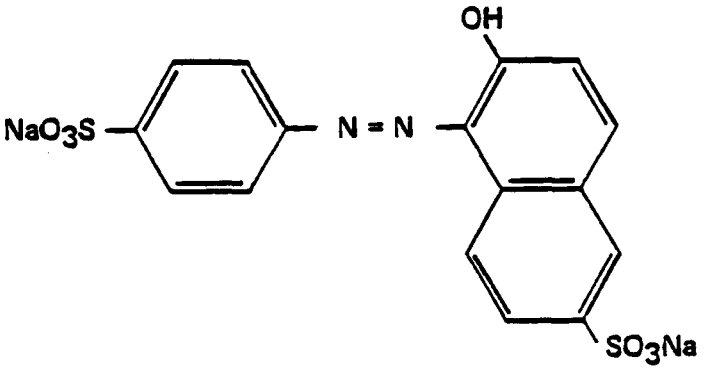


FD&C YELLOW NO. 6

$C_{16}H_{10}N_2Na_2O_7S_2$ Mol. Wt. 452.4

FD & C Yellow No. 6, Sunset Yellow FCF, (CAS No. 2783-94-0, Colour Index No. 15985) is a water soluble mono-azo dye certified by the U.S. Food and Drug Administration for use in food, drugs, or cosmetics (CFR, 1974). FD & C Yellow No. 6 has been used since 1929 to give a reddish-yellow color to various gelatin desserts, sherbets, carbonated beverages, candies, cereals, jams, pickles, smoked fish, puddings, drug solutions, tablets, capsules, toothpastes, and hair rinses (Kirk-Othmer, 1964 and 1978; Society of Dyers and Colourists, 1971; Goulden et al., 1972, Trautlein and Mann, 1978; and Khera and Munro, 1979). Production of FD & C Yellow No. 6 in the United States in 1978 was 488,000 kg (U.S. Int. Trade Commission, 1979).

The following acute LD_{50} values for the dye were reported (Gaunt et al., 1967) for CFE (Carworth Farm) rats and ICI (Alderley Park) mice:

Species	Sex	Route	LD_{50} (g/kg)
Rat	Male	Intraperitoneal	3.8
"	Female	"	5.5
"	Male	Oral	10
	Female	"	10
Mouse	Male	Intraperitoneal	5.5
"	Female	"	4.6
"	Male	Oral	6
"	Female	"	6

The toxic effects of large doses of FD & C Yellow No. 6 are dependent on the diet of the animals. No toxic effects were found in Sprague-Dawley rats fed laboratory chow containing 50,000 ppm FD & C Yellow No. 6 for 14 days; however, when the same dose was incorporated into a purified low fiber diet, growth retardation occurred in all animals and death in more than 50% of the animals (Ershoff, 1977). Diarrhea and enlarged ceca were observed by Gaunt et al. (1967) in CEF rats fed diets containing 20,000 or 30,000 ppm FD & C Yellow No. 6 for 90 days.

When 50 mg of FD & C Yellow No. 6 was administered by gavage to Osborne-Mendel rats, 3% was excreted unchanged in the urine and bile, whereas the remainder was reduced by intestinal bacteria and excreted in the urine after 48 hours as sulfanilic acid and 1-amino-2-hydroxy-6-naphthalene sulfonic acid (Radomski and Mellinger, 1962).

FD & C Yellow No. 6 was not mutagenic for Salmonella typhimurium TA 1538, TA 1535, TA 100, and TA 98, with or without metabolic activation (Garner and Nutman, 1977; Viola and Nosotti, 1978; and Brown et al., 1978). Also, three other azo dyes considered to be animal carcinogens (Sudan Yellow, Ponceau 3R, and Ponceau de Xylidine) were not mutagenic under similar test conditions (Garner and Nutman, 1977). Price et al. (1978) found that FD & C Yellow No. 6 did not transform cultured Fischer rat embryo cells. Concentrations of 2 to 5 x 10^{-3}M caused increased frequency of sister chromatid exchanges in cultured Chinese hamster cells but did not induce chromosome aberrations (Abe and Sasaki, 1977).

FD & C Yellow No. 6 was tested by the Carcinogenesis Testing Program because of its widespread use as a food colorant and because other studies using lower dose levels, smaller numbers of animals, and exposure periods of up to only 80 weeks were considered less than adequate for evaluative purposes (IARC, 1975; Gaunt et al., 1974; Bonser et al., 1956; Mannell et al., 1958).

II. MATERIALS AND METHODS

A. Chemical

FD & C Yellow No. 6 (NCI No. C 53907) --6-hydroxy-5-((4-sulfophenyl)azo)-2-naphthalene sulfonic acid disodium salt -- was obtained from Allied Chemical Corporation (Hawthorne, NJ). Lot No. Z7860 was used for the subchronic studies and for the first 44 weeks of the chronic study and Lot No. AA3448 for the final 60 weeks of the chronic study. Purity and identity analyses (Appendixes E and F) of both lots were performed at Midwest Research Institute (Kansas City, MO). The composition of both lots was similar.

Titration of reducible groups with titanous chloride indicated that Lot No. Z7860 was 91.9%±0.7% pure dye (Appendix E). Results of elemental analysis were consistent with a composition of 91.9% dye, 5.05% water, and 2.77% sodium chloride. Results of thin-layer chromatography indicated four impurities (three trace or slight trace and one minor impurity). Results of high-pressure liquid chromatography indicated three small impurities (0.3%, 0.2%, and 3.2%). The ultraviolet, visible, infrared, and nuclear magnetic resonance spectra were consistent with the structure and literature spectra. Titration of reducible groups with titanous chloride indicated Lot No. AA3448 was 91.8%±1.0% pure dye (Appendix F). Results of elemental analyses for carbon, sulfur, and sodium were slightly low, and hydrogen and nitrogen were consistent with a composition of 91.8% dye, 5.44% water, and 3.28% sodium chloride. Results of thin-layer chromatography indicated three trace impurities by one system and five trace impurities by a second system. One trace impurity and a second impurity that was 0.34% of the major peak were detected by high-pressure liquid chromatography. The ultraviolet, visible infrared, and nuclear magnetic resonance spectra were consistent with the structure and literature spectra.

The test chemical was stored at 23°±1°C.

B. Dietary Preparation

A 1-week supply of each diet was formulated no more than 4 days before use by mixing Purina® Laboratory Chow animal meal (Ralston Purina Company, Richmond, IN) and FD & C Yellow No. 6. Weighed amounts of animal meal were combined with weighed amounts of the test chemical and then mixed in a Patterson-Kelly® twin-shell blender for 15 minutes. Formulated diets were stored at 23°C for no longer than 10 days.

The concentration of dye in selected batches of formulated diets was measured every 8 weeks and was found to be within ±10% of the desired concentration (Appendix G). The stability of diets formulated with 100,000 ppm dye was determined by analyses performed after storage for 2 weeks at -20°, 5°, 25°, or 45°C. Spectrophotometric analysis of water extracts of the diets indicated that FD & C Yellow No. 6 was stable in feed for 2 weeks at temperatures up to 45°C (Appendix H).

C. Animals

Four-week-old male F344 rats, 3-week-old female F344 rats, and 5-week-old B6C3F1 mice were obtained from NCI Frederick Cancer Research Center, Frederick, Maryland, acclimated for 2 weeks, and assigned to control or dosed groups according to a table of random numbers.

D. Animal Maintenance

Rats and mice were housed five per cage in solid-bottom polycarbonate cages (Lab Products, Inc., Garfield, NJ) supplied with DuPont 2024 spin bond polyester filters and Absorb-dri® hardwood chip bedding (Lab Products, Inc.). Cages and bedding were changed twice weekly and feed hoppers once weekly. Water was supplied by an automatic watering system (Edstrom Industries, Waterford, WI), and the test diet for the dosed animals and Ralston Purina® Laboratory chow meal for the controls were available ad libitum. Temperature in the animal rooms was 22° to 24°C and the relative humidity was maintained at 45%-55%. Incoming air was passed through a filter

equipped with an electrostatic precipitator at a volume equivalent to 15 room changes per hour. Fluorescent lighting was provided 12 hours per day.

Rats and mice fed FD & C Yellow No. 6 were housed in the same room as animals of the same species on feeding studies of the following chemicals:

C.I. Acid Orange No. 10: CAS 1936-15-8

C.I. Acid Red 14: CAS 3567-69-9

E. Range-Finding and 14-Day Repeated Dose Studies

Single day dosing and 14-day repeated dose feed studies were conducted using F344 rats and B6C3F1 mice to determine the concentrations of FD & C Yellow No. 6 to be used in the subchronic studies.

In the single day dosing study, groups of five males and females of each species were fed diets containing 6,000, 12,500, 25,000, 50,000, 100,000, or 200,000 ppm FD & C Yellow No. 6 for 24 hours and then lab chow for 13 days. In the repeated dose study, similar groups of rats and mice were fed diets containing 6,000, 12,500, 25,000, 50,000 or 100,000 ppm FD & C Yellow No. 6 for 2 weeks. No deaths occurred among the rats or mice in either study, and other signs of toxicity were not observed. All animals were killed after 2 weeks.

F. Subchronic Studies

In the subchronic studies that were conducted to determine the concentrations to be used in the chronic studies, groups of 10 males and 10 females of each species were first fed diets containing 0, 6,000, 12,500, 25,000, 50,000, or 100,000 ppm FD & C Yellow No. 6 for 12 weeks and then given control diets for 1 week (Tables 1 and 2). Clinical observations were made twice daily and animals were weighed weekly. At the end of the 91-day period, survivors were killed, necropsies were performed on all animals, and selected tissues were taken for histopathologic analysis.

Rats: No deaths occurred among the rats. A dose-associated decrement in mean body weight gain was observed for both male and female rats. Weight gain was depressed 9.8% or more in male rats fed diets containing 25,000 ppm

Table 1. Doses, Survival, and Mean Body Weights of Rats Fed Diets Containing FD & C Yellow No. 6 for 12 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (%)
Male					
0	10/10	112.8	298.2	185.4	
6,000	10/10	106.2	281.3	175.1	-5.0
12,500	10/10	102.9	281.5	178.6	-3.7
25,000	10/10	103.5	270.8	167.3	-9.8
50,000	10/10	98.8	248.9	150.1	-19.0
100,000	10/10	102.8	208.6	105.8	-43.0
Female					
0	10/10	97.7	180.1	82.4	
6,000	10/10	92.9	174.7	81.8	-0.7
12,500	10/10	98.2	170.2	72.0	-12.6
25,000	10/10	92.2	165.8	73.6	-10.6
50,000	10/10	94.5	145.9	51.4	-38.0
100,000	10/10	85.9	148.2	62.3	-24.0

(a) Number surviving/number per group

(b) Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

Table 2. Dosage, Survival, and Mean Body Weights of Mice Fed Diets Containing FD & C Yellow No. 6 for 12 Weeks

Dose (ppm)	Survival (a)	Mean Body Weights (grams) Initial	Final	Gain	Weight Change Relative to Controls (b) (%)
Male					
0	10/10	18.1	30.2	12.1	
6,000	9/10	18.4	31.1	12.7	+5.0
12,500	10/10	18.3	30.8	12.5	+3.3
25,000	9/10	18.4	29.7	11.3	-6.6
50,000	10/10	18.5	29.6	11.1	-8.2
100,000	10/10	18.5	27.6	9.1	-25.0
Female					
0	9/10	18.1	24.6	6.3	
6,000	10/10	18.4	23.3	4.9	-22.0
12,500	10/10	18.3	23.6	5.3	-15.8
25,000	9/10	18.4	23.3	4.9	-22.0
50,000	10/10	18.5	23.0	4.5	-28.0
100,000	10/10	18.5	21.9	3.4	-46.0

(a) Number surviving/number per group

(b) Weight change relative to controls =

$$\frac{\text{Weight Gain (Dosed Group)} - \text{Weight Gain (Control Group)}}{\text{Weight Gain (Control Group)}} \times 100$$

or more FD & C Yellow No. 6 and in females fed diets containing 12,500 ppm or more.

Bone marrow hyperplasia, observed in all examined male and female rats fed diets containing 50,000 or 100,000 ppm, was not detected in animals fed diets containing 25,000 ppm or less.

The low and high dietary levels of FD & C Yellow No. 6 selected for the chronic study with rats were 12,500 and 25,000 ppm.

Mice: Deaths were not considered to be compound-related and occurred in one male mouse receiving a diet containing 6,000 ppm, one male mouse receiving 25,000 ppm, one female mouse receiving 25,000 ppm FD & C Yellow No. 6, and one female control mouse. Mean body weight gain among male mice was depressed by more than 10% only in those fed diets containing 100,000 ppm of the compound. Mean body weight gain was depressed by more than 10% in all dosed female mice. Although weight gain depression at 25,000 ppm was comparable with that at 6,000 ppm for female mice, depression of weight gain was dose related from 12,500 ppm to 100,000 ppm. Weight gain was depressed 46% in female mice receiving 100,000 ppm FD & C Yellow No. 6 in the diet.

No gross or microscopic lesions attributable to FD & C Yellow No. 6 were observed at any dose level.

The low and high dietary levels of FD & C Yellow No. 6 selected for the chronic study with mice were 12,500 and 25,000 ppm.

G. Chronic Studies

The number of animals in test groups, doses administered, and times on study are shown in Table 3.

H. Clinical Examinations and Pathology

All animals were observed twice daily, and observations of sick, tumor-bearing, and moribund animals were recorded. Clinical examination and palpation for masses were performed each month, and the animals were weighed at least monthly. Moribund animals and animals that survived to the end of the bioassay were killed using carbon dioxide and necropsied.

Table 3. Experimental Design of Chronic Feeding Studies with FD & C Yellow No. 6 in Rats and Mice

Test Group	Initial No. of Animals	FD & C Yellow No. 6 in feed (ppm)	Time on Study: Dosed (weeks)	Time on Study: Observed (weeks)
Male Rats				
Control	90 (a)	0	0	104
Low-Dose	50	12,500	103	1
High-Dose	50	25,000	103	1
Female Rats				
Control	90 (a)	0	0	104-105
Low-Dose	50	12,500	103	1
High-Dose	50	25,000	103	1
Male Mice				
Control	50	0	0	103
Low-Dose	49	12,500	103	1
High-Dose	50	25,000	103	1
Female Mice				
Control	50	0	0	104
Low-Dose	50	12,500	103	1
High-Dose	50	25,000	103	1

(a) Controls were shared with feeding studies of C.I. Acid Orange 10 and C.I. Red 14.

Gross and microscopic examinations were performed on major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The tissues were preserved in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. The following tissues were examined microscopically: skin (abdominal), lungs and bronchi, trachea, bone, bone marrow (femur) and thigh muscle, spleen, lymph nodes, thymus, heart, salivary glands, liver, pancreas, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain, epididymus, eye, and all tissue masses.

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

I. Data Recording and Statistical Analyses

Data on this experiment were recorded in the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). 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 reported only 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 (e.g., skin or mammary tumors) prior to histologic sampling or when lesions could have appeared at multiple sites (e.g.,lymphomas), the denominators consist of the 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.

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.

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 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 has occurred (P 0.025 one-tailed test when the control incidence is not zero, $P<0.050$ when the control incidence is zero). 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 high-dose male rats were slightly lower than those of the controls throughout the bioassay; those of high-dose female rats were comparable with mean body weights of controls (Figure 1). No other compound-related clinical signs were noted.

B. Survival (Rats)

Estimates of the probabilities of survival for male and female rats administered FD & C Yellow No. 6 in feed at the doses of this bioassay, and those of the controls, are shown by the Kaplan and Meier curves in Figure 2. The survival among all groups in either sex is comparable. In male rats, 70/90 (78%) of the controls, 36/50 (72%) of the low-dose group, and 38/50 (76%) of the high-dose group lived to the end of the study at week 104. In females, 66/88 (75%) of the control group, 40/50 (80%) of the low-dose group, and 37/50 (74%) of the high-dose group were alive at the end of the study at 104-105 weeks.

The number of animals at risk was sufficient to detect the development of late-appearing tumors.

C. Pathology (Rats)

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

A variety of neoplasms are represented among both dosed and control animals. Each type of tumor represented has been encountered previously as a spontaneous lesion in the rat and none appear to be related to administration of the chemical.

The nonneoplastic lesions found in both control and dosed animals have been encountered previously as spontaneous occurrences in aged laboratory rats.

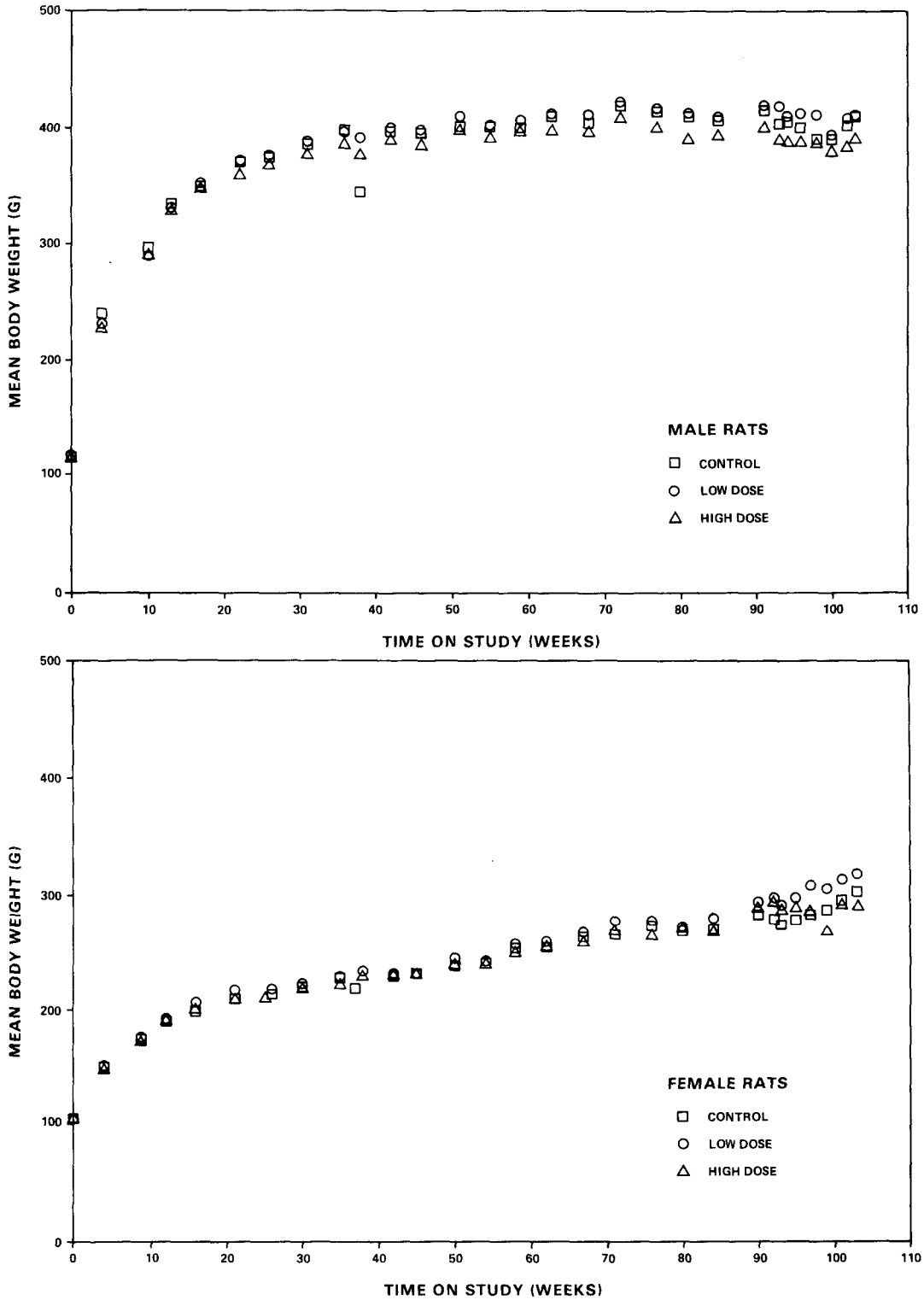


Figure 1. Growth Curves for Rats Fed Diets Containing FD&C Yellow No. 6

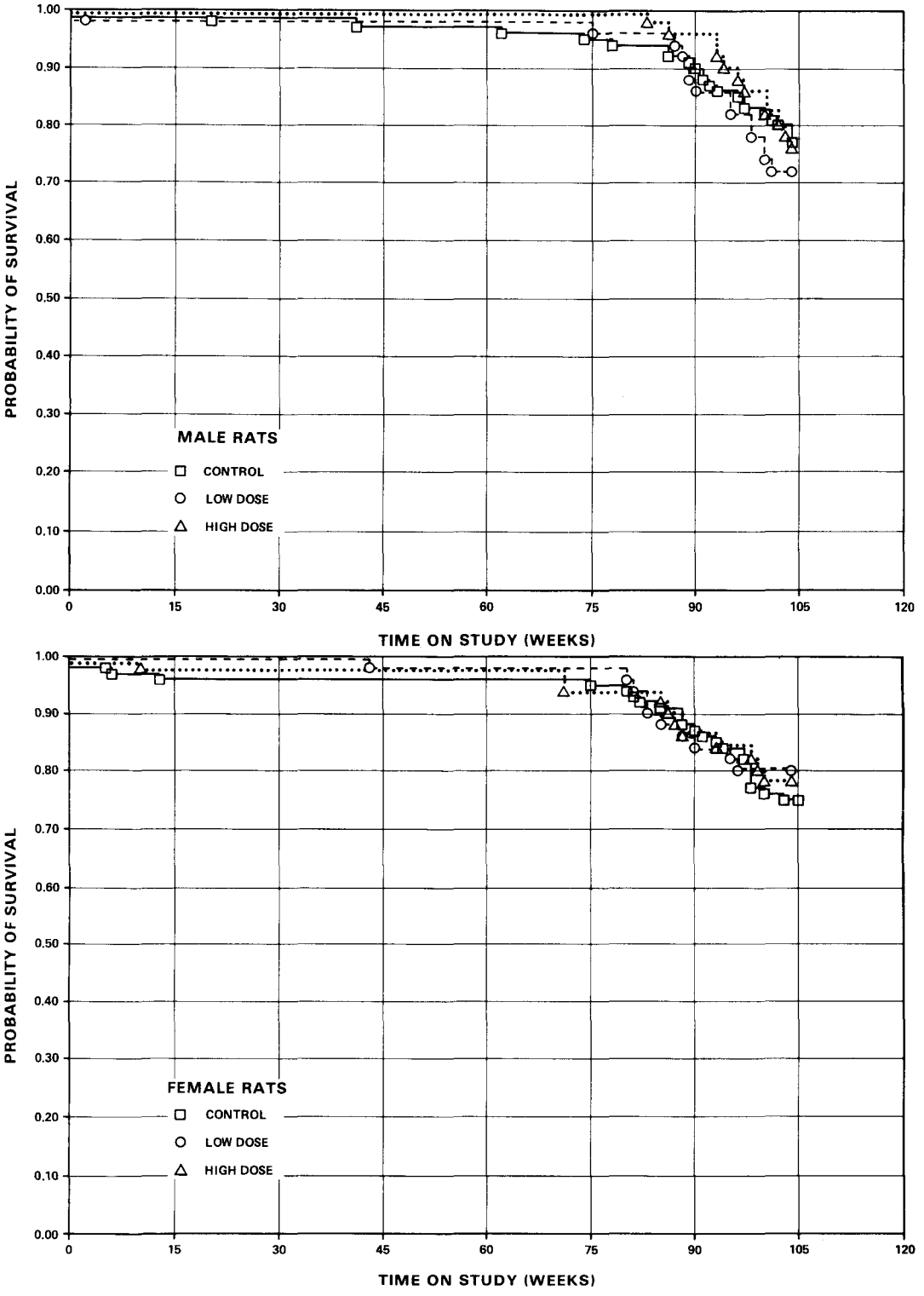


Figure 2. Survival Curves for Rats Fed Diets Containing FD&C Yellow No. 6

Results of histopathologic examination indicated that, under the conditions of this bioassay, there was no evidence for the carcinogenicity of FD & C Yellow No. 6 in F344 rats.

D. Statistical Analyses of Results (Rats)

Tables 4 and 5 contain the statistical analysis 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.

As a result of higher incidence in the low-dose group than in the other two groups, the Cochran-Armitage test indicates departures from linear trend in the incidences of animals with either neoplastic nodules of the liver or hepatocellular carcinomas (P=0.015) and with chromophobe carcinomas in the pituitary of male rats (P=0.022). However, the results of the Fisher exact test in the low-dose group are not significant in either instance. A departure from linear trend (P=0.028) is also indicated in the incidence of animals with either pheochromocytomas or malignant pheochromocytomas in the adrenal of males, due to a sharp decrease in the low-dose group incidence.

At no site was a significant positive increase in tumors observed. In each of the 95% confidence intervals for relative risk shown in the tables, the value of less than one is included: this indicates the absence of significant positive results. It should also be noted that each of the intervals has an upper limit greater than one, indicating the theoretical possibility of tumor induction by FD & C Yellow No. 6 which could not be detected under the conditions of this test.

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

Topography: Morphology	Control	Low Dose	High Dose
Subcutaneous Tissue:			
Fibroma (b)	4/90(4)	1/50(2)	3/50(6)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.450	1.350
Lower Limit		0.009	0.204
Upper Limit		4.365	7.621
Weeks to First Observed Tumor	104	100	103
Subcutaneous Tissue: Fibroma or			
Fibrosarcoma (b)	5/90(6)	2/50(4)	3/50(6)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.720	1.080
Lower Limit		0.070	0.173
Upper Limit		4.194	5.280
Weeks to First Observed Tumor	86	75	103
Hematopoietic System:			
Lymphocytic Leukemia (b)	22/90(24)	12/50(24)	17/50(34)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.982	1.391
Lower Limit		0.481	0.763
Upper Limit		1.871	2.442
Weeks to First Observed Tumor	74	2	86

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System:			
Leukemia or Lymphoma (b)	23/90(26)	12/50(24)	17/50(34)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.939	1.330
Lower Limit		0.463	0.735
Upper Limit		1.775	2.315
Weeks to First Observed Tumor	20	2	86
Liver: Neoplastic			
Nodule (b)	5/90(6)	6/50(12)	1/50(2)
P Values (c)	N.S.	N.S.	N.S.
Departure from Linear Trend (d)	P=0.042		
Relative Risk (Control) (e)		2.160	0.360
Lower Limit		0.575	0.008
Upper Limit		8.461	3.079
Weeks to First Observed Tumor	104	104	104
Liver: Neoplastic Nodule or			
Hepatocellular Carcinoma (b)	5/90(6)	7/50(14)	1/50(2)
P Values (c)	N.S.	N.S.	N.S.
Departure from Linear Trend (d)	P=0.015		
Relative Risk (Control) (e)		2.520	0.360
Lower Limit		0.724	0.008
Upper Limit		9.506	3.079
Weeks to First Observed Tumor	104	104	104

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma (b)	4/84(5)	2/46(4)	4/46(9)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.913	1.826
Lower Limit		0.085	0.354
Upper Limit		6.074	9.306
Weeks to First Observed Tumor	104	100	104
Pituitary: Chromophobe Carcinoma (b)	1/84(1)	3/46(7)	0/46(0)
P Values (c)	N.S.	N.S.	N.S.
Departure from Linear Trend (d)	P=0.022		
Relative Risk (Control) (e)		5.478	0.000
Lower Limit		0.453	0.000
Upper Limit		281.020	34.003
Weeks to First Observed Tumor	104	100	--
Pituitary: Chromophobe Adenoma or Carcinoma (b)	5/84(6)	5/46(11)	4/46(9)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.826	1.461
Lower Limit		0.440	0.302
Upper Limit		7.479	6.412
Weeks to First Observed Tumor	104	100	104

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	11/89(12)	3/50(6)	9/49(18)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control)(e)		0.485	1.486
Lower Limit		0.090	0.581
Upper Limit		1.727	3.636
Weeks to First Observed Tumor	86	90	97
Adrenal: Pheochromocytoma or Pheochromocytoma, malignant (b)	14/89(16)	3/50(6)	11/49(22)
P Values (c)	N.S.	N.S.	N.S.
Departure from Linear Trend (d)	P=0.028		
Relative Risk (Control) (e)		0.381	1.427
Lower Limit		0.073	0.631
Upper Limit		1.280	3.087
Weeks to First Observed Tumor	86	90	97
Thyroid: C-Cell Carcinoma (b)	2/89(2)	4/49(8)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		3.633	3.560
Lower Limit		0.539	0.528
Upper Limit		38.807	38.057
Weeks to First Observed Tumor	104	100	93

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pancreatic Islets: Islet-Cell Carcinoma or Adenoma (b)	3/88(3)	4/49(8)	2/49(4)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.395	1.197
Lower Limit		0.420	0.102
Upper Limit		15.662	10.044
Weeks to First Observed Tumor	104	100	104
Mammary Gland: Fibroadenoma (b)	2/90(2)	1/50(2)	3/50(6)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.900	2.700
Lower Limit		0.015	0.318
Upper Limit		16.768	31.289
Weeks to First Observed Tumor	97	104	104
Testis: Interstitial-Cell Tumor (b)	86/90(96)	48/50(96)	50/50(100)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.005	1.047
Lower Limit		0.922	0.970
Upper Limit		1.062	1.047
Weeks to First Observed Tumor	74	75	83

Table 4. Analyses of the Incidence of Primary Tumors in Male Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Testis: Interstitial-Cell Tumor or Malignant Tumor (b)	87/90(97)	48/50(96)	50/50(100)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.993	1.034
Lower Limit		0.923	0.968
Upper Limit		1.050	1.034
Weeks to First Observed Tumor	74	75	83

(a) Dosed groups received doses of 12,500 or 25,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less then 0.05; otherwise not significant (N.S.) is indicated.
(d) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphocytic Leukemia (b)	16/88(18)	12/50(24)	10/50(20)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.320	1.100
Lower Limit		0.616	0.480
Upper Limit		2.702	2.355
Weeks to First Observed Tumor	5	43	71
Hematopoietic System: All Leukemias (b)	16/88(18)	12/50(24)	10/50(20)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.320	1.100
Lower Limit		0.616	0.480
Upper Limit		2.702	2.355
Weeks to First Observed Tumor	5	43	71
Hematopoietic System: Leukemia or Lymphoma (b)	18/88(20)	13/50(26)	11/50(22)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.271	1.076
Lower Limit		0.622	0.496
Upper Limit		2.480	2.185
Weeks to First Observed Tumor	5	43	10

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Neoplastic Nodule (b)	3/88(3)	3/50(6)	0/50(0)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.760	0.000
Lower Limit		0.243	0.000
Upper Limit		12.617	2.933
Weeks to First Observed Tumor	88	104	--
Pituitary: Chromophobe Adenoma (b)	25/83(30)	18/45(40)	15/48(31)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.328	1.038
Lower Limit		0.764	0.562
Upper Limit		2.209	1.816
Weeks to First Observed Tumor	81	81	88
Pituitary: Chromophobe Carcinoma (b)	5/83(6)	0/45(0)	2/48(4)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.000	0.692
Lower Limit		0.000	0.068
Upper Limit		1.462	4.019
Weeks to First Observed Tumor	104	--	85

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma (b)	30/83(36)	18/45(40)	17/48(35)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.107	0.980
Lower Limit		0.653	0.566
Upper Limit		1.781	1.612
Weeks to First Observed Tumor	81	81	85
Adrenal: Cortical Adenoma (b)	6/86(7)	2/50(4)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.573	1.147
Lower Limit		0.058	0.248
Upper Limit		3.048	4.571
Weeks to First Observed Tumor	104	104	104
Adrenal: Cortical Adenoma or Carcinoma (b)	7/86(8)	2/50(4)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.491	0.983
Lower Limit		0.051	0.220
Upper Limit		2.449	3.644
Weeks to First Observed Tumor	104	104	104

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Adrenal: Pheochromocytoma (b)	3/86(3)	1/50(2)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.573	2.293
Lower Limit		0.011	0.402
Upper Limit		6.880	15.010
Weeks to First Observed Tumor	104	96	104
Adrenal: Pheochromocytoma or Pheochromocytoma, Malignant (b)	4/86(5)	1/50(2)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.430	1.720
Lower Limit		0.009	0.333
Upper Limit		4.170	8.800
Weeks to First Observed Tumor	104	96	104
Thyroid: C-Cell Adenoma or Carcinoma (b)	3/86(3)	2/50(4)	3/48(6)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.147	1.792
Lower Limit		0.098	0.248
Upper Limit		9.625	12.822
Weeks to First Observed Tumor	104	104	85

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Mammary Gland: Adenoma, NOS or Adenocarcinoma, NOS (b)	4/88(5)	0/50(0)	4/50(8)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.000	1.760
Lower Limit		0.000	0.340
Upper Limit		1.904	9.007
Weeks to First Observed Tumor	104	--	104
Mammary Gland: Fibroadenoma (b)	18/88(20)	6/50(12)	7/50(14)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		0.587	0.684
Lower Limit		0.202	0.257
Upper Limit		1.423	1.579
Weeks to First Observed Tumor	81	88	87
Uterus: Endometrial Stromal Polyp (b)	9/87(10)	6/49(12)	7/49(14)
P Values (c)	N.S.	N.S.	N.S.
Relative Risk (Control) (d)		1.184	1.381
Lower Limit		0.365	0.462
Upper Limit		3.472	3.876
Weeks to First Observed Tumor	88	104	104

Table 5. Analyses of the Incidence of Primary Tumors in Female Rats Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

(a) Dosed groups received doses of 12,500 or 25,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less then 0.05; otherwise not significant (N.S.) is indicated.
(d) The 95 percent confidence interval of the relative risk between each dosed group and the control group.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

Mean body weights of high-dose mice of either sex were slightly lower (less than 10%) than those of the controls throughout most of the bioassay. No other compound-related clinical signs were observed (Figure 3).

B. Survival (Mice)

Estimates of the probabilities of survival for male and female mice administered FD & C Yellow No. 6 in feed at the doses of this bioassay, and those of the controls, are shown by the Kaplan and Meier curves in Figure 4. Survival was comparable among all three groups of either sex. In males, 38/50 (76%) of the control group, 40/50 (80%) of the low-dose group, and 33/50 (66%) of the high-dose group lived to the end of the study at weeks 103-104. In female mice, 38/50 (76%) of the controls, 35/50 (70%) of the low-dose group, and 43/50 (86%) of the high-dose group lived to the end of the study at week 104.

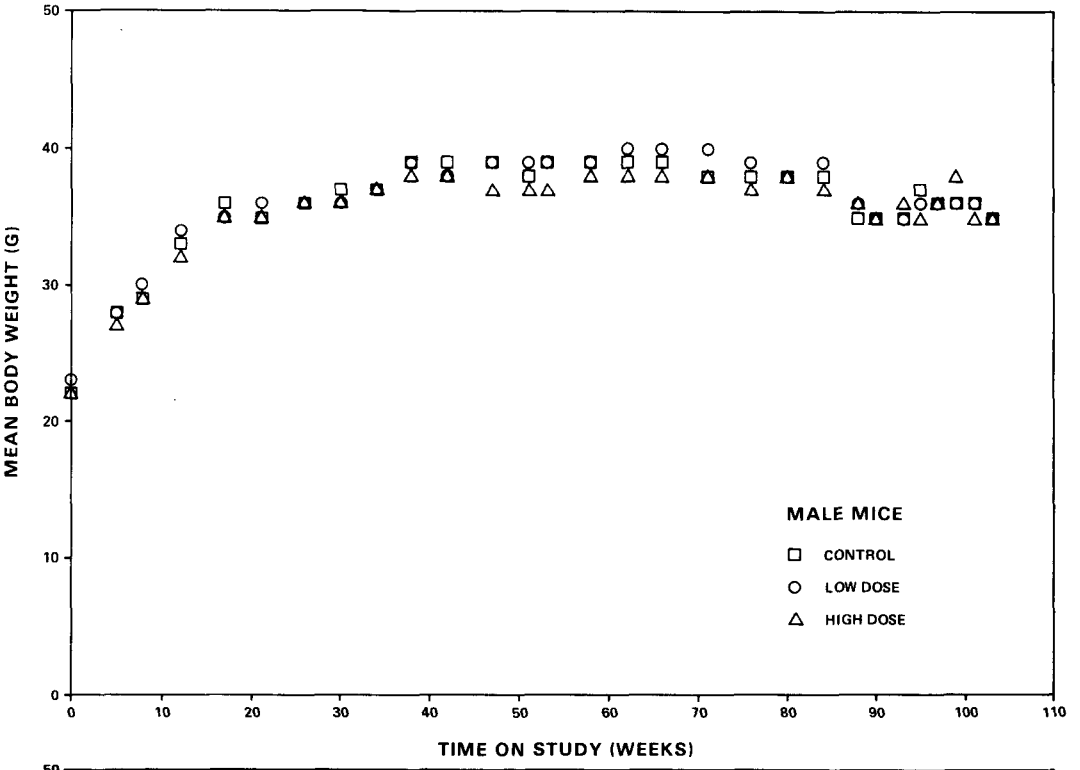
A sufficient number of animals were at risk for the development of late-appearing tumors in both sexes.

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.

A variety of neoplasms were identified among both dosed and control animals. Each type of tumor represented has been encountered previously as a spontaneous lesion in mice.

Increased incidences of hepatocellular carcinomas were observed in the low-dose males (46%) and high-dose males (32%) as compared with the control males (26%); no significant differences were observed among the females. The



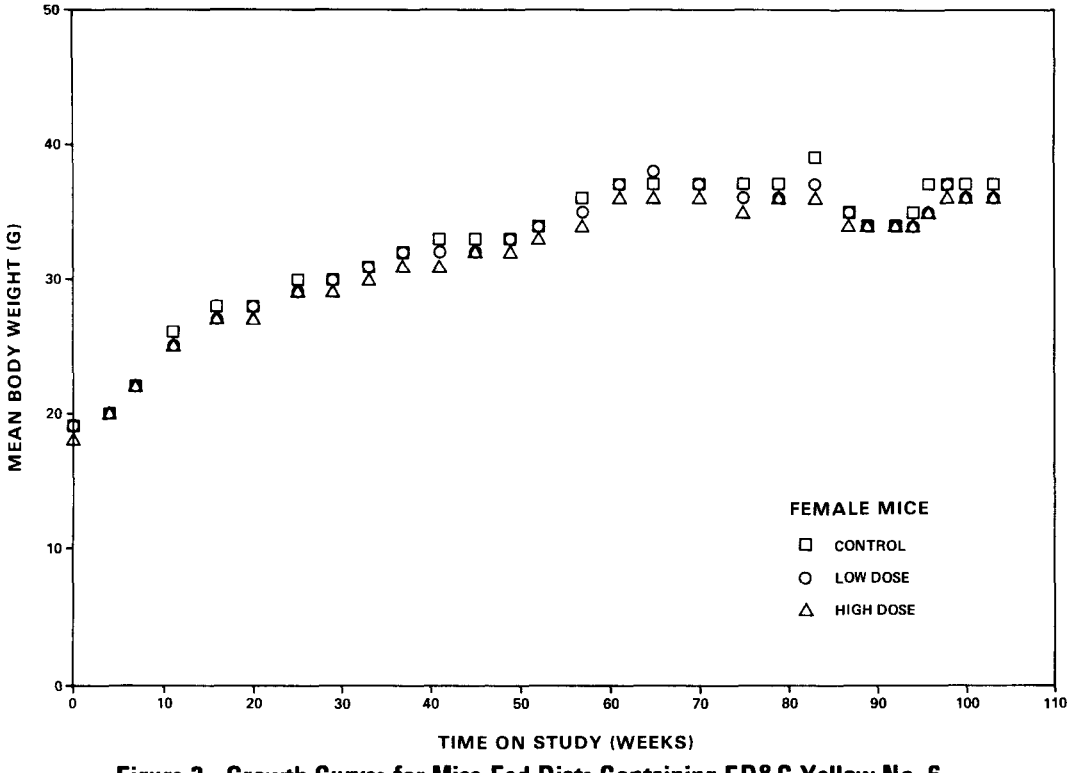


Figure 3. Growth Curves for Mice Fed Diets Containing FD&C Yellow No. 6

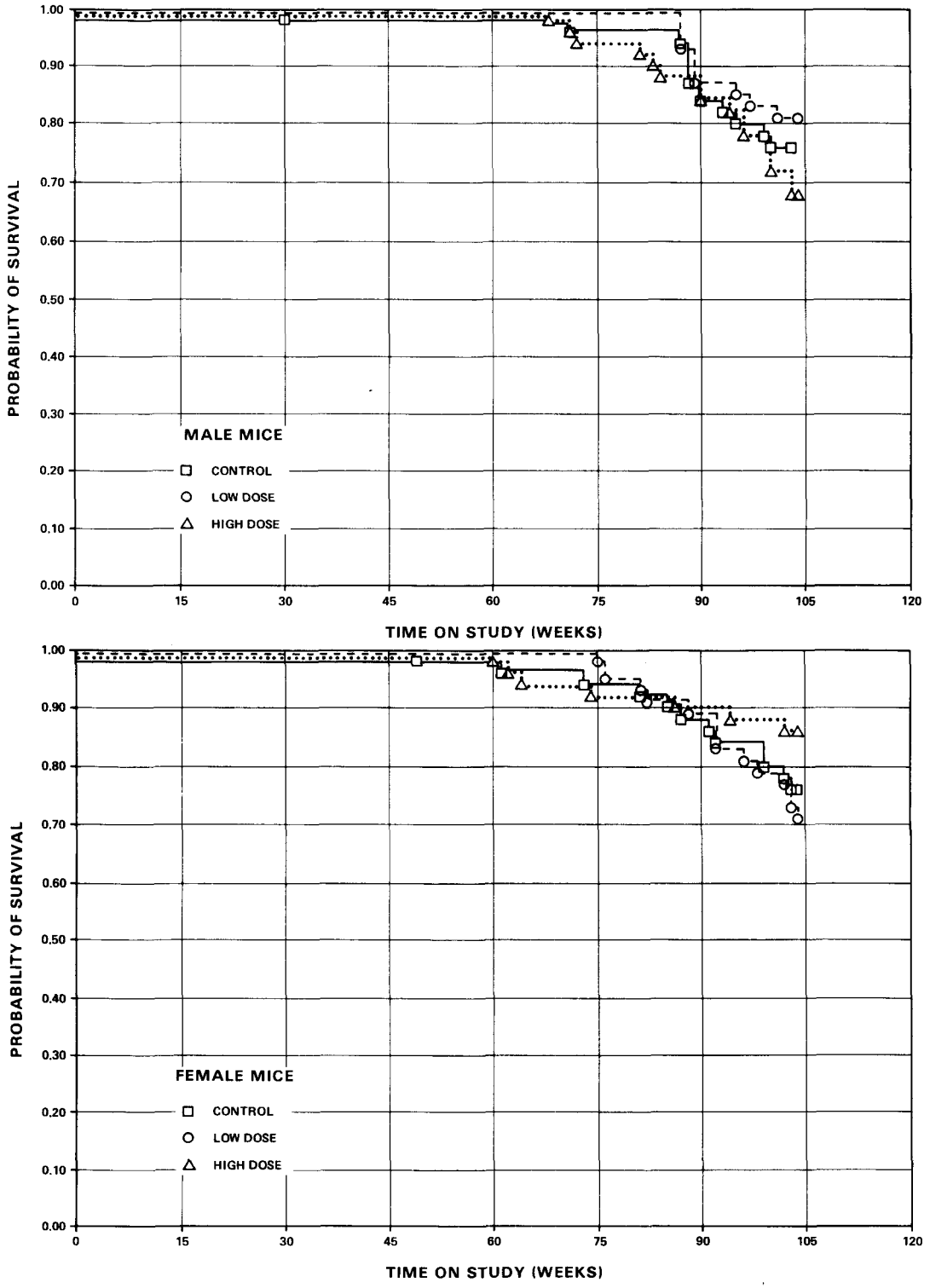


Figure 4. Survival Curves for Mice Fed Diets Containing FD&C Yellow No. 6

morphology of liver tumors in both control and dosed mice was similar.

A variety of nonneoplastic lesions are represented among both control and dosed animals. Such lesions have been encountered previously as spontaneous occurrences in aging laboratory mice.

The results of histopathologic examination indicated that no conclusive evidence for the carcinogenicity of FD & C Yellow No. 6 in B6C3F1 mice was detected under the conditions of this bioassay.

D. Statistical Analyses of Results (Mice)

Tables 6 and 7 contain the statistical analysis 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.

Hepatocellular carcinomas or adenomas were observed at a significantly higher incidence (P=0.020) in the low-dose males than in the controls. A departure from linear trend (P=0.024) is also indicated due to higher incidence in the low-dose group than in the other two groups. The historical incidence of 868/3,543 (24%) for these tumors in male B6C3F1 mice across all laboratories is comparable with the control-group incidence (13/50, 26%). No other control groups are available from this laboratory for comparison with the control group of this study, but at other laboratories incidences in groups of B6C3F1 mice have ranged as high as 50%. The Fisher exact test does not indicate a significantly higher incidence in the high-dose group than in the controls. In females, the incidence of hepatocellular carcinomas in the low-dose group is significantly lower (P=0.005) than that in the controls. The departure from linear trend (P=0.013) is due to the sharp fall in the low-dose group incidence. The variability in the occurrence of this type of tumor, the absence of significant results in the high-dose group of males, the comparability of survival and food consumption in this group with other male groups, and the absence of significant positive results in the female dosed groups are factors that suggest that there may not be a direct association of the liver tumors observed in the low-dose group of males with administration of the chemical.

The Cochran-Armitage test indicates a positive dose-related trend (P=0.038) in the incidence of male mice with either fibromas or fibrosarcomas in the skin, but the results of the Fisher exact test are not significant. Lymphomas in the hematopoietic system were found at an incidence significantly lower (P=0.009) in the low-dose group of males than in the controls. A departure from linear trend (P=0.005) is also indicated due to a sharp decline in the low-dose group incidence. In females, a negative trend (P=0.005) occurred in the incidence of malignant lymphomas (unspecified) in the hematopoietic system and significantly lower incidences in each of the dosed groups (P=0.015 and P=0.017, respectively) than in the controls.

The statistical conclusion is that no tumor at any site in mice can be clearly associated with the administration of FD & C Yellow No. 6 in this bioassay. In each of the 95% confidence intervals for relative risk shown in the tables, except for the incidence of hepatocellular carcinoma or adenoma in the low-dose group of males, 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 for the incidence of hematopoietic tumors in both sexes and for the incidence of hepatocellular carcinoma in the low-dose group of females, has an upper limit greater than one indicating the theoretical possibility of tumor induction by FD & C Yellow No. 6 which could not be detected under the conditions of this test.

Table 6. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing FD & C Yellow No. 6 (a)

Topography: Morphology	Control	Low Dose	High Dose
Skin: Fibrosarcoma or Fibroma (b)	0/50(0)	0/49(0)	3/50(6)
P Values (c), (d)	P=0.038	N.S.	N.S.
Relative Risk (Control) (e)		--	Infinite
Lower Limit		--	0.601
Upper Limit		--	Infinite
Weeks to First Observed Tumor	--	--	100
Subcutaneous Tissue: Fibrosarcoma (b)	4/50(8)	3/49(6)	3/50(6)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.765	0.750
Lower Limit		0.118	0.115
Upper Limit		4.288	4.206
Weeks to First Observed Tumor	71	104	90
Subcutaneous Tissue: Fibrosarcoma or Sarcoma, NOS (b)	4/50(8)	5/49(10)	3/50(6)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		1.276	0.750
Lower Limit		0.292	0.115
Upper Limit		6.070	4.206
Weeks to First Observed Tumor	71	89	90

Table 6. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Lung: Alveolar/Bronchiolar Adenoma (b)	6/50(12)	3/48(6)	2/50(4)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.521	0.333
Lower Limit		0.089	0.034
Upper Limit		2.288	1.758
Weeks to First Observed Tumor	87	104	104
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma (b)	6/50(12)	4/48(8)	3/50(6)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.694	0.500
Lower Limit		0.153	0.085
Upper Limit		2.739	2.200
Weeks to First Observed Tumor	87	95	103
Hematopoietic System: Malignant Lymphoma, NOS (b)	3/50(6)	0/49(0)	6/50(12)
P Values (c), (d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.030		
Relative Risk (Control) (e)		0.000	2.000
Lower Limit		0.000	0.454
Upper Limit		1.696	11.761
Weeks to First Observed Tumor	103	--	100

Table 6. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type (b)	1/50(2)	0/49(0)	3/50(6)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.000	3.000
Lower Limit		0.000	0.251
Upper Limit		19.032	154.270
Weeks to First Observed Tumor	90	--	96
Hematopoietic System: Malignant Lymphoma, Histiocytic Type (b)	3/50(6)	1/49(2)	1/50(2)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.340	0.333
Lower Limit		0.007	0.006
Upper Limit		4.062	3.983
Weeks to First Observed Tumor	88	97	104
Hematopoietic System: All Lymphomas (b)	9/50(18)	1/49(2)	10/50(20)
P Values (c), (d)	N.S.	P=0.009(N)	N.S.
Departure from Linear Trend (f)	P=0.005		
Relative Risk (Control) (e)		0.113	1.111
Lower Limit		0.003	0.445
Upper Limit		0.771	2.823
Weeks to First Observed Tumor	88	97	96

Table 6. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Lymphoma or Leukemia (b)	10/50(20)	2/49(4)	11/50(22)
P Values (c), (d)	N.S.	P=0.015(N)	N.S.
Departure from Linear Trend (f)	P=0.008		
Relative Risk (Control) (e)		0.204	1.100
Lower Limit		0.023	0.467
Upper Limit		0.894	2.624
Weeks to First Observed Tumor	88	87	96
Liver: Hepatocellular Carcinoma (b)	13/50(26)	22/48(46)	16/50(32)
P Values (c), (d)	N.S.	P=0.033	N.S.
Departure from Linear Trend (f)	P=0.043		
Relative Risk (Control) (e)		1.763	1.231
Lower Limit		0.969	0.624
Upper Limit		3.309	2.474
Weeks to First Observed Tumor	88	87	68
Liver: Hepatocellular Carcinoma or Adenoma (b)	13/50(26)	23/48(48)	16/50(32)
P Values (c), (d)	N.S.	P=0.020	N.S.
Departure from Linear Trend (f)	P=0.024		
Relative Risk (Control) (e)		1.843	1.231
Lower Limit		1.023	0.624
Upper Limit		3.428	2.474
Weeks to First Observed Tumor	88	87	68

Table 6. Analyses of the Incidence of Primary Tumors in Male Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

(a) Dosed groups received doses of 12,500 or 25,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the matched-control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

Table 7. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing FD & C Yellow No. 6 (a)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: Malignant Lymphoma, NOS (b)	8/50(16)	1/50(2)	1/49(2)
P Values (c), (d)	P=0.005(N)	P=0.015(N)	P=0.017(N)
Relative Risk (Control) (e)		0.125	0.128
Lower Limit		0.003	0.003
Upper Limit		0.880	0.898
Weeks to First Observed Tumor	85	104	104
Hematopoietic System: Malignant Lymphoma, Lymphocytic Type (b)	3/50(6)	2/50(4)	3/49(6)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.667	1.020
Lower Limit		0.058	0.143
Upper Limit		5.570	7.273
Weeks to First Observed Tumor	49	103	104
Hematopoietic System: Malignant Lymphoma, Histiocytic Type (b)	4/50(8)	1/50(2)	5/49(10)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.250	1.276
Lower Limit		0.005	0.292
Upper Limit		2.411	6.070
Weeks to First Observed Tumor	61	75	60

Table 7. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Hematopoietic System: All Lymphomas (b)	15/50(30)	5/50(10)	10/49(20)
P Values (c), (d)	N.S.	P=0.011(N)	N.S.
Departure from Linear Trend (f)	P=0.029		
Relative Risk (Control) (e)		0.333	0.680
Lower Limit		0.103	0.304
Upper Limit		0.881	1.453
Weeks to First Observed Tumor	49	75	60
Hematopoietic System: Lymphoma or Leukemia (b)	16/50(32)	7/50(14)	10/49(20)
P Values (c), (d)	N.S.	P=0.028(N)	N.S.
Relative Risk (Control) (e)		0.438	0.638
Lower Limit		0.167	0.288
Upper Limit		1.018	1.339
Weeks to First Observed Tumor	49	75	60
Liver: Hepatocellular Adenoma (b)	1/48(2)	3/50(6)	0/48(0)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		2.880	0.000
Lower Limit		0.241	0.000
Upper Limit		148.076	18.644
Weeks to First Observed Tumor	104	104	--

Table 7. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Liver: Hepatocellular Carcinoma (b)	7/48(15)	0/50(0)	4/48(8)
P Values (c), (d)	N.S.	P=0.005(N)	N.S.
Departure from Linear Trend (f)	P=0.013		
Relative Risk (Control) (e)		0.000	0.571
Lower Limit		0.000	0.131
Upper Limit		0.494	2.092
Weeks to First Observed Tumor	104	--	104
Liver: Hepatocellular Adenoma or Carcinoma (b)	7/48(15)	3/50(6)	4/48(8)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		0.411	0.571
Lower Limit		0.072	0.131
Upper Limit		1.687	2.092
Weeks to First Observed Tumor	104	104	104
Pituitary: Chromophobe Adenoma (b)	0/46(0)	3/43(7)	1/35(3)
P Values (c), (d)	N.S.	N.S.	N.S.
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.645	0.071
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	104

Table 7. Analyses of the Incidence of Primary Tumors in Female Mice Fed Diets Containing FD & C Yellow No. 6 (a)

(Continued)

Topography: Morphology	Control	Low Dose	High Dose
Pituitary: Chromophobe Adenoma or Carcinoma (b)	0/46(0)	4/43(9)	1/35(3)
P Values (c), (d)	N.S.	N.S.	N.S.
Departure from Linear Trend (f)	P=0.034		
Relative Risk (Control) (e)		Infinite	Infinite
Lower Limit		0.995	0.071
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	92	104

(a) Dosed groups received doses of 12,500 or 25,000 ppm in feed.
(b) Number of tumor-bearing animals/number of animals examined at site (percent).
(c) Beneath the incidence of tumors in the control group is the probability level for the Cochran-Armitage test when P is less than 0.05; otherwise, not significant (N.S.) is indicated. Beneath the incidence of tumors in a dosed group is the probability level for the Fisher exact test for the comparison of that dosed group with the control group when P is less than 0.05; otherwise not significant (N.S.) is indicated.
(d) A negative trend (N) indicates a lower incidence in a dosed group than in a control group.
(e) The 95 percent confidence interval of the relative risk between each dosed group and the control group.
(f) The probability level for departure from linear trend is given when P is less than 0.05 for any comparison.

V. DISCUSSION

Mean body weights of high-dose male rats and of high-dose mice of either sex were slightly lower (less than 10%) than those of the controls; mean body weights of all other groups were comparable with those of controls.

Weight gain depression in rats and mice and bone marrow hyperplasia in rats observed in the subchronic study influenced selection of the doses for the chronic study. However, no compound-related bone marrow hyperplasia was observed in the chronic study and the rats and mice may have been able to tolerate higher doses. Diarrhea and enlarged ceca, observed by Gaunt et al. (1967) in CFE rats fed doses comparable with those in the present study, were not seen in the present study.

Hepatocellular carcinomas occurred in low-dose male mice at an incidence significantly higher ($P=0.033$) than that in the controls. Because the incidence of these tumors in the high-dose group was not significantly higher than that in the controls and because of the variability of the occurrence of liver tumors in control B6C3F1 male mice, the increased incidence of hepatocellular carcinomas observed in low-dose male mice was not clearly related to administration of FD & C Yellow No. 6.

No carcinogenic effects were observed in previous bioassays of 52-80 weeks duration in which 500-16,000 ppm FD & C Yellow No. 6 was incorporated in the feed or drinking water of rats or mice (IARC, 1975; Gaunt et al., 1974; Bonser et al., 1956; Mannell et al., 1958).

VI. CONCLUSIONS

Under the conditions of this bioassay, there was no clear evidence of carcinogenicity of FD & C Yellow No. 6 in F344 rats or B6C3F1 mice of either sex.

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Appendix A

Summary of the Incidence of Neoplasms in Rats Fed Diets Containing FD & C Yellow No. 6

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS NECROPSIED	90	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	90	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(90)	(50)	(50)
SQUAMOUS CELL PAPILLOMA	3 (3%)	1 (2%)	1 (2%)
SQUAMOUS CELL CARCINOMA			1 (2%)
SEBACEOUS ADENOMA	1 (1%)		
FIBROMA	1 (1%)		
*SUBCUT TISSUE	(90)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL TUMOR		1 (2%)	
FIBROMA	4 (4%)	1 (2%)	3 (6%)
FIBROSARCOMA	1 (1%)	1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(89)	(50)	(49)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
ADENOCARCINOMA, NOS, METASTATIC			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (1%)		
PHEOCHROMOCYTOMA, METASTATIC	1 (1%)		
FIBROSARCOMA, METASTATIC	1 (1%)		
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(90)	(50)	(50)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (1%)		
LYMPHOCYTIC LEUKEMIA	21 (23%)	12 (24%)	17 (34%)
#BONE MARROW	(84)	(48)	(50)
OSTEOMA	1 (1%)		
#SPLEEN	(90)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	1 (1%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE OF THORAX	(89)	(47)	(46)
INTERSTITIAL-CELL TUMOR, METASTA	1 (1%)		
#MESENTERIC L. NODE	(89)	(47)	(46)
MUCINOUS ADENOCARCINOMA, METASTA	1 (1%)		
CIRCULATORY SYSTEM			
#HEART	(90)	(50)	(49)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (1%)		
NONCHROMAFFIN PARAGANGLIOMA	1 (1%)		
DIGESTIVE SYSTEM			
*INTESTINAL TRACT	(90)	(50)	(50)
MUCINOUS ADENOCARCINOMA	1 (1%)		
#SALIVARY GLAND	(89)	(49)	(48)
MIXED TUMOR, MALIGNANT	1 (1%)		
#LIVER	(90)	(50)	(50)
NEOPLASTIC NODULE	5 (6%)	6 (12%)	1 (2%)
HEPATOCELLULAR CARCINOMA		1 (2%)	
FIBROSARCOMA, METASTATIC	1 (1%)		
#CARDIAC STOMACH	(87)	(49)	(50)
SQUAMOUS CELL PAPILLOMA	1 (1%)		
#COLON	(87)	(47)	(40)
ADENOMATOUS POLYP, NOS	1 (1%)		
URINARY SYSTEM			
#KIDNEY	(90)	(50)	(50)
TUBULAR-CELL ADENOMA		1 (2%)	1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(84)	(46)	(46)
ADENOMA, NOS	1 (1%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CHROMOPHOBE ADENOMA	4 (5%)	2 (4%)	4 (9%)
CHROMOPHOBE CARCINOMA	1 (1%)	3 (7%)	
ACIDOPHIL ADENOMA	2 (2%)		
#ADRENAL	(89)	(50)	(49)
CORTICAL ADENOMA	3 (3%)	2 (4%)	
PHEOCHROMOCYTOMA	11 (12%)	3 (6%)	9 (18%)
PHEOCHROMOCYTOMA, MALIGNANT	3 (3%)		2 (4%)
GANGLIONEUROMA		1 (2%)	
#THYROID	(89)	(49)	(50)
FOLLICULAR-CELL ADENOMA	2 (2%)		
FOLLICULAR-CELL CARCINOMA	2 (2%)	1 (2%)	
C-CELL CARCINOMA	2 (2%)	4 (8%)	4 (8%)
#PANCREATIC ISLETS	(88)	(49)	(49)
ISLET-CELL ADENOMA		2 (4%)	
ISLET-CELL CARCINOMA	3 (3%)	2 (4%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(90)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
FIBROADENOMA	2 (2%)	1 (2%)	3 (6%)
*PREPUTIAL GLAND	(90)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
SEBACEOUS ADENOCARCINOMA	1 (1%)	1 (2%)	1 (2%)
#PROSTATE	(84)	(48)	(48)
ADENOMA, NOS		1 (2%)	
#TESTIS	(90)	(50)	(50)
INTERSTITIAL-CELL TUMOR	86 (96%)	48 (96%)	50 (100%)
INTERSTITIAL-CELL TUMOR, MALIGNA	1 (1%)		
NERVOUS SYSTEM			
#CEREBRUM	(90)	(50)	(50)
ASTROCYTOMA	1 (1%)		
#BRAIN	(90)	(50)	(50)
ASTROCYTOMA	1 (1%)		

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
OLIGODENDROGLIOMA	1 (1%)		
SPECIAL SENSE ORGANS			
*EAR CANAL	(90)	(50)	(50)
SQUAMOUS CELL PAPILLOMA			1 (2%)
MUSCULOSKELETAL SYSTEM			
*INTERCOSTAL MUSCLE	(90)	(50)	(50)
ALVEOLAR/BRONCHIOLAR CA, INVASIV	1 (1%)		
BODY CAVITIES			
*PERITONEUM	(90)	(50)	(50)
MESOTHELIOMA, MALIGNANT	1 (1%)		
*TUNICA VAGINALIS	(90)	(50)	(50)
MESOTHELIOMA, NOS		2 (4%)	
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(90)	(50)	(50)
MESOTHELIOMA, NOS	1 (1%)		
MESOTHELIOMA, MALIGNANT	1 (1%)		2 (4%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	90	50	50
NATURAL DEATH@	9	9	7
MORIBUND SACRIFICE	11	5	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	70	36	38
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	90	50	50
TOTAL PRIMARY TUMORS	175	97	105
TOTAL ANIMALS WITH BENIGN TUMORS	86	48	50
TOTAL BENIGN TUMORS	124	64	72
TOTAL ANIMALS WITH MALIGNANT TUMORS	40	22	24
TOTAL MALIGNANT TUMORS	45	25	32
TOTAL ANIMALS WITH SECONDARY TUMORS#	4		2
TOTAL SECONDARY TUMORS	7		2
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	6	8	1
TOTAL UNCERTAIN TUMORS	6	8	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 FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	88	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	88	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
SEBACEOUS ADENOMA		1 (2%)	
*SUBCUT TISSUE	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA			2 (4%)
FIBROMA	2 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(88)	(50)	(50)
CARCINOMA, NOS, METASTATIC		1 (2%)	
SQUAMOUS CELL CARCINOMA, METASTA			3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(88)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	2 (2%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
LYMPHOCYTIC LEUKEMIA	14 (16%)	11 (22%)	10 (20%)
#SPLEEN	(88)	(50)	(50)
LYMPHOCYTIC LEUKEMIA	2 (2%)	1 (2%)	
#LYMPH NODE	(86)	(45)	(41)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
#MANDIBULAR L. NODE	(86)	(45)	(41)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
#RENAL LYMPH NODE	(86)	(45)	(41)
TRANSITIONAL-CELL CARCINOMA, MET	1 (1%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
#HEART	(88)	(50)	(50)
NEURILEMOMA, MALIGNANT	1 (1%)	1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(88)	(50)	(50)
NEOPLASTIC NODULE	3 (3%)	3 (6%)	
#ILEUM	(85)	(49)	(49)
FIBROSARCOMA			1 (2%)
URINARY SYSTEM			
#KIDNEY/PELVIS	(88)	(50)	(50)
TRANSITIONAL-CELL CARCINOMA	1 (1%)		
ENDOCRINE SYSTEM			
#PITUITARY	(83)	(45)	(48)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
CHROMOPHOBE ADENOMA	25 (30%)	18 (40%)	15 (31%)
CHROMOPHOBE CARCINOMA	5 (6%)		2 (4%)
#ADRENAL	(86)	(50)	(50)
CORTICAL ADENOMA	6 (7%)	2 (4%)	4 (8%)
CORTICAL CARCINOMA	1 (1%)		
PHEOCHROMOCYTOMA	3 (3%)	1 (2%)	4 (8%)
PHEOCHROMOCYTOMA, MALIGNANT	1 (1%)		
#THYROID	(86)	(50)	(48)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
FOLLICULAR-CELL ADENOMA	1 (1%)		2 (4%)
C-CELL ADENOMA			1 (2%)
C-CELL CARCINOMA	3 (3%)	2 (4%)	2 (4%)
#PANCREATIC ISLETS	(83)	(50)	(50)
ISLET-CELL ADENOMA		1 (2%)	
ISLET-CELL CARCINOMA	1 (1%)		

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(88)	(50)	(50)
ADENOMA, NOS	2 (2%)		2 (4%)
ADENOCARCINOMA, NOS	2 (2%)		2 (4%)
CYSTADENOMA, NOS		2 (4%)	
CYSTADENOCARCINOMA, NOS			1 (2%)
ACINAR-CELL ADENOMA		1 (2%)	
FIBROADENOMA	18 (20%)	6 (12%)	7 (14%)
*PREPUTIAL GLAND	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA	1 (1%)		
*VAGINA	(88)	(50)	(50)
FIBROMA	1 (1%)	1 (2%)	
#UTERUS	(87)	(49)	(49)
FIBROMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	9 (10%)	6 (12%)	7 (14%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)
#BRAIN	(88)	(50)	(50)
EPENDYMOMA	1 (1%)		
ASTROCYTOMA			1 (2%)
#MEDULLA OBLONGATA	(88)	(50)	(50)
CHROMOPHOBE CARCINOMA, INVASIVE			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(88)	(50)	(50)
SQUAMOUS CELL CARCINOMA, METASTA			1 (2%)

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

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(88)	(50)	(50)
SARCOMA, NOS	1 (1%)		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	90	50	50
NATURAL DEATH@	11	4	8
MORIBUND SACRIFICE	11	6	5
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	66	40	37
ANIMAL MISSING	2		
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	68	37	42
TOTAL PRIMARY TUMORS	106	58	66
TOTAL ANIMALS WITH BENIGN TUMORS	50	30	31
TOTAL BENIGN TUMORS	67	39	43
TOTAL ANIMALS WITH MALIGNANT TUMORS	33	14	21
TOTAL MALIGNANT TUMORS	36	16	23
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	4
TOTAL SECONDARY TUMORS	1	1	10
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3	3	
TOTAL UNCERTAIN TUMORS	3	3	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX B

Summary of the Incidence of Neoplasms in Mice Fed Diets Containing FD & C Yellow No. 6

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
FIBROMA			1 (2%)
FIBROSARCOMA			2 (4%)
*SUBCUT TISSUE	(50)	(49)	(50)
SEBACEOUS ADENOMA	1 (2%)		
SARCOMA, NOS		2 (4%)	
FIBROSARCOMA	4 (8%)	3 (6%)	3 (6%)
CARCINOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(48)	(50)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)	3 (6%)	4 (8%)
ALVEOLAR/BRONCHIOLAR ADENOMA	6 (12%)	3 (6%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (2%)	1 (2%)
FIBROSARCOMA, METASTATIC	1 (2%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
MALIGNANT LYMPHOMA, NOS	2 (4%)		3 (6%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		1 (2%)
MALIGNANT LYMPHOMA, MIXED TYPE	2 (4%)		
GRANULOCYTIC LEUKEMIA	1 (2%)		
#SPLEEN	(50)	(48)	(50)
MALIGNANT LYMPHOMA, NOS			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			2 (4%)
GRANULOCYTIC LEUKEMIA			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(41)	(35)	(42)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#MESENTERIC L. NODE	(41)	(35)	(42)
MALIGNANT LYMPHOMA, NOS			2 (5%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)	1 (3%)	
#LUNG	(50)	(48)	(50)
LYMPHOCYTIC LEUKEMIA		1 (2%)	
CIRCULATORY SYSTEM			
*SKIN	(50)	(49)	(50)
HEMANGIOSARCOMA			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(50)
HEMANGIOMA	1 (2%)		
#HEART	(50)	(49)	(50)
FIBROSARCOMA, METASTATIC			1 (2%)
#LIVER	(50)	(48)	(50)
HEMANGIOMA			1 (2%)
ANGIOMA		2 (4%)	
DIGESTIVE SYSTEM			
#LIVER	(50)	(48)	(50)
HEPATOCELLULAR ADENOMA	1 (2%)	1 (2%)	
HEPATOCELLULAR CARCINOMA	13 (26%)	22 (46%)	16 (32%)
SARCOMA, NOS, METASTATIC			1 (2%)
#JEJUNAL MUCOUS MEMBR	(46)	(46)	(45)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
HEPATOCELLULAR CARCINOMA, METAST			1 (2%)
SARCOMA, NOS, METASTATIC			1 (2%)

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROSARCOMA, METASTATIC			1 (2%)
#KIDNEY/CORTEX	(50)	(49)	(50)
ALVEOLAR/BRONCHIOLAR CA, METASTA			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL	(48)	(47)	(48)
CORTICAL ADENOMA	2 (4%)	2 (4%)	1 (2%)
PHEOCHROMOCYTOMA	1 (2%)		1 (2%)
#THYROID	(49)	(47)	(46)
FOLLICULAR-CELL ADENOMA		2 (4%)	1 (2%)
#PANCREATIC ISLETS	(46)	(46)	(47)
ISLET-CELL ADENOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
NONE			
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEAL CAVITY	(50)	(49)	(50)
SARCOMA, NOS			1 (2%)
FIBROSARCOMA, INVASIVE	1 (2%)		
ALL OTHER SYSTEMS			
NONE			

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

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	10	8	13
MORIBUND SACRIFICE	2	1	4
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	38	40	33
ANIMAL MISSING			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	32	31	34
TOTAL PRIMARY TUMORS	40	42	42
TOTAL ANIMALS WITH BENIGN TUMORS	11	11	7
TOTAL BENIGN TUMORS	12	11	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	25	27	30
TOTAL MALIGNANT TUMORS	28	31	35
TOTAL ANIMALS WITH SECONDARY TUMORS#	3	3	6
TOTAL SECONDARY TUMORS	3	3	11
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

@ INCLUDES AUTOLYZED ANIMALS

* 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 FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE SITES	(50)	(50)	(49)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (2%)		
*MULTIPLE ORGANS	(50)	(50)	(49)
MALIGNANT LYMPHOMA, NOS	4 (8%)	1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	2 (4%)	2 (4%)	3 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	3 (6%)	1 (2%)	5 (10%)
MALIGNANT LYMPHOMA, MIXED TYPE		1 (2%)	1 (2%)
LYMPHOCYTIC LEUKEMIA	1 (2%)		
GRANULOCYTIC LEUKEMIA		2 (4%)	
#MESENTERIC L. NODE	(43)	(41)	(41)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#UTERUS	(48)	(48)	(46)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#THYMUS	(35)	(32)	(32)
THYMOMA		1 (3%)	
MALIGNANT LYMPHOMA, NOS	3 (9%)		

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CIRCULATORY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(49)
ANGIOMA	1 (2%)		1 (2%)
#SPLEEN	(47)	(49)	(47)
ANGIOMA		1 (2%)	
#HEART	(47)	(49)	(48)
RHABDOMYOSARCOMA		1 (2%)	
#LIVER	(48)	(50)	(48)
ANGIOMA	1 (2%)	1 (2%)	
#ENDOMETRIAL STROMA	(48)	(48)	(46)
ANGIOMA			1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(48)	(50)	(48)
HEPATOCELLULAR ADENOMA	1 (2%)	3 (6%)	
HEPATOCELLULAR CARCINOMA	7 (15%)		4 (8%)
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(43)	(35)
ADENOMA, NOS			1 (3%)
CHROMOPHOBE ADENOMA		3 (7%)	1 (3%)
CHROMOPHOBE CARCINOMA		1 (2%)	
#ADRENAL	(45)	(49)	(45)
CORTICAL ADENOMA		1 (2%)	1 (2%)
PHEOCHROMOCYTOMA		1 (2%)	
#THYROID	(49)	(48)	(46)
ADENOMA, NOS		1 (2%)	
FOLLICULAR-CELL ADENOMA			1 (2%)

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(47)	(50)	(46)
ISLET-CELL ADENOMA	1 (2%)		
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
ADENOMA, NOS	1 (2%)	1 (2%)	
#UTERUS	(48)	(48)	(46)
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
LEIOMYOMA			1 (2%)
ENDOMETRIAL STROMAL POLYP	1 (2%)	1 (2%)	
#ENDOMETRIAL STROMA	(48)	(48)	(46)
FIBROSARCOMA		1 (2%)	
GRANULAR-CELL TUMOR, NOS	2 (4%)		
#OVARY	(41)	(46)	(41)
NEOPLASM, NOS	1 (2%)		
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
LUTEOMA		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(48)	(50)	(46)
OSTEOSARCOMA, INVASIVE			1 (2%)
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(50)	(50)	(49)
ADENOMA, NOS		1 (2%)	
PAPILLARY ADENOMA	1 (2%)	1 (2%)	
PAPILLARY CYSTADENOMA, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*CRANIAL AND FACIAL B	(50)	(50)	(49)
OSTEOSARCOMA			1 (2%)
BODY CAVITIES			
NONE			

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

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
LOWER LEG			
OSTEOSARCOMA	1		
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	9	9	7
MORIBUND SACRIFICE	3	5	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED		1	
TERMINAL SACRIFICE	38	35	43
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	28	20	21
TOTAL PRIMARY TUMORS	37	29	23
TOTAL ANIMALS WITH BENIGN TUMORS	10	14	7
TOTAL BENIGN TUMORS	10	18	8
TOTAL ANIMALS WITH MALIGNANT TUMORS	21	10	15
TOTAL MALIGNANT TUMORS	24	11	15
TOTAL ANIMALS WITH SECONDARY TUMORS#			1
TOTAL SECONDARY TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	3		
TOTAL UNCERTAIN TUMORS	3		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

Summary of the Incidence of Nonneoplastic Lesions in Rats Fed Diets Containing FD & C Yellow No. 6

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS NECROPSIED	90	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	90	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(90)	(50)	(50)
EPIDERMAL INCLUSION CYST	2 (2%)	2 (4%)	
ULCER, ACUTE		1 (2%)	
FIBROSIS, FOCAL			1 (2%)
*SUBCUT TISSUE	(90)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
RESPIRATORY SYSTEM			
#PERITRACHEAL TISSUE	(88)	(47)	(50)
FIBROSIS			1 (2%)
#LUNG	(89)	(50)	(49)
HEMORRHAGE	1 (1%)		1 (2%)
INFLAMMATION, INTERSTITIAL	2 (2%)		
INFLAMMATION, ACUTE FOCAL			1 (2%)
PNEUMONIA INTERSTITIAL CHRONIC		1 (2%)	2 (4%)
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
NECROSIS, FOCAL	1 (1%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (1%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(84)	(48)	(50)
CONGESTION, ACUTE	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		
HYPOPLASIA, HEMATOPOIETIC	4 (5%)		2 (4%)
#SPLEEN	(90)	(50)	(50)
CONGESTION, NOS	1 (1%)		

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
CONGESTION, ACUTE			1 (2%)
FIBROSIS, FOCAL		1 (2%)	1 (2%)
FIBROSIS, DIFFUSE	1 (1%)		
INFARCT, FOCAL	1 (1%)		
LYMPHOID DEPLETION		1 (2%)	
#LYMPH NODE	(89)	(47)	(46)
EDEMA, NOS	1 (1%)		
LYMPHOID DEPLETION	1 (1%)		
#SUBMANDIBULAR L.NODE	(89)	(47)	(46)
HEMORRHAGE	1 (1%)		
#MANDIBULAR L. NODE	(89)	(47)	(46)
INFLAMMATION, ACUTE NECROTIZING			1 (2%)
LYMPHOID DEPLETION		1 (2%)	
#MESENTERIC L. NODE	(89)	(47)	(46)
LYMPHOID DEPLETION	1 (1%)	2 (4%)	
#RENAL LYMPH NODE	(89)	(47)	(46)
PLASMACYTOSIS		1 (2%)	
#LUNG	(89)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (1%)		
#THYMUS	(69)	(37)	(40)
HEMORRHAGE			1 (3%)
CIRCULATORY SYSTEM			
#MANDIBULAR L. NODE	(89)	(47)	(46)
LYMPHANGIECTASIS		1 (2%)	3 (7%)
#MEDIASTINAL L.NODE	(89)	(47)	(46)
LYMPHANGIECTASIS			1 (2%)
#MESENTERIC L. NODE	(89)	(47)	(46)
LYMPHANGIECTASIS		10 (21%)	2 (4%)
#HEART	(90)	(50)	(49)
MINERALIZATION	1 (1%)		
ENDOCARDITIS, BACTERIAL			1 (2%)
DEGENERATION, NOS			1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ENDOCARDIOSIS	1 (1%)		
#HEART/ATRIUM	(90)	(50)	(49)
THROMBOSIS, NOS	1 (1%)	1 (2%)	1 (2%)
THROMBUS, FIBRIN			1 (2%)
#LEFT ATRIUM	(90)	(50)	(49)
THROMBOSIS, NOS	1 (1%)		
#HEART/VENTRICLE	(90)	(50)	(49)
FIBROSIS, FOCAL			1 (2%)
#MYOCARDIUM	(90)	(50)	(49)
INFLAMMATION, ACUTE FOCAL			2 (4%)
INFLAMMATION, ACUTE/CHRONIC	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		1 (2%)
DEGENERATION, NOS	31 (34%)	27 (54%)	27 (55%)
INFARCT, FOCAL			1 (2%)
#CARDIAC VALVE	(90)	(50)	(49)
INFLAMMATION, CHRONIC FOCAL	1 (1%)		
FIBROSIS	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		
*PULMONARY ARTERY	(90)	(50)	(50)
MINERALIZATION			1 (2%)
*PANCREATIC ARTERY,	(90)	(50)	(50)
FIBROSIS	1 (1%)		
*MESENTERIC ARTERY	(90)	(50)	(50)
THROMBOSIS, NOS	1 (1%)		
INFLAMMATION, ACUTE/CHRONIC	1 (1%)		
*VEIN OF NECK	(90)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
*PULMONARY VEIN	(90)	(50)	(50)
THROMBOSIS, NOS			1 (2%)
#LIVER	(90)	(50)	(50)
THROMBOSIS, NOS	1 (1%)		
#PANCREAS	(88)	(49)	(49)
PERIARTERITIS		1 (2%)	3 (6%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*MESENTERY	(90)	(50)	(50)
PERIARTERITIS		3 (6%)	
#U.BLADDER/SEROSA	(82)	(49)	(49)
PERIARTERITIS	1 (1%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(89)	(49)	(48)
CYST, NOS			1 (2%)
INFLAMMATION, ACUTE FOCAL			1 (2%)
ATROPHY, FOCAL	2 (2%)		
#LIVER	(90)	(50)	(50)
CONGESTION, CHRONIC PASSIVE		1 (2%)	
INFLAMMATION, ACUTE FOCAL			1 (2%)
INFLAMMATION, CHRONIC FOCAL	2 (2%)	1 (2%)	
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
CIRRHOSIS, NOS	1 (1%)		
DEGENERATION, NOS	1 (1%)		
NECROSIS, FOCAL		1 (2%)	1 (2%)
BASOPHILIC CYTO CHANGE	63 (70%)	25 (50%)	28 (56%)
FOCAL CELLULAR CHANGE	2 (2%)	3 (6%)	1 (2%)
ANGIECTASIS		2 (4%)	
NODULAR REGENERATION			1 (2%)
#PORTA HEPATIS	(90)	(50)	(50)
FIBROSIS	1 (1%)		
#PORTAL TRACT	(90)	(50)	(50)
FIBROSIS, FOCAL			1 (2%)
FIBROSIS, MULTIFOCAL		1 (2%)	
#LIVER/CENTRILOBULAR	(90)	(50)	(50)
DEGENERATION, NOS	1 (1%)		2 (4%)
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL	1 (1%)	3 (6%)	1 (2%)
#BILE DUCT	(90)	(50)	(50)
HYPERPLASIA, NOS	7 (8%)	2 (4%)	4 (8%)
HYPERPLASIA, FOCAL	15 (17%)	12 (24%)	13 (26%)
HYPERPLASIA, DIFFUSE			1 (2%)
#PANCREATIC ACINUS	(88)	(49)	(49)
ATROPHY, NOS	2 (2%)	2 (4%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, FOCAL	12 (14%)	5 (10%)	2 (4%)
ATROPHY, DIFFUSE		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	
#PERIESOPHAGEAL TISSU	(89)	(49)	(50)
FIBROSIS			1 (2%)
#STOMACH	(87)	(49)	(50)
ULCER, FOCAL		1 (2%)	
INFLAMMATION, ACUTE FOCAL	1 (1%)		
HYPERPLASIA, BASAL CELL	1 (1%)		
#GASTRIC MUCOSA	(87)	(49)	(50)
MINERALIZATION	1 (1%)		
NECROSIS, FOCAL	2 (2%)		2 (4%)
#CARDIAC STOMACH	(87)	(49)	(50)
ECTOPIA	1 (1%)		
HYPERPLASIA, BASAL CELL	1 (1%)	1 (2%)	
#DUODENUM	(87)	(47)	(47)
ULCER, ACUTE		1 (2%)	
#JEJUNUM	(87)	(47)	(47)
ULCER, CHRONIC		1 (2%)	
#COLON	(87)	(47)	(40)
ULCER, CHRONIC		1 (2%)	
NEMATODIASIS	8 (9%)	4 (9%)	1 (3%)
URINARY SYSTEM			
#KIDNEY	(90)	(50)	(50)
CYST, NOS	1 (1%)	1 (2%)	
GLOMERULONEPHRITIS SUPPURATIVE			1 (2%)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
NEPHROPATHY	80 (89%)	38 (76%)	44 (88%)
DEGENERATION, HYALINE	1 (1%)		
PIGMENTATION, NOS	4 (4%)		
#KIDNEY/CORTEX	(90)	(50)	(50)
PIGMENTATION, NOS	2 (2%)	1 (2%)	2 (4%)
#KIDNEY/TUBULE	(90)	(50)	(50)
PIGMENTATION, NOS	1 (1%)		1 (2%)

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REGENERATION, NOS	1 (1%)		
#KIDNEY/PELVIS	(90)	(50)	(50)
MINERALIZATION			1 (2%)
HYPERPLASIA, DIFFUSE			1 (2%)
*PROSTATIC URETHRA	(90)	(50)	(50)
METAPLASIA, SQUAMOUS	1 (1%)		
ENDOCRINE SYSTEM			
#PITUITARY	(84)	(46)	(46)
HYPERPLASIA, FOCAL	1 (1%)		
HYPERPLASIA, CHROMOPHOBE-CELL	9 (11%)	3 (7%)	3 (7%)
#PITUITARY ACIDOPHIL	(84)	(46)	(46)
HYPERPLASIA, FOCAL	1 (1%)		2 (4%)
#PITUITARY/BASOPHIL	(84)	(46)	(46)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL	(89)	(50)	(49)
NECROSIS, HEMORRHAGIC		1 (2%)	
#ADRENAL CORTEX	(89)	(50)	(49)
LIPOIDOSIS	3 (3%)	6 (12%)	3 (6%)
CYTOPLASMIC VACUOLIZATION	1 (1%)		
HYPERPLASIA, FOCAL	5 (6%)	2 (4%)	9 (18%)
#ZONA FASCICULATA	(89)	(50)	(49)
HYPERPLASIA, NODULAR			1 (2%)
#ADRENAL MEDULLA	(89)	(50)	(49)
NECROSIS, NOS			1 (2%)
HYPERPLASIA, NOS		6 (12%)	5 (10%)
HYPERPLASIA, FOCAL	4 (4%)	1 (2%)	1 (2%)
ANGIECTASIS	1 (1%)		
#THYROID	(89)	(49)	(50)
THYROGLOSSAL DUCT CYST		1 (2%)	
MINERALIZATION	1 (1%)		
FOLLICULAR CYST, NOS			1 (2%)
HYPERPLASIA, C-CELL	17 (19%)	7 (14%)	13 (26%)
#PARATHYROID	(70)	(39)	(44)
HYPERPLASIA, FOCAL		1 (3%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(88)	(49)	(49)
HYPERPLASIA, NOS	2 (2%)	1 (2%)	4 (8%)
HYPERPLASIA, FOCAL	3 (3%)	5 (10%)	5 (10%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(90)	(50)	(50)
DILATATION, NOS	1 (1%)		
HYPERPLASIA, CYSTIC	1 (1%)	3 (6%)	
*MAMMARY ACINUS	(90)	(50)	(50)
CYST, NOS		1 (2%)	2 (4%)
HYPERPLASIA, NOS	5 (6%)		4 (8%)
*PREPUTIAL GLAND	(90)	(50)	(50)
CYST, NOS	1 (1%)		
#PROSTATE	(84)	(48)	(48)
INFLAMMATION, SUPPURATIVE	1 (1%)		
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, ACUTE DIFFUSE			1 (2%)
INFLAMMATION ACTIVE CHRONIC		1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	2 (2%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		1 (2%)
#PROSTATIC GLAND	(84)	(48)	(48)
DILATATION, NOS	1 (1%)		
HYPERPLASIA, EPITHELIAL	1 (1%)		
*SEMINAL VESICLE	(90)	(50)	(50)
CYST, NOS	1 (1%)		
#TESTIS	(90)	(50)	(50)
STEATITIS	4 (4%)		
NECROSIS, DIFFUSE			1 (2%)
NECROSIS, FAT		2 (4%)	
ATROPHY, NOS	1 (1%)		2 (4%)
HYPOSPERMATOGENESIS	1 (1%)		
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)
#TESTIS/TUBULE	(90)	(50)	(50)
DEGENERATION, NOS		1 (2%)	1 (2%)
*EPIDIDYMIS	(90)	(50)	(50)
MINERALIZATION		2 (4%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NERVOUS SYSTEM			
#BRAIN	(90)	(50)	(50)
HYDROCEPHALUS, NOS			1 (2%)
HEMORRHAGE	1 (1%)	1 (2%)	
NECROSIS, FOCAL	1 (1%)		
NECROSIS, HEMORRHAGIC		3 (6%)	2 (4%)
#HIPPOCAMPUS	(90)	(50)	(50)
NECROSIS, FOCAL	1 (1%)		2 (4%)
#HYPOTHALAMUS	(90)	(50)	(50)
ATROPHY, PRESSURE		1 (2%)	1 (2%)
#CEREBELLUM	(90)	(50)	(50)
NECROSIS, HEMORRHAGIC	1 (1%)		
#MEDULLA OBLONGATA	(90)	(50)	(50)
NECROSIS, HEMORRHAGIC	1 (1%)	1 (2%)	
SPECIAL SENSE ORGANS			
*EYE	(90)	(50)	(50)
SYNECHIA, POSTERIOR	1 (1%)		
*EYE/RETINA	(90)	(50)	(50)
DETACHMENT	1 (1%)		
*EYE/CRYSTALLINE LENS	(90)	(50)	(50)
DEGENERATION, NOS	1 (1%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(90)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (1%)		
*EPICARDIUM	(90)	(50)	(50)
HEMORRHAGE		1 (2%)	

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

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
*MESENTERY	(90)	(50)	(50)
STEATITIS	4 (4%)		
INFLAMMATION, CHRONIC		1 (2%)	
INFLAMMATION, GRANULOMATOUS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU		7 (14%)	4 (8%)
NECROSIS, FAT			3 (6%)
ALL OTHER SYSTEMS			
SITE UNKNOWN			
NECROSIS, FAT			1
CRANIOBUCCAL POUCH			
CYSTIC DUCTS	1		
SPECIAL MORPHOLOGY SUMMARY			
NONE			

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

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	90	50	50
ANIMALS MISSING	2		
ANIMALS NECROPSIED	88	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	88	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(88)	(50)	(50)
EPIDERMAL INCLUSION CYST			1 (2%)
*SUBCUT TISSUE	(88)	(50)	(50)
ABSCESS, CHRONIC	1 (1%)		
RESPIRATORY SYSTEM			
#PERITRACHEAL TISSUE	(86)	(50)	(48)
INFLAMMATION, CHRONIC	1 (1%)		
#LUNG	(88)	(50)	(50)
EDEMA, NOS	2 (2%)		1 (2%)
HEMORRHAGE	1 (1%)		
INFLAMMATION, INTERSTITIAL		3 (6%)	
PNEUMONIA INTERSTITIAL CHRONIC	1 (1%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(86)	(48)	(47)
DEPLETION	1 (1%)		
HYPERPLASIA, RETICULUM CELL	2 (2%)		
HYPOPLASIA, HEMATOPOIETIC	6 (7%)	2 (4%)	
#SPLEEN	(88)	(50)	(50)
CONGESTION, NOS	1 (1%)		
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
FIBROSIS, FOCAL	1 (1%)		
INFARCT, FOCAL	1 (1%)		
LYMPHOID DEPLETION	2 (2%)		
HEMATOPOIESIS	1 (1%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LYMPH NODE	(86)	(45)	(41)
LYMPHOID DEPLETION	1 (1%)		
#MANDIBULAR L. NODE	(86)	(45)	(41)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (1%)		
PLASMACYTOSIS	1 (1%)		
#PANCREATIC L.NODE	(86)	(45)	(41)
HEMORRHAGE		1 (2%)	
#MESENTERIC L. NODE	(86)	(45)	(41)
LYMPHOID DEPLETION	1 (1%)		
PLASMACYTOSIS	1 (1%)		
#LUNG	(88)	(50)	(50)
HYPERPLASIA, LYMPHOID	1 (1%)		
#SMALL INTESTINAL SUB	(85)	(49)	(49)
HYPERPLASIA, LYMPHOID		1 (2%)	
#THYMUS	(70)	(38)	(39)
CYST, NOS		1 (3%)	
HYPERPLASIA, EPITHELIAL			1 (3%)
CIRCULATORY SYSTEM			
#MANDIBULAR L. NODE	(86)	(45)	(41)
LYMPHANGIECTASIS			1 (2%)
#PANCREATIC L.NODE	(86)	(45)	(41)
LYMPHANGIECTASIS	1 (1%)		
#MESENTERIC L. NODE	(86)	(45)	(41)
LYMPHANGIECTASIS		4 (9%)	
#HEART	(88)	(50)	(50)
FIBROSIS, FOCAL	1 (1%)	1 (2%)	
#HEART/ATRIUM	(88)	(50)	(50)
THROMBOSIS, NOS	1 (1%)		
#MYOCARDIUM	(88)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (1%)		

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
FIBROSIS, DIFFUSE	1 (1%)		
DEGENERATION, NOS	11 (13%)	15 (30%)	8 (16%)
#CARDIAC VALVE	(88)	(50)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
*PORTAL VEIN	(88)	(50)	(50)
THROMBOSIS, NOS		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(87)	(49)	(49)
METAPLASIA, SQUAMOUS		1 (2%)	
#LIVER	(88)	(50)	(50)
INFLAMMATION, ACUTE/CHRONIC	2 (2%)		
INFLAMMATION, CHRONIC	3 (3%)		
INFLAMMATION, CHRONIC FOCAL	19 (22%)	7 (14%)	3 (6%)
INFLAMMATION, FOCAL GRANULOMATOU	1 (1%)		
DEGENERATION, NOS		1 (2%)	
NECROSIS, FOCAL	1 (1%)	2 (4%)	
NECROSIS, CENTRAL	1 (1%)		
BASOPHILIC CYTO CHANGE	62 (70%)	40 (80%)	36 (72%)
FOCAL CELLULAR CHANGE	3 (3%)	2 (4%)	
#PORTAL TRACT	(88)	(50)	(50)
INFLAMMATION, CHRONIC	1 (1%)		
#LIVER/CENTRILOBULAR	(88)	(50)	(50)
NECROSIS, FOCAL	1 (1%)		1 (2%)
PIGMENTATION, NOS	1 (1%)		
#LIVER/PERIPORTAL	(88)	(50)	(50)
CYTOPLASMIC VACUOLIZATION			1 (2%)
#BILE DUCT	(88)	(50)	(50)
HYPERPLASIA, NOS	6 (7%)	2 (4%)	
HYPERPLASIA, FOCAL	5 (6%)		2 (4%)
HYPERPLASIA, DIFFUSE			1 (2%)
#PANCREATIC ACINUS	(83)	(50)	(50)
DEGENERATION, NOS	1 (1%)		
ATROPHY, NOS	5 (6%)		
ATROPHY, FOCAL	1 (1%)	4 (8%)	6 (12%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ATROPHY, DIFFUSE	1 (1%)		
HYPERPLASIA, FOCAL		1 (2%)	
#PERIESOPHAGEAL TISSU	(87)	(50)	(48)
INFLAMMATION, CHRONIC	1 (1%)		
#STOMACH	(86)	(50)	(50)
INFLAMMATION, ACUTE FOCAL		1 (2%)	
FIBROSIS, DIFFUSE	1 (1%)		
#GASTRIC MUCOSA	(86)	(50)	(50)
NECROSIS, FOCAL	1 (1%)		
#CARDIAC STOMACH	(86)	(50)	(50)
EDEMA, NOS	1 (1%)		
INFLAMMATION, FOCAL	1 (1%)		
INFLAMMATION, VESICULAR	1 (1%)		
ULCER, CHRONIC	1 (1%)		
HYPERPLASIA, EPITHELIAL	1 (1%)		1 (2%)
#GASTRIC FUNDUS	(86)	(50)	(50)
NECROSIS, FOCAL		1 (2%)	
#COLON	(72)	(47)	(49)
NEMATODIASIS	6 (8%)	2 (4%)	1 (2%)
*RECTUM	(88)	(50)	(50)
NEMATODIASIS	1 (1%)		
*RECTAL MUCOUS MEMBRA	(88)	(50)	(50)
ATROPHY, NOS	1 (1%)		
URINARY SYSTEM			
#KIDNEY	(88)	(50)	(50)
CYST, NOS			1 (2%)
NEPHROPATHY	12 (14%)	2 (4%)	7 (14%)
INFARCT, NOS	1 (1%)		
PIGMENTATION, NOS	3 (3%)	4 (8%)	1 (2%)
#KIDNEY/CORTEX	(88)	(50)	(50)
PIGMENTATION, NOS	1 (1%)		
#KIDNEY/TUBULE	(88)	(50)	(50)
PIGMENTATION, NOS	2 (2%)		2 (4%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
REGENERATION, NOS	1 (1%)		
#KIDNEY/PELVIS	(88)	(50)	(50)
MINERALIZATION	2 (2%)	3 (6%)	3 (6%)
HYPERPLASIA, EPITHELIAL		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(83)	(45)	(48)
CYST, NOS			1 (2%)
HEMORRHAGE, CHRONIC			1 (2%)
HYPERPLASIA, CHROMOPHOBE-CELL	12 (14%)	8 (18%)	10 (21%)
ANGIECTASIS	2 (2%)	1 (2%)	
#PITUITARY ACIDOPHIL	(83)	(45)	(48)
HYPERPLASIA, NOS	1 (1%)		
#ADRENAL	(86)	(50)	(50)
CONGESTION, NOS			1 (2%)
HEMORRHAGE		1 (2%)	
HEMORRHAGE, CHRONIC		1 (2%)	
ABSCESS, CHRONIC	1 (1%)		
NECROSIS, FOCAL		1 (2%)	
ATROPHY, NOS	1 (1%)		
ANGIECTASIS	1 (1%)		1 (2%)
#ADRENAL CORTEX	(86)	(50)	(50)
DEGENERATION, NOS			1 (2%)
LIPOIDOSIS	15 (17%)	6 (12%)	11 (22%)
FOCAL CELLULAR CHANGE	1 (1%)		
HYPERPLASIA, NOS	5 (6%)	2 (4%)	
HYPERPLASIA, FOCAL	7 (8%)	8 (16%)	8 (16%)
ANGIECTASIS	1 (1%)		
#ZONA FASCICULATA	(86)	(50)	(50)
LIPOIDOSIS	1 (1%)		
#ADRENAL MEDULLA	(86)	(50)	(50)
HYPERPLASIA, FOCAL	1 (1%)		1 (2%)
#THYROID	(86)	(50)	(48)
HYPERPLASIA, C-CELL	16 (19%)	12 (24%)	10 (21%)
HYPERPLASIA, FOLLICULAR-CELL	1 (1%)		
#PARATHYROID	(69)	(40)	(37)
HYPERPLASIA, FOCAL			1 (3%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#PANCREATIC ISLETS	(83)	(50)	(50)
HYPERPLASIA, FOCAL		2 (4%)	1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(88)	(50)	(50)
DILATATION, NOS	2 (2%)		
DILATATION/DUCTS	3 (3%)		
CYST, NOS	2 (2%)		1 (2%)
CYSTIC DUCTS	2 (2%)		1 (2%)
HYPERPLASIA, NOS	1 (1%)		
HYPERPLASIA, EPITHELIAL	1 (1%)		
HYPERPLASIA, DIFFUSE			1 (2%)
HYPERPLASIA, CYSTIC	19 (22%)	1 (2%)	6 (12%)
*MAMMARY ACINUS	(88)	(50)	(50)
DILATATION, NOS	1 (1%)		
CYST, NOS	3 (3%)	8 (16%)	1 (2%)
MULTIPLE CYSTS	1 (1%)		
HYPERPLASIA, NOS	4 (5%)	2 (4%)	1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
HYPERPLASIA, CYSTIC	2 (2%)	8 (16%)	12 (24%)
*CLITORAL GLAND	(88)	(50)	(50)
CYST, NOS	1 (1%)		
*VAGINA	(88)	(50)	(50)
POLYP	1 (1%)		
#UTERUS/ENDOMETRIUM	(87)	(49)	(49)
HEMORRHAGE, CHRONIC			1 (2%)
HYPERPLASIA, NOS	1 (1%)		
#ENDOMETRIAL GLAND	(87)	(49)	(49)
CYST, NOS	4 (5%)	3 (6%)	3 (6%)
#OVARY	(86)	(50)	(49)
FOLLICULAR CYST, NOS	3 (3%)		
CORPUS LUTEUM CYST			1 (2%)
#OVARY/RETE OVARII	(86)	(50)	(49)
HYPERPLASIA, NOS	1 (1%)		
#MESOVARIUM	(86)	(50)	(49)
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FAT	1 (1%)	1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(88)	(50)	(50)
HYDROCEPHALUS, NOS	8 (9%)	3 (6%)	4 (8%)
HEMORRHAGE	1 (1%)	1 (2%)	1 (2%)
NECROSIS, FOCAL	1 (1%)	1 (2%)	
NECROSIS, HEMORRHAGIC			1 (2%)
ATROPHY, PRESSURE	2 (2%)	2 (4%)	1 (2%)
#HYPOTHALAMUS	(88)	(50)	(50)
ATROPHY, PRESSURE	6 (7%)	4 (8%)	9 (18%)
#MIDBRAIN	(88)	(50)	(50)
ATROPHY, PRESSURE			1 (2%)
#CEREBELLUM	(88)	(50)	(50)
MINERALIZATION	1 (1%)		
SPECIAL SENSE ORGANS			
*EYE	(88)	(50)	(50)
SYNECHIA, ANTERIOR	1 (1%)		
*EYE/RETINA	(88)	(50)	(50)
ATROPHY, NOS	1 (1%)	1 (2%)	
ATROPHY, DIFFUSE	1 (1%)		
*EYE/CRYSTALLINE LENS	(88)	(50)	(50)
DEGENERATION, NOS	2 (2%)		
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(88)	(50)	(50)
MINERALIZATION			1 (2%)
BODY CAVITIES			
*MESENTERY	(88)	(50)	(50)
NECROSIS, FAT	3 (3%)	1 (2%)	1 (2%)

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

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(88)	(50)	(50)
HEMORRHAGE	1 (1%)		
BROAD LIGAMENT			
STEATITIS	1		
SPECIAL MORPHOLOGY SUMMARY			
ANIMAL MISSING/NO NECROPSY	2		

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

APPENDIX D

Summary of the Incidence of Nonneoplastic Lesions
in Mice Fed Diets Containing FD & C Yellow No.6

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	49	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(50)
FOLLICULAR CYST, NOS	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
FIBROSIS			3 (6%)
FIBROSIS, FOCAL			1 (2%)
ALOPECIA		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
*SUBCUT TISSUE	(50)	(49)	(50)
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, FOCAL	1 (2%)		
FIBROSIS		1 (2%)	
NECROSIS, NOS		1 (2%)	
RESPIRATORY SYSTEM			
#LUNG/BRONCHIOLE	(50)	(48)	(50)
PERIVASCULAR CUFFING	1 (2%)		
#LUNG	(50)	(48)	(50)
CONGESTION, NOS	1 (2%)	1 (2%)	
EDEMA, NOS		1 (2%)	
BRONCHOPNEUMONIA, NOS		3 (6%)	
BRONCHOPNEUMONIA, FOCAL	4 (8%)		2 (4%)
INFLAMMATION, INTERSTITIAL	15 (30%)	15 (31%)	3 (6%)
PNEUMONIA INTERSTITIAL CHRONIC		4 (8%)	4 (8%)
BRONCHOPNEUMONIA, CHRONIC	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	20 (40%)	22 (46%)	14 (28%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(50)
HYPERPLASIA, LYMPHOID	1 (2%)		3 (6%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#BONE MARROW	(48)	(46)	(42)
HYPERPLASIA, GRANULOCYTIC	2 (4%)	2 (4%)	2 (5%)
#SPLEEN	(50)	(48)	(50)
HYPERPLASIA, LYMPHOID	17 (34%)	11 (23%)	16 (32%)
HEMATOPOIESIS		1 (2%)	1 (2%)
#SPLENIC RED PULP	(50)	(48)	(50)
HEMATOPOIESIS	2 (4%)	8 (17%)	1 (2%)
#MANDIBULAR L. NODE	(41)	(35)	(42)
HEMOSIDEROSIS		1 (3%)	
#MESENTERIC L. NODE	(41)	(35)	(42)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	1 (2%)	1 (3%)	1 (2%)
#AXILLARY LYMPH NODE	(41)	(35)	(42)
HYPERPLASIA, LYMPHOID	1 (2%)		
HEMATOPOIESIS	1 (2%)		
#FEMORAL LYMPH NODE	(41)	(35)	(42)
HEMOSIDEROSIS	1 (2%)		
#LUNG	(50)	(48)	(50)
HYPERPLASIA, LYMPHOID	4 (8%)	14 (29%)	9 (18%)
#SALIVARY GLAND	(50)	(49)	(47)
HYPERPLASIA, LYMPHOID	8 (16%)	9 (18%)	7 (15%)
#RIGHT PAROTID GLAND	(50)	(49)	(47)
HYPERPLASIA, LYMPHOID			1 (2%)
#PORTAL TRACT	(50)	(48)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#PANCREAS	(46)	(46)	(47)
HYPERPLASIA, LYMPHOID	1 (2%)		
#KIDNEY	(50)	(49)	(50)
HYPERPLASIA, LYMPHOID	22 (44%)	22 (45%)	14 (28%)
#URINARY BLADDER	(50)	(44)	(45)
HYPERPLASIA, LYMPHOID	3 (6%)	1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#U.BLADDER/SUBMUCOSA	(50)	(44)	(45)
HYPERPLASIA, LYMPHOID		1 (2%)	1 (2%)
*SEMINAL VESICLE	(50)	(49)	(50)
HYPERPLASIA, LYMPHOID			1 (2%)
#THYROID	(49)	(47)	(46)
HYPERPLASIA, LYMPHOID	1 (2%)		
CIRCULATORY SYSTEM			
#SPLENIC FOLLICLES	(50)	(48)	(50)
PERIARTERITIS	1 (2%)		
#MESENTERIC L. NODE	(41)	(35)	(42)
LYMPHANGIECTASIS			3 (7%)
#LUNG	(50)	(48)	(50)
PERIVASCULITIS	5 (10%)	1 (2%)	1 (2%)
#HEART	(50)	(49)	(50)
ATHEROSCLEROSIS	1 (2%)		
#MYOCARDIUM	(50)	(49)	(50)
DEGENERATION, NOS	1 (2%)	1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(49)	(47)
ATROPHY, FOCAL			1 (2%)
#LIVER	(50)	(48)	(50)
INFLAMMATION, GRANULOMATOUS	1 (2%)		
GRANULOMA, NOS			1 (2%)
INFLAMMATION, FOCAL GRANULOMATOU	3 (6%)	6 (13%)	3 (6%)
FIBROSIS, MULTIFOCAL	1 (2%)		
NECROSIS, NOS	1 (2%)		
NECROSIS, FOCAL			1 (2%)
INFARCT, NOS	1 (2%)		
ANGIECTASIS		1 (2%)	
#PORTAL TRACT	(50)	(48)	(50)
NECROSIS, FOCAL		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LIVER/CENTRILOBULAR	(50)	(48)	(50)
NECROSIS, FOCAL		1 (2%)	1 (2%)
#BILE DUCT	(50)	(48)	(50)
CYST, NOS		1 (2%)	
#PANCREAS	(46)	(46)	(47)
DILATATION/DUCTS	1 (2%)		
CYSTIC DUCTS		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
#PANCREATIC ACINUS	(46)	(46)	(47)
ATROPHY, NOS		1 (2%)	
#GASTRIC MUCOSA	(48)	(48)	(49)
DILATATION, NOS	1 (2%)		
#ILEUM	(46)	(46)	(45)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(50)	(49)	(50)
HYDRONEPHROSIS	1 (2%)		
CONGESTION, NOS			1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	
NEPHROSIS, NOS	1 (2%)	1 (2%)	1 (2%)
GLOMERULOSCLEROSIS, NOS	5 (10%)	8 (16%)	4 (8%)
#KIDNEY/CORTEX	(50)	(49)	(50)
MINERALIZATION		1 (2%)	
CONGESTION, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
INFARCT, FOCAL		1 (2%)	
REGENERATION, NOS			1 (2%)
#KIDNEY/MEDULLA	(50)	(49)	(50)
MINERALIZATION		1 (2%)	
#KIDNEY/TUBULE	(50)	(49)	(50)
DILATATION, NOS	2 (4%)		
DEGENERATION, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFARCT, NOS		1 (2%)	
HEMOSIDEROSIS			1 (2%)
REGENERATION, NOS	33 (66%)	32 (65%)	26 (52%)
#KIDNEY/PELVIS	(50)	(49)	(50)
INFLAMMATION, ACUTE			1 (2%)
*URETER	(50)	(49)	(50)
RETENTION FLUID	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#URINARY BLADDER	(50)	(44)	(45)
ULCER, ACUTE HEMORRHAGIC			1 (2%)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
*PROSTATIC URETHRA	(50)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)
ENDOCRINE SYSTEM			
#ADRENAL CORTEX	(48)	(47)	(48)
HYPERTROPHY, FOCAL			1 (2%)
#ADRENAL MEDULLA	(48)	(47)	(48)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(49)	(47)	(46)
FOLLICULAR CYST, NOS		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (2%)		
#THYROID FOLLICLE	(49)	(47)	(46)
HYPERPLASIA, CYSTIC		1 (2%)	
REPRODUCTIVE SYSTEM			
*PREPUCE	(50)	(49)	(50)
INFLAMMATION, ACUTE FOCAL			1 (2%)
*PREPUTIAL GLAND	(50)	(49)	(50)
INFLAMMATION, CHRONIC		1 (2%)	
GRANULOMA, NOS		1 (2%)	
#PROSTATE	(48)	(47)	(46)
INFLAMMATION, ACUTE DIFFUSE			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*SEMINAL VESICLE	(50)	(49)	(50)
HEMORRHAGE			1 (2%)
#TESTIS	(49)	(48)	(50)
MINERALIZATION		2 (4%)	
HYPERPLASIA, INTERSTITIAL CELL			1 (2%)
#TESTIS/TUBULE	(49)	(48)	(50)
MINERALIZATION	1 (2%)		
DEGENERATION, NOS	2 (4%)	2 (4%)	5 (10%)
*EPIDIDYMIS	(50)	(49)	(50)
MINERALIZATION	1 (2%)		
*VAS DEFERENS	(50)	(49)	(50)
RETENTION OF CONTENT			1 (2%)
DEGENERATION, NOS			1 (2%)
NERVOUS SYSTEM			
#CEREBRUM	(50)	(47)	(50)
MINERALIZATION	24 (48%)	20 (43%)	27 (54%)
DEGENERATION, NOS			1 (2%)
#BRAIN	(50)	(47)	(50)
MINERALIZATION			4 (8%)
PERIVASCULAR CUFFING	1 (2%)		
#CEREBRAL HEMISPHERE	(50)	(47)	(50)
MINERALIZATION	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(50)	(49)	(50)
INFLAMMATION, FOCAL			1 (2%)

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

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ALL OTHER SYSTEMS			
CRANIOBUCCAL POUCH			
CYSTIC DUCTS		1	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		2	

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

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE FED DIETS CONTAINING FD&C YELLOW NO. 6

	CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY	50	50	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(50)	(50)	(48)
CONGESTION, NOS	2 (4%)		
EDEMA, NOS	1 (2%)	1 (2%)	
HEMORRHAGE		2 (4%)	
BRONCHOPNEUMONIA, FOCAL	7 (14%)	9 (18%)	8 (17%)
INFLAMMATION, INTERSTITIAL	17 (34%)	15 (30%)	15 (31%)
HYPERPLASIA, ALVEOLAR EPITHELIUM	27 (54%)	24 (48%)	27 (56%)
HEMATOPOIETIC SYSTEM			
#BRAIN/MENINGES	(48)	(50)	(46)
HYPERPLASIA, LYMPHOID	1 (2%)		
*MULTIPLE ORGANS	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	4 (8%)	11 (22%)	5 (10%)
#BONE MARROW	(46)	(46)	(45)
HYPERPLASIA, GRANULOCYTIC			1 (2%)
#SPLEEN	(47)	(49)	(47)
FIBROSIS, FOCAL			1 (2%)
LEUKEMOID REACTION	1 (2%)		
HYPERPLASIA, LYMPHOID	14 (30%)	5 (10%)	9 (19%)
#SPLENIC RED PULP	(47)	(49)	(47)
HEMOSIDEROSIS	3 (6%)	1 (2%)	1 (2%)
LYMPHOID DEPLETION	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
ANGIECTASIS		1 (2%)	
HYPERPLASIA, RETICULUM CELL	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	
HEMATOPOIESIS	2 (4%)	1 (2%)	
#LYMPH NODE	(43)	(41)	(41)
GRANULOMA, NOS	1 (2%)		
HYPERPLASIA, LYMPHOID			1 (2%)
#MANDIBULAR L. NODE	(43)	(41)	(41)
HYPERPLASIA, PLASMA CELL			1 (2%)
HYPERPLASIA, LYMPHOID	1 (2%)	3 (7%)	
#LUNG	(50)	(50)	(48)
HYPERPLASIA, LYMPHOID	18 (36%)	11 (22%)	17 (35%)
#SALIVARY GLAND	(48)	(49)	(44)
HYPERPLASIA, LYMPHOID	9 (19%)	9 (18%)	12 (27%)
#LIVER	(48)	(50)	(48)
HYPERPLASIA, LYMPHOID	4 (8%)	3 (6%)	
*GALLBLADDER	(50)	(50)	(49)
HYPERPLASIA, LYMPHOID	1 (2%)		
#PANCREAS	(47)	(50)	(46)
HYPERPLASIA, LYMPHOID	3 (6%)	2 (4%)	
#KIDNEY	(49)	(50)	(48)
HYPERPLASIA, LYMPHOID	22 (45%)	15 (30%)	25 (52%)
#URINARY BLADDER	(45)	(45)	(43)
HYPERPLASIA, LYMPHOID	1 (2%)		
#U.BLADDER/SUBMUCOSA	(45)	(45)	(43)
HYPERPLASIA, LYMPHOID	17 (38%)	13 (29%)	18 (42%)
#THYMUS	(35)	(32)	(32)
NECROSIS, NOS		1 (3%)	
HYPERPLASIA, LYMPHOID			1 (3%)
#THYMIC MEDULLA	(35)	(32)	(32)
HYPERPLASIA, LYMPHOID		1 (3%)	
CIRCULATORY SYSTEM			
#CEREBRUM	(48)	(50)	(46)
THROMBOSIS, NOS	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#LUNG	(50)	(50)	(48)
PERIVASCULITIS	2 (4%)		
#HEART/ATRIUM	(47)	(49)	(48)
FIBROSIS, FOCAL		1 (2%)	
#INTERVENTRICULAR SEP	(47)	(49)	(48)
HEMOSIDEROSIS			1 (2%)
#MYOCARDIUM	(47)	(49)	(48)
INFLAMMATION, MULTIFOCAL	1 (2%)		
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
DEGENERATION, NOS	1 (2%)	1 (2%)	
#CARDIAC VALVE	(47)	(49)	(48)
PIGMENTATION, NOS	1 (2%)		
HEMOSIDEROSIS	3 (6%)	3 (6%)	6 (13%)
*UTERINE ARTERY	(50)	(50)	(49)
DEGENERATION, HYALINE	1 (2%)		
*OVARIAN ARTERY	(50)	(50)	(49)
THROMBUS, MURAL		1 (2%)	
#ADRENAL CORTEX	(45)	(49)	(45)
PERIARTERITIS	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(49)	(44)
INFLAMMATION, PYOGRANULOMATOUS		1 (2%)	
#LIVER	(48)	(50)	(48)
INFLAMMATION, ACUTE/CHRONIC		2 (4%)	
INFLAMMATION, FOCAL GRANULOMATOU	23 (48%)	16 (32%)	16 (33%)
NECROSIS, NOS	1 (2%)		
NECROSIS, FOCAL		2 (4%)	
INFARCT, NOS	1 (2%)		
FOCAL CELLULAR CHANGE	1 (2%)		
ANGIECTASIS		2 (4%)	
#LIVER/CENTRILOBULAR	(48)	(50)	(48)
CONGESTION, PASSIVE	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
NECROSIS, FOCAL		1 (2%)	
#LIVER/KUPFFER CELL	(48)	(50)	(48)
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREAS	(47)	(50)	(46)
DILATATION/DUCTS	2 (4%)	1 (2%)	
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		
#PANCREATIC ACINUS	(47)	(50)	(46)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
ATROPHY, NOS	1 (2%)		
ATROPHY, FOCAL	1 (2%)	1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(50)	(48)
ECTOPIA		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
NEPHROSIS, NOS	1 (2%)	5 (10%)	
GLOMERULOSCLEROSIS, NOS	9 (18%)	2 (4%)	6 (13%)
NECROSIS, FOCAL			1 (2%)
PIGMENTATION, NOS	1 (2%)		
#KIDNEY/CORTEX	(49)	(50)	(48)
HYPERPLASIA, FOCAL		2 (4%)	
#KIDNEY/TUBULE	(49)	(50)	(48)
MINERALIZATION	1 (2%)		
DILATATION, NOS	1 (2%)		
CYST, NOS		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
REGENERATION, NOS	13 (27%)	9 (18%)	13 (27%)
ENDOCRINE SYSTEM			
#PITUITARY	(46)	(43)	(35)
HYPERPLASIA, CHROMOPHOBE-CELL	3 (7%)	1 (2%)	3 (9%)
#ADRENAL CORTEX	(45)	(49)	(45)
DEGENERATION, HYALINE	1 (2%)		
FOCAL CELLULAR CHANGE			1 (2%)
#ZONA GLOMERULOSA	(45)	(49)	(45)
HYPERPLASIA, FOCAL	1 (2%)		

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
#ZONA RETICULARIS	(45)	(49)	(45)
CYTOPLASMIC VACUOLIZATION		1 (2%)	
#THYROID	(49)	(48)	(46)
FOLLICULAR CYST, NOS	1 (2%)		1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(49)
DILATATION/DUCTS	3 (6%)		2 (4%)
HYPERPLASIA, FOCAL		1 (2%)	
*PREPUTIAL GLAND	(50)	(50)	(49)
CYST, NOS		1 (2%)	
#UTERUS	(48)	(48)	(46)
PYOMETRA	1 (2%)		
#UTERUS/ENDOMETRIUM	(48)	(48)	(46)
HYPERPLASIA, CYSTIC	36 (75%)	35 (73%)	41 (89%)
#ENDOMETRIAL STROMA	(48)	(48)	(46)
ANGIECTASIS		1 (2%)	
#OVARY	(41)	(46)	(41)
CYST, NOS	3 (7%)	4 (9%)	10 (24%)
CORPUS LUTEUM CYST		1 (2%)	
MULTILOCULAR CYST	1 (2%)		
PAROVARIAN CYST		1 (2%)	
HEMORRHAGIC CYST	4 (10%)	3 (7%)	
HEMATOIDIN		1 (2%)	
HEMOSIDEROSIS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(48)	(50)	(46)
PERIVASCULAR CUFFING	1 (2%)		
#CEREBRUM	(48)	(50)	(46)
MINERALIZATION	17 (35%)	29 (58%)	21 (46%)
CORPORA AMYLACEA			1 (2%)
#BRAIN	(48)	(50)	(46)
MINERALIZATION		2 (4%)	

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

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	CONTROL	LOW DOSE	HIGH DOSE
PERIVASCULAR CUFFING		1 (2%)	1 (2%)
#HIPPOCAMPUS	(48)	(50)	(46)
DEGENERATION, NOS		1 (2%)	
#CEREBELLUM	(48)	(50)	(46)
HEMORRHAGE	1 (2%)		
NECROSIS, FOCAL	1 (2%)		
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(50)	(50)	(49)
HYPERPLASIA, CYSTIC	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(50)	(50)	(49)
GRANULOMA, NOS	1 (2%)		
INFLAMMATION, FOCAL GRANULOMATOU		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
AUTO/NECROPSY/HISTO PERF			1
AUTO/NECROPSY/NO HISTO			1
AUTOLYSIS/NO NECROPSY			1

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

APPENDIX E

Analysis of FD & C Yellow No. 6
(Lot No. Z7860)
Midwest Research Institute

Appendix E

Analysis of FD & C Yellow No. 6
Lot No. Z7860

A. ELEMENTAL ANALYSIS

Element	C	H	N	Na	S	Cl
Theory	42.48	2.23	6.19	10.17	14.18	0
Theory(a)	39.04	2.65	5.77	10.56	13.22	1.68
Determined	38.90	2.61	5.56	10.7±0.1(δ)	12.99	1.68±0.91(δ)
	39.03	2.63	5.70		13.07	

(a) Theory (91.9% compound; 5.05% water; 2.77% sodium chloride).

B. WATER ANALYSIS

(Karl Fisher) 5.05%±0.06δ%

C. TITRATION WITH TITANOUS CHLORIDE

91.9%±0.7(δ)% Reducible functions (relative to theory)

D. MELTING POINT

Determined	Literature Values
Decomposes without melting; decomposition begins at 390°C (visual, capillary)	No literature value found.

E. THIN-LAYER CHROMATOGRAPHY

Plates: Silica Gel 60 F254
Amount Spotted: 100 and 300 μg
Ref. Standard: Methyl red
Visualization: Visual and ultraviolet (254 and 366 nm)

System 1: n-Butanol:ethanol:water:ammonium hydroxide (40:30:20:10)
R_f: 0.49 (major) 0.44 (minor)
R_{st}: 0.73, 0.66

System 2: Ethyl acetate:isopropanol:water:concentrated ammonium hydroxide (35:35:10:20)
R_f: 0.65 (slight trace), 0.60 (slight trace, 254 nm only), 0.42 (major), 0.30 (trace), 0.22 (minor)
R_{st}: 1.08, 0.98, 0.69, 0.49, 0.36

F. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Instrument: Waters ALC 202 with Model 660 solvent programmer
Detector: Ultraviolet, 254 nm
Column: Partisil 10-strong anion exchange, 250 x 4 mm I.D.
Solvent Program: 0% to 30% B
A: 0.01M $Na_2B_4O_7$ in H_2O
B: 0.01M $Na_2B_4O_7$ and 0.5M $NaClO_4$ in H_2O
Program No.: 6 (linear)
Program Time: 15 min
Flow Rate: 1.5 ml/min
Results: Major peak and three minor impurities

Peak	Retention Time (min)	Retention Time (Relative to FD & C Yellow No. 6)	Area (Relative to FD & C Yellow No. 6)
1	11.0	0.51	0.3
2	16.0	0.74	0.2
3	21.6	1.0	100
4	23.8	1.1	3.2

G. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12
Cell: 1.5% potassium bromide pellet
Results: See Figure 5

Identical to literature spectrum (Sadtler Standard Spectra)

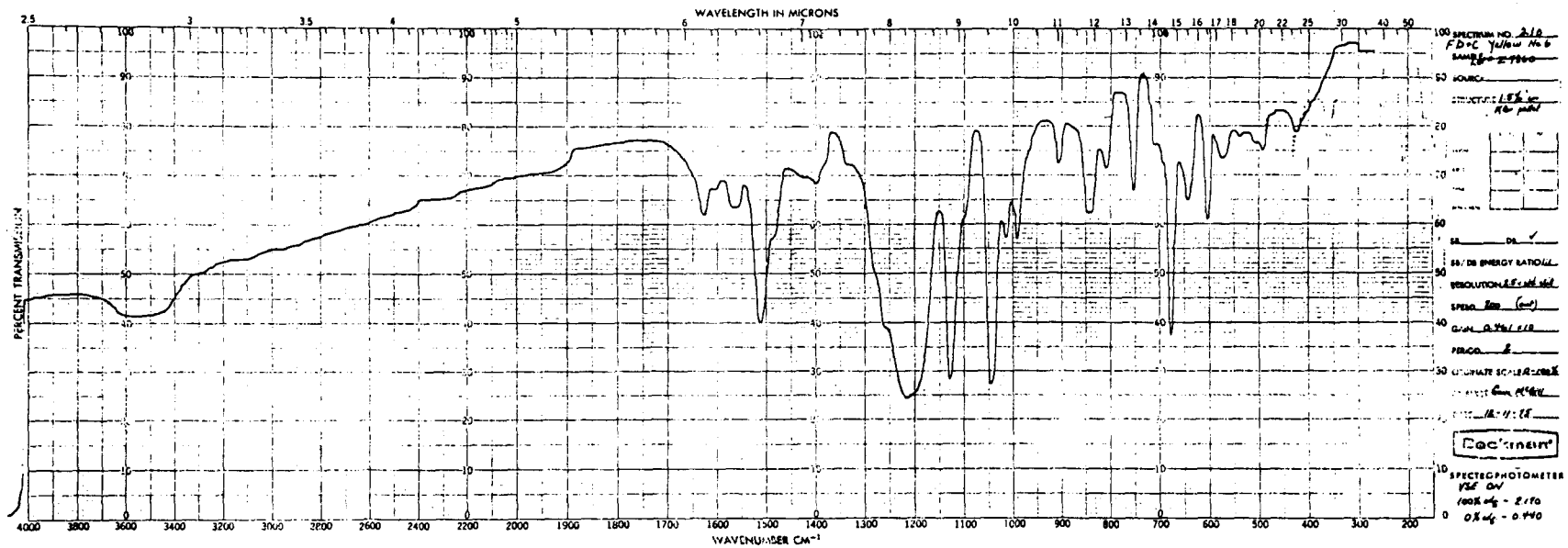

Figure 5. Infrared Absorption Spectrum of FD&C Yellow No. 6 (Lot No. Z7860)

(2) Ultraviolet/visible

λmax (nm)	ε x 10^3
500 shoulder	21.2±0.3(δ)
482	22.7±0.3(δ)
410 shoulder	8.9±0.2(δ)
314	11.04±0.09(δ)
261	14.2±0.1(δ)
254 shoulder	15.4±0.1(δ)
239 shoulder	31.3±0.3(δ)
234	33.0±0.3(δ)

Solvent: Water

λmax (nm)	ε x 10^3
500 shoulder	22.4
482	24.5
410 shoulder	9.9

Solvent: 0.02N $NH_4C_2H_3O_2$ (Stein, 1949)

λmax (nm)
481
310
228

No ε values given

Solvent: 0.02N $NH_4C_2H_3O_2$ (Gautier and Kiger, 1963)

(3) Nuclear Magnetic Resonance

Instrument: Varian HA-100
Solvent: DMSO-d_6 and D_2O (1:1) (v:v) with internal TSP
Assignments:
a = 6.54 ppm $J_{a,c}$ = 10 Hz f = 7.91 ppm
b = 7.47 ppm $J_{b,e}$ = 9 Hz g = 8.17 ppm
c = 7.60 ppm
d,e = 7.88 ppm $J_{d,g}$ = 9 Hz
Integration ratios:
a = 1.18 d,e = 2.88
b = 2.07 f = 1.18
c = 0.96 g = 0.96
(Figure 6)

Identical to literature spectrum (Marmion, 1974)

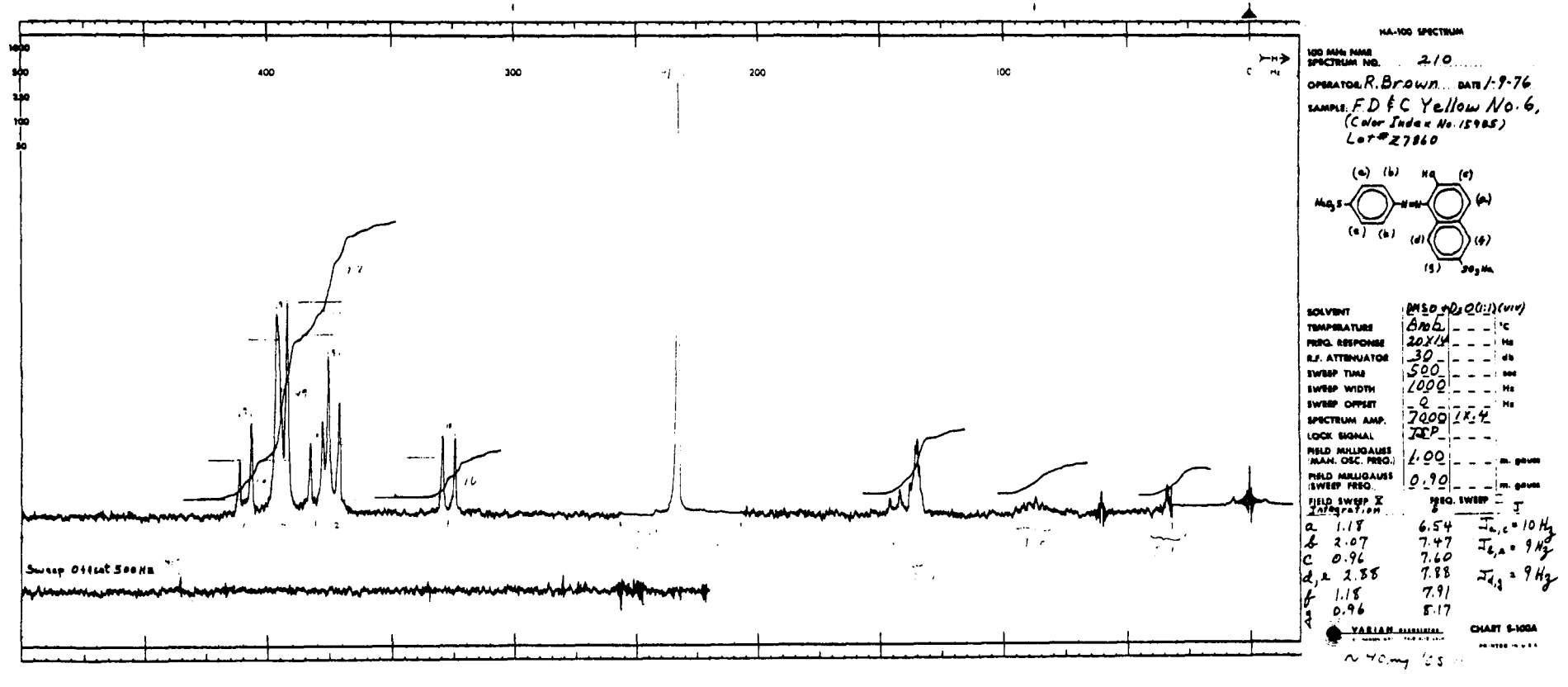

Figure 6. Nuclear Magnetic Resonance of FD&C Yellow No. 6 (Lot No. Z7860)

APPENDIX F

Analysis of FD & C Yellow No. 6
(Lot No. AA3448)
Midwest Research Institute

Appendix F

Analysis of FD & C Yellow No. 6
Lot No. AA3448

A. ELEMENTAL ANALYSIS

Element	C	H	N	Na	S	Cl
Theory	42.48	2.23	6.19	10.17	14.18	0
(a)	38.99	2.65	5.68	10.62	13.01	1.99
Determined	37.36 37.44	2.97 2.94	5.40 5.37	10.14 10.20	12.52 12.46	1.99±0.77(δ)

(a) Based on 91.8% compound; 5.44% water; 3.28% sodium chloride

B. WATER ANALYSIS

(Karl Fisher)

5.44%±0.06(δ)%

C. TITRATION WITH TITANOUS CHLORIDE

91.8%±1.0(δ)%

D. MELTING POINT

Determined	Literature Values
Decomposes without melting; decomposition begins at 390°C (visual, capillary)	No literature value found.

E. THIN LAYER CHROMATOGRAPHY

Plates: Sil G-25; UV_{254}
Amount Spotted: 100 and 300 μg (10.0 mg/ml in CH_3OH)
Ref. Standard: methyl red
Visualization: Visual and ultraviolet (254 and 366 nm)

System 1: n-Butanol:ethanol: water:concentrated ammonium hydroxide (40:30:20:10)

R_f: 0 82 (trace), 0.66 (trace, 366 nm only), 0.62 (trace), 0.52 (major)

R_{st}: 1,23,0.99,0.93,0.78

System 2: Ethyl acetate: isopropanol:water: concentrated ammonium hydroxide (35:35:10:20)

R_f: 0.68 (trace), 0.48 (trace, 366 nm only), 0.32 (major), 0.24 (trace), 0.14 (trace)

R_{st}: 1.21, 0.97, 0.87, 0.57, 0.43, 0.25

F. HIGH-PRESSURE LIQUID CHROMATOGRAPHY

Instrument: Waters ALC 202 with Model 660 solvent programmer
Detector: Ultraviolet, 254 nm
Column: μBondapak C_{18}
Solvent: 35% B, isocratic
A: 0.005M TBA (tetrabutyl ammonium hydroxide) in H_2O with H_3PO_4 to adjust pH to 7.4
B: 0.005M TBA (tetrabutyl ammonium hydroxide) in CH_3OH (Fisher HPLC) with equivalent amount of H_3PO_4 added as for solvent A.
Flow Rate: 1 ml/min
Results: Major peak and two impurities

Peak	Retention Time (min)	Retention Time (Relative to FD & C Yellow No. 6)	Area (Relative to FD & C Yellow No. 6)
1	2.0 (on solvent front)	0.30	Trace- Not calculable
2	9.8	1.0	100
3	14.2	1.45	0.34

G. SPECTRAL DATA

(1) Infrared

Instrument: Beckman IR-12

Consistent with literature spectrum (Sadtler Standard Spectra)

Cell: 0.7% potassium bromide pellet

See Figure 7.

(2) Ultraviolet/visible

λmax (nm)	ε x 10^{-3}
502 (shoulder)	14.5±0.4(δ)
483	15.6±0.4(δ)
396 (shoulder)	5.6±0.1(δ)
314.5	7.7±0.2(δ)
305 (shoulder)	7.2±0.3(δ)
262	10.1±0.3(δ)
255 (shoulder)	10.9±0.3(δ)
240 (shoulder)	21.7±0.6(δ)
234.5	23.0±0.6(δ)

Solvent: 0.02N $NH_4C_2H_3O_2$

λmax (nm)	ε x 10^{-3} (Values calculated from graphical data.)
500 (shoulder)	22.3
482	24.6
410 (shoulder)	9.9

Solvent: 0.02N $NH_4C_2H_3O_2$ (Stein, 1949)

λmax (nm)	No ε values given
481	
310	
228	

Solvent: 0.02N $NH_4C_2H_3O_2$ (Gautier and Kiger, 1963)

(3) Nuclear magnetic resonance

Determined

Instrument: Varian EM-360A

Solvent: DMSO-d_6: D_2O (1:1) (v:v) with TSP as internal standard

Assignments: (Figure 8)

(a) d, δ 6.53 ppm, J_{a-c} = 9 Hz
(b) d, δ 7.47 ppm, J_{b-e} = 9 Hz
(c) d, δ 7.62 ppm
(d,e) d, δ 7.78 ppm J_{d-g} = 9 Hz
(f) m, δ 7.80 ppm
(g) d, δ 8.20 ppm
(h) s, δ 3.26 ppm (impurity)

Integration ratios:

(a) 1.03
(b))
(c))
(d,e) } 7.02
(f))
(g) 0.95
(h) 0.08 (impurity)

Literature Values

Consistent with literature spectrum (Marmion, 1974), except for some differences attributed to elevated probe temperature and concentration in the literature spectrum.

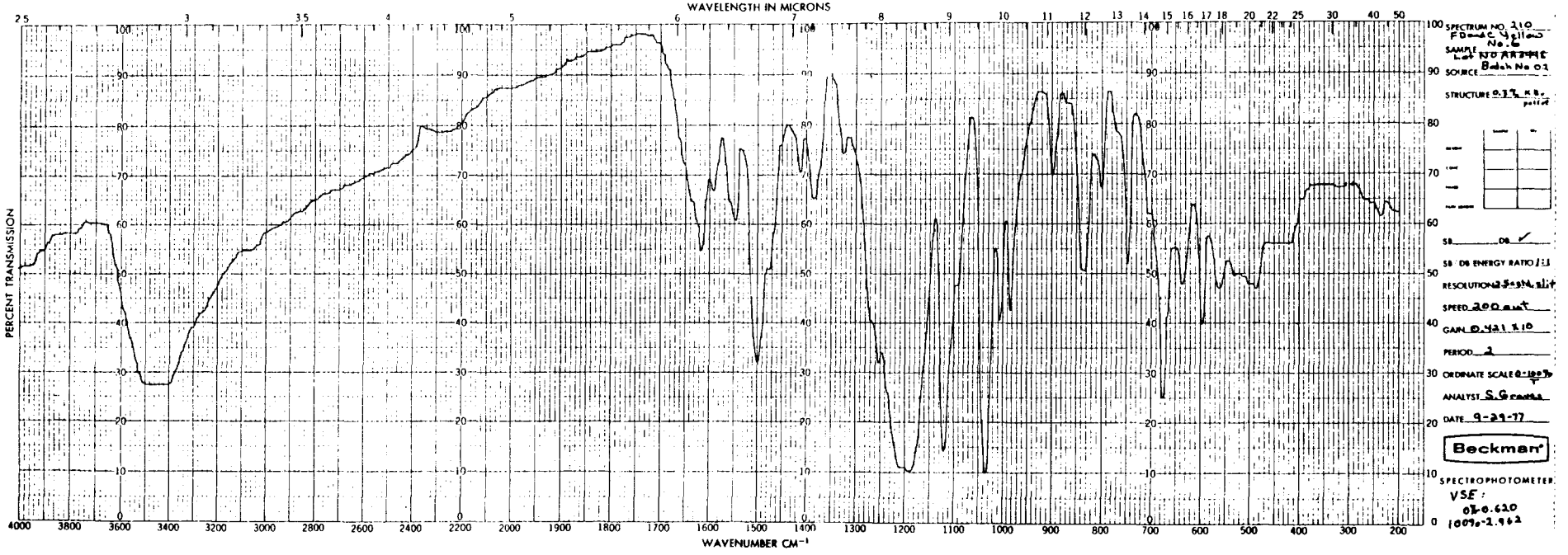

Figure 7. Infrared Absorption Spectrum of FD&C Yellow No. 6 (Lot No. AA3448)

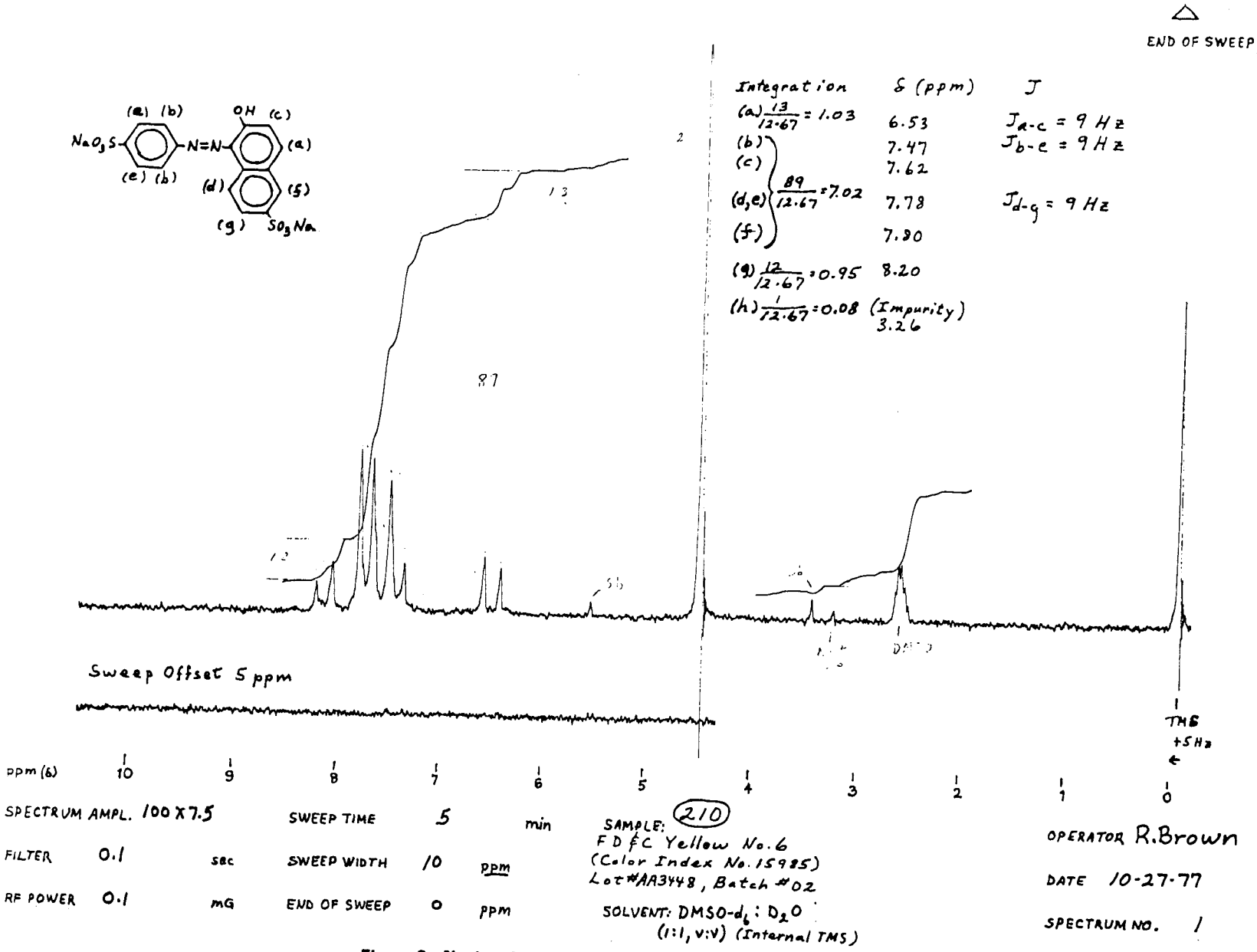

Figure 8. Nuclear Magnetic Resonance of FD&C Yellow No. 6 (Lot No. AA3448)

APPENDIX G

Analysis of Formulated Diets for Concentrations of FD & C Yellow No. 6

Appendix G

Analyses of Formulated Diets for Concentrations of FD & C Yellow No. 6

A 100-mg sample of the dye-feed mixture was mixed with 40 ml of distilled water and vortexed for 30 seconds. The suspension was centrifuged for 10 minutes at 10,000 rpm in a Sorvall RC-2B at 4°C. An appropriate volume of the supernatant was removed and diluted with distilled water to achieve a final concentration in the linear portion of the standard curve. Internal standards were prepared using control powdered feed and were assayed in the same manner. All samples and standards were run in triplicate. The absorbance was determined at 482 nm in a Gilford 2400-S spectrophotometer. The spectrophotometer was blanked with a 100-mg feed sample treated in the same manner as the samples. The standard curve developed with feed-dye standards (triplicate) automatically incorporates a correction for recovery. The concentration of dye in a feed sample can be read directly from the curve without any further adjustment for recovery.

Theoretical Dietary Level (ppm)	No. of Samples	Sample Analytical Mean (ppm)	Coefficient of Variation (%)	Range (ppm)
12,500	30	12,810	3.2	12,224-13,650
25,000	30	25,546	3.5	23,900-26,900

APPENDIX H

Analysis of FD & C Yellow No. 6 for Stability in Feed

Appendix H

Analysis of FD & C Yellow No. 6
for Stability in Feed

1. Mixing and storage: FD & C Yellow No. 6 (20 g) and Wayne Lab-Blox® Rodent Feed (180 g) were mixed in a mortar. Samples of the mixture were then removed and stored for 2 weeks at -20°, 5°, 25°, and 45°C, respectively. These samples were analyzed by the differential pulsed polarographic method.

2. Extraction and analysis: One-gram samples of each of the above stability mixtures were triturated twice with 50-ml portions of Fisher pH 10.4 buffer solution (THAM®). Ten milliliters of this resulting solution (made up exactly to 100 ml) was further diluted to 100 ml with buffer in a volumetric flask, and this constituted the test solution.

Instrument: Model 174 polarographic analyzer, Princeton Applied Research Corporation
Potential Scan: 2 mV/sec, range 1.5 V
Initial Potential: -0.35 mV
Modulation Amplitude: 25 mV
Current Range: 0.02 mA
Drop Time: 2 sec
Electrodes:
Working, dropping mercury
References, standard calomel
Counter, carbon

3. Results

Sample (°C)	Average% Compound Recovered(a)
-20	10.1±0.5
5	10.0±0.5
25	9.5±0.5
45	9.3±0.5

(a) Corrected for a spiked recovery value of 7.89%. Theoretical, 10.0%.

There is no significant difference between the samples stored at the various temperatures.

4. Conclusion: FD & C Yellow No. 6 mixed with feed is stable for 2 weeks at temperatures of up to 45°C.

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