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BIOASSAY OF

DIARYLANILIDE YELLOW

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
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REPORT ON THE BIOASSAY OF DIARYLANILIDE YELLOW
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS PROGRAM, DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

CONTRIBUTORS: This report presents the results of the bioassay of diarylanilide yellow conducted for the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This bioassay was conducted by Mason Research Institute, Worcester, Massachusetts, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc. (1), prime contractor for the NCI Carcinogenesis Bioassay Program.

The experimental design was determined by the NCI Project Officers, Dr. J. H. Weisburger (2,3) and Dr. E. K. Weisburger (2). The principal investigators for the contract were Dr. E. Smith (4) and Dr. A. Handler (4). Animal treatment and observation were supervised by Mr. G. Wade (4) and Ms. E. Zepp (4). Chemical analysis was performed by Midwest Research Institute (5).

Histopathology was performed by Dr. R. W. Fleischman (4) at the Mason Research Institute, and the diagnoses included in this report represent the interpretation of this pathologist.

Compilation of individual animal survival, pathology, and summary tables was performed by EG&G Mason Research Institute (6); the statistical analysis was performed by Dr. A. Chu (6) and Mr. W. W. Belew (7), using methods selected for the Bioassay Program by Dr. J. J. Gart (8).

This report was prepared at METREK, a Division of The MITRE Corporation (7) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (7), the task leader, Dr. M. R. Kornreich (7), and the senior biologist, Ms. P. Walker (7). The final report was reviewed by members of the participating organizations.

The statistical analysis was reviewed by a member or members of the Mathematical Statistics and Applied Mathematics Section of the NCI: Dr. J. J. Gart (8), Mr. J. Lam (8), Dr. H. M. Pettigrew (8), and Dr. R. E. Tarone (8).

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (2), Dr. C. Cueto, Jr. (2), Dr. J. F. Douglas (2), Dr. D. G. Goodman (2), Dr. R. A. Griesemer (2), Dr. T. W. Orme (2), Dr. R. A. Squire (9), and Dr. J. M. Ward (2).

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1. Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

2. Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

3. Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammon House Road, Valhalla, New York.

4. Mason Research Institute, 57 Union Street, Worcester, Massachusetts.

5. Midwest Research Institute, 425 Volker Boulevard, Kansas City, Montana.

6. EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

7. The MITRE Corporation, METREK Division, 1820 Dolley Madison Boulevard, McLean, Virginia.

8. Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

9. Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of technical-grade diarylanilide yellow for possible carcinogenicity was conducted using Fischer 344 rats and B6C3F1 mice. Diarylanilide yellow was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. The high and low dietary concentrations used in the chronic study for the male and female rats and mice were 5.0 and 2.5 percent, respectively, of the chemical in the feed. After a 78-week treatment period, observation of the rats continued for an additional 28 weeks and observation of the mice continued for an additional 19 weeks for high dose males and low and high dose females and 18 weeks for low dose males. For each species, 50 animals of each sex were placed on test as controls, and fed only the basal diet.

The high concentration administered to both species in this study was the maximum recommended in the Guidelines for Carcinogen Bioassay in Small Rodents (Sontag et al., 1976). These guidelines indicate that a chronic dietary level of 5 percent, or 50,000 ppm, should not be exceeded even when no signs of toxicity are observed during subchronic testing, except under special circumstances (e.g., when the compound is a major component of the human diet). No toxic effects were reported during subchronic testing and diarylanilide yellow did not qualify for exception; therefore, the highest permissible concentration (5 percent) was utilized in the chronic bioassay.

The dietary concentrations of diarylanilide yellow administered during the chronic bioassay had no significant effect on survival or body weight gain in either species. Except for yellow staining and some isolated neoplasms, the only adverse clinical sign or pathologic lesion observed in treated rats or mice was basophilic cytoplasm changes in hepatocytes of treated rats.

In both species the survival in all groups was adequate for statistical analysis of late-appearing tumors.

No treatment-related increase in the incidence of neoplasms or nonneoplastic lesions was evident in treated rats or mice. A few unusual findings were observed in both species, including single cases of metastatic chordoma and osteogenic sarcoma in rats, and single cases of squamous-cell carcinoma of the ear, infiltrating duct carcinoma of the mammary gland, and subcutaneous mastocytoma in mice.

The results of this study did not provide evidence for the carcinogenicity of diarylanilide yellow in Fischer 344 rats or B6C3F1 mice.

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I. INTRODUCTION

Diarylanilide yellow (NCI No. CO3269), one member of a family of organic azo pigments known as benzidine yellows, was selected for bioassay by the National Cancer Institute in an attempt to elucidate those dyes and dye intermediates which may be responsible for the increased incidence of bladder cancer observed among workers in the dye manufacturing industry (Wynder et al., 1963; Anthony et al., 1970). The structural relationship of this compound to the documented carcinogen 3,3'-dichlorobenzidine (Occupational Safety and Health Administration, 1973) was also a factor in its selection.

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 2,2'-[(3,3'-dichloro(1,1'-biphenyl)-4,4'-diyl)-bis (azo)] bis (3-oxo-N-phenyl)-butanamide.* It is also called Color Index (C.I.) Pigment Yellow 12 (C.I. 21090), diarylide yellow, and dichlorobenzidine coupled into acetoacetanilide. Diaryl-anilide yellow is an ingredient in industrial paints, most notably the paint applied to lead pencils (Weisburger, 1976). It is also an ingredient in printing inks and may sometimes be used to color plastics, rubber, linoleum, floor tiles, textiles, and wallpaper (Society of Dyers and Colourists, 1971; Hawley, 1971). According to the U.S. International Trade Commission (1977a), 6.028×10^6 pounds

*The CAS registry number is 6358-85-6.

of diarylanilide yellow were produced in the United States in 1975--the largest quantity of any single pigment produced in that year. U.S. imports of the pigment through principle U.S. customs districts amounted to 62,040 pounds in 1975 (U.S. International Trade Commission, 1977b).

The risk of exposure to diarylanilide yellow is greatest among workers in the dye manufacturing industry and at facilities where dyeing of textiles or production of inks, paints, and other commodities containing the pigment takes place. Additional occupational exposure may also occur among users of pigment-containing products (e.g., among printers, engravers, lithographers, textile workers, etc.).

Epidemiological studies suggest a relationship between occupational exposure to paints and increased incidences of cancer of the lung and bladder and between occupational exposure to printing inks and increased incidences of cancer of the liver and bladder (Hoover and Fraumeni, 1975). An increased incidence of bladder cancer has also been observed among textile workers and tailors (Anthony and Thomas, 1970).

Exposure of the general population to diarylanilide yellow is likely, due to the large variety of consumer products colored with this pigment. Chronic ingestion of the dye over long periods of time may result from habitual holding in the mouth or chewing of wooden pencils.

II. MATERIALS AND METHODS

A. Chemicals

Diarylanilide yellow was purchased from Chemtron Corporation and chemical analysis was performed by Midwest Research Institute. The melting point range (311° to 320°C) suggested the presence of impurities. Thin-layer chromatography was performed utilizing two different solvent systems (methylene chloride and 95:5 chloroform:diethylamine). Each plate was visualized with ultraviolet and visible light. One homogeneous spot was detected on each plate; however, the amounts of compound spotted on each plate (2.4 μg and 7.2 μg) were so low that only major impurities could have been detected by this technique. Infrared and mass spectrometry analyses were not inconsistent with the structure of the compound.

Throughout this report the term diarylanilide yellow is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both treated and control animals consisted of Wayne Lab-Blox® (Allied Mills, Inc.). Diarylanilide yellow was administered to the treated animals as a component of the diet. The chemical was mixed in the feed in a 6 kg capacity Patterson-Kelly standard model stainless steel twin-shell V-blender. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. Mixtures were prepared weekly and stored for not longer than 2 weeks.

C. Animals

Two animal species, rats and mice, were used in the carcinogenicity bioassay. The Fischer 344 rats and the B6C3F1 mice were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Animals of both species were supplied by Charles River Breeding Laboratories, Wilmington, Massachusetts. Treated animal groups of both species were received in separate shipments from their corresponding controls.

Upon arrival, a sample of animals was examined for parasites and other signs of disease. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals were assigned to groups and distributed among cages so that average body weight per cage was approximately equal for a given sex and species.

D. Animal Maintenance

All animals were housed by species in rooms having a temperature range of 23° to 34°C and a range in relative humidity of 10 to 85 percent. Incoming air was filtered through Tri-Dek® 15/40 denier Dacron® filters providing six changes of room air per hour. Fluorescent lighting was provided on a 12-hour-daily cycle.

Rats were housed five per cage by sex. During quarantine and for the first 6 weeks of study, they were kept in galvanized-steel wire-mesh cages suspended above newspapers. Newspapers were replaced daily, and cages and racks washed weekly. From week 6 rats were kept in suspended polycarbonate cages equipped with disposable nonwoven

filter sheets. Clean bedding and cages were provided twice weekly. Hardwood chips (Ab-sorb-dri®, Wilner Wood Products Co.) were used through the first 3 months of study, then corncob bedding (SAN-I-CEL®, Paxton Processing Co.) for the next 12 months, and then another type of corncob bedding (Bed-o'Cobs®, The Anderson's Cob Division) for the remainder of the bioassay. During the quarantine period Wayne Lab-Blox® was supplied in Alpine® aluminum feed cups (Curtin Matheson Scientific, Inc.) equipped with stainless steel baffles. The same feeding apparatus, containing treated Wayne Lab-Blox®, was utilized during the treatment period. The food assembly was replaced weekly. During the observation period following treatment, rats were supplied with pelleted Wayne Lab-Blox® on the cage floor.

Mice were housed by sex in polycarbonate cages. During quarantine and treatment periods, cages fitted with perforated stainless steel lids (Lab Products, Inc.) were used. During the observation period following treatment, stainless steel wire bar lids (Lab Products, Inc.) were used. Nonwoven fiber filter bonnets were used over cage lids. Treated mice were housed ten per cage for the first 12 months of the study and five per cage thereafter. Control mice were housed ten per cage for the first 13 months of study and five per cage thereafter. Cages, lids, filters, and bedding were provided three times per week when cage populations were ten and twice per week when cage populations were reduced to five. Bedding was of the same type as that used for rats. Reusable filter bonnets and pipe

racks were sanitized biweekly throughout the study. During the quarantine and test periods, Wayne Lab-Blox® was supplied in Alpine® aluminum feed cups equipped with stainless steel baffles. This food assembly was replaced weekly. During the observation period following treatment, mice were supplied with pelleted Wayne Lab-Blox® through a food hopper incorporated into the cage lid.

Water was available from 250 ml water bottles equipped with rubber stoppers and stainless steel sipper tubes. Bottles were replaced twice weekly. Food and water were available ad libitum to both rats and mice.

Treated and control rats used for this bioassay were housed with other rats treated with* fenaminosulf (140-56-7), 2,5-dithiobiurea (142-46-1), m-cresidine (102-50-1), and cupferron (135-20-6). After 6 weeks the diarylanilide yellow-treated rats were segregated from all other animals. The treated and control mice utilized in this bioassay were housed with other mice treated with 1-nitronaphthalene (86-57-7).

E. Selection of Initial Concentrations

In order to establish the maximum tolerated concentrations of diarylanilide yellow for administration to treated animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Rats were distributed among five groups and mice among six groups, each consisting of five males and five females.

*CAS registry numbers are given in parentheses.

Diarylanilide yellow was incorporated into the basal laboratory diet and fed ad libitum to four of the five rat groups in concentrations of 0.1, 0.3, 1.0, and 3.0 percent and to five of the six mouse groups in concentrations of 0.03, 0.1, 0.3, 1.0 and 3.0 percent. The remaining group of each species served as a control group, receiving only the basal laboratory diet. The dosed dietary preparations were administered for a period of 8 weeks.

A dosage inducing no mortality or body weight gain retardation in either sex was to be selected as the initial high dose in the chronic bioassay.

No decreases in food consumption or significant weight depression relative to controls were observed in any group. All animals survived until necropsy (week 8). Although the external surfaces of all animals at all concentrations were bright yellow, gross necropsy revealed no abnormalities or organ discoloration other than the mucosal surfaces of the intestinal tract, which appeared bright yellow due to direct contact with the test compound.

In the Guidelines for Carcinogen Bioassay in Small Rodents (Sontag et al., 1976) it is indicated that a chronic dietary concentration of 5 percent (50,000 ppm) should not be exceeded. This applies even if the compound causes no toxicity during subchronic testing. An exception can be made under special circumstances, e.g., if the chemical is a major component of the human diet. Because no toxic symptoms or gross abnormalities were observed clinically or at

necropsy in animals receiving the tested concentrations, 5.0 percent was selected as the concentration to be administered to the high dose groups of both species during the chronic bioassay.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

At initiation of the study animals of both species were approximately 7 weeks old. High and low dose animals of both species and sexes received concentrations of 5.0 and 2.5 percent, respectively, of the chemical in their food. Animals were treated for 78 weeks, followed by a 28-week observation period for rats and observation periods of 19 weeks for high dose male and low and high dose female mice and 18 weeks for low dose male mice, during which they received the basal laboratory diet. For both species, control animals were maintained and observed in the same manner as the treated animals.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. From the first day, all animals were inspected twice daily for mortality. Body weights were recorded twice weekly for the first 12 weeks of the study and at monthly intervals thereafter. Food consumption, for two cages from each group, was monitored for seven consecutive days once a month for the first nine months of

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
DIARYLANILIDE YELLOW FEEDING EXPERIMENT

	INITIAL GROUP SIZE	DIARYLANILIDE YELLOW CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	109
LOW DOSE	50	2.5 0	78	28
HIGH DOSE	50	5.0 0	78	28
FEMALE				
CONTROL	50	0	0	110
LOW DOSE	50	2.5 0	78	28
HIGH DOSE	50	5.0 0	78	28

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
DIARYLANILIDE YELLOW FEEDING EXPERIMENT

	INITIAL GROUP SIZE	DIARYLANILIDE YELLOW CONCENTRATION (PERCENT)	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	50	0	0	97
LOW DOSE	50	2.5 0	78	18
HIGH DOSE	50	5.0 0	78	19
FEMALE				
CONTROL	50	0	0	98
LOW DOSE	50	2.5 0	78	19
HIGH DOSE	50	5.0 0	78	19

the bioassay and for three consecutive days each month thereafter. The presence of tissue masses and lesions was determined by monthly observation and palpation of each animal.

A necropsy was performed on each animal regardless of whether it died, was killed when moribund, or was sacrificed at the end of the bioassay. The animals were euthanized by carbon dioxide inhalation, and were immediately necropsied. The histopathologic examination consisted of gross and microscopic examination of major tissues, organs, or gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice) and bile duct, pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, pancreatic islets, testis, prostate, brain, uterus, mammary gland, and ovary.

Tissues for which slides were prepared were preserved in 10 percent buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination. An occasional section was subjected to special staining techniques for more definitive diagnosis.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to

preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were placed on experiment in each group.

H. Data Recording and Statistical Analyses

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

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

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 used Tarone's (1975) extensions of Cox's methods for testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

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

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison

be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used. Under the assumption of a linear trend, this test determined 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 was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

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

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an

observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

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

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio

of the risk in a treated 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 (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

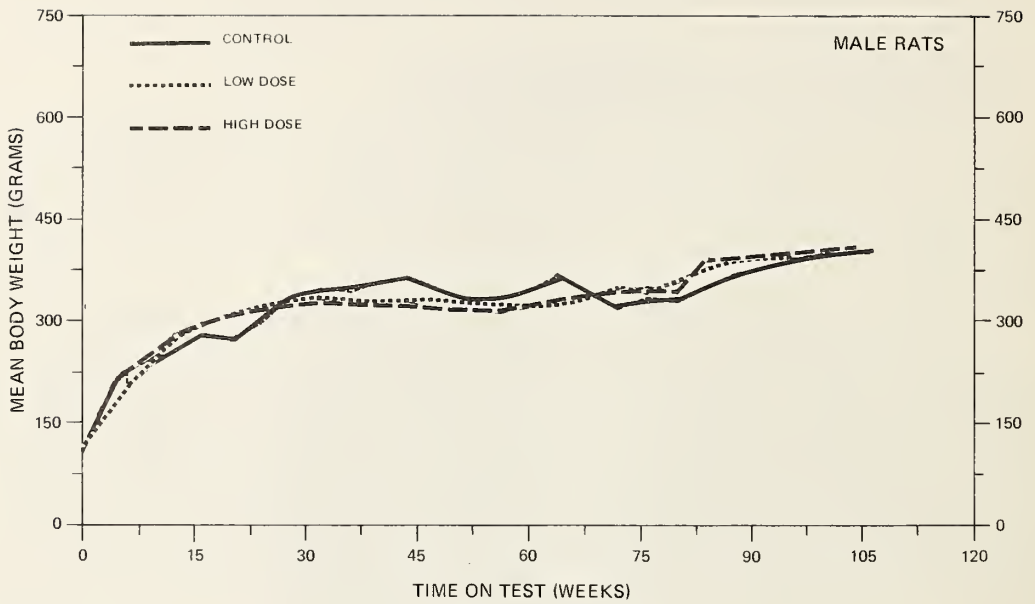
The body weight patterns for control and treated rat groups of both sexes were generally equivalent throughout the treatment period (Figure 1).

All the treated rats, both male and female, appeared bright yellow in color. In addition, the conjunctivas were faintly yellow as were most organs and internal mucosal surfaces. The only other clinical sign recorded for male or female rats was a hard crusted lesion on the back of one male control animal.

B. Survival

The estimated probabilities of survival for male and female rats in the control and diarylanilide yellow-treated groups are shown in Figure 2.

For both male and female rats the Tarone test detected no statistically significant positive association between dosage and mortality. In the males survival was quite high, as 74 percent of the high dose, 84 percent of the low dose, and 64 percent of the control rats survived until the end of the study, despite the sacrifice of five high dose and five control rats in week 78. In the females, 66 percent of the high dose, 80 percent of the low dose, and 72 percent of the control rats survived until the end of the study, despite the sacrifice of five high dose and five control rats in week 78.



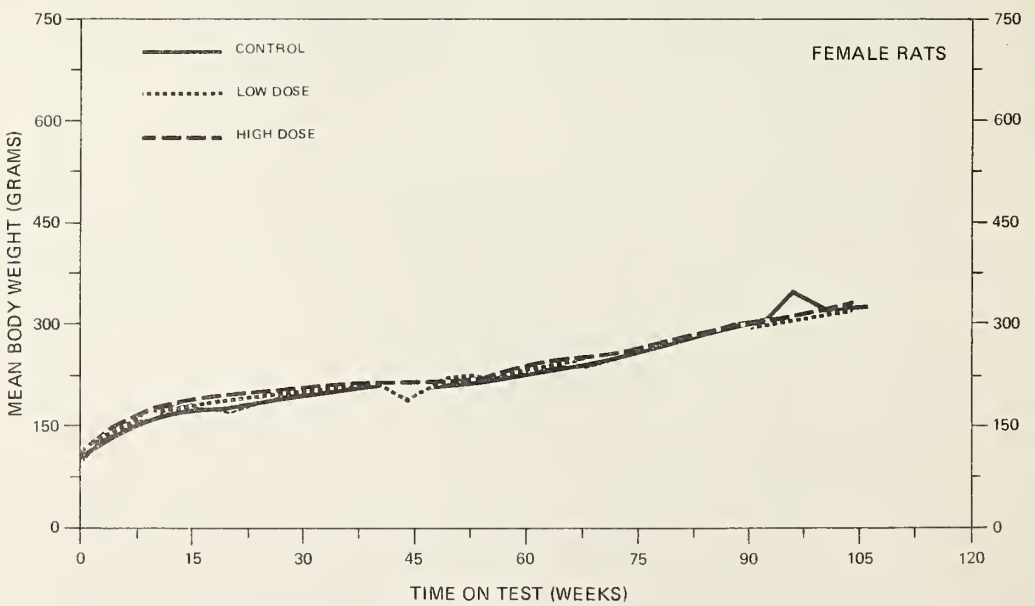
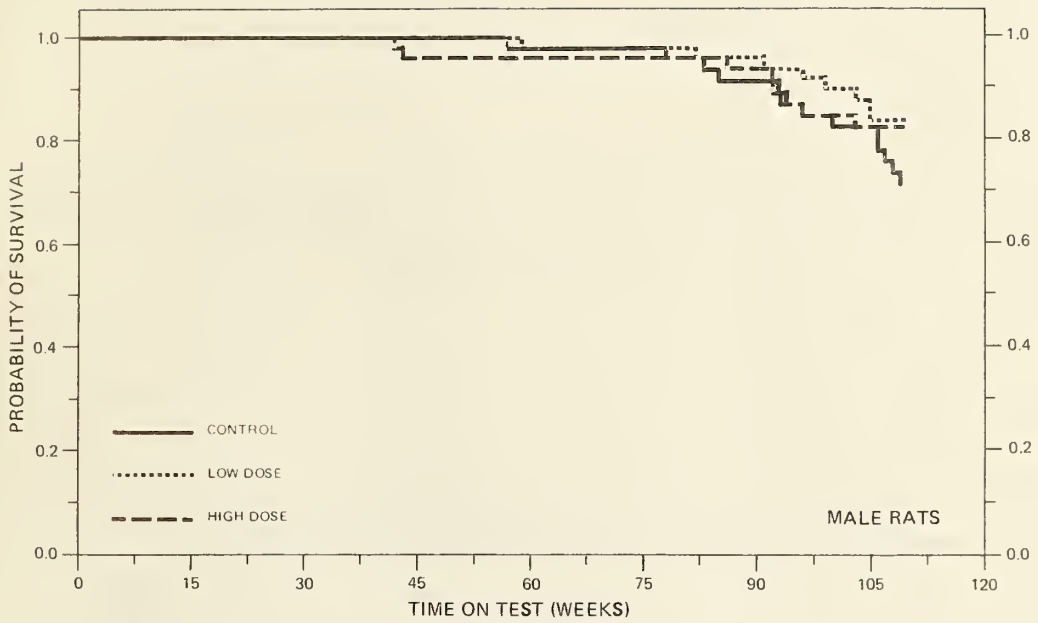


FIGURE 1
GROWTH CURVES FOR DIARYLANILIDE YELLOW CHRONIC STUDY RATS



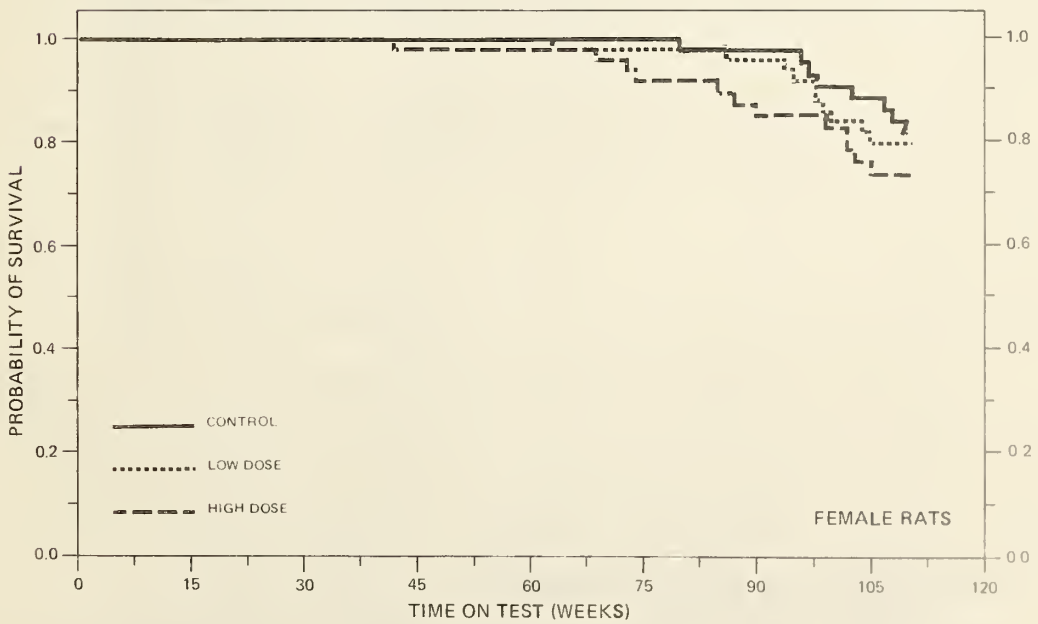


FIGURE 2
SURVIVAL COMPARISONS OF DIARYLANILIDE YELLOW CHRONIC STUDY RATS

In both sexes, survival was adequate for meaningful statistical analyses of tumor incidence.

C. Pathology

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

With a few exceptions, the same variety of neoplasms occurred sporadically and randomly in the chemically treated and control groups. No particular organ or system seemed to be the target of this chemical. Sporadic and unusual neoplasms that occurred in the treated but not in control animals were as follows: a metastatic chordoma of unknown origin occurred in the lung of 1/49 of the low dose males, and 1/49 of the low dose females had an osteogenic sarcoma.

The incidence and variety of nonneoplastic degenerative, proliferative, and inflammatory lesions were similar in the control and the chemically treated rats, except for treatment-related basophilic cytoplasm changes in hepatocytes of treated males and females.

The results of this histopathologic examination did not provide evidence for the carcinogenicity of diarylanilide yellow in Fischer 344 rats.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis for every type

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH DIARYLANILIDE YELLOW[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin: Fibroma or Basal-cell Carcinoma[b]	2/50(0.04)	4/50(0.08)	1/50(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	2.000	0.500
Lower Limit	---	0.301	0.009
Upper Limit	---	21.320	9.290
Weeks to First Observed Tumor	85	91	106
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	10/50(0.20)	2/50(0.04)	1/50(0.02)
P Values[c,d]	P = 0.001(N)	P = 0.014(N)	P = 0.004(N)
Relative Risk (Control)[e]	---	0.200	0.100
Lower Limit	---	0.022	0.002
Upper Limit	---	0.877	0.662
Weeks to First Observed Tumor	78	99	103
Pituitary: Adenoma[b]	7/45(0.16)	12/43(0.28)	5/45(0.11)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend	P = 0.041	---	---
Relative Risk (Control)[e]	---	1.794	0.714
Lower Limit	---	0.723	0.193
Upper Limit	---	4.856	2.414
Weeks to First Observed Tumor	78	106	106
Adrenal: Pheochromocytoma[b]	3/50(0.06)	3/47(0.06)	5/49(0.10)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.064	1.701
Lower Limit	---	0.149	0.351
Upper Limit	---	7.570	10.420
Weeks to First Observed Tumor	78	96	106

TABLE 3
(CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Thyroid: C-Cell Adenoma or Carcinoma[b]	3/37(0.08)	5/47(0.11)	1/48(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.312	0.257
Lower Limit	---	0.275	0.005
Upper Limit	---	7.994	3.055
Weeks to First Observed Tumor	109	96	106
Pancreatic Islets: Adenoma[b]	1/47(0.02)	2/47(0.04)	5/46(0.11)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	2.000	5.109
Lower Limit	---	0.108	0.603
Upper Limit	---	115.500	235.900
Weeks to First Observed Tumor	109	106	93
Testis: Interstitial-Cell Tumor[b]	42/50(0.84)	44/48(0.92)	39/49(0.80)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.091	0.948
Lower Limit	---	0.922	0.782
Upper Limit	---	1.240	1.161
Weeks to First Observed Tumor	78	96	78

[a]Dosed groups received concentrations of 2.5 and 5.0 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05; otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath each dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group to the control group when it is below 0.05, otherwise N.S. - not significant.

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

[e]Relative risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH DIARYLANILIDE YELLOW[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Hematopoietic System: Leukemia[b]	7/49(0.14)	4/49(0.08)	4/48(0.08)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.571	0.583
Lower Limit	---	0.130	0.133
Upper Limit	---	2.096	2.137
Weeks to First Observed Tumor	96	104	87
Pituitary: Adenoma[b]	17/39(0.44)	26/44(0.59)	14/42(0.33)
P Values[c,d]	N.S.	N.S.	N.S.
Departure from Linear Trend	P = 0.027	---	---
Relative Risk (Control)[e]	---	1.356	0.765
Lower Limit	---	0.852	0.411
Upper Limit	---	2.188	1.416
Weeks to First Observed Tumor	78	94	85
Adrenal: Pheochromocytoma[b]	3/49(0.06)	2/49(0.04)	1/47(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.667	0.348
Lower Limit	---	0.058	0.007
Upper Limit	---	5.564	4.144
Weeks to First Observed Tumor	110	106	74
Thyroid: C-Cell Carcinoma[b]	2/45(0.04)	2/42(0.05)	1/46(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	1.071	0.489
Lower Limit	---	0.081	0.009
Upper Limit	---	14.190	9.060
Weeks to First Observed Tumor	110	98	106

TABLE 4
(CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	12/49(0.24)	9/49(0.18)	10/48(0.21)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.750	0.851
Lower Limit	---	0.308	0.364
Upper Limit	1.757	1.757	1.938
Weeks to First Observed Tumor	103	86	73
Uterus: Endometrial Stromal Polyp[b]	6/46(0.13)	13/49(0.27)	7/47(0.15)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	2.034	1.142
Lower Limit	---	0.794	0.356
Upper Limit	---	5.984	3.807
Weeks to First Observed Tumor	78	94	78

[a]Dosed groups received concentrations of 2.5 and 5.0 percent in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]Beneath the incidence of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05; otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath each dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group to the control group when it is below 0.05, otherwise N.S. - not significant.

[d]A negative trend (N) indicates a lower incidence in a treated group than in the control group.

[e]Relative risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

of tumor that was observed in more than 5 percent of any of the diarylanilide yellow-dosed groups of either sex is included.

None of the statistical tests for rats of either sex indicated a significant positive association between dosage and tumor incidence.

To provide additional insight, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of a significantly increased rate of tumor incidence induced in rats by diarylanilide yellow that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

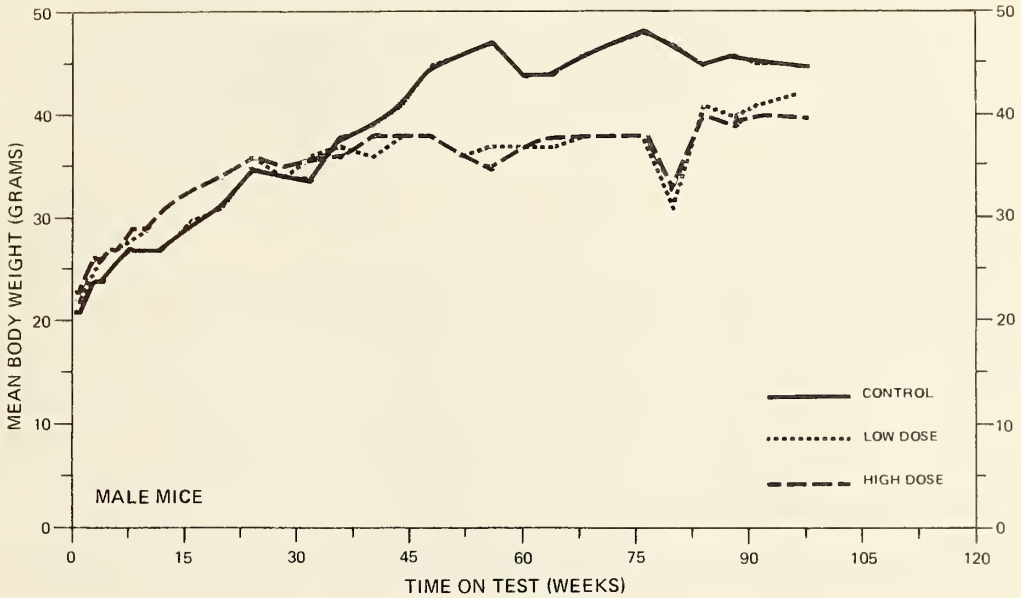
No differences between body weight gain patterns of high dose groups and low dose groups were evident in male or female mice during the 78-week treatment period (Figure 3). The control animals for both sexes began to experience marked weight gain beginning in week 36 when compared to the treated mice.

All the treated mice, both male and female, acquired a yellow discoloration of the hair coat during treatment. Because of the normal darker color of the B6C3F1 mice, the external appearance of the mice was not as strikingly affected as that of the rats, which are normally white. However, internal discoloration was as apparent in the mice as it was in the rats.

B. Survival

The estimated probabilities of survival for male and female mice in the control and diarylanilide yellow-treated groups are shown in Figure 4.

For both male and female mice the Tarone test did not detect a statistically significant positive association between dosage and mortality. In the male groups, 74 percent of the high dose, 88 percent of the low dose, and 84 percent of the control mice survived until the end of the study, despite the sacrifice of five high dose mice in week 78 and five control mice in week 79. In the female groups 68 percent of the high dose, 86 percent of the low dose, and



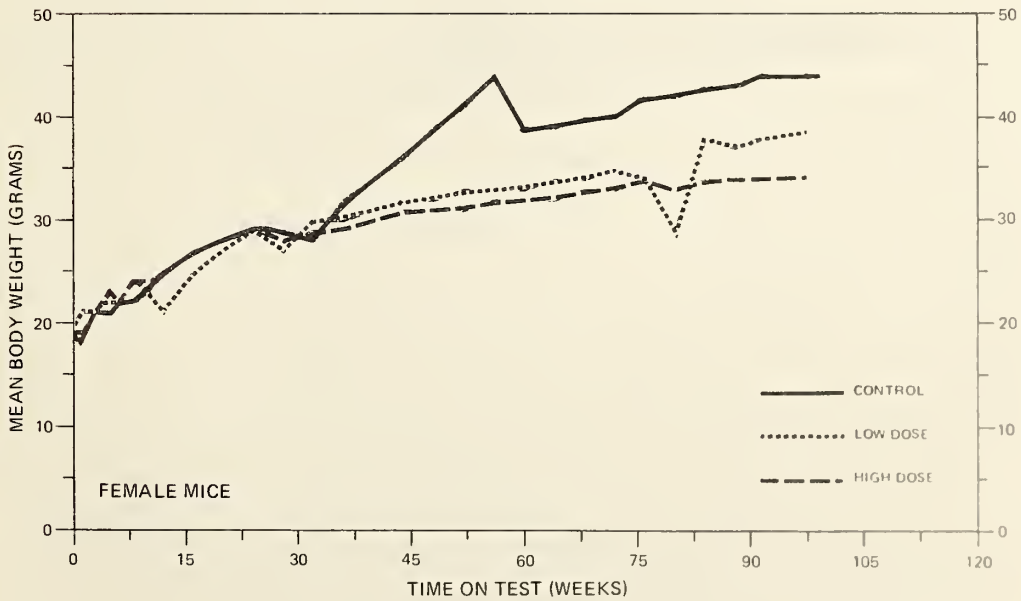
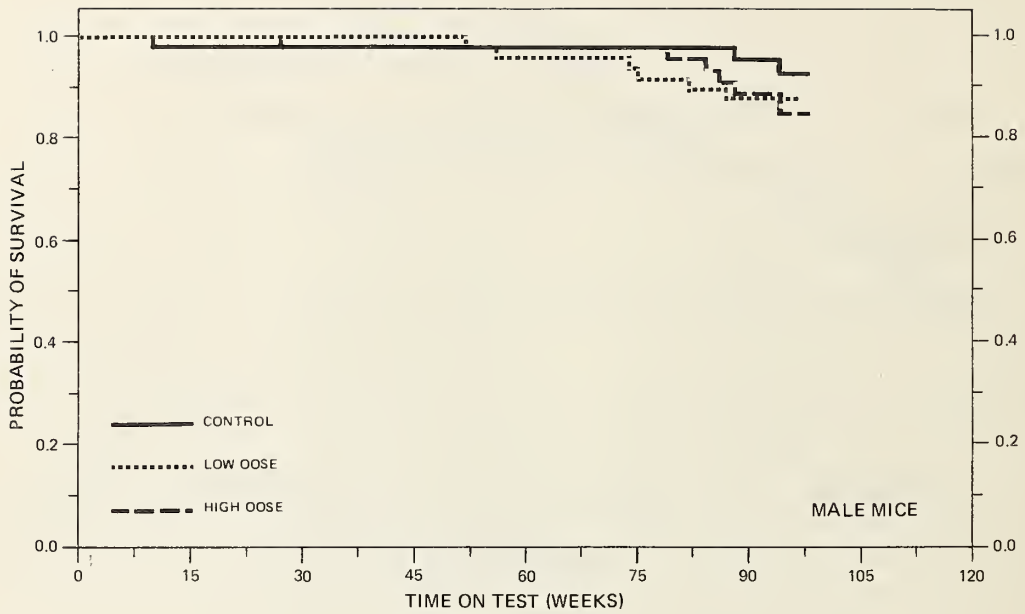


FIGURE 3
GROWTH CURVES FOR DIARYLANILIDE YELLOW CHRONIC STUDY MICE



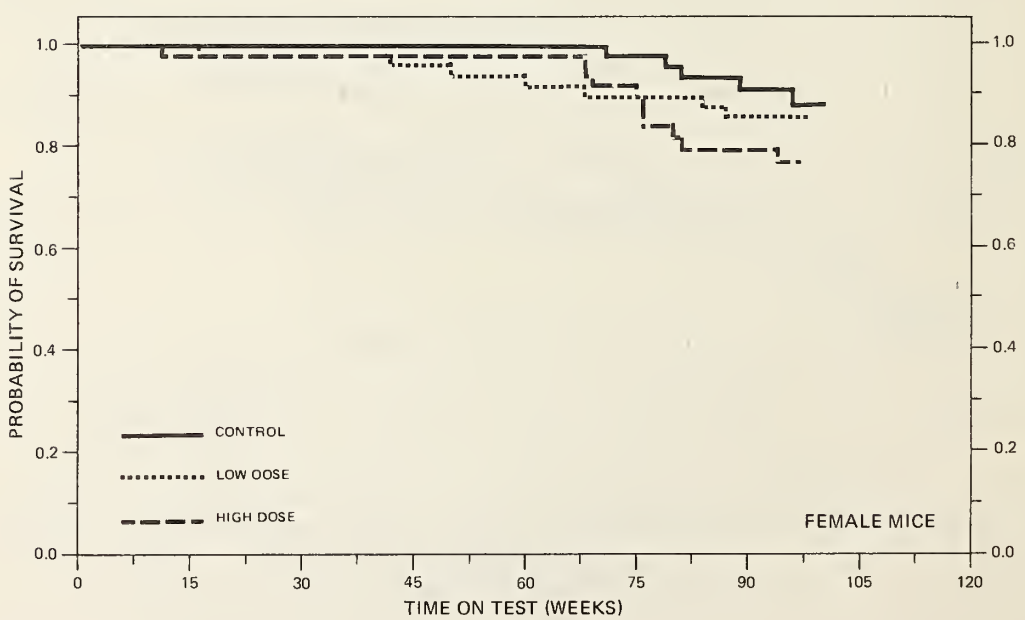


FIGURE 4
SURVIVAL COMPARISONS OF DIARYLANILIDE YELLOW CHRONIC STUDY MICE

80 percent of the control group survived until the end of the study, despite the sacrifice of five high dose mice in week 78 and five control mice in week 79.

In both sexes survival was adequate for meaningful statistical analyses of tumor incidence.

C. Pathology

Histopathologic findings on neoplasms in mice are tabulated in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are tabulated in Appendix D (Tables D1 and D2).

There appeared to be no dose- or sex-related increase in the incidence of neoplasms or toxic changes in the treated versus the control groups.

With a few exceptions, the same variety of neoplasms occurred sporadically and at random in the chemically treated and control groups. No particular organ or system seemed to be the target of this chemical. Sporadic and unusual problems that occurred in the treated but not in control animals were as follows: in the integumentary system, one mastocytoma affected the subcutaneous tissue of a high dose female; one squamous-cell carcinoma of the ear affected a low dose male; and one infiltrating duct carcinoma of the mammary gland affected one low dose female.

The incidence and variety of nonneoplastic degenerative, proliferative, and inflammatory lesions were similar in control and chemically treated mice.

The results of this histopathologic examination did not provide evidence for the carcinogenicity of diarylanilide yellow in B6C3F1 mice.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis for every type of tumor that was observed in more than 5 percent of any of the diarylanilide yellow-dosed groups of either sex is included.

None of the statistical tests for mice of either sex indicated a significant positive association between the administration of diarylanilide yellow and an increased tumor incidence in B6C3F1 mice.

To provide additional insight, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 5 and 6, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of a significantly increased rate of tumor incidence induced in mice by diarylanilide yellow that could not be established under the conditions of this test.

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH DIARYLANILIDE YELLOW[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	7/47(0.15)	5/49(0.10)	4/49(0.08)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.685	0.548
Lower Limit	---	0.184	0.125
Upper Limit	---	2.329	2.008
Weeks to First Observed Tumor	97	96	78
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	1/50(0.02)	3/49(0.06)	3/49(0.06)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	3.061	3.061
Lower Limit	---	0.256	0.256
Upper Limit	---	157.400	157.400
Weeks to First Observed Tumor	97	96	97
Liver: Hepatocellular Carcinoma[b]	15/49(0.31)	11/49(0.22)	4/46(0.09)
P Values[c,d]	P = 0.006(N)	N.S.	P = 0.007(N)
Relative Risk (Control)[e]	---	0.733	0.284
Lower Limit	---	0.341	0.074
Upper Limit	---	1.528	0.814
Weeks to First Observed Tumor	94	96	86

[a]Dosed groups received concentrations of 2.5 and 5.0 percent in feed.

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

[c]Beneath the incidence of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05; otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath each dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group to the control group when it is below 0.05, otherwise N.S. - not significant.

[d]A negative trend (N) indicates a lower incidence in s treated group than in a control group.

[e]Relative risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

TABLE 6

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH DIARYLANILIDE YELLOW[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Adenoma or Carcinoma[b]	4/50(0.08)	3/49(0.06)	1/48(0.02)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.765	0.260
Lower Limit	---	0.118	0.005
Upper Limit	---	4.288	2.508
Weeks to First Observed Tumor	79	96	97
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	6/50(0.12)	3/50(0.06)	6/50(0.12)
P Values[c,d]	N.S.	N.S.	N.S.
Relative Risk (Control)[e]	---	0.500	1.000
Lower Limit	---	0.085	0.287
Upper Limit	---	2.200	3.489
Weeks to First Observed Tumor	97	84	68

[a]Dosed groups received concentrations of 2.5 and 5.0 percent in feed.

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

[c]Beneath the incidence of the control is the probability level for the Cochran-Armitage test for dose-related trend in proportions when it is below 0.05; otherwise N.S. - not significant. Departure from linear trend is noted when it is below 0.05 for any comparison. Beneath each dose group incidence is the probability level for the Fisher exact (conditional) test for the comparison of that dose group to the control group when it is below 0.05, otherwise N.S. - not significant.

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

[e]Relative risk of the treated group versus the control group is shown along with the lower and upper limit of the 95% confidence interval for that relative risk.

V. DISCUSSION

Under the conditions of this bioassay, adequate numbers of chemically treated rats and mice survived for meaningful statistical analysis of the incidence of late-developing tumors. However, exposure to diarylanilide yellow did not result in a positive association between dietary concentration and increased incidence of any tumor in either species.

The high concentration administered to both species in the chronic bioassay was the highest permissible as indicated by the Guidelines for Carcinogen Bioassay in Small Rodents (Sontag et al., 1976). These guidelines indicate that a dietary concentration greater than 5 percent should not be administered except under special circumstances (e.g., when the compound is a major component of the human diet). As human exposure to diarylanilide yellow does not warrant special exemption, the 5 percent limit applied. Dietary administration of diarylanilide yellow had no significant effect on survival or body weight gain in rats or mice of either sex. The only clinical observation associated with chemical treatment was bright yellow staining of the fur and mucosal surfaces in both species and the only sign of toxicity observed during the histopathologic examination was basophilic cytoplasm changes in treated rats.

In rats, no treatment-related increase in the incidence of neoplasms, nonneoplastic lesions, or toxic effects was evident with the exception of basophilic changes in hepatocyte cytoplasm in treated

males and females. There were, however, two unusual findings: metastatic chordoma in 1/49 low dose males, and an osteogenic sarcoma in 1/49 low dose females.

In mice, no treatment-related increase in the incidence of neoplasms, nonneoplastic lesions, or toxic effects was evident. There were, however, three unusual findings: squamous-cell carcinoma of the ear in 1/49 low dose males, an infiltrating duct carcinoma of the mammary gland in 1/50 low dose females, and a mastocytoma of the subcutaneous tissue in 1/50 high dose females.

The results of this bioassay did not provide evidence for the carcinogenicity of diarylanilide yellow in Fischer 344 rats or B6C3F1 mice.

VI. BIBLIOGRAPHY

Anthony, H.M., and G.M. Thomas, "Tumors of the Urinary Bladder: An Analysis of the Occupations of 1,030 Patients in Leeds, England." Journal of the National Cancer Institute 45:879-895, 1970.

Armitage, P., Statistical Methods in Medical Research, Chapter 14. J. Wiley & Sons, New York, 1971.

Berenblum, I., editor, Carcinogenicity Testing. International Union Against Cancer, Technical Report Series, Vol. 2. International Union Against Cancer, Geneva, 1969.

Chemical Abstracts Service, The Chemical Abstracts Service (CAS) Ninth Collective Index, Volumes 76-85, 1972-1976. American Chemical Society, Washington, D.C., 1977.

Cox, D.R., Analysis of Binary Data, Chapters 4 and 5. Methuen and Co., Ltd., London, 1970.

Cox, D.R., "Regression Models and Life-Tables." Journal of the Royal Statistical Society, Series "B" 34:187-220, 1972.

Gart, J.J., "The Comparison of Proportion: A Review of Significance Tests, Confidence Limits, and Adjustments for Stratification." International Statistical Institute Review 39:148-169, 1971.

Hawley, G.G., The Condensed Chemical Dictionary, 8th edition. Van Nostrand Reinhold Company, New York, 1971.

Hoover, R. and J. Fraumeni, "Cancer Mortality in U.S. Counties with Chemical Industries." Environmental Research 9:196-207, 1975.

Kaplan, E.L., and P. Meier, "Nonparametric Estimation from Incomplete Observations." Journal of the American Statistical Association 53:457-481, 1958.

Linhart, M.S., J.A. Cooper, R.L. Martin, N.P. Page, and J.A. Peters, "Carcinogenesis Bioassay Data System." Computers and Biomedical Research 7:230-248, 1974.

Miller, R.G., Simultaneous Statistical Inference. McGraw-Hill Book Co., New York, 1966.

Occupational Safety and Health Administration, "Occupational Safety and Health Standards 1910.93c Carcinogens." Federal Register 38:974, 1973.

Saffiotti, U., R. Montesano, A.R. Sellakumar, F. Cefis, and D.G. Kaufman, "Respiratory Tract Carcinogenesis in Hamsters Induced by Different Numbers of Administration of Benzo (a) Pyrene and Ferric Oxide." Cancer Research 32:1073-1079, 1972.

Society of Dyers and Colourists, Colour Index, Third edition 4:3272-3305, 1971.

Sontag, J.M., N.P. Page, and U. Saffiotti, Guidelines for Carcinogen Bioassay in Small Rodents. Carcinogen Program, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, Maryland, NCI-CG-TR-1. DHEW Publication No. (NIH) 76-801, February 1976.

Tarone, R.E., "Tests for Trend in Life-Table Analysis." Biometrika 62:679-682, 1975.

U.S. International Trade Commission, Synthetic Organic Chemicals. United States Production and Sales, 1975. USITC Publication 804, U.S. Government Printing Office, Washington, D.C., 1977a.

U.S. International Trade Commission, Imports of Benzenoid Chemicals and Products, 1975. USITC Publication 806, U.S. Government Printing Office, Washington, D.C., 1977b.

Weisburger, E., Chief, Carcinogen and Toxicology Branch, National Cancer Institute, Bethesda, Maryland. Personal communication, December 17, 1976.

Wynder, E.L., J. Onderdonk, and N. Mantel, "An Epidemiological Investigation of Cancer of the Bladder." Cancer 16:1388-1407, 1963.

APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH DIARYLANILIDE YELLOW

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
BASAL-CELL CARCINOMA			1 (2%)
FIBROMA	2 (4%)	4 (8%)	
*SUBCUT TISSUE	(50)	(50)	(50)
UNDIFFERENTIATED CARCINOMA			1 (2%)
SARCOMA, NOS	1 (2%)		
FIBROMA	1 (2%)		
FIBROSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(49)	(50)
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (2%)	2 (4%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (2%)	1 (2%)	
CHORDOMA METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
LEUKEMIA,NOS	1 (2%)		
MYELOMONOCYTIC LEUKEMIA	9 (18%)	1 (2%)	
#SPLEEN	(50)	(49)	(48)
ANGIOSARCOMA		1 (2%)	
MYELOMONOCYTIC LEUKEMIA		1 (2%)	
#MESENTERIC L. NODE	(49)	(46)	(46)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
#HEART	(48)	(49)	(50)
SARCOMA, NOS, METASTATIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(50)
HEPATOCELLULAR CARCINOMA		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(50)
SARCOMA, NOS			1 (2%)
NEPHROBLASTOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(45)
ADENOMA, NOS	5 (11%)		
CHROMOPHOBE ADENOMA	2 (4%)	12 (28%)	5 (11%)
#ADRENAL	(50)	(47)	(49)
PHEOCHROMOCYTOMA	3 (6%)	3 (6%)	5 (10%)
#THYROID	(37)	(47)	(48)
FOLLICULAR-CELL CARCINOMA	1 (3%)		
C-CELL ADENOMA	1 (3%)	3 (6%)	1 (2%)
C-CELL CARCINOMA	2 (5%)	2 (4%)	
#PANCREATIC ISLETS	(47)	(47)	(46)
ISLET-CELL ADENOMA	1 (2%)	2 (4%)	5 (11%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
FIBROADENOMA			1 (2%)
*PREPUTIAL GLAND	(50)	(50)	(50)
CARCINOMA,NOS	2 (4%)		
SQUAMOUS CELL CARCINOMA			1 (2%)
ADENOMA, NOS			1 (2%)
#TESTIS	(50)	(48)	(49)
INTERSTITIAL-CELL TUMOR	42 (84%)	44 (92%)	39 (80%)

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

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
NERVOUS SYSTEM			
#BRAIN	(50)	(47)	(49)
GLIOMA, NOS			1 (2%)
#CEREBRAL CORTEX	(50)	(47)	(49)
GLIOMA, NOS	1 (2%)		
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(50)	(50)	(50)
MESOTHELIOMA, MALIGNANT			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	5	6	6
MORIBUND SACRIFICE	8	2	2
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	32	42	37
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A1 (CONCLUDED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	49	46	43
TOTAL PRIMARY TUMORS	76	77	68
TOTAL ANIMALS WITH BENIGN TUMORS	46	46	39
TOTAL BENIGN TUMORS	57	69	59
TOTAL ANIMALS WITH MALIGNANT TUMORS	16	7	8
TOTAL MALIGNANT TUMORS	19	8	8
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	
TOTAL SECONDARY TUMORS	1	1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	49	49	48
INTEGUMENTARY SYSTEM			
*SKIN	(49)	(49)	(48)
EPITHELIAL TUMOR, NOS, BENIGN	1 (2%)		
*SUBCUT TISSUE	(49)	(49)	(48)
FIBROMA	2 (4%)		
RESPIRATORY SYSTEM			
#LUNG	(49)	(48)	(48)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
PHEOCHROMOCYTOMA, METASTATIC		1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(49)	(49)	(48)
LEUKEMIA,NOS	1 (2%)		1 (2%)
MYELOMONOCYTIC LEUKEMIA	6 (12%)	4 (8%)	1 (2%)
#SPLEEN	(47)	(49)	(48)
MYELOMONOCYTIC LEUKEMIA			2 (4%)
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(48)	(49)	(48)
NEOPLASTIC NODULE			2 (4%)
HEPATOCELLULAR CARCINOMA	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
URINARY SYSTEM			
#KIDNEY	(48)	(49)	(48)
TUBULAR-CELL ADENOCARCINOMA			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(44)	(42)
ADENOMA, NOS	15 (38%)		
CHROMOPHOBE ADENOMA	2 (5%)	26 (59%)	14 (33%)
#ADRENAL	(49)	(49)	(47)
PHEOCHROMOCYTOMA	3 (6%)	1 (2%)	1 (2%)
PHEOCHROMOCYTOMA, MALIGNANT		1 (2%)	
#THYROID	(45)	(42)	(46)
FOLLICULAR-CELL CARCINOMA			1 (2%)
C-CELL CARCINOMA	2 (4%)	2 (5%)	1 (2%)
#PANCREATIC ISLETS	(49)	(46)	(47)
ISLET-CELL ADENOMA		1 (2%)	2 (4%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(48)
ADENOMA, NOS			2 (4%)
ADENOCARCINOMA, NOS		1 (2%)	
PAPILLARY ADENOCARCINOMA			1 (2%)
FIBROADENOMA	12 (24%)	9 (18%)	10 (21%)
*CLITORAL GLAND	(49)	(49)	(48)
ADENOMA, NOS	1 (2%)		1 (2%)
#UTERUS	(46)	(49)	(47)
ENDOMETRIAL STROMAL POLYP	6 (13%)	13 (27%)	7 (15%)
ENDOMETRIAL STROMAL SARCOMA			1 (2%)
#CERVIX UTERI	(46)	(49)	(47)
FIBROSARCOMA			1 (2%)
#OVARY	(47)	(47)	(48)
THECOMA			1 (2%)
GRANULOSA-CELL TUMOR			1 (2%)

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

TABLE A2 (CONTINUED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
GRANULOSA-CELL CARCINOMA			1 (2%)
NERVOUS SYSTEM			
*BRAIN	(49)	(48)	(48)
ASTROCYTOMA	1 (2%)		
SPECIAL SENSE ORGANS			
*ZYMBAL'S GLAND	(49)	(49)	(48)
CERUMINOUS CARCINOMA	1 (2%)		
MUSCULOSKELETAL SYSTEM			
*BONE	(49)	(49)	(48)
OSTEOSARCOMA		1 (2%)	
BODY CAVITIES			
*BODY CAVITIES	(49)	(49)	(48)
MESOTHELIOMA, MALIGNANT			1 (2%)
*ABDOMINAL CAVITY	(49)	(49)	(48)
SARCOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	2	6	6
MORIBUND SACRIFICE	6	4	6
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	36	40	33
ANIMAL MISSING	1		

@ INCLUDES AUTOLYZED ANIMALS

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

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	32	40	34
TOTAL PRIMARY TUMORS	54	59	55
TOTAL ANIMALS WITH BENIGN TUMORS	28	36	26
TOTAL BENIGN TUMORS	42	50	38
TOTAL ANIMALS WITH MALIGNANT TUMORS	10	8	13
TOTAL MALIGNANT TUMORS	12	9	14
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 B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH DIARYLANILIDE YELLOW

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(49)
SQUAMOUS CELL CARCINOMA		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(49)
HEMANGIOSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(47)	(49)	(49)
HEPATOCELLULAR CARCINOMA, METAST	2 (4%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	4 (9%)	1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	4 (8%)	3 (6%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(49)	(49)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
#SPLEEN	(49)	(46)	(47)
HEMANGIOSARCOMA	1 (2%)		1 (2%)
ANGIOSARCOMA		1 (2%)	1 (2%)
#MESENTERIC L. NODE	(40)	(35)	(43)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (3%)	
#PEYERS PATCH	(49)	(48)	(49)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (2%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL(UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
DIGESTIVE SYSTEM			
#LIVER	(49)	(49)	(46)
HEPATOCELLULAR CARCINOMA	15 (31%)	11 (22%)	4 (9%)
HEMANGIOSARCOMA		1 (2%)	
ANGIOSARCOMA			1 (2%)
#STOMACH	(49)	(47)	(46)
ADENOMATOUS POLYP, NOS	1 (2%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(36)	(40)
CHROMOPHOBE ADENOMA		2 (6%)	
#ADRENAL	(47)	(45)	(45)
PHEOCHROMOCYTOMA			1 (2%)
#THYROID	(42)	(34)	(43)
FOLLICULAR-CELL CARCINOMA		2 (6%)	
REPRODUCTIVE SYSTEM			
#TESTIS	(49)	(49)	(48)
INTERSTITIAL-CELL TUMOR	1 (2%)		
SEMINOMA/DYSGERMINOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE B1 (CONCLUDED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	5	5
MORIBUND SACRIFICE		1	2
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	42	44	37
ANIMAL MISSING			1
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	21	13
TOTAL PRIMARY TUMORS	26	26	17
TOTAL ANIMALS WITH BENIGN TUMORS	6	3	1
TOTAL BENIGN TUMORS	6	3	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	18	20	12
TOTAL MALIGNANT TUMORS	20	23	15
TOTAL ANIMALS WITH SECONDARY TUMORS#	2		
TOTAL SECONDARY TUMORS	2		
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(50)	(50)	(50)
FIBROSARCOMA	1 (2%)		
HEMANGIOSARCOMA	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(50)	(49)	(48)
HEPATOCELLULAR CARCINOMA, METAST	1 (2%)		
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (2%)	1 (2%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	3 (6%)	2 (4%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(50)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	3 (6%)		1 (2%)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			2 (4%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
UNDIFFERENTIATED LEUKEMIA		1 (2%)	
*SUBCUTANEOUS TISSUE	(50)	(50)	(50)
MAST-CELL TUMOR			1 (2%)
#SPLEEN	(49)	(49)	(47)
HEMANGIOSARCOMA	1 (2%)		
MALIG.LYMPHOMA, HISTIOCYTIC TYPE	1 (2%)		
#MANDIBULAR L. NODE	(40)	(36)	(45)
MALIGNANT LYMPHOMA, NOS	1 (3%)		
#MESENTERIC L. NODE	(40)	(36)	(45)
MALIGNANT LYMPHOMA, MIXED TYPE			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
#PEYERS PATCH	(49)	(46)	(47)
MALIGNANT LYMPHOMA, NOS	1 (2%)		
#JEJUNUM	(49)	(46)	(47)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(49)	(47)	(46)
HEPATOCELLULAR CARCINOMA	2 (4%)		
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(39)	(44)
ADENOMA, NOS		1 (3%)	
CHROMOPHOBE ADENOMA			1 (2%)
#ADRENAL	(47)	(46)	(45)
PHEOCHROMOCYTOMA		1 (2%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(50)	(50)	(50)
INFILTRATING DUCT CARCINOMA		1 (2%)	
#UTERUS	(49)	(47)	(46)
ENDOMETRIAL STROMAL SARCOMA		1 (2%)	
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			

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

TABLE B2 (CONCLUDED)

	CONTROL(UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	50	50	50
NATURAL DEATH@	3	6	8
MORIBUND SACRIFICE	2	1	3
SCHEDULED SACRIFICE	5		5
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	40	43	34
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	12	10	9
TOTAL PRIMARY TUMORS	15	10	9
TOTAL ANIMALS WITH BENIGN TUMORS	1	3	2
TOTAL BENIGN TUMORS	1	3	2
TOTAL ANIMALS WITH MALIGNANT TUMORS	11	7	6
TOTAL MALIGNANT TUMORS	14	7	6
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			1
TOTAL UNCERTAIN TUMORS			1
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

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

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH DIARYLANILIDE YELLOW

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS
TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	50
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(50)	(50)
INFLAMMATION, SUPPURATIVE	1 (2%)		
RESPIRATORY SYSTEM			
#TRACHEA	(49)	(48)	(47)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
#LUNG/BRONCHUS	(49)	(49)	(50)
BRONCHIECTASIS			1 (2%)
#LUNG	(49)	(49)	(50)
CONGESTION, CHRONIC PASSIVE	1 (2%)		
INFLAMMATION, INTERSTITIAL	4 (8%)		
FIBROSIS, DIFFUSE	1 (2%)		
HYPERPLASIA, NOS	1 (2%)		
HYPERPLASIA, ALVEOLAR EPITHELIUM	1 (2%)	1 (2%)	
#LUNG/ALVEOLI	(49)	(49)	(50)
HEMORRHAGE	1 (2%)		
HYPERTROPHY, FOCAL		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(50)	(49)	(48)
FIBROSIS	1 (2%)		
HEMOSIDEROSIS	2 (4%)		
LYMPHOID DEPLETION			1 (2%)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, ERYTHROID			1 (2%)
#MANDIBULAR L. NODE	(49)	(46)	(46)
HYPERPLASIA, PLASMA CELL	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
#MESENTERIC L. NODE	(49)	(46)	(46)
HYPERPLASIA, PLASMA CELL	1 (2%)		
CIRCULATORY SYSTEM			
#HEART	(48)	(49)	(50)
PERIARTERITIS			2 (4%)
#MYOCARDIUM	(48)	(49)	(50)
INFLAMMATION, FOCAL		1 (2%)	
INFLAMMATION, INTERSTITIAL	1 (2%)		
FIBROSIS	2 (4%)		
DEGENERATION, NOS	1 (2%)		
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(50)	(47)	(47)
HYPERPLASIA, INTRADUCTAL	1 (2%)		
#LIVER	(49)	(49)	(50)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, FOCAL	3 (6%)		
NECROSIS, DIFFUSE			1 (2%)
NECROSIS, HEMORRHAGIC		1 (2%)	
METAMORPHOSIS FATTY			2 (4%)
BASOPHILIC CYTO CHANGE		5 (10%)	11 (22%)
ECSINOPHILIC CYTO CHANGE		1 (2%)	
HYPERPLASIA, FOCAL	1 (2%)		
#LIVER/CENTRILOBULAR	(49)	(49)	(50)
CONGESTION, PASSIVE	1 (2%)		
*BILE DUCT	(50)	(50)	(50)
HYPERPLASIA, NOS	2 (4%)	3 (6%)	
#PANCREAS	(47)	(47)	(46)
INFLAMMATION, NOS	2 (4%)		2 (4%)
INFLAMMATION, CHRONIC		2 (4%)	
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#STOMACH	(49)	(48)	(48)
HYPERKERATOSIS	1 (2%)		
ACANTHOSIS	1 (2%)		

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
#GASTRIC MUCOSA	(49)	(48)	(48)
NECROSIS, FOCAL		1 (2%)	
#PEYERS PATCH	(49)	(48)	(49)
HYPERPLASIA, NOS	1 (2%)		
#COLON	(48)	(48)	(47)
NEMATODIASIS		2 (4%)	3 (6%)
URINARY SYSTEM			
#KIDNEY	(50)	(48)	(50)
CYST, NOS		1 (2%)	
CONGESTION, NOS	1 (2%)		
GLOMERULONEPHRITIS, NOS	4 (8%)		
INFLAMMATION, CHRONIC		1 (2%)	
GLOMERULONEPHRITIS, CHRONIC		5 (10%)	
NEPHROPATHY		3 (6%)	9 (18%)
NEPHROSIS, NOS	35 (70%)		
ENDOCRINE SYSTEM			
#PITUITARY	(45)	(43)	(45)
CYST, NOS		1 (2%)	
CONGESTION, NOS	1 (2%)		
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	2 (4%)
ANGIECTASIS		1 (2%)	
#ADRENAL CORTEX	(50)	(47)	(49)
HYPERPLASIA, FOCAL		1 (2%)	
#ADRENAL MEDULLA	(50)	(47)	(49)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	
#THYROID	(37)	(47)	(48)
CYSTIC FOLLICLES	1 (3%)		
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, C-CELL	2 (5%)	2 (4%)	1 (2%)
#PANCREATIC ISLETS	(47)	(47)	(46)
HYPERPLASIA, NOS			1 (2%)
HYPERPLASIA, FOCAL		1 (2%)	

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

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(50)	(50)
ABSCESS, NOS			1 (2%)
#PROSTATE	(48)	(47)	(48)
INFLAMMATION, NOS	3 (6%)		
INFLAMMATION, ACUTE		3 (6%)	6 (13%)
INFLAMMATION, ACUTE FOCAL		8 (17%)	11 (23%)
#TESTIS	(50)	(48)	(49)
HYDROCELE			1 (2%)
PERIVASCULITIS	1 (2%)		
DEGENERATION, NOS		15 (31%)	
CALCIFICATION, NOS	3 (6%)		
CALCIFICATION, FOCAL	1 (2%)		
ATROPHY, NOS	11 (22%)		
HYPERPLASIA, INTERSTITIAL CELL	4 (8%)	1 (2%)	2 (4%)
*EPIDIDYMIS	(50)	(50)	(50)
GRANULOMA, NOS			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PLEURA	(50)	(50)	(50)
FIBROSIS, DIFFUSE	1 (2%)		
ALL OTHER SYSTEMS			
OMENTUM			
STEATITIS		1	

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

TABLE C1 (CONCLUDED)

	CONTROL(UNTR) 01-0160	LOW DOSE 01-0195	HIGH DOSE 01-0200
NECROSIS, FAT		1	

SPECIAL MORPHOLOGY SUMMARY

NO LESION REPORTED			1
AUTO/NECROPSY/HISTO PERF		1	1
AUTO/NECROPSY/NO HISTO		1	

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS
TREATED WITH DIARYLANILIDE YELLOW

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING	1		
ANIMALS NECROPSIED	49	49	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	49	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG/BRONCHUS	(49)	(48)	(48)
BRONCHIECTASIS			1 (2%)
#LUNG	(49)	(48)	(48)
CONGESTION, ACUTE PASSIVE	1 (2%)		
INFLAMMATION, FOCAL GRANULOMATOU			1 (2%)
HEMATOPOIETIC SYSTEM			
#SPLEEN	(47)	(49)	(48)
HEMOSIDEROSIS	3 (6%)		
HYPERPLASIA, HEMATOPOIETIC			2 (4%)
HYPERPLASIA, ERYTHROID			2 (4%)
CIRCULATORY SYSTEM			
#HEART	(48)	(48)	(48)
PERIARTERITIS		1 (2%)	
#MYOCARDIUM	(48)	(48)	(48)
FIBROSIS	1 (2%)		
*AORTA	(49)	(49)	(48)
PERIARTERITIS		1 (2%)	
DIGESTIVE SYSTEM			
#LIVER	(48)	(49)	(48)
INFLAMMATION, ACUTE/CHRONIC	1 (2%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
NECROSIS, FOCAL	1 (2%)	3 (6%)	1 (2%)
METAMORPHOSIS FATTY	1 (2%)		3 (6%)
BASOPHILIC CYTO CHANGE	2 (4%)	42 (86%)	40 (83%)
HYPERPLASIA, FOCAL	1 (2%)		
*BILE DUCT	(49)	(49)	(48)
HYPERPLASIA, NOS	2 (4%)		
HYPERPLASIA, FOCAL	1 (2%)		
#PANCREAS	(49)	(46)	(47)
INFLAMMATION, NOS		2 (4%)	
INFLAMMATION, FOCAL		1 (2%)	
#STOMACH	(49)	(47)	(48)
INFLAMMATION, NOS	1 (2%)		
#GASTRIC SUBMUCOSA	(49)	(47)	(48)
EDEMA, NOS	1 (2%)		
#PEYERS PATCH	(49)	(47)	(47)
HYPERPLASIA, NOS	2 (4%)		
#COLON	(49)	(45)	(48)
NEMATODIASIS		3 (7%)	3 (6%)
PARASITISM	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(48)	(49)	(48)
CYST, NOS			1 (2%)
POLYCYSTIC KIDNEY			1 (2%)
GLOMERULONEPHRITIS, NOS	4 (8%)		
NEPHROPATHY		4 (8%)	3 (6%)
NEPHROSIS, NOS	29 (60%)		
#KIDNEY/CORTEX	(48)	(49)	(48)
METAMORPHOSIS FATTY	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(39)	(44)	(42)
CYST, NOS		1 (2%)	
HYPERPLASIA, CHROMOPHOBE-CELL		1 (2%)	1 (2%)

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

TABLE C2 (CONTINUED)

	CONTROL(UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
#ADRENAL	(49)	(49)	(47)
METAMORPHOSIS FATTY			2 (4%)
#ADRENAL MEDULLA	(49)	(49)	(47)
CYST, NOS			1 (2%)
HYPERPLASIA, NOS			1 (2%)
#THYROID	(45)	(42)	(46)
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, C-CELL	2 (4%)	1 (2%)	4 (9%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(49)	(49)	(48)
DILATATION/DUCTS	1 (2%)		
GALACTOCELE		5 (10%)	6 (13%)
*MAMMARY DUCT	(49)	(49)	(48)
HYPERPLASIA, CYSTIC	1 (2%)		
*VAGINA	(49)	(49)	(48)
EPIDERMAL INCLUSION CYST		1 (2%)	
#UTERUS	(46)	(49)	(47)
HYDROMETRA	1 (2%)	1 (2%)	2 (4%)
HEMATOMA, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(46)	(49)	(47)
INFLAMMATION, ACUTE			1 (2%)
ABSCESS, NOS		1 (2%)	
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, CYSTIC			7 (15%)
#OVARY/OVIDUCT	(46)	(49)	(47)
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
#OVARY	(47)	(47)	(48)
ABSCESS, NOS		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
NERVOUS SYSTEM			
NONE			

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

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 02-0160	LOW DOSE 02-0195	HIGH DOSE 02-0200
SPECIAL SENSE ORGANS			
*EYE	(49)	(49)	(48)
CATARACT			1 (2%)
*LENS CAPSULE	(49)	(49)	(48)
CALCIFICATION, NOS	1 (2%)		
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			
ALL OTHER SYSTEMS			
OMENTUM			
NECROSIS, FAT		3	
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2		
ANIMAL MISSING/NO NECROPSY	1		
AUTOLYSIS/NO NECROPSY		1	2

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

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH DIARYLANILIDE YELLOW

TABLE D1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE
TREATED WITH DIARYLANILIDE YELLOW

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS MISSING			1
ANIMALS NECROPSIED	50	49	49
ANIMALS EXAMINED HISTOPATHOLOGICALLY **	49	49	49
INTEGUMENTARY SYSTEM			
*SKIN	(50)	(49)	(49)
ABSCESS, NOS		1 (2%)	1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
*SUBCUT TISSUE	(50)	(49)	(49)
HEMATOMA, NOS	1 (2%)		
INFLAMMATION, NECROTIZING			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
ABSCESS, NOS	1 (2%)		
RESPIRATORY SYSTEM			
#LUNG	(47)	(49)	(49)
INFLAMMATION, NOS		1 (2%)	
#LUNG/ALVEOLI	(47)	(49)	(49)
HYPERTROPHY, NOS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#SPLEEN	(49)	(46)	(47)
INFARCT, NOS			1 (2%)
HYPERPLASIA, RETICULUM CELL		3 (7%)	
ERYTHROPOIESIS	2 (4%)		
#MANDIBULAR L. NODE	(40)	(35)	(43)
ATROPHY, NOS			1 (2%)
#MESENTERIC L. NODE	(40)	(35)	(43)
HYPERPLASIA, RETICULUM CELL		1 (3%)	
#RENAL LYMPH NODE	(40)	(35)	(43)
HYPERPLASIA, NOS	2 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
CIRCULATORY SYSTEM			
#HEART	(49)	(49)	(49)
PERIARTERITIS		1 (2%)	
*AORTA	(50)	(49)	(49)
PERIARTERITIS		2 (4%)	1 (2%)
*VESICAL ARTERY	(50)	(49)	(49)
PERIVASCULITIS		1 (2%)	
DIGESTIVE SYSTEM			
#SUBMAXILLARY GLAND	(47)	(46)	(49)
ATROPHY, NOS		1 (2%)	
#LIVER	(49)	(49)	(46)
NECROSIS, FOCAL		2 (4%)	
METAMORPHOSIS FATTY	1 (2%)		
ANGIECTASIS	1 (2%)		
#LIVER/KUPFFER CELL	(49)	(49)	(46)
HYPERPLASIA, NOS	1 (2%)		
#PANCREAS	(46)	(46)	(48)
CYSTIC DUCTS	1 (2%)		
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
PERIVASCULITIS	1 (2%)		
DEGENERATION, CYSTIC		1 (2%)	
NECROSIS, FOCAL		1 (2%)	
NECROSIS, FAT	1 (2%)		
#PANCREATIC ACINUS	(46)	(46)	(48)
DEGENERATION, NOS			1 (2%)
#PEYERS PATCH	(49)	(48)	(49)
INFLAMMATION, ACUTE	1 (2%)		
HYPERPLASIA, LYMPHOID	1 (2%)		1 (2%)
#COLON	(48)	(43)	(45)
NEMATODIASIS		1 (2%)	
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(49)
HYDRONEPHROSIS	2 (4%)		

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

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
PYELONEPHRITIS, NOS		1 (2%)	
PYELONEPHRITIS, FOCAL		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, CHRONIC	1 (2%)		
#URINARY BLADDER	(49)	(49)	(49)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(36)	(40)
CYST, NOS			2 (5%)
#THYROID	(42)	(34)	(43)
PERIARTERITIS			1 (2%)
HYPERPLASIA, FOCAL	1 (2%)		
REPRODUCTIVE SYSTEM			
*PREPUTIAL GLAND	(50)	(49)	(49)
DILATATION, NOS	1 (2%)		
#PROSTATE	(49)	(48)	(46)
INFLAMMATION, ACUTE			1 (2%)
*SEMINAL VESICLE	(50)	(49)	(49)
INFLAMMATION WITH FIBROSIS			1 (2%)
#TESTIS	(49)	(49)	(48)
DEGENERATION, NOS			1 (2%)
#TESTIS/TUBULE	(49)	(49)	(48)
NECROSIS, FOCAL	1 (2%)		
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			

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

TABLE D1 (CONCLUDED)

	CONTROL(UNTR) 05-0160	LOW DOSE 05-0195	HIGH DOSE 05-0200
BODY CAVITIES			
*ABDOMINAL CAVITY	(50)	(49)	(49)
ADHESION, NOS	1 (2%)		
*PERITONEUM	(50)	(49)	(49)
INFLAMMATION, ACUTE			1 (2%)
*MESENTERY	(50)	(49)	(49)
STEATITIS	1 (2%)		
ABSCESS, NOS	1 (2%)		
ALL OTHER SYSTEMS			
ADIPOSE TISSUE			
STEATITIS	1		
NECROSIS, FAT	2		
OMENTUM			
STEATITIS			1
NECROSIS, NOS			1
NECROSIS, FAT			1
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	17	18	31
ANIMAL MISSING/NO NECROPSY			1
AUTO/NECROPSY/HISTO PERF	1	2	
AUTO/NECROPSY/NO HISTO	1		
AUTOLYSIS/NO NECROPSY		1	

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

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE
TREATED WITH DIARYLANILIDE YELLOW

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
ANIMALS INITIALLY IN STUDY	50	50	50
ANIMALS NECROPSIED	50	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	50	49	48
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
NONE			
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(49)	(49)	(47)
MYELOFIBROSIS		2 (4%)	1 (2%)
#SPLEEN	(49)	(49)	(47)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
HYPERPLASIA, RETICULUM CELL		1 (2%)	
HYPERPLASIA, LYMPHOID	1 (2%)	1 (2%)	1 (2%)
HEMATOPOIESIS		1 (2%)	
ERYTHROPOIESIS	1 (2%)		
#MANDIBULAR L. NODE	(40)	(36)	(45)
INFLAMMATION, NOS			1 (2%)
HYPERPLASIA, PLASMA CELL	1 (3%)		
#BRONCHIAL LYMPH NODE	(40)	(36)	(45)
INFLAMMATION, ACUTE			1 (2%)
#MEDIASTINAL L.NODE	(40)	(36)	(45)
HYPERPLASIA, NOS	1 (3%)		
#LUMBAR LYMPH NODE	(40)	(36)	(45)
HYPERPLASIA, NOS	1 (3%)		
#MESENTERIC L. NODE	(40)	(36)	(45)
HYPERPLASIA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
#RENAL LYMPH NODE	(40)	(36)	(45)
HYPERPLASIA, NOS	1 (3%)		
HYPERPLASIA, PLASMA CELL	1 (3%)		
CIRCULATORY SYSTEM			
#MYOCARDIUM	(50)	(49)	(47)
INFLAMMATION, FOCAL			2 (4%)
INFLAMMATION, ACUTE DIFFUSE	1 (2%)		
*AORTA	(50)	(50)	(50)
PERIARTERITIS			1 (2%)
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(48)	(48)	(46)
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	
#LIVER	(49)	(47)	(46)
ECTOPIA			1 (2%)
INFLAMMATION, NOS			2 (4%)
NECROSIS, NOS	1 (2%)		
NECROSIS, FOCAL	1 (2%)	1 (2%)	1 (2%)
INFARCT, NOS	1 (2%)		
BASOPHILIC CYTO CHANGE		1 (2%)	
HYPERPLASIA, RETICULUM CELL			1 (2%)
HEMATOPOIESIS		1 (2%)	
*BILE DUCT	(50)	(50)	(50)
INFLAMMATION, CHRONIC FOCAL	2 (4%)		
#PANCREAS	(47)	(48)	(43)
CYSTIC DUCTS		1 (2%)	
INFLAMMATION, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
INFLAMMATION, CHRONIC FOCAL			1 (2%)
ABSCESS, CHRONIC		1 (2%)	
HYPERPLASIA, FOCAL		1 (2%)	
#PANCREATIC ACINUS	(47)	(48)	(43)
DEGENERATION, NOS		1 (2%)	
#STOMACH	(49)	(45)	(44)
INFLAMMATION, NOS			1 (2%)

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE FOCAL	1 (2%)		
INFLAMMATION, CHRONIC	1 (2%)		
#PEYERS PATCH	(49)	(46)	(47)
HYPERPLASIA, LYMPHOID	1 (2%)		
#COLON	(50)	(41)	(45)
NEMATODIASIS	1 (2%)		
URINARY SYSTEM			
#KIDNEY	(49)	(49)	(47)
GLOMERULONEPHRITIS, NOS		4 (8%)	
LYMPHOCYTIC INFILTRATE	1 (2%)		
INFLAMMATION, INTERSTITIAL			1 (2%)
INFLAMMATION, CHRONIC	2 (4%)		
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
GLOMERULOSCLEROSIS, NOS	1 (2%)		
#URINARY BLADDER	(50)	(44)	(45)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC FOCAL	1 (2%)		
#U.BLADDER/SUBMUCOSA	(50)	(44)	(45)
INFLAMMATION, CHRONIC	1 (2%)		
INFLAMMATION, CHRONIC FOCAL	16 (32%)		
PERIVASCULITIS	1 (2%)		
#U.BLADDER/MUSCULARIS	(50)	(44)	(45)
CALCIUM DEPOSIT	1 (2%)		
ENDOCRINE SYSTEM			
#PITUITARY	(42)	(39)	(44)
CYST, NOS		2 (5%)	
HYPERPLASIA, CHROMOPHOBE-CELL		1 (3%)	
#THYROID	(41)	(41)	(44)
HYPERPLASIA, EPITHELIAL		1 (2%)	
HYPERPLASIA, FOCAL			1 (2%)
HYPERPLASIA, C-CELL	2 (5%)		
HYPERPLASIA, FOLLICULAR-CELL	1 (2%)	1 (2%)	

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

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
REPRODUCTIVE SYSTEM			
#UTERUS	(49)	(47)	(46)
HYDROMETRA	5 (10%)	7 (15%)	7 (15%)
ABSCESS, NOS		1 (2%)	
NECROSIS, FAT	1 (2%)		
CALCIFICATION, NOS	1 (2%)		
#UTERUS/ENDOMETRIUM	(49)	(47)	(46)
INFLAMMATION, SUPPURATIVE	2 (4%)		
INFLAMMATION, ACUTE		1 (2%)	3 (7%)
HYPERPLASIA, NOS		1 (2%)	1 (2%)
HYPERPLASIA, CYSTIC	32 (65%)	34 (72%)	24 (52%)
#OVARY/OVIDUCT	(49)	(47)	(46)
INFLAMMATION, NOS			1 (2%)
ABSCESS, NOS		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
#OVARY/PAROVARIAN	(49)	(47)	(46)
STEATITIS			1 (2%)
ABSCESS, NOS		2 (4%)	
INFLAMMATION, ACUTE/CHRONIC		1 (2%)	
#OVARY	(48)	(42)	(46)
CYST, NOS	6 (13%)	7 (17%)	7 (15%)
INFLAMMATION, NOS			2 (4%)
INFLAMMATION, SUPPURATIVE	1 (2%)		
ABSCESS, NOS			1 (2%)
INFLAMMATION, ACUTE/CHRONIC		2 (5%)	
INFLAMMATION, CHRONIC	1 (2%)		
HYPERPLASIA, EPITHELIAL		1 (2%)	
NERVOUS SYSTEM			
#BRAIN/MENINGES	(49)	(46)	(47)
LYMPHOCYTIC INFILTRATE			2 (4%)
#BRAIN	(49)	(46)	(47)
HYDROCEPHALUS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
*EYE/LACRIMAL GLAND	(50)	(50)	(50)
HYPERPLASIA, NOS		1 (2%)	

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

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 06-0160	LOW DOSE 06-0195	HIGH DOSE 06-0200
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MEDIASTINUM	(50)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
NECROSIS, NOS		1 (2%)	
*PERITONEUM	(50)	(50)	(50)
INFLAMMATION, NOS			1 (2%)
ABSCESS, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(50)	(50)	(50)
AMYLOIDOSIS	1 (2%)		
CMENTUM			
PERIVASCULITIS	1		
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	5	3
AUTO/NECROPSY/HISTO PERF	2		
AUTO/NECROPSY/NO HISTO		1	2

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

Review of the Bioassay of Diarylanilide Yellow*
for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup of the
Clearinghouse on Environmental Carcinogens

September 26, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which Diarylanilide Yellow was reviewed.

The primary reviewer said that the compound is used as a dye coating for yellow lead pencils. It could be a public health concern from the standpoint of people ingesting the dye by chewing on their pencils. Diarylanilide Yellow belongs to the chemical class of diazobenzidines. Some members of this class are reduced by hepatic enzymes to free amines which may be carcinogenic. It was noted that certain bladder carcinogens were not identified until they were tested in appropriate animal models.

The primary reviewer said that the conclusion drawn in the bioassay report was that the study did not provide evidence for the carcinogenicity of Diarylanilide Yellow in either rats or mice. He pointed out, however, that the incidence of pituitary chromophobe adenomas in the treated

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

rats was statistically significant when compared to the controls. A staff pathologist commented that in this particular laboratory, the pituitary tumors were sub-classified. If they were considered simply as pituitary adenomas NOS, or had the control pituitary adenomas been sub-classified, they would not have been statistically significant.

The primary reviewer also noted a finding of a single squamous-cell ear carcinoma in a mouse and that this lesion was unreported among the historical control animals. In addition, he commented on a number of other "odd tumors" found in the treated animals. The primary reviewer was critical of the report for not pointing out these tumors in the treated animals since it could mislead readers to believe that there should be no concern about the dye. Another Subgroup member noted a significant increase in the incidence of leukemias and lymphomas in the treated male rats, as well as a decrease in the incidence of hepatocellular carcinomas in the treated male mice. He said that consideration should be given to this phenomenon in evaluating the biological potential of the test compound. Another Subgroup member commented that a survival analysis would be necessary to determine whether there was a true reduction in tumor incidence among the treated animals.

A motion was made that Diarylanilide Yellow was not carcinogenic under the conditions of test. It was further moved that metabolism studies be done to determine if the compound is reduced to a free amine and if so, consideration be given to a retest in an animal model appropriate for studying bladder carcinogenesis. The motion was seconded and accepted unanimously.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Arnold L. Brown, Mayo Clinic
Lawrence Garfinkel, American Cancer Society
Joseph H. Highland, Environmental Defense Fund
George Roush, Jr., Monsanto Company
Sheldon Samuels, Industrial Union Department, AFL-CIO
Michael Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
John Weisburger, American Health Foundation
Sidney Wolfe, Health Research Group

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