BIOASSAY OF

PROFLAVINE

FOR POSSIBLE CARCINOGENICITY

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Carcinogenesis Program, Division of Cancer Cause and Prevention

CONTRIBUTORS: This report presents the results of the carcinogenesis bioassay of proflavine (CO4137) conducted under the direction of the Carcinogen Bioassay and Program Resources Branch, Carcinogenesis Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. Tests were conducted by the Dow Chemical Company, Indianapolis, Indiana, initially under direct contract with the NCI, and currently under a subcontract with Tracor Jitco, Inc., the prime contractor for the Carcinogen Bioassay Program.

The principal investigator for this study was Dr. J. L. Emerson. Dr. C. G. Gergig supervised animal care and the laboratory procedures involved in the preparation of test diets. The protocols for the chronic test were established by Dr. E. K. Weisburger.

Histopathologic examinations were performed by Drs. J. L. Emerson, J. A. Molello, S. D. Warner and R. A. Renne; the diagnoses included in this report represent the interpretation of these pathologists. NCI and Tracor Jitco pathologists have reviewed selected slides and concur with the overall pathologic evaluation of the study. All chemicals were analyzed under the direction of Dr. E. Murrill, and chemical analyses were reviewed by Dr. S. S. Olin. Statistical analyses were made by Dr. J. R. Joiner, using procedures selected by Dr. J. J. Gart. Pathology tables in this report were generated from data in the Carcinogenesis Bioassay Data System under the supervision of Mr. D. Tidwell. This report was written under the direction of Dr. J. F. Robens with the assistance of L. A. Waitz. The results
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SUMMARY

A bioassay of the carcinogenicity of proflavine monohydrochloride hemihydrate was conducted using Fischer 344/CR rats and B6C3F1 mice. The compound was administered in the diet at concentrations of 300 and 600 ppm to groups of 50 rats for 109 weeks and at concentrations of 200 and 400 ppm to groups of 50 mice for 104 weeks. The animals were subjected to necropsy and histopathologic evaluation as they died or at the end of their periods of treatment.

Average weights attained by high-dose groups were consistently lower than those of control groups; weights of low-dose groups showed essentially no differences from those of the controls. Survival rates of the treated rats and mice did not differ from those of the controls except for a lower rate among the female mice.

Five malignant neoplasms of the intestinal tract consisting of three leiomyosarcomas of the small intestine, a sarcoma near the colon area, and an adenocarcinoma of the small intestine were observed in five of the high-dose male rats. None were observed in other treatment or control groups. If these five intestinal neoplasms are considered together, they are significant at the \( P = 0.026 \) level using the Fisher exact test. A positive dose-related trend \( (P = 0.034) \) was also present for the three leiomyosarcomas.

The observed incidence of hepatocellular carcinoma in female mice was 4/50 (8\%) in the control group, 20/49 (41\%) in the low-dose group, and 22/50 (44\%) in the high-dose group. The test for dose-related trend showed a level of significance of \( P < 0.001 \). In male mice, the observed incidence of hepatocellular carcinoma was 20/49 (41\%) in the control group 28/49 (57\%) in the low-dose group, and 30/50 (60\%) in the high-dose group. The dose-related trend was significant at \( P = 0.057 \), and the high dose was significant at \( P = 0.044 \).

The unusually high incidence of hepatocellular carcinomas and hemangiosarcomas in control male mice and the unusually high
incidence of malignant lymphomas in all groups of female mice in conjunction with the fact that a positive-control carcinogen was tested in the same room with these animals, raises a question of the validity of these bioassay results.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Introduction</td>
<td>1</td>
</tr>
<tr>
<td>II. Materials and Methods</td>
<td>3</td>
</tr>
<tr>
<td>A. Chemicals</td>
<td>3</td>
</tr>
<tr>
<td>B. Dietary Preparation</td>
<td>4</td>
</tr>
<tr>
<td>C. Animals</td>
<td>4</td>
</tr>
<tr>
<td>D. Animal Maintenance</td>
<td>5</td>
</tr>
<tr>
<td>E. Subchronic Toxicity Tests</td>
<td>6</td>
</tr>
<tr>
<td>F. Design of the Chronic Studies</td>
<td>8</td>
</tr>
<tr>
<td>G. Clinical and Pathologic Examinations</td>
<td>8</td>
</tr>
<tr>
<td>H. Data Recording and Statistical Analyses</td>
<td>10</td>
</tr>
<tr>
<td>III. Results - Rats</td>
<td>15</td>
</tr>
<tr>
<td>A. Body Weights and Clinical Signs</td>
<td>15</td>
</tr>
<tr>
<td>B. Survival</td>
<td>15</td>
</tr>
<tr>
<td>C. Pathology</td>
<td>17</td>
</tr>
<tr>
<td>D. Statistical Analyses of Results</td>
<td>28</td>
</tr>
<tr>
<td>IV. Results - Mice</td>
<td>31</td>
</tr>
<tr>
<td>A. Body Weights and Clinical Signs</td>
<td>31</td>
</tr>
<tr>
<td>B. Survival</td>
<td>31</td>
</tr>
<tr>
<td>C. Pathology</td>
<td>34</td>
</tr>
<tr>
<td>D. Statistical Analyses of Results</td>
<td>44</td>
</tr>
<tr>
<td>V. Discussion</td>
<td>49</td>
</tr>
<tr>
<td>VI. Bibliography</td>
<td>53</td>
</tr>
</tbody>
</table>

## APPENDICES

Appendix A Summary of the Incidence of Neoplasms and Proliferative Lesions in Rats Fed Proflavine in the Diet.. 55

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table A1</td>
<td>Proliferative Endocrine Lesions</td>
<td>57</td>
</tr>
<tr>
<td>Table A2</td>
<td>Proliferative Lesions: Digestive System</td>
<td>58</td>
</tr>
<tr>
<td>Table A3</td>
<td>Hematopoietic Neoplasms</td>
<td>59</td>
</tr>
<tr>
<td>Table A4</td>
<td>Proliferative Lesions: Urinary Tract</td>
<td>60</td>
</tr>
<tr>
<td>Table A5</td>
<td>Proliferative Lesions: Respiratory Tract</td>
<td>61</td>
</tr>
<tr>
<td>Table A6</td>
<td>Proliferative Lesions: Reproductive System and Mammary Gland</td>
<td>62</td>
</tr>
<tr>
<td>Table A7</td>
<td>Proliferative Lesions: Skin and Subcutis</td>
<td>63</td>
</tr>
</tbody>
</table>
Table Aβ Miscellaneous Proliferative Lesions 64
Table A9 Analyses of the Incidence of Primary Tumors at Specific Sites in Rats, Proflavine 65
Appendix B Summary of the Incidence of Neoplasms and Proliferative Lesions in Mice Fed Proflavine in the Diet 67
Table B1 Proliferative Endocrine Lesions 69
Table B2 Proliferative Lesions: Digestive System 70
Table B3 Hematopoietic Neoplasms 71
Table B4 Proliferative Lesions: Urinary Tract 71
Table B5 Cardiovascular Neoplasms 72
Table B6 Genital and Mammary Neoplasms 73
Table B7 Proliferative Lesions: Respiratory System 73
Table B8 Miscellaneous Neoplasms 74
Table B9 Analyses of the Incidence of Primary Tumors at Specific Sites in Mice, Proflavine 75
Appendix C Summary of the Incidence of Nontumor Pathology in Rats Fed Proflavine in the Diet 77
Appendix D Summary of the Incidence of Nontumor Pathology in Mice Fed Proflavine in the Diet 91

TABLES

Table 1 Experimental Design - Proflavine 9

FIGURES

Figure 1 Growth Curves for Rats - Proflavine 16
Figure 2 Survival Curves for Rats - Proflavine 18
Figure 3 Growth Curves for Mice - Proflavine 32
Figure 4 Survival Curves for Mice - Proflavine 33
Figure 5 Kaplan and Meier Curves of the Proportion of Mice Surviving Without Observed Hepatocellular Adenoma or Carcinoma (Proflavine Test) 46
I. INTRODUCTION

Proflavine (3,6-diaminoacridine) is a synthetic acridine dye which early in this century was found to have bacteriostatic and bacteriocidal properties when administered topically (Goodman and Gilman, 1965). During World War II it was widely used as a wound antiseptic (Mitchell et al., 1942). With the advent of more specific and less toxic antibiotics, its clinical importance declined (Giarman, 1958), until it was reintroduced recently in combination with ultraviolet light for the treatment of psoriasis (Weisburger, 1976) and type-II herpesvirus infection (Amstey, 1973).
II. MATERIALS AND METHODS

A. Chemicals

The proflavine used for the prechronic toxicity tests was purchased as Lot No. V1571 from Schwarz/Mann (Orangeburg, N.Y.) in the form of proflavine dihydrochloride. The chronic phase, however, was conducted with proflavine monohydrochloride hemihydrate because of its availability. Unless otherwise noted, the term "proflavine" in this report refers to proflavine monohydrochloride hemihydrate. The following analyses were performed on the monohydrochloride hemihydrate of proflavine, which was manufactured and supplied as Lot No. 032937 by Aldrich Chemical Company (Milwaukee, Wis.). Elemental analyses were in agreement with theoretical values for the monohydrochloride hemihydrate of proflavine. The percentage of amine, as determined by nonaqueous titration of the free amine group, was 103.4 ± 0.3% of theoretical for the monohydrochloride hemihydrate. High-pressure liquid chromatography indicated one impurity (0.6% of total peak area, uv detector). Infrared, ultraviolet, visible, and nuclear magnetic resonance spectra also conformed to expectations for this structure. Thin-layer chromatography in a 1,4-dioxane : ammonium hydroxide solvent system, as visualized by ultraviolet light and furfural, showed three trace impurities. A second analysis in a pyridine : ammonium hydroxide solvent system showed
a single trace impurity. No attempt was made to further characterize or identify these minor impurities.

B. Dietary Preparation

Test diets were prepared every 2 weeks and used within a 2-week period. A 10% premix of proflavine in Wayne Lab Blox reground meal was prepared first and then blended in a Patterson-Kelly Twin Shell blender with additional feed to obtain the appropriate dose concentration. All dietary preparations were stored in plastic-lined fiber drums and refrigerated at 40° F.

Analysis of two batches of the 10% premix several weeks after mixing gave 10.6 ± 0.21% and 10.93 ± 0.27% proflavine, corrected for recovery losses, indicating adequate stability of the compound in feed.

C. Animals

Fischer 344/CR rats were obtained from A. R. Schmidt/Sprague Dawley, Madison, Wisconsin and Harlan Industries, Cumberland, Indiana. Hybrid B6C3F1 mice were obtained from Charles River Breeding Laboratories, Wilmington, Massachusetts. Animal suppliers were under contract with the Division of Cancer Treatment, NCI, to provide the animals used for testing. Rats and mice were received at approximately 28 days of age and quarantined for 7 to 14 days. Those determined to be free from
observable disease or parasites were assigned to treatment
groups. Because the rats were received from two different
suppliers, they were distributed so that 3/4 of the rats in each
treatment and control group were from A. R. Schmidt and 1/4 were
from Harlan Industries.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled
rooms. The temperature was maintained at 23° C with variations
from 22°-25° C, and the humidity ranged from 45-55%. The rooms
had 15 complete air changes per hour. All rooms were equipped
with automatic timers which controlled lighting and provided a
14-hour-per-day light cycle. Wayne® Lab Blox ground meal (Allied
Mills, Inc., Chicago, Ill.) and water (deionized chlorinated well
water) were consumed ad libitum.

Rats in the chronic study were first housed individually in
suspended cages made of stainless-steel wire mesh (Ford Fence
Co., Indianapolis, Ind.). At week 45 all rats were transferred
to suspended polycarbonate cages (Maryland Plastics,
Federalsburg, Md.) equipped with filters and automatic waterers
and lined with autoclaved Ab-Sorb® Bedding (Lab Products,
Inc., Garfield, N.J.). The cages were changed, washed, and
sanitized at 82° C twice weekly. The feeders were changed,
washed, and sterilized weekly, and the filters were changed every 2 weeks.

Mice were housed five per cage in filtered prebedded cages made of disposable polypropylene (Lab Products, Inc., Garfield, N.J.). The cages were changed twice weekly and the used cages were incinerated. Feeders, water bottles, and cage lids also were changed twice weekly, and filters were changed weekly. Feeders and sipper tubes were washed and sterilized prior to use. Water bottles and cage lids were sanitized at 82°C.

Rats and mice were housed in separate rooms. The racks were rotated weekly and the cages were kept in fixed positions on the racks. The rats being fed proflavine were in the same room as rats being fed N,N’-dicyclohexylthiourea, 1,3-dichloro-5,5-dimethylhydantoin, and the positive control, N-2-fluorenylacetamide. Mice treated with proflavine were housed in a room in which 2-amino-5-nitrothiazole, 3-nitropropionic acid, N,N’-dicyclohexylthiourea, 1,3-dichloro-5,5-dimethylhydantoin, and N-2-fluorenylacetamide were also on test. Untreated controls were housed in the same room with their respective test animals.

E. **Subchronic Toxicity Tests**

In the subchronic toxicity tests, proflavine dihydrochloride was administered to rats in the diet at dose concentrations ranging
from 500 to 2,000 ppm for six weeks. Following the treatment period, there was a 2-week observation period. The gain in body weight of female rats was 95% of control values at a dose of 500 ppm, 82% at 750 ppm, 69% at 1,000 ppm, 53% at 1,500 ppm, and 50% at 2,000 ppm. The gain in body weight of male rats followed the same trend: 90% at 500 ppm, 97% at 750 ppm, 82% at 1,000 ppm, 57% at 1,500 ppm, and 35% at 2,000 ppm. Two males and one female rat died at the 2,000 ppm concentration. At concentrations above 750 ppm, animals displayed rough hair coats; yellow discoloration in the skin, hair, and urine; and diarrhea. Pneumonia was diagnosed in one animal, and males receiving a dose of 2,000 ppm had small testes, although spermatogenesis was normal. The results indicated a level of 600 ppm as the MTD for male and female rats.

Proflavine dihydrochloride was administered to mice in the diet at five dose concentrations ranging from 200 to 1,400 ppm on the same schedule. The gain in body weight of females was unaffected at doses of 200 and 400 ppm; the gain in weight at 600 ppm was 73% of that of controls. The gain in weight of males was unaffected at doses below 1,400 ppm, while at 1,400 the gain in weight was 81% of that of controls. Only one mouse died during the subchronic tests — a control animal. Necropsy findings included hydronephrosis in two mice (at 400 ppm), pyelonephritis in one (at 600 ppm), and a hyperplastic nodule in the liver of
one mouse (at 1,400 ppm). On the basis of these findings, the MTD for mice of both sexes was set at 400 ppm.

F. Design of the Chronic Studies

During the chronic study, proflavine was administered to Fischer 344/CR rats and B6C3F1 mice at either of two concentrations in the diet (see table 1). Rats and mice were killed 2 days after the treatments were concluded.

G. Clinical and Pathologic Examinations

Body weights were recorded every 14 days for the first 3 months and every 28 days thereafter. Animals were inspected twice daily, 7 days a week, for clinical signs and mortality. The general physical condition of the animals and the nature, extent, and location of any gross abnormalities were noted and recorded at weekly intervals. Animals appearing moribund when examined were killed and immediately necropsied, although some moribund animals were isolated from their cage-mates for a few days prior to killing. All animals, regardless of whether they were killed early or survived to termination, were subjected to a complete gross necropsy. Animals were killed by inhalation of carbon dioxide, exsanguinated, and immediately necropsied.

Tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, stained with hematoxylin and eosin, and
Table 1. Experimental Design – Proflavine

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<thead>
<tr>
<th></th>
<th>Dietary Concentrations</th>
<th>Treatment Period (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Animals (ppm)</td>
<td></td>
</tr>
</tbody>
</table>

### RATS

#### Male

- Matched Control: 50 0 109
- Low Dose: 50 300 109
- High Dose: 50 600 109

#### Female

- Matched Control: 50 0 109
- Low Dose: 50 300 109
- High Dose: 50 600 109

### MICE

#### Male

- Matched Control: 50 0 104
- Low Dose: 50 200 104
- High Dose: 50 400 104

#### Female

- Matched Control: 50 0 104
- Low Dose: 50 200 104
- High Dose: 50 400 104

*No observation period.*
examined microscopically. Histopathologic evaluation consisted of examination of the following: gross lesions, tissue masses or suspect tumors and regional lymph node(s), blood smear (if anemia, enlarged thymus, lymphadenopathy, or hepatosplenomegaly were present), mandibular lymph node, mammary gland, salivary gland, sternebrae including marrow, thymus, trachea, lungs and mainstem bronchi, heart, thyroids, parathyroids, esophagus, stomach, small intestine (one section), colon, liver, gall-bladder, pancreas, spleen, kidney, adrenals, bladder, prostate, testes, ovaries, uterus, brain (three sections including frontal cortex and basal ganglia, parietal cortex and thalamus, and cerebellum and pons), pituitary, eyes (if grossly abnormal), and spinal cord (if neurologic signs were present).

The intent was to evaluate all organs, tissues, and gross lesions for every animal as specified in the pathology protocol for the Bioassay Program. However, a few tissues (especially small organs) were lost during the necropsy and the process of histologic preparation; therefore, the denominator used for a particular organ, tissue, or lesion in Appendixes A and B may not necessarily equal the number of animals 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 included descriptive information on the chemicals, animals, experimental design, clinical observations, survival, animal 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.

Probabilities of survival were estimated by the product limit procedure of Kaplan and Meier (1958) and presented in this report in the form of graphs. Deaths due to accident or scheduled deaths are treated as censored observations and all other deaths are uncensored. Statistical tests of differences in survival between groups are compared using the method of Cox (1972) for two groups and an extension of this method by Tarone (1975) for more than two groups.

The number of animals with tumors was analyzed as a percentage of the number of animals pathologically examined. For specific anatomic sites, the animal is not included in the denominator if that particular site was not histologically examined. For tumors which required gross detection, e.g. skin tumors, the denominator included all animals necropsied. For tumors that may appear at several sites, e.g. lymphoma, any animal that had at least one
involved site histologically examined is entered in the denominator of the proportions given for that tumor.

Statistical analysis of the incidence of tumors was made using the Fisher exact test (Cox, 1970) to compare a control group to a group of treated animals at each dose level. In addition, the Armitage and Cochran test for linear trend in proportions, with continuity correction (Armitage, 1971), was used. This test, assuming a linear trend, determines if the slope of the dose-response curve is different from zero, at the 0.05 level of significance. The method also calculates the level of probability of a departure from linear trend.

A conservative adjustment, the Bonferroni inequality (Miller, 1966), was used for simultaneous comparison of several treated groups with a control group. For the comparison of results obtained with k different test doses with those for a control, this correction requires a level of significance less than or equal to 0.05/k for the overall comparison to be significant at the 0.05 level. This adjustment was not made in the tables where the Fisher exact test results are shown but is discussed in the analysis when appropriate.

As an additional analysis, the exact 95% confidence interval for the odds ratio (Gart, 1970) between each of the dose groups and its control was calculated. The odds ratio is \( \frac{p_t(1-p_c)}{p_c(1-p_t)} \)
where $p_T$ is the true binomial probability of tumor in a dosed animal and $p_C$ is the true spontaneous tumor probability in the controls. The hypothesis of equality between the true proportion of a specific tumor in a dosed group and that in a control is expressed by an odds ratio of 1 (one). Values in excess of 1 (one) represent the condition of a larger proportion in the dosed group than in the control. The confidence interval entries in the statistical tables of this report represent the conversion of each odds ratio to the difference in probabilities, $p_T-p_C$, where $p_T-p_C = 0$ implies an odds ratio of 1 (one).
III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Gains in body weight in both male and female tested rats were not decreased greatly at the 3,000 ppm concentration and were not less than 85% of control values at the 6,000 ppm concentration (see figure 1).

During the 2-year period of the study, the appearance and behavior of the treated and control rats were generally comparable. The first palpable mass recorded for a control male rat was on day 377 and for a control female rat on day 580. The first palpable masses in the treated rats were recorded for a low-dose male and a high-dose female on day 491. Palpable masses were recorded on day 519 for a low-dose female and on day 659 for a high-dose male. During the second year of the study the control group and the treated groups began to exhibit similar incidences of masses. Unilateral and occasionally bilateral cataracts were observed at the end of the first year and through the second year in both controls and treated rats.

B. Survival (Rats)

The survival rate of the dosed male rats was similar to that of the matched-control group, and 80% of the high-dose males lived
Figure 1. Growth Curves for Rats - Proflavine
to termination of the study at 109 weeks. Figure 2 shows the Kaplan and Meier survival curves for male and female rats.

In female rats, the matched-controls and the high-dose group exhibited similar death rates, but the low-dose group experienced a higher rate of survival than either of the other two groups. Consequently the statistics on survival show a departure from linear trend (P = 0.018). Seventy percent of the high-dose females were alive at the end of the study.

One low-dose male rat was reported lost due to accidental death at 6 weeks; otherwise all deaths were due to natural causes.

C. Pathology (Rats)

Histopathologic findings are tabulated in Appendix A, tables Al-A8.

Numerous inflammatory, degenerative, and proliferative lesions commonly observed in aged Fischer rats occurred with approximately equal frequency in test and control rats. These lesions included chronic tracheitis; multifocal alveolar macrophage aggregates in lung parenchyma; alveolar epithelial-cell hyperplasia (table A5); chronic nephritis with scarring, tubular dilatation, and tubular regeneration; hyperplasia of epithelium of the renal pelvis and urinary bladder (table A4); suppurative endometritis and oophoritis; cystic endometrial hyperplasia
Figure 2. Survival Curves for Rats - Proflavine
(table A6); testicular atrophy; and C-cell hyperplasia of thyroid (table A1).

Other nonneoplastic proliferative lesions included hyperplasia of thyroid follicular cells (table A1), adrenal medulla (table A1), parathyroid (table A1), endometrial stroma (table A6), mesothelium of vaginal tunic (table A6), mammary epithelium (table A6), gastric mucosa (table A2), and hepatocytes (table A2).

With regard to liver lesions, the term "focal hyperplasia" was used in this study to indicate the presence of one or more foci of hepatocytes with increased cytoplasmic basophilia and a slight increase in the amount of nuclear chromatin; many of these hepatocytes also had a slight increase in nuclear:cytoplasmic ratio when compared with adjacent normal hepatocytes, and infrequently mitotic figures or hepatocytes with double nuclei were observed. These foci of hyperbasophilic hepatocytes were thought to represent areas of hyperplasia and were diagnosed as such in this study. They did not compress adjacent hepatic parenchyma. These lesions are similar morphologically to those described by Squire and Levitt (1975) as "basophilic foci". The one lesion classified as nodular hyperplasia was similar to the hyperplastic foci, but was larger, more discrete, and compressed adjacent hepatic parenchyma, thus was classified as a "nodule" of hyperplasia.
Lesions classified as "hepatocytomegaly" consisted of foci of enlarged hepatocytes, many of which contained large, vesicular nuclei and numerous fine cytoplasmic vacuoles which gave the cytoplasm a "ground-glass" appearance. Distortion of lobular architecture in these foci was minimal, and trabeculae were continuous with adjacent normal hepatocytes. These lesions correspond morphologically to those described by Squire and Levitt as "eosinophilic foci," "ground-glass foci," or "clear-cell foci."

Lesions classified in this study (table A2) as "neoplastic nodules" (Squire and Levitt, 1975) had many similarities to all three of the previously described proliferative hepatocytic lesions. However, the lesions classified as neoplastic nodules were larger, and contained more distinct abnormality of lobular architecture; liver cords at the periphery of the neoplastic nodules were oriented perpendicular to cords of adjacent normal hepatic parenchyma, and distinct compression of adjacent normal liver was evident. Hepatocellular carcinoma was diagnosed when the lesion contained areas of complete loss of normal lobular architecture. The most frequent abnormality in this type of lesion was the presence of widely dilated sinusoids lined by rows or nests of hepatocytes several cells thick, sometimes with papillary projections of hepatocytes into the sinusoidal space.
Formation of pseudo-acini or solid sheets of hepatocytes was less frequently observed.

Endocrine tissues were the most frequent sites of neoplasms in both treated and control rats in this study. Interstitial-cell tumors of the testis were observed in nearly all male rats in all groups (table A6); a high spontaneous incidence of this tumor is characteristic of aged Fischer 344 rats. Pituitary adenomas were also found in a high incidence in all groups, especially females (table A1). Other endocrine neoplasms seen with approximately equal frequency in test and control rats included follicular-cell and C-cell tumors of thyroid, islet-cell tumors of pancreas and pheochromocytomas of adrenal (table A1). In some proliferative endocrine lesions, differentiation between benign and malignant neoplasms was difficult. Thyroid C-cell lesions were classified as adenomas when the proliferating C-cells were present in nodular masses which widely separated thyroid follicles and distorted follicular architecture. In many of the larger adenomas, the C-cells were present in interlacing bundles of elongated, spindling cells, rather than the polyhedral to spherical shape characteristic of normal C-cells. When invasion of thyroid capsule, adjacent tissues, or vessels was present, or when metastasis was detected, the lesion was classified as C-cell carcinoma. Pulmonary metastasis occurred in four of six C-cell
carcinomas, and invasion of trachea and adjacent soft tissue was observed in one of these four tumors.

Follicular-cell neoplasms occurred less frequently than C-cell neoplasms (table A1). The follicular-cell adenoma appeared microscopically as a well-circumscribed mass composed of enlarged follicles lined by hyperbasophilic follicular cells which were increased in number per unit area by papillary infolding of simple cuboidal or columnar epithelium into the follicular lumen and stratification of follicular cells surrounding the lumen. Distinct compression of adjacent normal thyroid parenchyma, with some evidence of fibrous encapsulation, was present. Follicular-cell lesions were classified as carcinoma based upon the presence of anaplasia and histologic arrangement in disorderly nests and/or sheets. Areas with papillary patterns were also present. Fibrous stroma often intermingled with, but did not encapsulate, the follicular-cell carcinomas.

Pheochromocytomas of adrenal medulla and islet-cell tumors of the pancreas both occurred more frequently in male rats (table A1). The diagnosis of pheochromocytoma was made when the adrenal medullary lesion was present as a discrete mass which compressed adjacent normal adrenal parenchyma. These neoplasms were composed of sheets, nests, and/or cords of polyhedral to spherical cells with abundant, slightly basophilic, cytoplasm and
large nuclei with abundant chromatin. Islet-cell adenomas appeared as discrete, encapsulated nodules of islet cells which compressed adjacent normal pancreas; diagnosis of islet-cell carcinoma was based on invasion of the capsule surrounding the neoplasm, the size of the neoplasm, a high mitotic index, and atypia of the neoplastic cells.

Malignant neoplasms of the intestinal tract occurred in five male rats from the high-dose group. Three of these were diagnosed as leiomyosarcomas, all in small intestine. Two of these three lesions were noted grossly as masses approximately one inch in diameter in the wall of the ileum. The third lesion was described grossly as a mass in mesenteric lymph node, but at microscopic examination the neoplasm was observed in the wall of the small intestine, mesentery, and pancreas. Microscopically, all three of these neoplasms were composed of a pleomorphic population of cells, with a predominance of spindling cells with elongated, blunt-ended nuclei, occurring in interlacing bundles, and lesser numbers of plump, oval to round cells occurring in sheets and nests. Bone formation within the neoplasm was evident in one rat. Staining with Masson's trichrome stain demonstrated moderate amounts of finely divided collagen in the neoplasm in one rat; the same technique demonstrated only scanty amounts of collagen in the other two neoplasms. Diagnosis of leiomyosarcoma was based on the location of the neoplasm in the intestinal wall,
size and shape of the predominant neoplastic cell type, the predominance of blunt-ended nuclei in neoplastic cells, and the relatively small amount of collagen demonstrated with Masson's trichrome stain. Differential diagnoses in these lesions would include fibrosarcoma, other spindle-cell sarcomas, and malignant histiocytic tumors.

The histologic characteristics of another tumor observed in the area of the colon were partially masked by autolysis; no good landmarks were present histologically to identify location of the neoplasm, but fatty tissue was present adjacent to the mass, which was described grossly as "involving the colon 5 cm from the cecum". This neoplasm also was made up of a pleomorphic population of cells, varying from spindle-shaped to round. Many mitotic figures were present, and numerous foci of necrosis and acute inflammation were evident. This neoplasm was diagnosed as a sarcoma, but not further classified due to autolysis, lack of evident differentiation of cells, and inability to determine the exact anatomic location of the neoplasm.

The other neoplasm occurring in the intestinal tract in this group was a 4-cm diameter mass in the duodenum, which on cut section had a cystic, fluid-filled center. On histologic examination, this mass was found to be composed of numerous cystic spaces partially filled with mucus, lined by a thickened,
papillary epithelium with a loose stroma containing foci of inflammatory cells and numerous nests of proliferating epithelial cells. Numerous mitotic figures and atypical cells were present within the epithelium lining the cysts and in the stroma. This neoplasm was classified as an adenocarcinoma of the small intestine.

As noted previously, focal hyperplasia of gastric mucosa was observed in two rats (table A2); both of these were males from the high-dose group. The relatively low spontaneous incidence of gastrointestinal tract neoplasms reported in laboratory rats (Rowlatt, 1967) makes the observation of these lesions only in test animals more noteworthy.

Malignant lymphomas occurred rather frequently in both control and test groups (table A3). Most of these neoplasms were composed of relatively undifferentiated lymphoreticular cells, and many were generalized, i.e., involved numerous organs and tissues throughout the body. In those lymphomas that were not generalized, the organ most frequently affected was the spleen. Liver, lymph nodes, and thymus were also frequently affected. Evidence of leukemia (masses of neoplastic lymphoreticular cells in vessel lumens) was seen in some cases of generalized lymphoma. Two cases of granulocytic neoplasia were observed (table A3).

Neoplasms of urinary tract epithelium, although occurring infre-
quently, were observed in both control and test groups and in
both sexes in approximately equal frequency (table A4). Carcinomas of transitional epithelium occurred in both renal
pelvis and urinary bladder, and were characterized by formation
of solid nest and sheets of transitional cells interspersed with
small cystic spaces filled with necrotic debris. The carcinoma
in the renal pelvis in a high-dose female not only proliferated
outward into the lumen, but invaded deeply into the renal
parenchyma. The tubular adenoma observed in a low-dose male was
a very small encapsulated mass composed of large, well differen-
tiated tubular cells.

Pulmonary neoplasms were observed only in males, with approxi-
mately equal frequency in test and control groups (table A5). Differen-
tiation between adenoma and carcinoma was based on the
degree of anaplasia, mitotic index, size of the neoplasm, and
presence of apparent invasion of adjacent pulmonary parenchyma in
carcinoma, as opposed to mere compression of adjacent parenchyma
and thus a more discrete lesion seen in adenoma. One poorly
differentiated adenocarcinoma of the submucosal glands of the
larynx was observed in a control female rat.

The most frequently occurring reproductive tract neoplasm, other
than the previously mentioned interstitial-cell tumor of the
testis, was the endometrial stromal polyp of the uterus (table
A6). This lesion was present as a discrete mass protruding into the lumen of the uterus, lined by endometrium, and sometimes associated with suppurative endometritis and/or cystic endometrial hyperplasia. The stroma was usually proliferating in a rather loosely woven pattern, with numerous small vessels interdispersed among stromal cells. Endometrial stromal sarcoma, a less frequent lesion, was similar to stromal polyp except larger and more highly cellular, with more numerous mitoses, anaplasia, and evidence of invasion of adjacent tissues. One leiomyoma of uterine musculature was observed in a high-dose female rat.

Adenocarcinoma of the endometrium occurred in four rats: three in controls and one in the low-dose group (table A6). These neoplasms proliferated into the lumen of the uterus, and were composed of poorly formed small acini or nests of endometrial cells invading stroma. Numerous mitotic figures were present.

The most common neoplasm of the mammary gland was the fibroadenoma; these lesions occurred much more frequently in females, were often multiple, and were seen in both test and control groups (table A6). Mammary carcinomas were also more common in females (table A6). No metastasis of any mammary carcinoma was observed.

Various other types of malignant and benign neoplasms were observed in low incidence in sections of skin and subcutis (table
A7), and in other organs and tissues throughout the body (table A8). No apparent difference in incidence of these neoplasms between test and control groups was present.

There were instances in this study, as noted above and in the summary tables, where neoplastic or hyperplastic lesions occurred only in test animals, or with increased frequency when compared to control groups. In the judgement of the pathologist, the nature, incidence, and severity of the lesions observed provide no clear evidence of carcinogenic effect.

For a summary of the incidence of nontumor pathology in rats exposed to proflavine, see Appendix C.

D. Statistical Analyses of Results (Rats)

Table A9 contains the statistical analyses of the incidence of those tumors which appeared in over 5% of any dosed group. In male rats, the only tumor with a statistically significant (P = 0.034) positive dose-related trend was leiomyosarcoma, which was seen in 3/45 (7%) of the high-dose group and in none of the other male or female groups. Two other high-dose males had tumors of the intestinal tract. When the Fisher exact test is applied to the total incidence 5/45, it shows statistical significance at the 0.026 probability level. No other Fisher exact tests between the high- or low-dose groups and the controls were significant.
(P > 0.05) for positive dose-related tumors, nor is there any apparent significance in the analyses of the time of observation of any particular neoplasm. Although experience of the observation of tumors from this laboratory is insufficient to compare with the observations reported in the control group matched to the dosed group, historic results on this strain of rats from all laboratories in the Bioassay Program show that the male matched controls on this study showed a greater incidence of pituitary tumors (14/49 [29%] compared with 86/846 [10%] in the historic results) and in hematopoietic neoplasms (17/50 [34%] compared with 55/846 [6.5%] in the historic results). As a result of these disparities, both these tumor sites show a negative dose relationship in the proportions of tumors observed.

No positive dose-related trends were present in the tumor proportions reported in the female rats, and in these rats a significantly higher proportion (15/50, 30%) of hematopoietic tumors were reported in the matched control compared with the historic control data from all laboratories in the Bioassay Program (45/840, 5.4%).
A. Body Weights and Clinical Signs (Mice)

Gains in body weight in male and female tested mice were not different from those of the control groups at any dose concentration during either the first or second year of the test (see figure 3).

During the first year of the study, the appearance and behavior of the treated and control mice were generally comparable. Focal alopecia, focal dermatitis, and small palpable masses in the perineal area were observed in increasing numbers of male mice after 7 months on test. These lesions were associated with male animals fighting. The first palpable mass for a control male mouse was recorded on day 294 and for a control female mouse on day 629. The first palpable mass in a treated mouse was recorded for a high-dose male on day 546 and for a high-dose female on day 574. Palpable masses were recorded on day 519 for both male and female low-dose mice.

B. Survival (Mice)

All three groups of male mice had similar survival rates, and 68% of the high-dose males lived to the termination of the study at 103 weeks. Figure 4 shows the Kaplan and Meier survival curves for male and female mice. In the female mice, survival curves
Figure 3. Growth Curves for Mice - Proflavine
Figure 4. Survival Curves for Mice - Proflavine
were comparable up to 90 weeks, but after that time the matched-control group had a higher survival rate than the dosed groups. The poorest survival was experienced by the low-dose group, in which 54% of the animals survived to the termination of the test.

In neither sex were the statistics for positive dose association significant ($P > 0.05$). There were no animals reported missing or accidentally killed, and no statistically significant relationship of tumors to early deaths was apparent.

**C. Pathology (Mice)**

Histopathologic findings are tabulated in Appendix B, tables Bl-B8. Several chronic inflammatory, degenerative, and proliferative lesions which often occur spontaneously in aged laboratory mice were observed with approximately equal frequency and severity in test and control animals. These lesions included cystic ovaries, suppurative oophoritis and endometritis, cystic endometrial hyperplasia, and chronic nephritis.

The incidence of proliferative endocrine lesions is summarized in table Bl. The thyroid carcinoma observed in a control male mouse was a poorly differentiated neoplasm with some microscopic features of a C-cell tumor (sheets and nests of pleomorphic cells); however, the most differentiated portion of the neoplasm contained areas of cuboidal epithelial cells forming follicles,
thus the neoplasm was considered to be of follicular-cell origin. Follicular-cell adenomas and hyperplastic foci of follicular cells were similar histologically in that they were both composed of several follicles lined by large, hyperbasophilic follicular cells which were increased in number per unit area by papillary infolding of simple cuboidal or columnar epithelium into the follicular lumen and stratification of follicular cells surrounding the lumen. Differentiation of follicular-cell adenoma from hyperplasia was based largely on presence of distinct compression of adjacent normal thyroid parenchyma with some evidence of encapsulation in adenoma; other criteria were the size of the lesion, the degree of difference in cellular morphology and follicular architecture between the mass and adjacent normal thyroid, and the presence of a single lesion in adenoma as opposed to multiple foci in hyperplasia.

A total of six primary neoplasms occurred in sections of adrenal glands from all groups. Four of these were pheochromocytomas, one was a cortical carcinoma, and one lesion was diagnosed as a carcinoma, but not further classified due to extensive autolysis. The latter neoplasm, found in low-dose female, was described grossly as a large mass in the area of the left adrenal. It had no normal tissue attached to it when examined microscopically, and the histologic pattern was partially masked by autolysis. A mass of similar tissue was present in the lung section. Despite
the autolytic changes and the uncertainty as to location, the most likely primary site of this tumor was considered to be the adrenal gland; the histologic features were those of carcinoma; therefore it was classified as carcinoma, N.O.S., adrenal gland, with metastasis to lung.

Table B2 summarizes the incidence of pertinent lesions of the digestive system, including the liver. Numerous hepatocellular carcinomas were observed, the morphologic pattern varying from small, discrete, compressing nodules of enlarged hepatocytes with moderate deviation from normal hepatic architecture to very large masses involving entire lobes of liver, composed of anaplastic hepatocytes forming pseudoacini, solid sheets of cells, or cords separated by angiectatic sinusoids, and in some cases containing foci of necrosis. Pulmonary metastasis of hepatocellular carcinoma occurred only in male mice: 5/20 in controls, 1/28 in low-dose animals, and 4/30 in the high-dose group.

Numerous hemangiosarcomas were also observed in liver parenchyma (table B2), as well as in other sites (table B5). Hemangiosarcoma and hepatocellular carcinoma occurred concurrently in four low-dose male mice, one control male mouse, and two high-dose female mice. Foci of necrosis were also seen in some liver sections containing hemangiosarcoma (two control males, one control female, and three low-dose males).
Diagnosis of hepatic hemangiosarcoma was based on the presence of atypical endothelial cells lining sinusoids or appearing in solid masses. Most hemangiosarcomas were composed of (1) areas of angiectatic sinusoids lined by plump endothelial cells with large, hyperchromatic nuclei, (2) densely cellular areas composed of endothelial cells similar to those described in (1) with only a few small vascular spaces, and (3) areas with a morphology intermediate between (1) and (2).

Hepatic lesions classified as nodular hyperplasia consisted of discrete nodules of proliferating hepatocytes which compressed adjacent liver parenchyma, but which did not have sufficient variation in cellular morphology or lobular architecture to warrant diagnosis of neoplasia. The liver section from one low-dose female contained a hyperplastic nodule as well as a hepatocellular carcinoma. The diagnosis of hepatocytomegaly indicates the presence of one or more foci of enlarged hepatocytes containing large amounts of finely vacuolated cytoplasm. Compression of adjacent parenchyma by these foci was minimal or absent.

Although no neoplasms were observed in sections of stomach, several hyperplastic lesions of the glandular or squamous mucosa were seen, all occurring in test groups (table B2). These were small, focal lesions. Hyperkeratosis of the squamous gastric
mucosa was noted in three mice, two of which were controls. One papillary adenoma of the duodenum was observed in a high-dose female mouse. Microscopically, this was a focal, polypoid mass protruding into the lumen of the bowel, near the pylorus.

Numerous hematopoietic neoplasms occurred in all groups; the vast majority were malignant lymphomas (table B3). These were classified as "generalized malignant lymphoma" when they involved numerous organs and tissues, and as "malignant lymphoma" when only a few organs were involved. The organs most frequently involved with malignant lymphoma were the mesenteric lymph nodes, liver, Peyer's patches, spleen, and thymus. Several morphologic types were observed, including the mixed type, which contained a mixture of reticulum cells, lymphoblasts, and a lesser number of other leukocytes; and the undifferentiated type, in which the vast majority of neoplastic cells were large, undifferentiated reticulum cells. The mixed type and undifferentiated type of lymphomas correspond morphologically to Dunn's reticulum cell sarcoma, types B and A, respectively (Dunn, 1954). In some cases, subclassification of lymphoma was not practical due to extensive autolysis; these are classified as malignant lymphoma, N.O.S. Two generalized granulocytic sarcomas were observed. Two mast-cell sarcomas were also observed, one of which involved numerous organs and one of which involved only spleen and liver. Both lesions diagnosed as mast-cell sarcoma were composed of
large oval cells with round central nuclei, abundant cytoplasm, and a distinct cytoplasmic membrane. Although stains for metachromasia were negative (Dr. J. L. Emerson, personal communication), mast-cell sarcoma remains the most plausible diagnosis, based on cellular morphology and anatomic location. Differential diagnoses include lymphoma and granulocytic sarcoma.

Table B4 summarizes the incidence of proliferative urinary tract lesions. Two carcinomas of renal tubular epithelial cells were observed, in a low-dose female mouse and in a high-dose female mouse. Both these neoplasms occurred in renal cortex, as papillary fronds and nests of large epithelial cells with large hyperchromatic nuclei, numerous mitotic figures and foci of necrosis. Neither of the neoplasms was encapsulated. A low incidence of epithelial hyperplasia of urinary bladder was observed, and epithelial hyperplasia of urethra was noted in two control male mice.

The incidence and morphology of hepatic hemangiosarcomas have already been discussed; other primary cardiovascular neoplasms are summarized as to location and incidence in table B5. Hemangiosarcoma of the spleen was observed concurrently with hepatic hemangiosarcoma in six cases. The morphology of the lesion in the spleen was somewhat similar to that described for hepatic hemangiosarcoma, except for a decreased incidence of
angiectatic areas in the splenic neoplasms. The hemangiosarcomas observed in the subcutis and the bone marrow both were predominantly cavernous; plump endothelial cells with hyperchromatic nuclei lined the cavernous spaces. Hemangiomas, which occurred in subcutis and myocardium were also cavernous in architecture.

The incidence of genital and mammary neoplasms is summarized in table B6. Several types of primary ovarian neoplasms were observed, all in females from the test groups. Four of these were papillary adenomas, which appeared microscopically as nodules composed of well-differentiated epithelial cells in rows, small nests, and papillae, located within or bulging from the surface of the ovary. One papillary carcinoma was observed. This neoplasm had some similarities to the papillary adenomas, but was composed of epithelial cells in solid sheets as well as papillae, with numerous mitoses. This tumor appeared to infiltrate the adjacent bursa at one point. Four granulosa-cell tumors occurred; these tumors had a prominent stromal component, but also contained more rounded cells and rosette-like structures reminiscent of Call-Exner bodies. A large, infiltrating spindle-cell neoplasm was observed in the wall of the uterus of a low-dose female mouse. This lesion was diagnosed as leiomyosarcoma based upon the densely cellular, whorling pattern and the blunt-ended nuclei seen in many neoplastic cells. Differential
diagnoses would include fibrosarcoma, other spindle cell neoplasms, and endometrial stromal sarcoma.

Only two mammary neoplasms were observed, both adenocarcinomas; one in a low-dose female and one in a high-dose female. The latter neoplasm invaded adjacent skeletal muscle.

Primary pulmonary neoplasms were observed in all test and control groups (table B7). Twenty-two of these were alveolar/bronchiolar carcinomas, and 19 were alveolar/bronchiolar adenomas. Differentiation between adenoma and carcinoma was based on the degree of anaplasia, mitotic index, size of the neoplasm, and microscopic evidence of invasion of adjacent pulmonary parenchyma (carcinoma), as opposed to mere compression of adjacent parenchyma, and thus a more discrete lesion (adenoma). Incidence of hyperplasia of pulmonary epithelium is also summarized in table B7. This lesion usually consisted of one to several small foci of alveolar epithelial-cell hyperplasia.

Miscellaneous neoplasms encountered in low incidence are summarized in table B8. Lacrimal gland adenomas were often noted grossly, and consisted of papillary proliferation of lacrimal-gland epithelium. The location of the bone involved with osteochondrosarcoma is not known. The osteoma was located in a vertebra, and was a small lesion composed of well-formed bone in
a dense trabecular arrangement, with numerous well-differentiated osteoblasts lining the trabeculae.

There were instances in this study, as noted above and in the summary tables, where neoplastic or hyperplastic lesions occurred only in test animals, or with increased frequency when compared to control groups. Particularly striking is the high incidence of hepatocellular carcinoma, especially in test female mice, when compared with controls of the same sex. These differences undoubtedly will have significance statistically. The incidence of nodular hyperplasia of the liver in the test females in the absence of this lesion in female controls is supportive evidence of an effect of the compound on the liver of the female mouse, since this lesion is considered by many to represent a precursor to neoplasia of hepatocytes. Several factors are present, however, which cast doubt upon the biologic significance of these differences in incidence. Especially significant is the unusually high incidence of primary hepatic neoplasms observed in the male control group. It is difficult to justify declaring this compound a carcinogen in female mice based on an incidence of hepatocellular carcinoma of 41-44% in exposed animals, when the incidence of the same tumor in the controls of the opposite sex is 41%. Another factor is the occurrence of hemangiosarcomas in rather high incidence in the controls as well as the test mice; these neoplasms rarely occur spontaneously in mice.
Data were obtained on another group of B6C3F1 control mice from another bioassay study (2-amino-5-nitrothiazole) which was carried out in the same room (Dr. J. L. Emerson, personal communication); these mice were procured from the same source as those used in the proflavine study, although at a different time. The incidences of hepatocellular carcinoma and hepatic hemangiosarcomas in these mice were as follows:

<table>
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<tr>
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<th>Control Males</th>
<th>Control Females</th>
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<tr>
<td>Hepatocellular Carcinoma</td>
<td>5/50 (10%)</td>
<td>1/50 (2%)</td>
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<td>Hemangiosarcoma, Liver</td>
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</table>

The unusually high incidence of these two types of neoplasms in the controls as well as test mice in the proflavine study leads one to consider several possible explanations: (1) some stimulus other than exposure to the test compound induced these lesions in both the test and control mice in the proflavine study; (2) this particular group of mice had an unusually high incidence of spontaneously occurring hepatocellular carcinoma and hepatic hemangiosarcoma; (3) the proflavine control mice were inadvertently exposed to the test compound, which did indeed induce these lesions. The latter explanation seems rather unlikely, since stringent procedures were in effect to prevent
this specific event from happening (Dr. J. L. Emerson, personal communication). In any event, unless some explanation of the inconsistency of the data is forthcoming, the high incidence of these neoplasms in the control mice would seem to preclude consideration of the data as conclusive evidence of a carcinogenic effect of proflavine on the liver of the mouse.

For a summary of the incidence of nontumor pathology in mice exposed to proflavine, see Appendix D.

D. Statistical Analyses of Results (Mice)

Table B9 contains the statistical analyses of those tumors which were observed in more than 5% of any dosed group. The male matched controls exhibited significantly higher tumor proportions than those seen in the 1,132 control mice whose results have been recorded in the Bioassay Program in tumors of the lung (19% vs. 9.2%), hematopoietic system (22% vs. 2%), liver (41% vs. 15.6%), and endocrine system (10% vs. 4%). In female mice, only the incidence of hematopoietic tumors differed statistically from the results on the entire Bioassay Program (46% vs. 6.8%). In both sexes, at all tumor sites except the liver, the proportion of tumors seen in the dosed groups were not statistically different (P > 0.05) than the control groups.

In an experiment with 2-amino-5-nitrothiazole which was made at

44
the same time in the same laboratory, the incidences of hematopoietic tumors and liver tumors in control mice were not significantly different from the combined historic controls of 1,132 B6C3F1 mice across all laboratories in the Bioassay Program. Therefore, the incidence in the controls for proflavine of the tumors listed above is higher than that expected from the experience available.

The proportions of hepatocellular carcinoma in both dosed groups of female mice have probability levels of less than 0.001 by the Fisher exact test for positive dose-related effects, and the Cochran-Armitage test for positive dose-related trend is significant ($P < 0.001$). In male mice the high-dose group differs from the matched controls ($P = 0.044$) and the Cochran-Armitage test for trend has probability level $P = 0.057$. Figure 5 shows that in both sexes of mice the observation of these hepatic tumors occurred mainly at the end of the study, and prior to that time there is no statistically significant difference between the dosed groups and their controls. In conclusion, the statistical inference is made that in female mice, there is a strong association of carcinoma of the liver with the dosage of proflavine given. In male mice, there is a slight suggestion of such a relationship shown in the high-dose group, but the data on these
Figure 5. Kaplan and Meier Curves of the Proportion of Mice Surviving Without Observed Hepatocellular Adenoma or Carcinoma (Proflavine Test)
groups are inconclusive due to the high proportion of carcinoma of the liver seen in the matched control.
V. DISCUSSION

The doses of proflavine used in this study were slightly toxic, since comparisons of average group weights (figures 1 and 3, above) show that animals on the high doses (600 ppm for rats and 400 ppm for mice) consistently gained less weight than their respective controls, while average weights of low-dose animals were indistinguishable from those of the controls. Thus, the doses which were used were high enough to provide an acceptable bioassay of carcinogenicity. The survival rates of the treated rats and mice did not differ from those of the controls except for lower survival among the female mice.

Proflavine is absorbed from the gastrointestinal tract, since (1) yellow discoloration of the urine was noted in the subchronic toxicity studies using doses of 1,000 ppm or higher and (2) in vivo staining of nuclei of mammalian cells has been reported following parenteral or oral administration (de Bruyn et al., 1951).

Five malignant neoplasms of the intestinal tract consisting of three leiomyosarcomas of the small intestine, a sarcoma near the colon area, and an adenocarcinoma of the small intestine were observed only in five of the high-dose male rats. The relatively low spontaneous incidence of gastrointestinal tract neoplasms
usually found in laboratory rats, in contrast to these data, suggests but does not provide clear evidence of a proflavine induced carcinogenic effect in these animals. This is supported by the statistical evaluation of the combined intestinal tumor. Although the $P$ value given in table A9 for these tumors is 0.026, correction for simultaneous use of controls raises the effective $P$ value above 0.05.

The results of the present study are suggestive of, but do not provide strong evidence for the carcinogenicity of proflavine in mice. Fisher exact tests for individual doses showed significant increases in proportions of tumor-bearing animals for hepatocellular carcinoma in female mice administered proflavine at concentrations of 200 ppm ($P < 0.001$) or 400 ppm ($P < 0.001$). This effect was of borderline significance in male mice given 400 ppm ($P = 0.044$). Armitage and Cochran tests for dose-related trend showed levels of significance of $P < 0.001$ for female mice, and $P = 0.057$ for male mice. However, the control mice in this bioassay had an unusually high incidence of tumors. For example, the male control mice had a 41% incidence of hepatocellular carcinoma and an unusually high incidence of hemangiosarcoma (12%), while female control mice showed an abnormally high incidence of lymphoma (46%). The unusually high incidence of these tumors may be due to the fact that the mice were housed in
the same room as those treated with a positive-control compound
N-2-fluorenylacacetamide.

Previous studies have demonstrated that proflavine has disruptive
influences on cellular processes, such as chromatid breakage in
leukocytes, fibroblasts, and HeLa cells (Ostertag and Kersten,
1965), inhibition of cellular uptake of amino acids (Birkmayer
and Balda, 1971), and aggregation of nucleoli with decrease in
their size (Recher et al., 1971). However, in vivo staining of
cell nuclei is possible without affecting the vitality of the
cells, as evidenced by lack of toxicity of doses of proflavine
used for the staining and by normal regeneration of liver in
animals treated with proflavine and subjected to partial
hepatectomy (de Bruyn et al., 1951). Work of Salaman and
Glendenning (1951) demonstrated the promoting action of repeated
intradermal injections of proflavine on tumor development at or
near sites of topical DMBA application in mice. Repeated
applications of proflavine alone to the skin, or subcutaneous
implantation of the compound, showed neither promoting nor
carcinogenic action.

Although the present bioassay may suggest that proflavine is
carcinogenic in female mice, serious questions have been
identified regarding the validity of the bioassay. These
consisted of an unusually high incidence of hepatocellular
carcinoma in the control male mice and an unusually high incidence of lymphomas in all female mouse groups including the controls. Furthermore, the positive control compound N-2-fluorenylacacetamide, was tested in the same room as proflavine and may have contributed to the findings.
VI. BIBLIOGRAPHY


SUMMARY OF THE INCIDENCE OF NEOPLASMS AND PROLIFERATIVE LESIONS IN RATS FED PROFLAVINE IN THE DIET
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<tr>
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<tr>
<td><strong>LIVER</strong></td>
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<tr>
<td>Hepatocytomegaly</td>
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<td>11/50 (22%)</td>
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<td>0/49</td>
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<tr>
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<tr>
<td>Leiomyosarcoma</td>
<td>0/41</td>
<td>0/48</td>
</tr>
<tr>
<td>Adenocarcinoma</td>
<td>0/42</td>
<td>0/48</td>
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<tr>
<td>Generalized Malignant Lymphoma*</td>
<td>10/47 (21%)</td>
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<tr>
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</tr>
<tr>
<td>Granulocytic Sarcoma/Leukemia</td>
<td>0/50 (2%)</td>
<td>1/50 (2%)</td>
</tr>
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</table>

*Tabulation of incidence of this neoplasm is divided into those animals in which the neoplasm was generalized, i.e., involved numerous organs and tissues, and those animals in which it was seen in only a few organs. Since the spleen is the organ most often involved with this lesion in the Fischer rat, the number of spleens examined microscopically was used as the denominator.
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<tr>
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<td>High Dose</td>
<td>Control</td>
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<td>0/49</td>
<td>0/50</td>
<td>1/50</td>
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<td>1/49</td>
<td>0/50</td>
<td>0/50</td>
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<td>1/49</td>
<td>1/50</td>
<td>0/50</td>
</tr>
<tr>
<td>plasia</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Renal Pelvis</td>
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<td>(2%)</td>
<td>(2%)</td>
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<td><strong>BLADDER</strong></td>
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<td>0/44</td>
<td>1/45</td>
<td>1/42</td>
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<td>Carcinoma</td>
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<td>0/44</td>
<td>2/45</td>
<td>3/42</td>
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60
### TABLE A5
PROLIFERATIVE LESIONS
RESPIRATORY TRACT

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<td><strong>LUNG</strong></td>
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<td>(2%)</td>
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<td>0/50</td>
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<td><strong>LARYNX</strong></td>
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<td>0/49</td>
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<td>----------------------</td>
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<tr>
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<td>46/49</td>
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<td>0/49</td>
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<td>(27%)</td>
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<td>(7%)</td>
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<table>
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<td>1/50</td>
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<tr>
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<td>(2%)</td>
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<td>Glioma, Brain</td>
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<tr>
<td>Granular-Cell Tumor, Brain</td>
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<td>Papilloma, Tongue</td>
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<tr>
<td>Liposarcoma, Skeletal Muscle</td>
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<td>0/50</td>
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<tr>
<td>Osteogenic Sarcoma, Bone</td>
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<td>1/50 (2%)</td>
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<tr>
<td>Heart, Metastatic Adenocarcinoma, Primary Unknown</td>
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<td>0/50 (2%)</td>
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<tr>
<td>Hemangiosarcoma, Spleen</td>
<td>0/47</td>
<td>0/50</td>
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<td>Topography: Morphology</td>
<td>Untreated Controls</td>
<td>Male Low Dose</td>
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<tr>
<td>Integumentary System: All Tumors</td>
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<td>4/50 (8)</td>
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<td>P Values</td>
<td>N.S.</td>
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<tr>
<td>First Tumor Incidence (weeks)</td>
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<td>85</td>
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<tr>
<td>Reproductive System: All Tumors</td>
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<tr>
<td>Thyroid: C-cell Adenoma or Carcinoma</td>
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<td>P Values</td>
<td>N.S.</td>
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<td>First Tumor Incidence (weeks)</td>
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<td>109</td>
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<tr>
<td>Pancreatic Islets: Adenoma or Carcinoma</td>
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<td>5/50 (10)</td>
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<td>First Tumor Incidence (weeks)</td>
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Table A9. Analyses of the Incidence of Primary Tumors at Specific Sites in Rats, Proflavine

continued

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<th>Topography: Morphology</th>
<th>Untreated Controls</th>
<th>Low Dose</th>
<th>High Dose</th>
<th>Untreated Controls</th>
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<th>High Dose</th>
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<tbody>
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<td>Hematopoietic System: All Tumors</td>
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<td>10/50(20)</td>
<td>5/50(10)</td>
<td>15/50(30)</td>
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<td>7/50(14)</td>
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<td>1/50(2)</td>
<td>3/49(6)</td>
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<td>2/50(4)</td>
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<td>--</td>
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aDosed groups received time-weighted average dose of 300 and 600 ppm in feed.
bNumber of tumor-bearing animals/number of animals examined at site (percent).
cBeneath the incidence of each of the controls is the probability level for the Armitage test for dose-related trend in proportions when it is below 0.10 and when the response of both dose groups is not zero; otherwise N.S. - not significant.
dDeparture from linear trend is noted when it is below 0.05 for any comparison.
eBeneath the dose group incidence is the probability level for the Fisher exact (conditional) test for increased proportion in that dose group compared with the untreated-control group when it is below 0.05.
fCorrection for simultaneous use of controls raises the effective P value above 0.05.
gFive intestinal tumors combined: one sarcoma N.O.S. in the colon, three leiomyosarcomas in the small intestine and one adenocarcinoma in the duodenum.
SUMMARY OF THE INCIDENCE OF NEOPLASMS AND PROLIFERATIVE LESIONS IN MICE FED PROFLAVINE IN THE DIET
## TABLE B1
PROLIFERATIVE ENDOCRINE LESIONS

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<td>High Dose</td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
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<td></td>
<td></td>
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<td></td>
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</tr>
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<td>1/39 (3%)</td>
<td>0/46</td>
<td>0/46</td>
<td>0/38</td>
<td>0/45</td>
<td>0/46</td>
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<td>Follicular-Cell Adenoma</td>
<td>2/39 (5%)</td>
<td>1/46</td>
<td>0/46</td>
<td>1/38 (3%)</td>
<td>2/45</td>
<td>1/46 (2%)</td>
</tr>
<tr>
<td>Follicular-Cell Hyperplasia</td>
<td>2/39 (5%)</td>
<td>2/46</td>
<td>2/46</td>
<td>5/38 (13%)</td>
<td>10/45</td>
<td>4/46 (9%)</td>
</tr>
<tr>
<td>PARATHYROID</td>
<td></td>
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<tr>
<td>Hyperplasia</td>
<td>0/15</td>
<td>0/27</td>
<td>1/31 (3%)</td>
<td>1/12</td>
<td>0/32</td>
<td>0/31</td>
</tr>
<tr>
<td>Adenoma</td>
<td>0/15</td>
<td>0/27</td>
<td>1/31 (3%)</td>
<td>0/12</td>
<td>0/32</td>
<td>0/31</td>
</tr>
<tr>
<td>PITUITARY</td>
<td></td>
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<tr>
<td>Chromophobe Adenoma</td>
<td>0/36</td>
<td>0/13</td>
<td>0/15 (9%)</td>
<td>3/34 (9%)</td>
<td>2/24</td>
<td>2/8 (25%)</td>
</tr>
<tr>
<td>ADRENAL</td>
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<td></td>
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</tr>
<tr>
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<td>2/45 (4%)</td>
<td>1/46 (2%)</td>
<td>0/50</td>
<td>0/45</td>
<td>1/49 (2%)</td>
<td>0/48</td>
</tr>
<tr>
<td>Cortical Carcinoma</td>
<td>0/45</td>
<td>0/46</td>
<td>0/50</td>
<td>0/45</td>
<td>0/49</td>
<td>1/48 (2%)</td>
</tr>
<tr>
<td>Carcinoma, N.O.S.</td>
<td>0/45</td>
<td>0/46</td>
<td>0/50</td>
<td>0/45</td>
<td>1/49 (2%)</td>
<td>0/48</td>
</tr>
<tr>
<td>PANCREAS</td>
<td>Islet-Cell Hyperplasia</td>
<td>3/48 (6%)</td>
<td>9/44 (20%)</td>
<td>2/44 (5%)</td>
<td>0/44</td>
<td>0/46</td>
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TABLE B2
PROLIFERATIVE LESIONS
DIGESTIVE SYSTEM

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<td>High Dose</td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
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<tr>
<td><strong>LIVER</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular</td>
<td>20/49</td>
<td>28/49</td>
<td>30/50</td>
<td>4/50</td>
<td>20/49</td>
<td>22/50</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>(41%)</td>
<td>(57%)</td>
<td>(60%)</td>
<td>(8%)</td>
<td>(41%)</td>
<td>(44%)</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>6/49</td>
<td>6/49</td>
<td>3/50</td>
<td>1/50</td>
<td>0/49</td>
<td>2/50</td>
</tr>
<tr>
<td></td>
<td>(12%)</td>
<td>(12%)</td>
<td>(6%)</td>
<td>(2%)</td>
<td></td>
<td>(4%)</td>
</tr>
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<td>26/49</td>
<td>34/49</td>
<td>33/50</td>
<td>5/50</td>
<td>20/49</td>
<td>24/50</td>
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<tr>
<td></td>
<td>(53%)</td>
<td>(69%)</td>
<td>(66%)</td>
<td>(10%)</td>
<td>(41%)</td>
<td>(48%)</td>
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<tr>
<td>Nodular Hepatocyte Hyperplasia</td>
<td>3/49</td>
<td>4/49</td>
<td>4/50</td>
<td>0/50</td>
<td>8/49</td>
<td>9/50</td>
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<tr>
<td></td>
<td>(6%)</td>
<td>(8%)</td>
<td>(8%)</td>
<td></td>
<td>(16%)</td>
<td>(18%)</td>
</tr>
<tr>
<td>Hepatocytomegaly</td>
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<td>1/50</td>
<td>1/50</td>
<td>0/49</td>
<td>0/50</td>
</tr>
<tr>
<td></td>
<td>(10%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td></td>
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</tr>
<tr>
<td>Angiectasis</td>
<td>1/49</td>
<td>10/49</td>
<td>8/50</td>
<td>2/50</td>
<td>4/49</td>
<td>5/50</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td>(20%)</td>
<td>(16%)</td>
<td>(4%)</td>
<td>(8%)</td>
<td>(10%)</td>
</tr>
<tr>
<td></td>
<td>(4%)</td>
<td>(18%)</td>
<td>(12%)</td>
<td>(10%)</td>
<td>(20%)</td>
<td>(6%)</td>
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</tr>
<tr>
<td>Hyperplasia, Glandular mucosa</td>
<td>0/47</td>
<td>2/46</td>
<td>0/48</td>
<td>0/45</td>
<td>1/49</td>
<td>0/45</td>
</tr>
<tr>
<td>Hyperplasia, Squamous mucosa</td>
<td>0/47</td>
<td>1/46</td>
<td>1/48</td>
<td>0/45</td>
<td>0/49</td>
<td>1/45</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>1/47</td>
<td>0/46</td>
<td>1/48</td>
<td>1/45</td>
<td>0/49</td>
<td>0/45</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td></td>
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</tr>
<tr>
<td><strong>SMALL INTESTINE</strong></td>
<td>Papillary Adenoma</td>
<td>0/46</td>
<td>0/41</td>
<td>0/45</td>
<td>0/44</td>
<td>0/44</td>
</tr>
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70
### TABLE B3
HEMATOPOIETIC NEOPLASMS

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</thead>
<tbody>
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<td>Low Dose</td>
<td>High Dose</td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
</tr>
<tr>
<td>Generalized Malignant Lymphoma*</td>
<td>2/50</td>
<td>9/50 (4%)</td>
<td>3/50 (6%)</td>
<td>15/50</td>
<td>3/50 (20%)</td>
<td>13/50 (26%)</td>
</tr>
<tr>
<td>Malignant Lymphoma*</td>
<td>9/50 (18%)</td>
<td>2/50 (4%)</td>
<td>10/50 (20%)</td>
<td>8/50 (16%)</td>
<td>8/50 (16%)</td>
<td>7/50 (14%)</td>
</tr>
<tr>
<td>Generalized Granulocytic Sarcoma</td>
<td>1/50 (2%)</td>
<td>0/50</td>
<td>0/50</td>
<td>1/50 (2%)</td>
<td>0/50</td>
<td>0/50</td>
</tr>
<tr>
<td>Mast-Cell Sarcoma</td>
<td>1/50 (2%)</td>
<td>0/50</td>
<td>0/50</td>
<td>1/50 (2%)</td>
<td>0/50</td>
<td>0/50</td>
</tr>
</tbody>
</table>

*Tabulation of incidence of this neoplasm is divided into those animals in which the neoplasm was generalized, i.e., involved numerous organs, and those animals in which it was observed in only a few organs. The number of animals necropsied was used as the denominator.

### TABLE B4
PROLIFERATIVE LESIONS
URINARY TRACT

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<tr>
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<th>FEMALE MICE</th>
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<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
</tr>
<tr>
<td>KIDNEY</td>
<td>Tubular-Cell Carcinoma</td>
<td>0/50</td>
<td>0/49</td>
<td>0/47</td>
<td>0/49</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>BLADDER</td>
<td>Epithelial Hyperplasia</td>
<td>4/42 (10%)</td>
<td>3/43 (7%)</td>
<td>0/39</td>
<td>2/38 (5%)</td>
<td>2/40 (5%)</td>
</tr>
<tr>
<td>URETHRA**</td>
<td>Epithelial Hyperplasia</td>
<td>2/50 (4%)</td>
<td>0/50</td>
<td>0/50</td>
<td>0/49</td>
<td>0/50</td>
</tr>
</tbody>
</table>

**Samples of urethra are not routinely examined histologically; therefore the number of mice from the group necropsied was used as the denominator.
<table>
<thead>
<tr>
<th></th>
<th>MALE MICE</th>
<th>FEMALE MICE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low Dose</td>
</tr>
<tr>
<td>LIVER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma*</td>
<td>6/49</td>
<td>6/49</td>
</tr>
<tr>
<td></td>
<td>(12%)</td>
<td>(12%)</td>
</tr>
<tr>
<td>SPLEEN</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>2/46</td>
<td>1/45</td>
</tr>
<tr>
<td></td>
<td>(4%)</td>
<td>(2%)</td>
</tr>
<tr>
<td>SUBCUTIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>1/50</td>
<td>0/50</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td></td>
</tr>
<tr>
<td>Hemangioma</td>
<td>0/50</td>
<td>0/50</td>
</tr>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>HEART</td>
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</tr>
<tr>
<td>Sarcoma, N.O.S.</td>
<td>0/48</td>
<td>1/49</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td></td>
</tr>
<tr>
<td>Hemangioma</td>
<td>1/48</td>
<td>0/49</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td></td>
</tr>
<tr>
<td>BONE MARROW</td>
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<tr>
<td>Hemangiosarcoma</td>
<td>0/48</td>
<td>1/49</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
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</table>

*Incidence of hepatic hemangiosarcoma is also tabulated in table B2.
### TABLE B6
**GENITAL AND MAMMARY NEOPLASMS**

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</tr>
</thead>
<tbody>
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<td></td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
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</tr>
<tr>
<td><strong>OVARY</strong></td>
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<td></td>
</tr>
<tr>
<td>Papillary Carcinoma</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>0/43 (2%)</td>
</tr>
<tr>
<td>Granulosa-Cell Tumor</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>2/42 (4%), 2/43 (5%)</td>
</tr>
<tr>
<td>Papillary Adenoma</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>2/42 (5%), 2/43 (4%)</td>
</tr>
<tr>
<td>Total Primary</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>5/42 (12%), 4/43 (9%)</td>
</tr>
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<td>Ovarian Tumors</td>
<td>0/43</td>
<td>5/42 (12%)</td>
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<td><strong>UTERUS</strong></td>
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<tr>
<td>Leiomyosarcoma</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>1/46 (2%)</td>
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<tr>
<td>Adenocarcinoma</td>
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<td>0/50</td>
<td>0/50</td>
<td>0/49 (2%), 1/50 (2%)</td>
</tr>
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</table>

### TABLE B7
**PROLIFERATIVE LESIONS**
**RESPIRATORY SYSTEM**

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<thead>
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<th><strong>FEMALE MICE</strong></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Low Dose</td>
<td>High Dose</td>
<td></td>
</tr>
<tr>
<td><strong>LUNG</strong></td>
<td></td>
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</tr>
<tr>
<td>Alveolar/Bronchiolar Carcinoma</td>
<td>4/48 (8%)</td>
<td>6/50 (12%)</td>
<td>6/50 (12%)</td>
<td>0/49 2/50 (4%), 4/49 (8%)</td>
</tr>
<tr>
<td>Alveolar/Bronchiolar Adenoma</td>
<td>5/48 (10%)</td>
<td>5/50 (10%), 2/50 (4%)</td>
<td></td>
<td>4/49 1/50 (2%), 2/49 (4%)</td>
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<tr>
<td>Sarcoma, N.O.S.</td>
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<td>1/50</td>
<td>0/50</td>
<td>0/49 0/50 (2%)</td>
</tr>
<tr>
<td>Alveolar/Bronchiolar Hyperplasia</td>
<td>2/48 (4%)</td>
<td>6/50 (12%), 5/50 (10%)</td>
<td></td>
<td>0/49 1/50 (2%)</td>
</tr>
</tbody>
</table>

73
<table>
<thead>
<tr>
<th></th>
<th>MALE MICE</th>
<th></th>
<th>FEMALE MICE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
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<td>High Dose</td>
<td>Control</td>
</tr>
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<td><strong>FAT</strong></td>
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<td>0/50</td>
<td>0/50</td>
<td>0/49</td>
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</tr>
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<td>1/50</td>
<td>0/50</td>
<td>1/49</td>
</tr>
<tr>
<td></td>
<td>(2%)</td>
<td>(2%)</td>
<td>(2%)</td>
<td>(2%)</td>
</tr>
<tr>
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<td>0/50</td>
<td>0/49</td>
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<tr>
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<td>(2%)</td>
<td></td>
<td>(2%)</td>
<td></td>
</tr>
<tr>
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<td>0/50</td>
<td>0/49</td>
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<td>0/50</td>
<td>1/50</td>
<td>0/49</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(2%)</td>
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</tr>
<tr>
<td><strong>SUBCUTIS</strong></td>
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<td>0/50</td>
<td>1/50</td>
<td>0/49</td>
</tr>
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<td>(2%)</td>
<td></td>
</tr>
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<td>1/50</td>
<td>0/50</td>
<td>0/49</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
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<td>0/50</td>
<td>0/50</td>
<td>1/50</td>
<td>0/49</td>
</tr>
<tr>
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<td></td>
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<td>(2%)</td>
<td></td>
</tr>
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<td>Topography: Morphology</td>
<td>Untreated Controls</td>
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<td>High Dose</td>
<td>Untreated Controls</td>
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<tr>
<td>------------------------</td>
<td>-------------------</td>
<td>----------</td>
<td>----------</td>
<td>-------------------</td>
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<tr>
<td>Lung: Alveolar/Bronchiolar Adenoma or Carcinoma⁵</td>
<td>9/48(19)</td>
<td>11/49(22)</td>
<td>8/49(16)</td>
<td>4/49(8)</td>
</tr>
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<td>N.S.</td>
<td>N.S.</td>
</tr>
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<td>46</td>
<td>86</td>
<td>77</td>
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<tr>
<td>Hematopoietic System: Lymphomas⁷</td>
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<td>11/50(22)</td>
<td>13/50(26)</td>
<td>23/50(46)</td>
</tr>
<tr>
<td>P Values⁸</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
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<td>First Tumor Incidence (weeks)</td>
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<td>44</td>
<td>55</td>
<td>51</td>
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<tr>
<td>Liver: Hepatocellular Adenoma or Carcinoma⁷</td>
<td>20/49(41)</td>
<td>28/49(57)</td>
<td>30/50(60)</td>
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<tr>
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<td>78</td>
<td>64</td>
<td>47</td>
<td>103</td>
</tr>
<tr>
<td>Any Site: Hemangiosarcoma⁷</td>
<td>9/50(18)</td>
<td>8/50(16)</td>
<td>8/50(16)</td>
<td>2/50(4)</td>
</tr>
<tr>
<td>P Values⁸</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
<td>N.S.</td>
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<tr>
<td>First Tumor Incidence (weeks)</td>
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<td>95</td>
<td>98</td>
<td>103</td>
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<tr>
<td>Endocrine System: All Tumors⁷</td>
<td>5/49(10)</td>
<td>3/49(6)</td>
<td>1/50(2)</td>
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<tr>
<td>P Values⁸</td>
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<td>104</td>
<td>104</td>
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<td>Reproductive System: All Tumors⁷</td>
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<td>0/50(0)</td>
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<td>N.S.</td>
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⁵Untreated controls, dosed groups received time-weighted average dose of 200 and 400 ppm in feed.
⁶Number of tumor-bearing animals/number of animals examined at site (percent).
⁷Beneath the incidence of each of the controls is the probability level for the Armitage test for dose-related trend in proportions when it is below 0.10, otherwise N.S. = not significant. Departure from linear trend is noted when it is below 0.05 for any comparison.
⁸Beneath the dose group incidence is the probability level for the Fisher exact (conditional) test for increased proportion in that dose group compared with the untreated control group when it is below 0.05.
APPENDIX C

SUMMARY OF THE INCIDENCE OF NONTUMOR PATHOLOGY IN RATS FED PROFLAVINE IN THE DIET
TABLE C1
SUMMARY OF THE INCIDENCE OF NONTUMOR PATHOLOGY IN MALE RATS TREATED WITH PROFLAVINE

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<tbody>
<tr>
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<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
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<td>50 (100%)</td>
<td>50 (100%)</td>
<td>50 (100%)</td>
</tr>
<tr>
<td>ANIMALS EXAMINED HISTOPATHOLOGICALLY</td>
<td>50</td>
<td>50</td>
<td>50</td>
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<tr>
<td>ANIMALS WITH NON-TUMOR PATHOLOGY</td>
<td>50 (100%)</td>
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**INTEGUMENTARY SYSTEM**

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**RESPIRATORY SYSTEM**

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**HEMATOPOIETIC SYSTEM**

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<td>4</td>
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*System percentages are based on the number of animals necropsied.*
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**Urinary System**

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<td>47 (94%)</td>
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<td>47</td>
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*System percentages are based on the number of animals necropsied.*
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*System percentages are based on the number of animals necropsied
TABLE C1 MALE RATS: NONTUMOR PATHOLOGY (CONT.)

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<tr>
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<tr>
<td></td>
<td>37 (74%)</td>
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<td>46 (92%)</td>
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<tr>
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<tr>
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*System percentages are based on the number of animals necropsied.

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<td>Body Cavities</td>
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<td>All Other Systems</td>
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<td>Necrosis Fat</td>
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SPECIAL MORPHOLOGY SUMMARY

None
### Table C2

**Summary of the Incidence of Nontumor Pathology in Female Rats Treated with Proflavine**

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<td>50</td>
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<td>50 (100%)</td>
<td>50 (100%)</td>
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<td><strong>Animals Examined Histopathologically</strong></td>
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<td>50</td>
<td>50</td>
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<tr>
<td><strong>Animals with Non-Tumor Pathology</strong></td>
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<td>50 (100%)</td>
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#### Integumentary System

- **Skin**
  - Lacerated Wound: 1
  - Inflammation Suppurative: 1

#### Respiratory System

- **Trachea**
  - Inflammation Suppurative: 20
  - Inflammation Chronic: 5

- **Lung/Bronchus**
  - Bronchiectasis: 1

- **Lung/Bronchiole**
  - Foreign Body: 1
  - Inflammation Suppurative: 3

- **Lung**
  - Congestion: 22
  - Edema: 7
  - Hemorrhage: 4
  - Inflammation: 1
  - Abscess: 2
  - Alveolar Macrophages: 12
  - Hyperplasia Alveolar-Cell: 3

#### Hematopoietic System

- **Blood**
  - Leukocytosis: 1

*System percentages are based on the number of animals necropsied.*
TABLE C2 FEMALE RATS: NONTUMOR PATHOLOGY (CONT.)

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<tr>
<td>HEART/PERICARDIUM</td>
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<tr>
<td>THROMBOSIS</td>
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<td>7</td>
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*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED
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*System percentages are based on the number of animals necropsied.*
TABLE C2 FEMALE RATS: NONTUMOR PATHOLOGY (CONT.)

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*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED.
TABLE C2 FEMALE RATS: NONTUMOR PATHOLOGY (CONT.)

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*System percentages are based on the number of animals necropsied.
SUMMARY OF THE INCIDENCE OF NONTUMOR PATHOLOGY IN MICE FED PROFLAVINE IN THE DIET
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<td>50</td>
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<td>50 (100%)</td>
<td>50 (100%)</td>
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<td>50</td>
<td>50</td>
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<tr>
<td><strong>ANIMALS WITH NON-TUMOR PATHOLOGY</strong></td>
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<td>44 (88%)</td>
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**INTEGUMENTARY SYSTEM***

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**RESPIRATORY SYSTEM**

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**HEMATOPOIETIC SYSTEM**

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<th>5 (10%)</th>
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**SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED**
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<td>4</td>
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*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED.
### Table D1 Male Mice: Nontumor Pathology (Cont.)

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<tr>
<td>Hyperplasia epithelial</td>
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<tr>
<td><strong>Bile duct</strong></td>
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<tr>
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**Urinary system***

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*System percentages are based on the number of animals necropsied.

95
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*System Percentages are based on the number of animals necropsied.
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<td>26</td>
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*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED
### TABLE D1 MALE MICE: NONTUMOR PATHOLOGY (CONT.)

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**SPECIAL MORPHOLOGY SUMMARY**

| AUTO/NECROPSY PERF/HISTO PERF | 2 | 2 |

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*System percentages are based on the number of animals necropsied
TABLE D2 FEMALE MICE: NONTUMOR PATHOLOGY (CONT.)

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CIRCULATORY SYSTEM*

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DIGESTIVE SYSTEM

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*SYSTEM PERCENTAGES ARE BASED ON THE NUMBER OF ANIMALS NECROPSIED

100
TABLE D2 FEMALE MICE: NONTUMOR PATHOLOGY (CONT.)

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<td>INFLAMMATION FOCAL</td>
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*System percentages are based on the number of animals necropsied.*

102
<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>LOW DOSE</th>
<th>HIGH DOSE</th>
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<tbody>
<tr>
<td>GRANULATION TISSUE</td>
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<tr>
<td>EYE/LACRIMAL GLAND</td>
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<tr>
<td>HYPERPLASIA EPITHELIAL</td>
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<tr>
<td>MUSCULOSKELETAL SYSTEM*</td>
<td>1 (2%)</td>
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<tr>
<td>SKELETAL MUSCLE</td>
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<tr>
<td>PERIVASCULAR CUFFING</td>
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<tr>
<td>BODY CAVITIES</td>
<td>NONE</td>
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<tr>
<td>ALL OTHER SYSTEMS</td>
<td>5 (10%)</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
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<tr>
<td>MULTIPLE ORGANS</td>
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<td>LYMPHOID HYPERPLASIA</td>
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<tr>
<td>MESENTERY</td>
<td>2</td>
<td>4</td>
<td>1</td>
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<td>CYST</td>
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<td>NECROSIS FAT</td>
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