FOREWORD

The National Toxicology Program (NTP) is an interagency program within the Public Health Service (PHS) of the Department of Health and Human Services (HHS) and is headquartered at the National Institute of Environmental Health Sciences of the National Institutes of Health (NIEHS/NIH). Three agencies contribute resources to the program: NIEHS/NIH, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention (NIOSH/CDC), and the National Center for Toxicological Research of the Food and Drug Administration (NCTR/FDA). Established in 1978, the NTP is charged with coordinating toxicological testing activities, strengthening the science base in toxicology, developing and validating improved testing methods, and providing information about potentially toxic substances to health regulatory and research agencies, scientific and medical communities, and the public.

The Technical Report series began in 1976 with carcinogenesis studies conducted by the National Cancer Institute. In 1981, this bioassay program was transferred to the NTP. The studies described in the Technical Report series are designed and conducted to characterize and evaluate the toxicologic potential, including carcinogenic activity, of selected substances in laboratory animals (usually two species, rats and mice). Substances selected for NTP toxicity and carcinogenicity studies are chosen primarily on the basis of human exposure, level of production, and chemical structure. The interpretive conclusions presented in NTP Technical Reports are based only on the results of these NTP studies. Extrapolation of these results to other species, including characterization of hazards and risks to humans, requires analyses beyond the intent of these reports. Selection per se is not an indicator of a substance’s carcinogenic potential.

The NTP conducts its studies in compliance with its laboratory health and safety guidelines and FDA Good Laboratory Practice Regulations and must meet or exceed all applicable federal, state, and local health and safety regulations. Animal care and use are in accordance with the Public Health Service Policy on Humane Care and Use of Animals. Studies are subjected to retrospective quality assurance audits before being presented for public review.

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NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDIES OF ANDROSTENEDIONE

(CAS NO. 63-05-8)

IN F344/N RATS AND B6C3F1 MICE

(GAVAGE STUDIES)

NATIONAL TOXICOLOGY PROGRAM
P.O. Box 12233
Research Triangle Park, NC  27709

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National Institutes of Health
Public Health Service
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
CONTRIBUTORS

National Toxicology Program
Evaluated and interpreted results and reported findings
C.R. Blystone, Ph.D., Study Scientist
S.A. Elmore, D.V.M., M.S., Study Pathologist
J.B. Bishop, Ph.D.
D.W. Bristol, Ph.D.
J.R. Bucher, Ph.D.
R.S. Chhabra, Ph.D.
P.M. Foster, Ph.D.
R.A. Herbert, D.V.M., Ph.D.
M.J. Hooth, Ph.D.
A.P. King-Herbert, D.V.M.
G.E. Kissling, Ph.D.
D.E. Malarkey, D.V.M., Ph.D.
J.H. Roycroft, Ph.D.
J.M. Sanders, Ph.D.
C.S. Smith, Ph.D.
G.S. Travlos, D.V.M.
N.J. Walker, Ph.D.
K.L. Witt, M.S.

Battelle Columbus Operations
Conducted 2-week studies and evaluated pathology findings
M.R. Hejtmancik, Ph.D., Principal Investigator
D.K. Gerken, D.V.M., Ph.D.

Southern Research Institute
Conducted 3-month and 2-year studies and evaluated pathology findings
C.D. Hébert, Ph.D., Principal Investigator
J.E. Heath, D.V.M.
R.B. Thompson, D.V.M., Ph.D.

Experimental Pathology Laboratories, Inc.
Provided pathology review
M.H. Hamlin, II, D.V.M., Principal Investigator
E.T. Adams, D.V.M., Ph.D.
H.M. Kolenda-Roberts, D.V.M., Ph.D.

TherImmune Research Corporation
Provided SMVCE analysis
G.W. Wolfe, Ph.D., Principal Investigator
H.S. Seung, M.S.

Dynamic Corporation
Prepared quality assessment audits
S. Brecher, Ph.D., Principal Investigator
S. Iyer, B.S.
V.S. Tharakan, D.V.M.

NTP Pathology Working Group
Evaluated slides and contributed to pathology report on rats
(October 23, 2007)
L.H. Kooistra, D.V.M., Ph.D., Coordinator
Pathology Associates International, A Charles River Company
M.F. Cesta, D.V.M.
National Toxicology Program
D. Dixon, D.V.M., Ph.D.
National Toxicology Program
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
H.M. Kolenda-Roberts, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
R.R. Maronpot, D.V.M.
Consultant
J.B. Nold, D.V.M.
GlaxoSmithKline
B.P. Singh, D.V.M., M.S.
National Toxicology Program
NTP Pathology Working Group
Evaluated slides and contributed to pathology report on mice
(July 26 and September 18, 2007)

L.H. Kooistra, D.V.M., Ph.D., Coordinator
Pathology Associates International, A Charles River Company
E.T. Adams, D.V.M., Ph.D.
Experimental Pathology Laboratories, Inc.
M.F. Cesta, D.V.M.
National Toxicology Program
J.M. Cullen, V.M.D., Ph.D.
North Carolina State University
S.A. Elmore, D.V.M., M.S.
National Toxicology Program
G.P. Flake, M.D.
National Toxicology Program
R.A. Herbert, D.V.M., Ph.D.
National Toxicology Program
T. Koujitani, D.V.M., Ph.D.
National Toxicology Program
D.E. Malarkey, D.V.M., Ph.D.
National Toxicology Program
J.B. Nold, D.V.M.
GlaxoSmithKline

SRA International, Inc.
Provided statistical analyses

P.W. Crockett, Ph.D., Principal Investigator
L.J. Betz, M.S.
K.P. McGowan, M.B.A.

Biotechnical Services, Inc.
Prepared Technical Report

S.R. Gunnels, M.A., Principal Investigator
L.M. Harper, B.S.
P.C. Rathman, B.S.E.
D.C. Serbus, Ph.D.
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SUMMARY

Background
Androstenedione is a natural androgen steroid hormone that is synthesized in men and women. Commercially it is used as an intermediate in the production of other steroids including oral contraceptives and anti-inflammatory products. Until its over-the-counter sales were banned in 2004, androstenedione was marketed as a supplement to aid athletes in gaining muscle mass. We studied the effects of androstenedione on male and female rats and mice to identify potential toxic or cancer-related hazards.

Methods
We deposited androstenedione dissolved in methylcellulose solutions through a tube directly into the stomach to groups of 50 male and female rats and mice for two years. Male and female rats and male mice received 10, 20, or 50 milligrams of androstenedione per kilogram of body weight each day; female mice received 2,10, or 50 mg/kg. Control animals received methylcellulose solutions with no chemical added by the same method. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
A few adenomas and one carcinoma of the lung were seen in male rats receiving androstenedione and there was a slight increase in the rate of mononuclear cell leukemia in exposed female rats. Male and female mice given androstenedione had marked increases in a variety of liver tumors, including adenomas, carcinomas and hepatoblastomas. There were also increases in the rates of pancreatic islet adenomas in male and female mice. Female rats also had increased rates of hyperplasia of the pancreatic islets and atrophy of the exocrine pancreas. Female mice had very marked increases in the rates of hyperplasia of the clitoral gland, metaplasia in the kidney, and cytoplasmic alteration of the salivary gland.

Conclusions
We conclude that androstenedione caused liver cancer and pancreatic islet cancer in male and female mice. The occurrence of lung tumors in male rats and mononuclear cell leukemia in female rats may have been related to androstenedione exposure. Increases in nonneoplastic lesions of the pancreas in female rats and of the clitoral gland, kidney, and salivary gland in female mice were attributed to androstenedione exposure.
ABSTRACT

Androstenedione is an androgen steroid that is normally synthesized within men and women and may be metabolized to a more potent androgen or estrogen hormone. It was nominated to the National Toxicology Program for study due to concern for adverse health effects associated with its chronic use as a dietary supplement by athletes (prior to the banning of its over the counter sales). In order to evaluate its subchronic and chronic toxicity, male and female F344/N rats and B6C3F1 mice were administered androstenedione (98% pure) by gavage for 2 weeks, 3 months, or 2 years. Genetic toxicology studies were conducted in Salmonella typhimurium, Escherichia coli, rat bone marrow cells, and mouse peripheral blood erythrocytes.

2-WEEK STUDY IN RATS

Groups of five male and five female rats were administered 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for 12 days. All rats survived to the end of the study, and the mean body weights of dosed groups were similar to those of the vehicle control groups. The development of cytoplasmic vacuoles within centrilobular hepatocytes in male rats was the only treatment-related effect observed.

2-WEEK STUDY IN MICE

Groups of five male and five female mice were administered 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for 12 days. One vehicle control female, one 20 mg/kg female, and one 50 mg/kg female died early due to gavage accidents. There were no significant chemical-related histopathological or mean body weight changes.

3-MONTH STUDY IN RATS

Groups of 10 male and 10 female core study rats were administered 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for 14 weeks; additional groups of 10 male and 10 female clinical pathology study rats received the same doses for 23 days. All
rats survived to the end of the study. The mean body weights of the 20 mg/kg female group was significantly greater than those of the vehicle control group and there was significant increased weight gain in the 1, 20, and 50 mg/kg female groups. Female thymus weights were significantly increased in the 20 and 50 mg/kg groups, which may be related to the increase in mean body weight. The numbers of sperm per mg cauda epididymis in the 10, 20, and 50 mg/kg male groups and the total number of sperm per cauda epididymis in 50 mg/kg males were significantly less than those of the vehicle controls. No treatment-related histological lesions were observed in males or females.

3-MONTH STUDY IN MICE
Groups of 10 male and 10 female mice were administered 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for 14 weeks. Except for one 10 mg/kg female that died early due to a dosing accident, all mice survived to the end of the study. The mean body weights of dosed groups were similar to those of the vehicle control groups. The number of spermatids per mg testis and the total number of spermatids per testis in 20 mg/kg males were significantly greater than those of the vehicle controls. Sperm motility in 50 mg/kg males was significantly lower than that in the vehicle controls.

The incidences of x-zone atrophy of the adrenal cortex, an androgen-sensitive endpoint, were significantly increased in females administered 5 mg/kg or greater. There were also significant decreases in the incidences of x-zone cytoplasmic vacuolization in 20 and 50 mg/kg females. The incidences of bone marrow hyperplasia were significantly increased in 5 and 50 mg/kg males.

2-YEAR STUDY IN RATS
Groups of 50 male and 50 female rats were administered 0, 10, 20, or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for at least 104 weeks. Survival of 10 mg/kg males was significantly greater than that of the vehicle controls. The mean body weights of 20 and 50 mg/kg females were greater than those of the vehicle controls after weeks 17 and 9, respectively.

The incidences of mononuclear cell leukemia were significantly increased in 20 and 50 mg/kg females and significantly decreased in 20 and 50 mg/kg males.

Incidence of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in 20 mg/kg males.

The incidence of testicular interstitial cell adenoma (including bilateral) was significantly decreased in 50 mg/kg males. In females, the incidences of mammary gland fibroadenoma were significantly decreased in the 20 and 50 mg/kg groups, the incidences of mammary gland hyperplasia were significantly decreased in all dosed groups, and the incidences of mammary gland cyst were significantly decreased in the 10 and 50 mg/kg groups.

In the liver of males, the incidences of basophilic focus in all dosed groups, the incidence of clear cell focus in the 20 mg/kg group, and the incidence of eosinophilic focus in the 50 mg/kg group were significantly increased.

The incidences of pancreatic islet hyperplasia and atrophy of the exocrine pancreas were significantly increased in 50 mg/kg females.

2-YEAR STUDY IN MICE
Groups of 50 male and 50 female mice were administered 0, 2 (females only), 10, 20 (males only), or 50 mg androstenedione/kg body weight in a 0.5% aqueous methylcellulose solution by gavage, 5 days per week for at least 104 weeks. Survival of dosed groups was similar to that of the vehicle control groups. Mean body weights of 10 and 50 mg/kg females were generally less than those of the vehicle controls after weeks 81 and 17, respectively.

The incidences of hepatocellular adenoma in males and females were significantly increased in the 50 mg/kg groups. In females, the incidences of hepatocellular carcinoma were significantly increased in all dosed groups. Incidences of hepatocellular adenoma or carcinoma (combined) in males and females were significantly increased in the 50 mg/kg groups. Incidences of hepatoblastoma were marginally increased in dosed males.
Incidences of multiple hepatocellular adenomas and carcinomas were significantly increased in 10 and 50 mg/kg males, and there was an increased incidence of multiple hepatocellular adenomas in 50 mg/kg females. The incidence of eosinophilic focus was significantly increased in 50 mg/kg males, and the incidences of mixed cell focus and cytoplasmic vacuolization were significantly increased in 50 mg/kg females.

There was a marginally increased incidence of pancreatic islet adenoma in 50 mg/kg males and in 10 and 50 mg/kg females, with an earlier day of first incidence in males. The incidences of clitoral gland hyperplasia and clitoral gland duct dilatation were significantly increased in 10 and 50 mg/kg females. The incidence of glomerular metaplasia of the kidney was significantly increased in 50 mg/kg females, and the incidences of cytoplasmic alteration of the submandibular salivary gland were significantly increased in all dosed female groups. The increased incidences of cytoplasmic alteration of the submandibular salivary gland and glomerular metaplasia of the kidney in female mice indicated a masculinizing effect from androstenedione treatment.

In 50 mg/kg females, the incidence of malignant lymphoma was significantly decreased.

**Genetic Toxicology**
Androstenedione was not mutagenic in either of two independent bacterial mutation assays conducted with and without exogenous metabolic activation. No significant increases in the frequencies of micronucleated polychromatic erythrocytes, indicators of chromosomal damage, were observed in bone marrow of male rats administered androstenedione by gavage once daily for 3 consecutive days. Results of a peripheral blood erythrocyte micronucleus test in mice, in which androstenedione was administered by gavage for 3 months, were negative in males but judged to be equivocal in females due to a small increase (twofold over background) in micronucleated normochromatic erythrocytes observed at the highest dose administered (50 mg/kg).

**Conclusions**
Under the conditions of these 2-year gavage studies, there was equivocal evidence of carcinogenic activity* of androstenedione in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was equivocal evidence of carcinogenic activity of androstenedione in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was clear evidence of carcinogenic activity of androstenedione in male B6C3F1 mice based on increased incidences of multiple hepatocellular adenoma and hepatocellular carcinoma and increased incidence of hepatoblastoma. There was clear evidence of carcinogenic activity of androstenedione in female B6C3F1 mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma. Increased incidences of pancreatic islet adenoma in male and female mice were also considered chemical related.

Androstenedione administration caused increased incidences in nonneoplastic lesions of the liver in male and female rats and mice; pancreatic islets and exocrine pancreas of female rats; and clitoral gland, kidney, and submandibular salivary gland of female mice. Decreases in the incidences of testicular interstitial cell adenoma in male rats, mammary gland fibroadenoma, cysts, and hyperplasia in female rats, and malignant lymphoma in female mice were considered related to androstenedione administration.

*Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.*
## Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Male F344/N Rats</th>
<th>Female F344/N Rats</th>
<th>Male B6C3F1 Mice</th>
<th>Female B6C3F1 Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Doses in methylcellulose solution by gavage</strong></td>
<td>0, 10, 20, or 50 mg/kg</td>
<td>0, 10, 20, or 50 mg/kg</td>
<td>0, 10, 20, or 50 mg/kg</td>
<td>0, 2, 10, or 50 mg/kg</td>
</tr>
<tr>
<td><strong>Body weights</strong></td>
<td>Dosed groups similar to the vehicle control group</td>
<td>20 mg/kg group 6% greater than the vehicle control group after week 17; 50 mg/kg group 7% greater than the vehicle control group after week 9</td>
<td>Dosed groups similar to the vehicle control group</td>
<td>10 mg/kg group 7% less than the vehicle control group after week 81; 50 mg/kg group 7% less than the vehicle control group after week 17</td>
</tr>
<tr>
<td><strong>Survival rates</strong></td>
<td>21/50, 33/50, 29/50, 27/50</td>
<td>38/50, 37/50, 33/50, 37/50</td>
<td>36/50, 44/50, 34/50, 37/50</td>
<td>35/50, 40/50, 40/50, 40/50</td>
</tr>
<tr>
<td><strong>Nonneoplastic effects</strong></td>
<td>Liver: basophilic focus (17/50, 29/50, 29/50, 33/50); clear cell focus (13/50, 21/50, 23/50, 17/50); eosinophilic focus (3/50, 10/50, 7/50, 13/50)</td>
<td>Pancreatic islets: hyperplasia (0/50, 4/50, 1/50, 11/50)</td>
<td>Liver: eosinophilic focus (13/50, 10/50, 11/50, 25/50)</td>
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<td>Exocrine pancreas: atrophy (10/50, 10/50, 16/50, 26/50)</td>
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<th>Male B6C3F1 Mice</th>
<th>Female B6C3F1 Mice</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Neoplastic effects</strong></td>
<td>None</td>
<td>None</td>
<td>Liver: hepatocellular adenoma, multiple (16/50, 27/50, 23/50, 34/50); hepatocellular adenoma (includes multiples) (32/50, 38/50, 29/50, 43/50); hepatocellular carcinoma, multiple (7/50, 12/50, 10/50, 17/50); hepatocellular adenoma or hepatocellular carcinoma (combined; includes multiples) (41/50, 47/50, 42/50, 48/50); hepatoblastoma (3/50, 8/50, 7/50, 8/50); hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) (41/50, 47/50, 43/50, 48/50)</td>
<td>Liver: hepatocellular adenoma (14/50, 16/50, 18/50, 28/50); hepatocellular carcinoma (5/50, 13/50, 15/50, 15/50); hepatocellular adenoma or carcinoma (17/50, 23/50, 27/50, 32/50)</td>
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<td><strong>Equivocal findings</strong></td>
<td>Lung: alveolar/bronchiolar adenoma (0/50, 0/50, 5/50, 2/50); alveolar/bronchiolar adenoma or carcinoma (combined) (0/50, 0/50, 5/50, 3/50)</td>
<td>Mononuclear cell leukemia: (5/50, 11/50, 18/50, T5/50)</td>
<td>None</td>
<td>None</td>
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<tr>
<td><strong>Decreased incidences</strong></td>
<td>Testis: interstitial cell adenoma (42/50, 39/50, 36/50, 26/50)</td>
<td>Mammary gland: fibroadenoma (35/50, 31/50, 22/50, 12/50)</td>
<td>None</td>
<td>Malignant lymphoma: (14/50, 15/50, 11/50, 2/50)</td>
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<tr>
<td><strong>Level of evidence of carcinogenic activity</strong></td>
<td>Equivocal evidence</td>
<td>Equivocal evidence</td>
<td>Clear evidence</td>
<td>Clear evidence</td>
</tr>
</tbody>
</table>

**Genetic toxicology**

- *Salmonella typhimurium* gene mutations: Negative in strains TA97, TA98, TA100, and TA1535 and in *Escherichia coli* WP2 *uvrA/pKM101* with and without S9

- Micronucleated erythrocytes

- Rat bone marrow *in vivo*: Negative in males

- Mouse peripheral blood *in vivo*: Negative in males; equivocal in females
EXPLANATION OF LEVELS OF EVIDENCE OF CARCINOGENIC ACTIVITY

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

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

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

For studies showing multiple chemical-related neoplastic effects that if considered individually would be assigned to different levels of evidence categories, the following convention has been adopted to convey completely the study results. In a study with clear evidence of carcinogenic activity at some tissue sites, other responses that alone might be deemed some evidence are indicated as “were also related” to chemical exposure. In studies with clear or some evidence of carcinogenic activity, other responses that alone might be termed equivocal evidence are indicated as “may have been” related to chemical exposure.

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

- adequacy of the experimental design and conduct;
- occurrence of common versus uncommon neoplasia;
- progression (or lack thereof) from benign to malignant neoplasia as well as from preneoplastic to neoplastic lesions;
- some benign neoplasms have the capacity to regress but others (of the same morphologic type) progress. At present, it is impossible to identify the difference. Therefore, where progression is known to be a possibility, the most prudent course is to assume that benign neoplasms of those types have the potential to become malignant;
- combining benign and malignant tumor incidence known or thought to represent stages of progression in the same organ or tissue;
- latency in tumor induction;
- multiplicity in site-specific neoplasia;
- metastases;
- supporting information from proliferative lesions (hyperplasia) in the same site of neoplasia or in other experiments (same lesion in another sex or species);
- presence or absence of dose relationships;
- statistical significance of the observed tumor increase;
- concurrent control tumor incidence as well as the historical control rate and variability for a specific neoplasm;
- survival-adjusted analyses and false positive or false negative concerns;
- structure-activity correlations; and
- in some cases, genetic toxicology.
The members of the Technical Reports Review Subcommittee who evaluated the draft NTP Technical Report on androstenedione on February 25, 2009, are listed below. Subcommittee members serve as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, subcommittee members have five major responsibilities in reviewing the NTP studies:

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

Raymond F. Novak, Ph.D., Chairperson
Institute of Environmental Health Sciences
Wayne State University
Detroit, MI

Tracie E. Bunton, D.V.M., Ph.D.
Toxicology Consultant
Eicarte LLC
Gettysburg, PA

Russell C. Cattley, V.M.D., Ph.D.
Amgen
Thousand Oaks, CA

Michael V. Pino, D.V.M., Ph.D.
Drug Safety Evaluation
Sanofi-aventis
Aifierville, France

Kenneth M. Portier, Ph.D.
American Cancer Society
Atlanta, GA

Jim E. Riviere, D.V.M., Ph.D.
College of Veterinary Medicine
North Carolina State University
Raleigh, NC

Special Ad Hoc Reviewers

David A. Eastmond, Ph.D.
Department of Cell Biology and Neuroscience
University of California
Riverside, CA

Stephen W. Looney, Ph.D.
Department of Biostatistics
Medical College of Georgia
Augusta, GA

Mitzi Nagarkatti, Ph.D.
Department of Pathology, Microbiology, and Immunology
University of South Carolina School of Medicine
Columbia, SC

James L. Sherley, M.D., Ph.D.
Programs in Regenerative Biology and Cancer
Boston Biomedical Research Institute
Watertown, MA

Justin G. Teeguarden, Ph.D.
Pacific Northwest National Laboratory
Richland, WA
SUMMARY OF TECHNICAL REPORTS REVIEW SUBCOMMITTEE COMMENTS

On February 25, 2009, the draft Technical Report on the toxicology and carcinogenesis studies of androstenedione received public review by the National Toxicology Program’s Board of Scientific Counselors Technical Report Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences, Research Triangle Park, NC.

Dr. Chad Blystone, NIEHS, introduced the studies on androstenedione by describing its former use as a dietary supplement, the study design and dose selection for the rodent studies, the results of genetic toxicity assays, the effects of the chemical on body weight and reproductive tissues, and the incidence of lesions in the three-month and two-year studies. The proposed conclusions were:

Under the conditions of these 2-year gavage studies, there was equivocal evidence of carcinogenic activity of androstenedione in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was equivocal evidence of carcinogenic activity of androstenedione in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was clear evidence of carcinogenic activity in male B6C3F1 mice based on increased incidences of liver neoplasms. There was clear evidence of carcinogenic activity in female B6C3F1 mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma. Increased incidences of pancreatic islet adenoma in male and female mice were also considered chemical related.

Androstenedione administration caused increased incidences in nonneoplastic lesions of the liver in male rats and male and female mice; pancreatic islets and exocrine pancreas of female rats; and clitoral gland, kidney and submandibular salivary gland of female mice.

Decreases in the incidences of testicular interstitial cell adenoma and mononuclear cell leukemia in male rats, mammary gland fibroadenoma, cysts, and hyperplasia in female rats, and malignant lymphoma in female mice were considered related to androstenedione administration.

Dr. Bunton, the first primary reviewer, felt the studies were limited by not having achieved a maximum tolerated dose level. She agreed with the proposed conclusions.

Dr. Eastmond, the second primary reviewer, raised questions about interpreting the statistical significance of tumors with high variability and high background rates. In particular, regarding liver tumors in male mice, he inquired if a rate of 48/50 of treated animals with tumors compared with 41/50 in controls could be deemed clear evidence of an effect. Dr. Grace Kissling, NIEHS, replied that in addition to statistical significance, knowledge of historical background rates and biological plausibility were a factor in study interpretation. Dr. Blystone noted that in addition to overall incidence, the increases in tumor multiplicity and the incidences of malignant carcinomas and hepatoblastomas added to the strength of clear evidence.

Dr. Teeguarden, the third primary reviewer, also agreed with the proposed conclusions.

Dr. Eastmond suggested that the conclusion for liver tumors in male mice specify the tumor types and multiplicity for liver neoplasms. The revised sentence for male mice was “There was clear evidence of carcinogenic activity of androstenedione in male B6C3F1 mice based on increased incidences of liver neoplasms, particularly multiple adenomas and carcinomas, and hepatoblastomas.”

Dr. Eastmond moved and Dr. Mitzi Nagarkatti seconded that the conclusions be accepted with the suggested revision. The motion was approved unanimously with 8 yes votes, 0 no votes, and 0 abstentions.
**INTRODUCTION**

![Chemical Structure of Androstenedione]

**ANDROSTENEDIONE**

CAS No. 63-05-8

Chemical Formula: $C_{19}H_{26}O_2$  Molecular Weight: 286.4

**Synonyms:** Andro; androst-4-ene-3,17-dione; 4-androstene-3,17-dione; delta-4-androstene-3,17-dione; delta-4-androstenedione; 3,17-dioxoandrost-4-ene; 17-ketotestosterone; SKF 2170

**Trade names:** Androtex, Fecundin

**CHEMICAL AND PHYSICAL PROPERTIES**

Androstenedione in its solid state occurs as needles when crystallized from acetone or crystals when crystallized from hexane. The needle form has a melting point of 142° to 144° C and the crystalline form has a melting point of 173° to 174° C (Merck, 1996). Androstenedione has a log P (octanol-water partition coefficient) of 2.75 and water solubility of 58.7 mg/L (SRC, 2008).

**PRODUCTION, USE, AND HUMAN EXPOSURE**

Androstenedione is a natural androgen steroid hormone synthesized within men and women. It is produced commercially for use as an intermediate in the synthesis of testosterone and other pharmaceutical steroids, including oral contraceptives and topical anti-inflammatory products. The concentrations of residual androstenedione in the final product are expected to be very low, and the level of exposure in the human population by this route is likely minimal. Androstenedione has been detected in water samples obtained downstream of paper mills which suggests possible environmental exposure (Jenkins et al., 2001), although fractions of this effluent that were androgenic *in vitro* did not contain androstenedione (Durhan et al., 2002).

Androstenedione was marketed as a supplement to aid athletes in producing muscle mass during training in order to optimize performance. The compound was available for oral, sublingual, or dermal administration. Exposure levels during androstenedione use are not available, but likely varied across users and may have followed a cyclic pattern of prolonged use with a short recovery period. Recommended oral doses of androstenedione ranged from 100 to as high as 1,200 mg/day, which equates to 1.4 to 17.1 mg/kg per day for a 70 kg person (Bahrke and Yesalis, 2004). Supplements containing androstenedione were banned for over-the-counter sale by The Anabolic Steroid Control Act (2004), which defined androstenedione as an anabolic steroid thus adding it to Schedule III of the Controlled Substances Act (21 CFR, Part 1300; 21 USC, Chapter 13). Androstenedione use among young men has likely declined dramatically since this legislative action.
**ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION**

**Experimental Animals**

Androstenedione can be metabolized to more potent hormones, which are known carcinogens, testosterone or estrone and ultimately to estradiol by the enzymes 17β-hydroxysteroid dehydrogenase (17β-HSD) and aromatase (CYP19) (Figure 1). Endogenous androstenedione production through the steroidogenic pathway involves multiple enzymes, including CYP17, an enzyme with hydroxylase and lyase activities. In the rat, CYP17 preferentially catalyzes the formation of androstenedione from progesterone in the delta 4 biosynthetic pathway, whereas in humans, androstenedione arises preferentially from CYP17 catalyzed dehydroepiandrosterone (DHEA) from pregnenolone in the delta 5 pathway (Brock and Waterman, 1999). Androstenedione undergoes extensive metabolism in humans and animals, including hydroxylation, reduction, and conjugation to glucuronic acid or sulfate.

The metabolism and disposition of 14C-labeled androstenedione were investigated in male and female F344/N rats, B6C3F1 mice, and beagle dogs in studies sponsored by the NTP (Green and Catz, 2007). Androstenedione was readily absorbed following oral administration to rodents. Approximately 80 to 90% of single oral doses of 1, 10, or 100 mg/kg (in 0.5% methylcellulose) was excreted in urine of rats and mice within 72 hours. The remaining dose (in all treatment groups) was mostly excreted in feces, with less than 1% remaining in tissues after 72 hours. The bioavailability of the oral doses of androstenedione in rats was low due to extensive metabolism. Several unidentified metabolites, but not androstenedione, were detected in rat plasma samples collected from 0.5 to 24 hours after dosing. The half-lives of the 14C in rat plasma ranged from 4.4 to 7.1 hours among the three doses, and were highest in the female treatment groups. No 14C-derived androstenedione, estradiol, estrone, or testosterone was detected in the urine of these animals. Although the metabolic profile of the 14C in urine differed significantly between male and female rats, the metabolites were poorly resolved and were not identified. Metabolism of androstenedione was not characterized in vivo in the mouse. Dogs excreted most, if not all, of an oral dose of either 1 or 100 mg/kg within 120 hours of dosing; however, in contrast to rodents, the 14C was equally excreted in urine and feces.

In the Green and Catz (2007) studies, the half-lives of the 14C in plasma were similar between male and female rats (approximately 6 hours) following intravenous (IV) administration, were higher following oral administration than IV administration, and were higher in females than in males (16 to 17 hours for females versus 11 hours for males) following dermal administration. Three minutes after IV administration, the 14C in rat plasma consisted primarily of androstenedione (80%). Testosterone, 6β-hydroxyandrostenedione, epiandrosterone, and 5α-androstenedione were also identified in plasma by co-elution with authentic standards. Other unidentified metabolites were present at later timepoints. In dogs, the half-lives of the parent chemical after 1 mg/kg IV administration were 0.9 hours for males and 0.4 hours for females. In dogs receiving 100 mg/kg androstenedione by oral administration, the compound was detected in plasma samples and the half-lives were 0.4 and 0.2 hours for males and females, respectively. As in rats, plasma and urine of orally treated dogs contained many poorly resolved metabolites that were not identified. No 14C estrone or estradiol was detected in plasma or urine and no 14C androstenedione or testosterone was detected in urine after IV or oral administration. Differences in the 14C metabolic profile in urine of male and female dogs were minor.

The metabolism of androstenedione was further investigated in hepatocytes of male and female F344/N rats, B6C3F1 mice, and beagle dogs in the Green and Catz (2007) studies. The rate of androstenedione metabolism was highest in the rat and lowest in the dog. There was a clear sex difference in androstenedione metabolism in rat hepatocytes. Mass spectrometry indicated that the major metabolite formed by male rat hepatocytes incubated with 100 µM androstenedione for 4 hours was 16α-hydroxyandrostenedione. Other identified metabolites were 6β-hydroxyandrostenedione, 16α-hydroxyandrosterone, epiandrosterone, and androsterone glucuronide. Female rat hepatocytes metabolized androstenedione predominantly via a 5α-reduced pathway as indicated by the formation of two major metabolites, 5α-androstenedione and androsterone, in the 4 hour incubation. Metabolites detected in female hepatocytes 24 hours after incubation of 100 µM androstenedione included 5α-dihydrotestosterone glucuronide, 5α-androstenediol glucuronide, androsterone glucuronide,
Androstenedione, NTP TR 560

Figure 1
Formation of Endogenous Sex Steroids
(The intermediate from CYP17’s two-step reaction is not shown.)

epiandrosterone sulfate, and androsterone sulfate. In contrast to rats, the metabolic profiles were similar in male and female mouse liver cells. The primary pathway of androstenedione metabolism in mouse hepatocytes appeared to be conversion to testosterone followed by glucuronidation, resulting in the formation of the major metabolite, testosterone glucuronide. Female mouse hepatocytes metabolized androstenedione at a faster rate, but had metabolites common to both sexes: testosterone, 5α-dihydrotestosterone glucuronide, and 6β-hydroxyandrostenedione. Approximately 50% of 100 µM androstenedione was unmetabolized at 4 hours by male and female dog hepatocytes. Metabolites common to males and females at 4 hours were testosterone glucuronide, androsterone glucuronide, 6α-hydroxyandrostenedione, 6β-hydroxyandrostenedione, and 16α-hydroxyandrostenedione.

Humans
The absorption, distribution, metabolism, and excretion of exogenous androstenedione in humans are not well characterized and are complicated by androstenedione transformation in various tissues to other hormones. Androstenedione has a half-life of 30 minutes in pregnant and nonpregnant women (Belisle et al., 1980). Circulating levels and excretion rates of several hormones increase after androstenedione dosing. Administration of 100 to 300 mg androstenedione/day
for up to 28 days to men increased serum concentrations of androstenedione, testosterone, dihydrotestosterone, estradiol, and increased excretion of testosterone glucuronide, dihydrotestosterone, etiocholanolone, and androsterone (Leder et al., 2001, 2002; Brown et al., 2004a). The increase in circulating testosterone after androstenedione exposure is not a consistent effect and may be related to androstenedione dose, exposure length, and age of the individual. Testosterone was not elevated after a 28-day exposure to 200 mg androstenedione/day or 8 weeks of exposure to 300 mg androstenedione/day (King et al., 1999; Beckham and Earnest, 2003). Prolonged androstenedione exposure (200 mg/day for 12 weeks) elevated androstenedione, estradiol, and estrone serum concentrations, but not serum testosterone concentrations, which may be due to a negative endocrine feedback loop (Broeder et al., 2000). However, a large dose (1,500 mg/day) of androstenedione given to hypogonadal men increased circulating levels of androstenedione and testosterone after a 12-week exposure (Jasuja et al., 2005). Serum androstenedione and testosterone concentrations rise considerably in women compared to men after administration, likely due to the low concentrations of androgens normally circulating in women (Brown et al., 2004b). Androstenedione metabolism in human hepatocytes consists of hydroxylation and reduction resulting in multiple metabolites that may remain free or conjugated to glucuronide or sulfate (Lévesque et al., 2002). In the human hepatocyte studies conducted by the NTP (Green and Catz, 2007), androstenedione metabolism in male and female donors was generally similar with 5α-reduction and conjugation forming the two major metabolites androsterone glucuronide and epiandrosterone sulfate. In these studies, testosterone was a minor metabolite in male and female cultures, and mono-hydroxylated metabolites of androstenedione and testosterone were highest in the young male donor (20 years old).

**TOXICITY**

**Experimental Animals**

The data of androstenedione toxicity (non-reproductive) within the peer reviewed literature are limited. Androstenedione treatment did alter the distribution and abundance of some fatty acids in tissues of nonpregnant and pregnant female rats and reduced liver ATP levels in pregnant rats (Wiesenfeld et al., 2006; Kim et al., 2007), and data indicate that 60 mg androstenedione/kg per day upregulates female rat liver cytochrome P450s (Flynn et al., 2005).

**Humans**

Androgen use has been associated with psychiatric effects, hirsutism, and acne (Snyder, 2001). There are suggestions that cardiovascular toxicity occurs from androgen use due to changes in lipid metabolism, coagulation, and direct myocardial injury, and androgen use has been associated with health problems ranging from hypertension to sudden death (Dhar et al., 2005). Women diagnosed with polycystic ovarian syndrome have elevated androgen levels, reduced fertility, and often exhibit hirsutism, acne, and female pattern alopecia (Norman et al., 2007).

**REPRODUCTIVE AND DEVELOPMENTAL TOXICITY**

**Experimental Animals**

In rodents, exposure to androstenedione via maternal injection affects reproductive behavior and development in female offspring. Perinatal exposure to androstenedione reduced the number of mammillary buds, vaginal patency, and sexual behavior in female rats and induced the formation of male reproductive organs in females also exposed in utero (Popolow and Ward, 1978). Female mice, rats, and hamsters exposed postnatally to androstenedione via injection display decreased ovulation and sexual behavior (Edwards, 1971; Boris et al., 1972; Paup et al., 1972). Gestational exposure to androstenedione via an oral route (<60 mg/kg per day) did not affect fetal rat skeletal development, but 10 and 30 mg/kg per day did alter the estrous cycle of F0 females before mating (Sprando et al., 2004, 2005). Anogenital distance, a sexually dimorphic trait, was not affected in female rat pups after in utero androstenedione exposure (via maternal gavage up to 30 mg/kg per day), but administration of androstenedione via intramuscular injection increased anogenital distance (Popolow and Ward, 1978; Sprando et al., 2004). The development of
a male-like fin in female mosquitofish exposed to androstenedione suggests that the chemical can masculinize females of this species (Stanko and Angus, 2007).

**Humans**

Evidence of reproductive and developmental toxicity of androstenedione in humans was not found in the literature except for case studies associating androstenedione use with altered reproductive function. Individual bodybuilders displayed loss of libido and oligospermia (Ritter et al., 2005) or priapism (Kachhi and Henderson, 2000) after androstenedione use. High circulating concentrations of androstenedione have been associated with gynecomastia likely due to metabolism to estrogens (Hemsell et al., 1977; Castro-Magana et al., 1991; Blackett and Freeman, 1996). The abuse of androgens in young men may lead to premature closure of the epiphyseal plates during development (stunted growth) due to the conversion of androgens to estrogens (Snyder, 2001).

**CARCINOGENICITY**

**Experimental Animals**

Only a few studies have examined the carcinogenic or tumor promotor activity of androstenedione. Male Marsh-Buffalo mice injected subcutaneously two to three times over a 2-month period, for a total of 15 to 20 mg of androstenedione per mouse, had increased numbers of fibrosarcomas of the skin (Bischoff, 1957). Following the induction of mammary gland carcinoma in ovariectomized rats with the known carcinogen 7,12-dimethylbenz[a]anthracene, administration of androstenedione led to increased tumor size compared to that in induced ovariectomized controls (Dauvois and Labrie, 1989). This effect was blocked by an aromatase inhibitor, indicating that conversion of androstenedione to estradiol was required.

Androstenedione metabolic precursors or metabolites are reported to produce tumors. DHEA produces hepatocellular carcinomas in rats and rainbow trout (Rao et al., 1992; Metzger et al., 1995; Orner et al., 1995). There is sufficient evidence of testosterone carcinogenicity in animals based upon studies showing testosterone propionate induces cervical-uterine tumors in female mice and prostatic adenocarcinomas in male rats, and testosterone treatment induces mammary tumors in mice (IARC, 1987). Testosterone and DHEA also increase ovarian granulosa cell tumor incidence in SWXJ-9 female mice, a strain that is genetically susceptible to these tumors (Beamer et al., 1988). Oxymetholone, an androgenic anabolic steroid, was carcinogenic in female rats based upon increased neoplasms in the liver, lung, and skin, but there were no certain neoplastic effects in male rats (NTP, 1999). Furthermore, estrogens, which are downstream metabolites of androstenedione, are known carcinogens (IARC, 1987; NTP, 2002).

**Humans**

Epidemiological studies linking androstenedione use to increased cancer outcome were not found in the literature. Recent studies that examined endogenous hormone concentrations have not found an association with serum androstenedione or androgen concentrations and prostate cancer (Roddam et al., 2008; Weiss et al., 2008). A case study of two bodybuilders did associate anabolic steroid use with the occurrence of hepatocellular adenoma, but androstenedione was not one of the steroids frequently used (Socas et al., 2005). The use of oxymetholone to treat anemia has been associated with liver tumors in patients, which regress upon stopping treatment (IARC, 1987; Pavlatos et al., 2001).

**GENETIC TOXICITY**

Androgens are generally not considered to be mutagenic, clastogenic, or aneugenic. Only one published report of genetic toxicity data for androstenedione was identified in the open literature. This report described results of a Salmonella typhimurium spot test conducted with several bile acid and cholesterol derivatives, including androstenedione (500 µg/disc), in strain TA1538 with and without induced Wistar rat liver S9 enzymes; no mutagenicity was detected with androstenedione in this assay (McKillop et al., 1983). Testosterone (500 µg/plate) was also tested for mutagenicity in S. typhimurium strains TA100, TA1535, and TA1538, with and without rat liver S9; as with androstenedione, no mutagenicity was detected (Ingerowski et al., 1981). Testosterone (100 µM) was tested for induction of mitotic disruption in cultured Chinese hamster Don cells; no mitotic disturbances were
noted following a 7-hour treatment period (Wheeler et al., 1986). Tsutsui et al. (1995) tested testosterone and testosterone propionate in mammalian cell assays for induction of chromosomal aberrations, aneuploidy, and gene mutations at the hypoxanthine phosphoribosyl transferase or Na+/K+ ATPase loci, and all tests results were negative. However, these two compounds were shown to induce cell transformation in cultured Syrian hamster embryo cells over a concentration range of 1 to 30 µg/mL, in a dose-related manner (Tsutsui et al., 1995).

**STUDY RATIONALE**

Androstenedione was nominated for study by the National Cancer Institute (prior to the 2004 regulation) due to its widespread use in individuals interested in bodybuilding or maximizing athletic performance and concern for potential adverse health effects after prolonged use. Due to the possible long-term exposure to androstenedione in athletes and a lack of chronic data to identify potential carcinogenicity, 2-week, 3-month, and 2-year gavage studies in rats and mice were selected by the NTP.
MATERIALS AND METHODS

PROCUREMENT AND CHARACTERIZATION

Androstenedione
Androstenedione was obtained from Steraloids, Inc. (Newport, RI), in one lot (H408) which was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory (Battelle Memorial Institute, Columbus, OH), Research Triangle Institute (Research Triangle Park, NC), and the study laboratory that conducted the 3-month and 2-year studies (Southern Research Institute, Birmingham, AL) (Appendix J). Elemental analyses and Karl Fischer titration were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on the analyses performed in support of the androstenedione studies are on file at the National Institute of Environmental Health Sciences.

Lot H408 of androstenedione, a white, crystalline solid, was identified as androstenedione by infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the literature spectra (Sigma, 1986; Simova et al., 1997) of androstenedione.

The moisture content of lot H408 was determined using Karl Fischer titration. The purity of lot H408 was determined by gas chromatography (GC) and high-performance liquid chromatography (HPLC).

For lot H408, Karl Fischer titration indicated an average water content of 0.11%. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for androstenedione. GC indicated one major peak and two impurities with a combined area of 2.3% of the total peak area. The largest impurity, 2.2% of the total peak area, was identified as an isomer of androstenedione by mass spectrometry. HPLC analysis indicated one impurity with a relative area of 0.8%, confirmed to be testosterone by mass spectrometry. The overall purity of lot H408 was determined to be 98% or greater.

Stability studies demonstrated that the bulk chemical could be stored protected from light at room temperature (25°C). Periodic analysis of the bulk chemical was performed using HPLC; no degradation of the bulk chemical was observed.

Methylcellulose
For the 2-week study, methylcellulose was obtained from Fisher Scientific, Inc. (Pittsburgh, PA), in one lot (984735). Identity was confirmed using IR. The average methoxyl content (29.1%) was determined by Galbraith Laboratories, Inc. Methylcellulose was obtained from Sigma-Aldrich (St. Louis, MO) in one lot (31K0155) for the 3-month study and in two lots (31K0155 and 113K0078) for the 2-year studies. Identity was confirmed using IR; spectra were consistent with the structure of methylcellulose. The average methoxyl content (31.0% and 32.1%, respectively) was determined by Galbraith Laboratories, Inc.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The vehicle was prepared by mixing methylcellulose with heated, deionized water (Table J2).

The analytical chemistry laboratory conducted homogeneity, resuspendability, and stability studies using HPLC, and a gavageability study using a 20-gauge gavage needle. Homogeneity, gavageability, and resuspendability were confirmed. Stability was confirmed for up to 35 days for dose formulations stored in sealed amber glass bottles protected from light at room temperature or 5°C.

Prior to the 2-week studies, the study laboratory performed homogeneity and gavageability studies. Homogeneity and gavageability were confirmed. Prior to the
3-month and 2-year studies, the study laboratory conducted homogeneity studies using HPLC. Homogeneity was confirmed in both instances.

Periodic analyses of the dose formulations of androstenedione in 0.5% methylcellulose were conducted at the study laboratories using HPLC. Dose formulations were analyzed once for the 2-week studies; animal room samples were also analyzed. All dose formulations were within 10% of the target concentrations; three of five rat animal room samples and three of five mouse animal room samples were within 10% of target concentrations. Dose formulations were analyzed three times during the 3-month studies; animal room samples were also analyzed. All 31 dose formulations analyzed were within 10% of the target concentrations; 8 of 15 rat animal room samples and 11 of 15 mouse animal room samples were within 10% of the target concentrations. Dose formulations were analyzed every 2 to 3 months during the 2-year studies; animal room samples were also analyzed. All 81 dose formulations were within 10% of the target concentrations; 16 of 21 rat animal room samples and 29 of 32 mouse animal room samples were within 10% of target concentrations. Difficulties in resuspending the formulations from the animal rooms for analysis caused some results to be farther from target values than expected based on the original analyses. Improvements in handling the samples minimized this problem in the 2-year studies.

2-WEEK STUDIES
Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 4-5 weeks old. Animals were quarantined for 11 days and were 5-6 weeks old on the first day of the studies. Groups of five male and five female rats and mice were administered androstenedione by gavage in a 0.5% aqueous methylcellulose solution at doses of 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight 5 days per week for 12 days; vehicle control animals received only the methylcellulose solution. Doses were selected for the 2-week studies to cover the higher range of levels that may occur from severe abuse among athletes. The top dose of 50 mg/kg per day reached the limit of gavageability. Higher doses of androstenedione (i.e., >10 mg/mL) required longer times to administer through a 20-gauge gavage needle, which was not acceptable. Feed and water were available ad libitum. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

At the end of the 2-week studies, necropsies were performed on all rats and mice. The heart, right kidney, liver, lung, right testis, and thymus were weighed. In order to evaluate peroxisome and cell proliferation, a portion of each rat and mouse liver was homogenized for protein, peroxisomal enzyme, and cell cycle biomarker analyses. Liver protein concentrations were determined using bicinchoninic acid and copper (Smith et al., 1985). Proliferation of peroxisomes was determined by measuring acyl-CoA oxidase activity by the methods of Small et al. (1985). Proliferating cell nuclear antigen and cyclin-dependent kinase concentrations were measured using ELISA kits from Paracelsian, Inc. (Ithaca, NY). Histopathologic examinations were performed on vehicle control and 50 mg/kg rats and mice, and the liver was examined in all groups of male rats. Table 1 lists the tissues and organs examined.

3-MONTH STUDIES
The 3-month studies were conducted to evaluate the cumulative toxic effects of repeated exposure to androstenedione and to determine the appropriate doses to be used in the 2-year studies.

Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY). On receipt, the rats and mice were 3 to 4 weeks old. Animals were quarantined for 12 to 15 days and were 5 to 6 weeks old on the first day of the studies. Before the studies began, five male and five female rats and mice were randomly selected for parasite evaluation and gross observation for evidence of disease. At the end of the studies, serologic analyses were performed on five male and five female vehicle control rats and mice using the protocols of the NTP Sentinel Animal Program (Appendix L).

Groups of 10 male and 10 female core study rats and mice were administered androstenedione by gavage in a 0.5% aqueous methylcellulose solution at doses of 0, 1, 5, 10, 20, or 50 mg androstenedione/kg body weight 5 days per week for 12 days; vehicle control animals received only the methylcellulose solution. Doses were selected for the 2-week studies to cover the higher range of levels that may occur from severe abuse among athletes. The top dose of 50 mg/kg per day reached the limit of gavageability. Higher doses of androstenedione (i.e., >10 mg/mL) required longer times to administer through a 20-gauge gavage needle, which was not acceptable. Feed and water were available ad libitum. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded daily for rats and mice. The animals were weighed initially, on day 8, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

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3-MONTH STUDIES
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tum. Rats and female mice were housed five per cage; male mice were housed individually. Clinical findings were recorded weekly for core study rats and mice. The animals were weighed initially, on day 2 (female mice), day 3 (male rats and mice), day 4 (female rats), weekly thereafter, and at the end of the studies. Details of the study design and animal maintenance are summarized in Table 1.

Animals were anesthetized with a carbon dioxide/oxygen mixture, and blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 24 and from core study rats at study termination for hematology and clinical chemistry analyses. Blood was collected from the retroorbital sinus of mice at the end of the study for hematology analyses. Samples for hematology analyses were placed in collection tubes containing EDTA; samples for clinical chemistry evaluations were placed in similar tubes containing no anticoagulant. Packed cell volume; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; leukocyte count and differentials; mean cell volume; mean cell hemoglobin; and mean cell hemoglobin concentration were determined with an ADVIA® 120 hematology system analyzer (Bayer Diagnostics, Tarrytown, NY) with reagents supplied by Bayer, Inc. (Tarrytown, NY), or Fisher Scientific (Norcross, GA). Manual hematocrit values were determined using a Model MB micro-capillary centrifuge (International Equipment Company, Needham Heights, MA) for comparison to ADVIA® values for packed cell volume. Blood smears were prepared and stained for determination of nucleated erythrocyte counts using an Ames Hema-Tek™ slide stainer (Miles Laboratory, Inc., Elkhart, IN) and a modified Wright’s stain. For clinical chemistry analyses, serum samples were analyzed using a Hitachi 911 automated analyzer (Boehringer Mannheim, Indianapolis, IN) and reagents supplied by Sigma Diagnostics (St. Louis, MO) or Roche Diagnostics (Indianapolis, IN). The parameters measured are listed in Table 1.

At the end of the 3-month studies, samples were collected for sperm motility and vaginal cytology evaluations on rats and mice administered 0, 10, 20, or 50 mg/kg. The parameters evaluated are listed in Table 1. For 12 consecutive days prior to scheduled terminal sacrifice, the vaginal vaults of the females were moistened with saline, if necessary, and samples of vaginal fluid and cells were stained. Relative numbers of leukocytes, nucleated epithelial cells, and large squamous epithelial cells were determined and used to ascertain estrous cycle stage (i.e., diestrus, proestrus, estrus, and metestrus). Male animals were evaluated for sperm count and motility. The left testis and left epididymis were isolated and weighed. The tail of the epididymis (cauda epididymis) was then removed from the epididymal body (corpus epididymis) and weighed. Test yolk (rats) or modified Tyrode’s buffer (mice) was applied to slides, and a small incision was made at the distal border of the cauda epididymis. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the numbers of motile and nonmotile spermatozoa were counted for five fields per slide by two observers. Following completion of sperm motility estimates, each left cauda epididymis was placed in buffered saline solution. Caudae were finely minced, and the tissue was incubated in the saline solution and then heat fixed at 65°C. Sperm density was then determined microscopically with the aid of a hemacytometer. To quantify spermatogenesis, the testicular spermatid head count was determined by removing the tunica albuginea and homogenizing the left testis in phosphate-buffered saline containing 10% dimethyl sulfoxide. Homogenization-resistant spermatid nuclei were counted with a hemacytometer.

Necropsies were performed on all core study animals. The heart, right kidney, liver, lung, right testis, thymus, and uterus were weighed. Tissues for microscopic examination were fixed and preserved in 10% neutral buffered formalin (eyes were initially fixed in Davidson’s solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin. Complete histopathologic examinations were performed on vehicle control and 50 mg/kg core study rats and mice. In addition, the adrenal gland, heart, liver, mammary gland, ovary, prostate gland, and thyroid gland of rats; the bone marrow, liver, mammary gland, mandibular and mesenteric lymph nodes, ovary, prostate gland, spleen, and thymus of mice; and the adrenal gland and heart of female mice were examined in the remaining dosed groups. Table 1 lists the tissues and organs routinely examined.

2-YEAR STUDIES

Study Design

Groups of 50 male and 50 female rats were administered androstenedione by gavage in a 0.5% aqueous methylcellulose solution at doses of 0, 10, 20, or 50 mg/kg 5 days per week for at least 104 weeks. Groups of 50 male and 50 female mice were administered
androstenedione by gavage in a 0.5% aqueous methylcellulose solution at doses of 0, 2 (females only), 10, 20 (males only), or 50 mg/kg 5 days per week for at least 104 weeks. Vehicle control groups received the methylcellulose solution alone.

Source and Specification of Animals
Male and female F344/N rats and B6C3F1 mice were obtained from Taconic Farms, Inc. (Germantown, NY), for use in the 2-year studies. Rats and mice were quarantined for 12 days before the beginning of the studies. Five male and five female rats and mice were randomly selected for parasite evaluation and gross observation of disease. Rats and mice were 5 to 6 weeks old at the beginning of the studies. The health of the animals was monitored during the studies according to the protocols of the NTP Sentinel Animal Program (Appendix L).

Animal Maintenance
Rats were housed three (males) or five (females) per cage, and mice were housed individually (males) or five per cage (females). Feed and water were available ad libitum. Cages were changed once (male mice) or twice weekly, and cages and racks were rotated every 2 weeks. Further details of animal maintenance are given in Table 1. Information on feed composition and contaminants is provided in Appendix K.

Clinical Examinations and Pathology
All animals were observed twice daily. Clinical findings were recorded every 4 weeks; body weights were recorded on day 1, day 4 (males), day 5 (females), weekly for 13 weeks, monthly thereafter, and at the end of the studies.

Complete necropsies and microscopic examinations were performed on all rats and mice. At necropsy, all organs and tissues were examined for grossly visible lesions, and all major tissues were fixed and preserved in 10% neutral buffered formalin (eyes were first fixed in Davidson’s solution), processed and trimmed, embedded in paraffin, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. For all paired organs (e.g., adrenal gland, kidney, ovary), samples from each organ were examined. Tissues examined microscopically are listed in Table 1.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the Toxicology Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to the NTP Archives for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, and pathology tables were evaluated by an independent quality assessment laboratory. The individual animal records and tables were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. For the 2-year studies, a quality assessment pathologist evaluated slides from all tumors and all potential target organs, which included the liver and kidney of male rats, the hematopoietic system and pancreas of female rats, the liver of male and female mice, and the adrenal gland of female mice.

The quality assessment report and the reviewed slides were submitted to the NTP Pathology Working Group (PWG) coordinator, who reviewed the selected tissues and addressed any inconsistencies in the diagnoses made by the laboratory and quality assessment pathologists. Representative histopathology slides containing examples of lesions related to chemical administration, examples of disagreements in diagnoses between the laboratory and quality assessment pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. The PWG consisted of the quality assessment pathologist and other pathologists experienced in rodent toxicologic pathology. This group examined the tissues without any knowledge of dose groups or previously rendered diagnoses. When the PWG consensus differed from the opinion of the laboratory pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus between the laboratory pathologist, reviewing pathologist(s), and the PWG. Details of these review procedures have been described, in part, by Maronpot and Boorman (1982) and Boorman et al. (1985). For subsequent analyses of the pathology data, the decision of whether to evaluate the diagnosed lesions for each tissue type separately or combined was generally based on the guidelines of McConnell et al. (1986).

Statistical Methods
Survival Analyses
The probability of survival was estimated by the product-limit procedure of Kaplan and Meier (1958) and is presented in the form of graphs. Animals found dead of other than natural causes were censored from the survival analyses; animals dying from natural causes were
### TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

<table>
<thead>
<tr>
<th>Study Laboratory</th>
<th>2-Week Studies</th>
<th>3-Month Studies</th>
<th>2-Year Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Battelle Columbus Operations (Columbus, OH)</td>
<td>Southern Research Institute (Birmingham, AL)</td>
<td>Southern Research Institute (Birmingham, AL)</td>
<td></td>
</tr>
<tr>
<td>Strain and Species</td>
<td>F344/N rats</td>
<td>F344/N rats</td>
<td>F344/N rats</td>
</tr>
<tr>
<td></td>
<td>B6C3F1 mice</td>
<td>B6C3F1 mice</td>
<td>B6C3F1 mice</td>
</tr>
<tr>
<td>Animal Source</td>
<td>Taconic Farms, Inc. (Germantown, NY)</td>
<td>Taconic Farms, Inc. (Germantown, NY)</td>
<td>Taconic Farms, Inc. (Germantown, NY)</td>
</tr>
<tr>
<td>Time Held Before Studies</td>
<td>11 days</td>
<td>Rats: 13 (males) or 12 (females) days</td>
<td>12 days</td>
</tr>
<tr>
<td></td>
<td>Mice: 14 (males) or 15 (females) days</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age When Studies Began</td>
<td>5-6 weeks</td>
<td>5 to 6 weeks</td>
<td>5 to 6 weeks</td>
</tr>
<tr>
<td>Date of First Dose</td>
<td>Rats: August 14, 2000</td>
<td>Rats: April 23 (males) or 22 (females), 2002</td>
<td>Rats: February 3, 2003</td>
</tr>
<tr>
<td></td>
<td>Mice: August 21, 2000</td>
<td>Mice: April 24 (males) or 25 (females), 2002</td>
<td>Mice: December 16, 2002</td>
</tr>
<tr>
<td>Duration of Dosing</td>
<td>5 days/week for 12 days</td>
<td>5 days/week for 14 weeks</td>
<td>5 days/week for 104 to 105 weeks (rats) or 104 to 106 weeks (mice)</td>
</tr>
<tr>
<td>Date of Last Dose</td>
<td>Rats: August 29, 2000</td>
<td>Rats: July 23 (males) or 22 (females), 2002 (core study)</td>
<td>Rats: January 30 to February 6, 2005</td>
</tr>
<tr>
<td></td>
<td>Mice: September 6, 2000</td>
<td>May 15 (males) or 14 (females), 2002 (clinical pathology study)</td>
<td>Mice: December 12-20, 2004</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mice: July 24 (males) or 25 (females), 2002</td>
<td></td>
</tr>
<tr>
<td>Necropsy Dates</td>
<td>Rats: August 30, 2000</td>
<td>Rats: July 24 (males) or 23 (females), 2002 (core study)</td>
<td>Rats: January 31 to February 7, 2005</td>
</tr>
<tr>
<td></td>
<td>Mice: September 7, 2000</td>
<td>Mice: July 25 (males) or 26 (females), 2002</td>
<td>Mice: December 13-21, 2004</td>
</tr>
<tr>
<td>Age at Necropsy</td>
<td>8-9 weeks</td>
<td>18 to 19 weeks</td>
<td>109 to 111 weeks</td>
</tr>
<tr>
<td>Size of Study Groups</td>
<td>5 males and 5 females</td>
<td>10 males and 10 females</td>
<td>50 males and 50 females</td>
</tr>
</tbody>
</table>
TABLE 1  
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

<table>
<thead>
<tr>
<th>Method of Distribution</th>
<th>2-Week Studies</th>
<th>3-Month Studies</th>
<th>2-Year Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rats were distributed randomly into groups of approximately equal initial mean body weights. Due to an error, mice were distributed into groups of decreasing body weight.</td>
<td>Animals were distributed randomly into groups of approximately equal initial mean body weights.</td>
<td>Same as 3-month studies</td>
<td></td>
</tr>
<tr>
<td>Animals per Cage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rats: 5</td>
<td>Rats: 5</td>
<td>Rats: 3 (males) or 5 (females)</td>
<td></td>
</tr>
<tr>
<td>Mice: 1 (males) or 5 (females)</td>
<td>Mice: 1 (males) or 5 (females)</td>
<td>Mice: 1 (males) or 5 (females)</td>
<td></td>
</tr>
<tr>
<td>Method of Animal Identification</td>
<td>Tail tattoo</td>
<td>Tail tattoo</td>
<td>Tail tattoo</td>
</tr>
<tr>
<td>Diet</td>
<td>NTP-2000 irradiated wafers (Zeigler Brothers, Inc., Gardners, PA), available <em>ad libitum</em>, changed weekly</td>
<td>Same as 2-week studies</td>
<td>Same as 2-week studies</td>
</tr>
<tr>
<td>Water</td>
<td>Tap water (Columbus, OH, municipal supply) via automatic watering system (Edstrom Industries, Inc., Waterford, WI), available <em>ad libitum</em></td>
<td>Same as 2-week studies, except Birmingham, AL, municipal supply</td>
<td>Same as 3-month studies</td>
</tr>
<tr>
<td>Cages</td>
<td>Solid bottom polycarbonate (Lab Products, Inc., Maywood, NJ), changed once (male mice) or twice (rats and female mice) weekly and rotated every 2 weeks</td>
<td>Same as 2-week studies except racks not rotated</td>
<td>Same as 2-week studies</td>
</tr>
<tr>
<td>Bedding</td>
<td>Irradiated Sani-Chips® hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ)</td>
<td>Same as 2-week studies</td>
<td>Same as 2-week studies</td>
</tr>
<tr>
<td>Cage Filters</td>
<td>Dupont 2024 spunbonded polyester (Snow Filtration Co., Cincinnati, OH), changed every 2 weeks</td>
<td>Reemay® spunbonded polyester (Andico, Birmingham, AL), changed every 2 weeks</td>
<td>Same as 3-month studies</td>
</tr>
<tr>
<td>Racks</td>
<td>Stainless steel (Lab Products, Inc., Maywood, NJ), changed and rotated every 2 weeks</td>
<td>Same as 2-week studies</td>
<td>Same as 2-week studies</td>
</tr>
<tr>
<td>Animal Room Environment</td>
<td>Temperature: 72° ± 3° F</td>
<td>Temperature: 72° ± 3° F</td>
<td>Temperature: 72° ± 3° F</td>
</tr>
<tr>
<td>Relative humidity: 50% ± 15%</td>
<td>Relative humidity: 50% ± 15%</td>
<td>Relative humidity: 50% ± 15%</td>
<td></td>
</tr>
<tr>
<td>Room fluorescent light: 12 hours/day</td>
<td>Room fluorescent light: 12 hours/day</td>
<td>Room fluorescent light: 12 hours/day</td>
<td></td>
</tr>
<tr>
<td>Room air changes: minimum of 10/hour</td>
<td>Room air changes: 10/hour</td>
<td>Room air changes: 10/hour</td>
<td></td>
</tr>
</tbody>
</table>
**Table 1**  
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

<table>
<thead>
<tr>
<th>Doses</th>
<th>2-Week Studies</th>
<th>3-Month Studies</th>
<th>2-Year Studies</th>
</tr>
</thead>
</table>
| 0, 1, 5, 10, 20, or 50 mg/kg in 0.5% methylcellulose by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice) | 0, 1, 5, 10, 20, or 50 mg/kg in 0.5% methylcellulose by gavage (dosing volumes 5 mL/kg for rats and 10 mL/kg for mice) | Rats: 0, 10, 20, or 50 mg/kg in 0.5% methylcellulose by gavage (dosing volume 5 mL/kg)  
Mice: 0, 2 (females), 10, 20 (males), or 50 mg/kg in 0.5% methylcellulose by gavage (dosing volume 10 mL/kg) |

**Type and Frequency of Observation**

- **2-Week Studies**: Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.
- **3-Month Studies**: Observed twice daily; animals were weighed initially, on day 2 (female mice), day 3 (males), day 4 (female rats), weekly, and at the end of the studies; clinical findings were recorded weekly for core study animals.
- **2-Year Studies**: Observed twice daily; animals were weighed on day 1, day 4 (males), day 5 (females), weekly for 13 weeks, monthly thereafter, and at the end of the studies. Clinical findings were recorded every 4 weeks.

**Method of Sacrifice**

- **2-Week Studies**: Carbon dioxide asphyxiation
- **3-Month Studies**: Same as 2-week studies
- **2-Year Studies**: Same as 2-week studies

**Necropsy**

- **2-Week Studies**: Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.
- **3-Month Studies**: Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, thymus, and uterus.
- **2-Year Studies**: Necropsies were performed on all animals.

**Clinical Pathology**

- **2-Week Studies**: None
- **3-Month Studies**: None
- **2-Year Studies**: None

Blood was collected from the retroorbital sinus of clinical pathology study rats on days 4 and 24 and from core study rats and mice at the end of the studies for hematology and clinical chemistry (rats only).  
**Hematology**: automated and manual hematocrit; hemoglobin concentration; erythrocyte, nucleated erythrocytes, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; leukocyte count and differentials  
**Clinical chemistry**: urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, creatine kinase, sorbitol dehydrogenase, and total bile acids
2-Week Studies | 3-Month Studies | 2-Year Studies
---|---|---
**Histopathology**
Histopathology was performed on vehicle control and 50 mg/kg rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, heart, kidney, liver, lung, mammary gland, ovary, pituitary gland, prostate gland, seminal vesicle, testis (with epididymis), uterus (with cervix), and vagina. The liver was examined in all male rats.

Complete histopathology was performed on vehicle control and 50 mg/kg core study rats and mice. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, eye, gallbladder (mice only), hardier gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus. In addition, the adrenal gland, heart, liver, mammary gland, ovary, prostate gland, and thyroid gland of rats; the bone marrow, liver, mammary gland, mandibular and mesenteric lymph nodes, ovary, prostate gland, spleen, and thymus of mice; and the adrenal gland and heart of female mice were examined in the remaining dosed groups.

Complete histopathology was performed on all animals. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone, brain, clitoral gland, esophagus, eye, gallbladder (mice only), hardier gland, heart, large intestine (cecum, colon, rectum), small intestine (duodenum, jejunum, ileum), kidney, liver, lung, lymph nodes (mandibular and mesenteric), mammary gland, nose, ovary, pancreas, parathyroid gland, pituitary gland, preputial gland, prostate gland, salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis with epididymis, thymus, thyroid gland, trachea, urinary bladder, and uterus.

**Sperm Motility and Vaginal Cytology**
None

At the end of the studies, sperm samples were collected from male animals in the 0, 10, 20, and 50 mg/kg groups for sperm motility evaluations. The following parameters were evaluated: spermatid heads per testis and per gram testis, spermatid counts, and epididymal spermatotozoal motility and concentration. The left cauda, left epididymis, and left testis were weighed. Vaginal samples were collected for up to 12 consecutive days prior to the end of the studies from females administered 0, 10, 20, or 50 mg/kg for vaginal cytology evaluations. The percentage of time spent in the various estrous cycle stages and estrous cycle length were evaluated.

None

**Hepatic Biomarkers**
At necropsy, a portion of each liver was homogenized immediately after weighing for peroxisomal enzyme and cell cycle biomarker analyses. Parameters measured were acyl-CoA oxidase activity, cyclin-dependent kinase, and proliferating cell nuclear antigen concentrations.

None

None
Androstenedione, NTP TR 560

not censored. Statistical analyses for possible dose-related effects on survival used Cox’s (1972) method for testing two groups for equality and Tarone’s (1975) life table test to identify dose-related trends. All reported P values for the survival analyses are two sided.

Calculation of Incidence
The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1, A4, B1, B4, C1, C4, D1, and D4 as the numbers of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. For calculation of statistical significance, the incidences of most neoplasms (Tables A2, B2, C2, and D2) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. However, when macroscopic examination was required to detect neoplasms in certain tissues (e.g., hardener gland, intestine, mammary gland, and skin) before microscopic evaluation, or when neoplasms had multiple potential sites of occurrence (e.g., leukemia or lymphoma), the denominators consist of the number of animals on which a necropsy was performed. Tables A2, B2, C2, and D2 also give the survival-adjusted neoplasm rate for each group and each site-specific neoplasm. This survival-adjusted rate (based on the Poly-3 method described below) accounts for differential mortality by assigning a reduced risk of neoplasm, proportional to the third power of the fraction of time on study, only to site-specific, lesion-free animals that do not reach terminal sacrifice.

Analysis of Neoplasm and Nonneoplastic Lesion Incidences
The Poly-k test (Bailer and Portier, 1988; Portier and Baier, 1989; Piegorsch and Baier, 1997) was used to assess neoplasm and nonneoplastic lesion prevalence. This test is a survival-adjusted quantal-response procedure that modifies the Cochran-Armitage linear trend test to take survival differences into account. More specifically, this method modifies the denominator in the quantal estimate of lesion incidence to approximate more closely the total number of animal years at risk. For analysis of a given site, each animal is assigned a risk weight. This value is one if the animal had a lesion at that site or if it survived until terminal sacrifice; if the animal died prior to terminal sacrifice and did not have a lesion at that site, its risk weight is the fraction of the entire study time that it survived, raised to the kth power. This method yields a lesion prevalence rate that depends only upon the choice of a shape parameter for a Weibull hazard function describing cumulative lesion incidence over time (Bailer and Portier, 1988). Unless otherwise specified, a value of k=3 was used in the analysis of site-specific lesions. This value was recommended by Bailer and Portier (1988) following an evaluation of neoplasm onset time distributions for a variety of site-specific neoplasms in control F344 rats and B6C3F1 mice (Portier et al., 1986). Bailer and Portier (1988) showed that the Poly-3 test gave valid results if the true value of k was anywhere in the range from 1 to 5. A further advantage of the Poly-3 method is that it does not require lesion lethality assumptions. Variation introduced by the use of risk weights, which reflect differential mortality, was accommodated by adjusting the variance of the Poly-3 statistic as recommended by Bieler and Williams (1993).

Tests of significance included pairwise comparisons of each dosed group with controls and a test for an overall dose-related trend. Continuity-corrected Poly-3 tests were used in the analysis of lesion incidence, and reported P values are one sided. The significance of lower incidences or decreasing trends in lesions is represented as 1–P with the letter N added (e.g., P=0.99 is presented as P=0.01N).

Analysis of Continuous Variables
Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which historically have approximately normal distributions, were analyzed with the parametric multiple comparison procedures of Dunnett (1955) and Williams (1971, 1972). Hematology, clinical chemistry, spermatid, and epididymal spermatozoal data, which have typically skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) (as modified by Williams, 1986) and Dunn (1964). Jonckheere’s test (Jonckheere, 1954) was used to assess the significance of the dose-related trends and to determine whether a trend-sensitive test (Williams’ or Shirley’s test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-related trend (Dunnett’s or Dunn’s test). Prior to statistical analysis, extreme values identified by the outlier test of Dixon and Massey (1957) were examined by NTP personnel, and implausible values were eliminated from the analysis.
Because vaginal cytology data are proportions (the proportion of the observation period that an animal was in a given estrous stage), an arcsine transformation was used to bring the data into closer conformance with a normality assumption. Treatment effects were investigated by applying a multivariate analysis of variance (Morrison, 1976) to the transformed data to test for simultaneous equality of measurements across doses. Proportions of regularly cycling females in each dosed group were compared to the control group using Fisher’s exact test (Gart et al., 1979). Tests for extended periods of estrus and diestrus were constructed based on a Markov chain model proposed by Girard and Sager (1987). For each dose group, a transition probability matrix was estimated for transitions among the proestrus, estrus, metestrus, and diestrus stages, with provision for extended stays within estrus and diestrus. Equality of transition matrices among dose groups and between the control group and each dosed group was tested using chi-square statistics.

**Historical Control Data**

The concurrent control group represents the most valid comparison to the treated groups and is the only group analyzed statistically in NTP bioassays. However, historical control data are often helpful in interpreting potential treatment-related effects, particularly for uncommon or rare neoplasm types. For meaningful comparisons, the conditions for studies in the historical database must be generally similar. One significant factor affecting the background incidence of neoplasms at a variety of sites is diet. In 1995, the NTP incorporated a new diet (NTP-2000) that contains less protein and more fiber and fat than the NIH-07 diet previously used in toxicity and carcinogenicity studies (Rao, 1996, 1997). The current NTP historical database contains all studies that use the NTP-2000 diet with histopathology findings completed within the most recent 5-year period. A second potential source of variability is route of administration. In general, the historical database for a given study will include studies using the same route of administration, and the overall incidences of neoplasms for all routes of administration are included for comparison, including the present study.

**QUALITY ASSURANCE METHODS**

The 3-month and 2-year studies were conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). In addition, as records from the 2-year studies were submitted to the NTP Archives, these studies were audited retrospectively by an independent quality assurance contractor. Separate audits covered completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report. Audit procedures and findings are presented in the reports and are on file at NIEHS. The audit findings were reviewed and assessed by NTP staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

**GENETIC TOXICOLOGY**

The genetic toxicity of androstenedione was assessed by testing the ability of the chemical to induce mutations in various strains of *Salmonella typhimurium* and *Escherichia coli*, micronucleated erythrocytes in rat bone marrow, and increases in the frequency of micronucleated erythrocytes in mouse peripheral blood. Micronuclei (literally “small nuclei” or Howell-Jolly bodies) are biomarkers of induced structural or numerical chromosomal alterations and are formed when acen- tric fragments or whole chromosomes fail to incorporate into either of two daughter nuclei during cell division (Schmid, 1975; Heddle et al., 1983). The protocols for these studies and the results are given in Appendix E.

The genetic toxicity studies have evolved from an earlier effort by the NTP to develop a comprehensive database permitting a critical anticipation of a chemical’s carcinogenicity in experimental animals based on numerous considerations, including the molecular structure of the chemical and its observed effects in short-term *in vitro* and *in vivo* genetic toxicity tests (structure-activity relationships). The short-term tests were origi-
Finally developed to clarify proposed mechanisms of chemical-induced DNA damage based on the relationship between electrophilicity and mutagenicity (Miller and Miller, 1977) and the somatic mutation theory of cancer (Straus, 1981; Crawford, 1985). However, it should be noted that not all cancers arise through genotoxic mechanisms.

DNA reactivity combined with Salmonella mutagenicity is highly correlated with induction of carcinogenicity in multiple species/sexes of rodents and at multiple tissue sites (Ashby and Tennant, 1991). A positive response in the Salmonella test was shown to be the most predictive \textit{in vitro} indicator for rodent carcinogenicity (89\% of the Salmonella mutagens are rodent carcinogens) (Tennant \textit{et al.}, 1987; Zeiger \textit{et al.}, 1990). Additionally, no battery of tests that included the Salmonella test improved the predictivity of the Salmonella test alone. However, these other tests can provide useful information on the types of DNA and chromosomal damage induced by the chemical under investigation.

The predictivity for carcinogenicity of a positive response in acute \textit{in vivo} bone marrow chromosome aberration or micronucleus tests appears to be less than that in the Salmonella test (Shelby \textit{et al.}, 1993; Shelby and Witt, 1995). However, clearly positive results in long-term peripheral blood micronucleus tests have high predictivity for rodent carcinogenicity (Witt \textit{et al.}, 2000); negative results in this assay do not correlate well with either negative or positive results in rodent carcinogenicity studies. Because of the theoretical and observed associations between induced genetic damage and adverse effects in somatic and germ cells, the determination of \textit{in vivo} genetic effects is important to the overall understanding of the risks associated with exposure to a particular chemical. Most organic chemicals that are identified by the International Agency for Research on Cancer as human carcinogens, other than hormones, are genotoxic. The vast majority of these are detected by both the Salmonella assay and rodent bone marrow cytogenetics tests (Shelby, 1988; Shelby and Zeiger, 1990).
RESULTS

RATS

2-WEEK STUDY

All rats survived to the end of the study (Table 2). Final mean body weights and body weight gains of all dosed groups were similar to those of the vehicle control groups. There were no clinical findings attributed to androstenedione administration.

There were no significant differences in acyl coenzyme A oxidase activity, proliferating cell nuclear antigen concentration, or cyclin-dependent kinase concentration between the dosed groups and the vehicle control groups, suggesting that treatment did not induce peroxisome or cell proliferation (Table G1).

There were no significant differences in organ weights between dosed groups and the vehicle control groups (Table H1).

The only histologic change associated with treatment was the development of cytoplasmic vacuoles within centrilobular hepatocytes in male rats (0 mg/kg, 0/5; 1 mg/kg, 1/5; 5 mg/kg, 2/5; 10 mg/kg, 2/5; 20 mg/kg, 3/5; 50 mg/kg, 3/5). Morphologically, cytoplasmic alteration consisted of irregular lacy clear spaces adjacent to the hepatocyte nucleus, and/or larger round vacuoles also adjacent to the nucleus. The larger round vacuoles often stained lightly eosinophilic. The overall appearance was similar to that generally interpreted to be due to cytoplasmic glycogen.

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Survival</th>
<th>Mean Body Weight</th>
<th>Final Weight</th>
<th>Final Weight</th>
</tr>
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<tbody>
<tr>
<td></td>
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<td>Initial</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5/5</td>
<td>113 ± 5</td>
<td>184 ± 9</td>
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</tr>
<tr>
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<td>5/5</td>
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<td>181 ± 7</td>
<td>71 ± 6</td>
</tr>
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<td>5</td>
<td>5/5</td>
<td>111 ± 3</td>
<td>185 ± 7</td>
<td>74 ± 4</td>
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<td>5/5</td>
<td>110 ± 2</td>
<td>183 ± 3</td>
<td>73 ± 1</td>
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<tr>
<td>50</td>
<td>5/5</td>
<td>110 ± 3</td>
<td>182 ± 3</td>
<td>71 ± 2</td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>5/5</td>
<td>96 ± 2</td>
<td>131 ± 2</td>
<td>36 ± 2</td>
</tr>
<tr>
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<td>127 ± 1</td>
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<td>30 ± 1</td>
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<td>5/5</td>
<td>96 ± 2</td>
<td>137 ± 3</td>
<td>41 ± 3</td>
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<tr>
<td>20</td>
<td>5/5</td>
<td>95 ± 1</td>
<td>132 ± 1</td>
<td>37 ± 2</td>
</tr>
<tr>
<td>50</td>
<td>5/5</td>
<td>95 ± 2</td>
<td>130 ± 2</td>
<td>35 ± 2</td>
</tr>
</tbody>
</table>

a Number of animals surviving at 2 weeks/number initially in group

b Weights and weight changes are given as mean ± standard error.
Dose Selection Rationale: Because there were no effects of androstenedione on survival, mean body weight, or other dose-limiting factors in the 2-week study in rats, doses selected for the 3-month gavage study in rats were 1, 5, 10, 20, and 50 mg/kg. The top dose of 50 mg/kg was the limit of acceptable gavage-ability.

3-Month Study
All rats survived to the end of the study (Table 3). The final mean body weight of the 20 mg/kg female group was significantly greater than the control group. The body weight change was significantly increased from control in the 1, 20, and 50 mg/kg female groups; the final mean body weights of dosed male rats were similar to those of the vehicle control group. There were no clinical findings attributed to androstenedione administration.

There were no changes in hematology or clinical chemistry variables that were considered attributable to androstenedione administration (Table F1).

Absolute thymus weights of 20 and 50 mg/kg females were significantly greater than those of the vehicle controls, but relative to body weight, there was no significant difference in the thymus weight compared to the vehicle controls (Table H2). The numbers of sperm per mg cauda epididymis in the 10, 20, and 50 mg/kg groups were significantly less than that of the vehicle controls,

**Table 3**
Survival and Body Weights of Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Survivala</th>
<th>Mean Body Weightb (g)</th>
<th>Final Weight Relative to Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Male</td>
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<td></td>
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<tr>
<td>0</td>
<td>10/10</td>
<td>101 ± 2</td>
<td>333 ± 7</td>
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<tr>
<td>1</td>
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<td>10/10</td>
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<td>101 ± 2</td>
<td>340 ± 5</td>
</tr>
<tr>
<td>Female</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10/10</td>
<td>92 ± 1</td>
<td>189 ± 2</td>
</tr>
<tr>
<td>1</td>
<td>10/10</td>
<td>89 ± 1</td>
<td>200 ± 4*</td>
</tr>
<tr>
<td>5</td>
<td>10/10</td>
<td>92 ± 1</td>
<td>198 ± 2*</td>
</tr>
<tr>
<td>10</td>
<td>10/10</td>
<td>93 ± 1</td>
<td>198 ± 2*</td>
</tr>
<tr>
<td>20</td>
<td>10/10</td>
<td>90 ± 2</td>
<td>206 ± 3**</td>
</tr>
<tr>
<td>50</td>
<td>10/10</td>
<td>89 ± 1</td>
<td>199 ± 4**</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05) from the vehicle control group by Williams’ test
** P<0.01
a Number of animals surviving at 3 months/number initially in group
b Weights and weight changes are given as mean ± standard error.
and the total number of sperm per cauda epididymis in 50 mg/kg males was significantly less than that of the vehicle controls (Table II). Androstenedione administration for 3 months elicited changes in the male reproductive system of rats that would indicate potential to produce adverse effects in studies of fertility and reproductive performance.

No lesions were observed through gross or histopathologic observation that could be attributed to the administration of androstenedione.

**Dose Selection Rationale:** Because there were no effects on survival, body weights, clinical pathology, or other toxicity parameters indicating an intolerable dose in the 3-month study, the doses selected for the 2-year gavage study in rats were 10, 20, and 50 mg/kg. The top dose of 50 mg/kg was the limit of acceptable gavage-ability.

### 2-YEAR STUDY

#### Survival

Estimates of 2-year survival probabilities for male and female rats are shown in Table 4 and in the Kaplan-Meier survival curves (Figure 2). Survival of 10 mg/kg males was significantly greater than that of the vehicle controls; survival of dosed groups of females was similar to that of the vehicle controls.

#### Body Weights and Clinical Findings

Mean body weights of all dosed groups of male rats were similar to those of the vehicle controls throughout the study (Table 5 and Figure 3). The mean body weights of 20 mg/kg female rats were generally greater than those of the vehicle controls after week 17, and those of 50 mg/kg females were greater after week 9 (Table 6 and Figure 3). There were no clinical findings related to chemical administration.

### Table 4

**Survival of Rats in the 2-Year Gavage Study of Androstenedione**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Moribund</td>
<td>22</td>
<td>9</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Animals surviving to study termination</td>
<td>21&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Percent probability of survival at end of study&lt;sup&gt;a&lt;/sup&gt;</td>
<td>42</td>
<td>66</td>
<td>58</td>
<td>54</td>
</tr>
<tr>
<td>Mean survival (days)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>661</td>
<td>693</td>
<td>684</td>
<td>690</td>
</tr>
<tr>
<td>Survival analysis&lt;sup&gt;c&lt;/sup&gt;</td>
<td>P=0.658N</td>
<td>P=0.018N</td>
<td>P=0.132N</td>
<td>P=0.255N</td>
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<tr>
<td><strong>Female</strong></td>
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<td></td>
<td></td>
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<tr>
<td>Animals initially in study</td>
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<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Moribund</td>
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<td>7</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Natural deaths</td>
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<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Animals surviving to study termination</td>
<td>38</td>
<td>37&lt;sup&gt;d&lt;/sup&gt;</td>
<td>33</td>
<td>37</td>
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<tr>
<td>Percent probability of survival at end of study</td>
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<td>74</td>
<td>66</td>
<td>74</td>
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<tr>
<td>Mean survival (days)</td>
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<td>693</td>
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<td>Survival analysis</td>
<td>P=0.754</td>
<td>P=0.967</td>
<td>P=0.273</td>
<td>P=0.804</td>
</tr>
</tbody>
</table>

<sup>a</sup> Kaplan-Meier determinations  
<sup>b</sup> Mean of all deaths (uncensored, censored, and terminal sacrifice).  
<sup>c</sup> The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.  
<sup>d</sup> Includes one animal that died during the last week of the study.
FIGURE 2
Kaplan-Meier Survival Curves for Male and Female Rats
Administered Androstenedione by Gavage for 2 Years
Table 5
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Weeks on Study</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. Wt. (g)</td>
<td>No. of Survivors</td>
<td>Av. Wt. (g)</td>
<td>Wt. (% of controls)</td>
</tr>
<tr>
<td>1</td>
<td>121</td>
<td>50</td>
<td>121</td>
<td>100</td>
</tr>
<tr>
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<td>99</td>
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<td>35</td>
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Mean for weeks
1-13 271 268 99 269 99 268 99
14-52 451 447 99 440 98 433 96
53-101 508 511 101 504 99 500 98
# Table 6
Mean Body Weights and Survival of Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Weeks on Study</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tbody>
<tr>
<td></td>
<td>Av. Wt. (g)</td>
<td>Av. Wt. (g)</td>
<td>Wt. (% of controls)</td>
<td>No. of Survivors</td>
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<tr>
<td>1</td>
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<td>34</td>
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</table>

Mean for weeks

<table>
<thead>
<tr>
<th>Weeks on Study</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>1-13</td>
<td>169</td>
<td>100</td>
<td>50</td>
<td>171</td>
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<td>14-52</td>
<td>244</td>
<td>103</td>
<td>50</td>
<td>267</td>
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<td>53-101</td>
<td>332</td>
<td>106</td>
<td>50</td>
<td>351</td>
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</tbody>
</table>
Figure 3
Growth Curves for Male and Female Rats
Administered Androstenedione by Gavage for 2 Years
Pathology and Statistical Analyses
This section describes the statistically significant or biologically noteworthy changes in the incidences of mononuclear cell leukemia and neoplasms and/or nonneoplastic lesions of the lung, testes, mammary gland, liver, pancreatic islets, exocrine pancreas, spleen, thyroid gland, and adrenal cortex. Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix A for male rats and Appendix B for female rats.

Mononuclear Cell Leukemia: The incidences of mononuclear cell leukemia were significantly increased in 20 and 50 mg/kg females, while the incidences were significantly decreased in 20 and 50 mg/kg males (Tables 7, A1, A2, B1, and B2). Mononuclear cell leukemia was characterized by a proliferation of neoplastic mononuclear cells in the spleen and liver and in the blood vessels of many other tissues.

Lung: The incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined) were significantly increased in 20 mg/kg males (Tables 8, A1, and A2). Mononuclear cell leukemia was characterized by a proliferation of neoplastic mononuclear cells in the spleen and liver and in the blood vessels of many other tissues.

Testes: The incidences of testicular interstitial cell adenoma (including bilateral) occurred with a negative trend, and the incidence was significantly decreased in 50 mg/kg males (Tables 9, A1, and A2). Interstitial cell adenomas were characterized by small to large nodules of interstitial cells that compressed the surrounding seminiferous tubules; larger neoplasms effaced much of the testicular architecture. Cystic areas filled with an eosinophilic homogenous fluid material and areas of hemorrhage and necrosis were seen in larger adenomas. Neoplastic cells had an abundant, pale, eosinophilic to amphophilic, finely vacuolated cytoplasm, fairly well defined cell borders, and a central nucleus. Mild anisocytosis and anisokaryosis were noted. Mitotic figures were present although usually at less than one per high-power field. A rim of smaller basophilic cells with scant cytoplasm was often located at the margin of the nodules. In some adenomas, large areas were composed of these basophilic cells, having a smaller round to oval nucleus with stippled chromatin and a sparse amount of eosinophilic cytoplasm with indistinct borders. Mitotic figures were rare in this population. Cystic areas containing eosinophilic fluid were also noted in this population.

Mammary Gland: The incidences of mammary gland fibroadenoma occurred with a negative trend, and the incidences were significantly decreased in 20 and 50 mg/kg females (Tables 10, B1, and B2). Furthermore, the incidences of multiple fibroadenomas were decreased. The incidences of fibroadenoma, adenoma, or carcinoma (combined) were significantly decreased in 20 and 50 mg/kg females, mainly due to the decreased incidences of fibroadenoma. The incidences of mammary gland hyperplasia were significantly decreased in all dosed female groups, and the incidences of mammary gland cyst were significantly decreased in 10 and 50 mg/kg females (Tables 10 and B4).

Fibroadenomas consisted of both ductular and/or alveolar epithelium and fibrous connective tissue. Smaller neoplasms usually contained a higher proportion of glandular tissue, and larger ones consisted almost entirely of connective tissue. Smaller fibroadenomas often had a lobular growth pattern; the alveoli within the lobules were usually well formed and composed of a single layer of epithelium that contained clear lipid vacuoles.
Table 7
Incidences of Mononuclear Cell Leukemia in Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>26/50 (52%)</td>
<td>22/50 (44%)</td>
<td>18/50 (36%)</td>
<td>18/50 (36%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>59.0%</td>
<td>47.8%</td>
<td>39.9%</td>
<td>39.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>9/21 (43%)</td>
<td>14/33 (42%)</td>
<td>8/29 (28%)</td>
<td>7/27 (26%)</td>
</tr>
<tr>
<td>First incidence</td>
<td>526</td>
<td>629</td>
<td>558</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.052N</td>
<td>P=0.191N</td>
<td>P=0.050N</td>
<td>P=0.042N</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>11/50 (22%)</td>
<td>18/50 (36%)</td>
<td>15/50 (30%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>10.4%</td>
<td>23.3%</td>
<td>38.4%</td>
<td>30.3%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/38 (3%)</td>
<td>5/37 (14%)</td>
<td>8/33 (24%)</td>
<td>3/37 (8%)</td>
</tr>
<tr>
<td>First incidence</td>
<td>512</td>
<td>610</td>
<td>442</td>
<td>510</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.029</td>
<td>P=0.079</td>
<td>P=0.001</td>
<td>P=0.013</td>
</tr>
</tbody>
</table>

a Number of animals with mononuclear cell leukemia per number of animals necropsied
b Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 49/100 (49.0% ± 4.2%), range 46%-52%; all routes: 553/1,399 (39.5% ± 12.5%), range 8%-58%
c Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
d Observed incidence at terminal kill
e Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.
f Historical incidence for methylcellulose gavage studies: 17/100 (17.0% ± 9.9%), range 10%-24%; all routes: 297/1,350 (22.0% ± 8.8%), range 8%-40%

Alveolar lumens contained secretory material. Fibrous connective tissue consisting of well-differentiated fibrocytes and abundant collagen was distributed within and between lobules. Occasionally, alveoli within lobules were separated by a scant stroma, while broad, dense bands of connective tissue separated the individual lobules. Large dilated ducts filled with secretory product occurred in some fibroadenomas. In larger neoplasms, the majority of the neoplasm was composed of fibrous tissue with only a few atrophied glands remaining. The central portions of larger neoplasms were often necrotic, and only faint outlines of glands were identified.

Mammary gland hyperplasia was characterized by increased layers of ductular and/or alveolar epithelial cells, enlarged lobules, and enlarged ducts filled with secretory product. Alveolar epithelial cells often had a vacuolated cytoplasm. Mammary gland cysts were characterized by large dilated ducts that were more prominently distended with secretory product than the majority of the enlarged ducts in the hyperplastic mammary glands.

Liver: The incidences of basophilic focus were significantly increased in all dosed male groups, the incidence...
### Table 8
Incidences of Neoplasms of the Lung in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Examined Microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Alveolar/bronchiolar Adenoma&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>5/50 (10%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0%</td>
<td>0.0%</td>
<td>11.6%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0/21 (0%)</td>
<td>0/33 (0%)</td>
<td>2/29 (7%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>—&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td>631</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test&lt;sup&gt;e&lt;/sup&gt;</td>
<td>P=0.195</td>
<td>—</td>
<td>P=0.039</td>
<td>P=0.258</td>
</tr>
<tr>
<td>Alveolar/bronchiolar Carcinoma</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Alveolar/bronchiolar Adenoma or Carcinoma&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>5/50 (10%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>0.0%</td>
<td>0.0%</td>
<td>11.6%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/21 (0%)</td>
<td>0/33 (0%)</td>
<td>2/29 (7%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>—&lt;sup&gt;f&lt;/sup&gt;</td>
<td>—</td>
<td>631</td>
<td>687</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.083</td>
<td>—</td>
<td>P=0.039</td>
<td>P=0.137</td>
</tr>
</tbody>
</table>

(T) Terminal sacrifice

<sup>a</sup> Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 1/100 (1.0% ± 1.4%), range 0%-2%; all routes: 34/1,399 (2.4% ± 2.8%), range 0%-8%

<sup>b</sup> Number of animals with neoplasm per number of animals with lung examined microscopically

<sup>c</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice.

<sup>f</sup> Not applicable; no neoplasms in animal group

<sup>g</sup> Value of statistic cannot be computed

<sup>h</sup> Historical incidence for methylcellulose gavage studies: 1/100 (1.0% ± 1.4%), range 0%-2%; all routes: 47/1,399 (3.4% ± 3.0%), range 0%-10%

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The incidence of clear cell foci was significantly increased in 20 mg/kg males, and the incidence of eosinophilic focus was significantly increased in 50 mg/kg males (Tables 11 and A4). The incidences of cytoplasmic vacuolization were significantly decreased in 20 and 50 mg/kg males. The incidence of bile duct hyperplasia was significantly increased in 50 mg/kg females, and the incidences of mixed cell infiltrates were significantly increased in all dosed female groups (Tables 11 and B4). In contrast to males, basophilic foci incidences were decreased in dosed females.

Basophilic foci were randomly distributed in liver sections. Foci were generally oval to round with irregular but distinct margins, and hepatic cords were arranged in a relatively normal pattern that merged with the surrounding hepatic cords. Hepatocytes sometimes extended beneath the endothelium of a central vein. Basophilic foci consisted of small basophilic hepatocytes; basophilia was characterized by the presence of dense linear aggregates (tigroid pattern) in the cytoplasm.

Clear cell foci were composed of distinct groups of hepatocytes in which the cytoplasm was less dense or clear. The hepatocytes were generally of normal size or slightly enlarged and had a centrally located nucleus. Some hepatocytes containing discrete vacuoles consistent with lipid were sometimes found within clear cell foci.
### TABLE 9
Incidences of Testicular Interstitial Cell Adenoma in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>Number Examined Microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Bilateral Interstitial Cell Adenoma</td>
<td>29</td>
<td>29</td>
<td>28</td>
<td>10**</td>
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<tr>
<td><strong>Significantly different (P&lt;0.01) from the vehicle control group by the Poly-3 test</strong></td>
<td></td>
<td></td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Tool</th>
<th>Adusted rate</th>
<th>Terminal rate</th>
<th>First incidence (days)</th>
<th>Poly-3 test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>42/50 (84%)</td>
<td>39/50 (78%)</td>
<td>36/50 (72%)</td>
<td>26/50 (52%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>91.1%</td>
<td>83.8%</td>
<td>79.2%</td>
<td>58.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>20/21 (95%)</td>
<td>30/33 (91%)</td>
<td>24/29 (83%)</td>
<td>19/27 (70%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>464</td>
<td>540</td>
<td>558</td>
<td>607</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.001N</td>
<td>P=0.203N</td>
<td>P=0.070N</td>
<td>P=0.001N</td>
</tr>
</tbody>
</table>

** Significantly different (P<0.01) from the vehicle control group by the Poly-3 test

| a | Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 83/100 (83.0% ± 1.4%), range 82%-84%; all routes: 1,170/1,399 (83.6% ± 11.5%), range 58%-98% |
| b | Number of animals with neoplasm per number of animals with testis examined microscopically |
| c | Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality |
| d | Observed incidence at terminal kill |
| e | Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N. |

Although hepatocytes in clear cell foci were somewhat disorganized, they merged gradually with the cords of the surrounding hepatic parenchyma.

Eosinophilic foci were well-circumscribed lesions, one to two lobules in diameter, and consisted of enlarged hepatocytes with distinct granular eosinophilic cytoplasm. Minimal to slight compression of surrounding hepatocytes was noted. Architecture of the liver lobule was retained as is typical of foci of cellular alteration in general.

Hepatocellular vacuolization was characterized by the presence of small to moderately large, well-delineated intracytoplasmic clear vacuoles. These vacuoles occurred more often in the centrilobular and midzonal areas with sparing of the periportal areas.

Bile duct hyperplasia was characterized by either a few, small, well-circumscribed groups of small bile ducts in the triad areas randomly extending into the surrounding parenchyma. The latter type of hyperplasia was more commonly seen in livers that were also affected with mononuclear cell leukemia.

Mixed cell infiltrates were characterized by randomly scattered small foci of mixed inflammatory cells consisting of macrophages, lymphocytes, and an occasional neutrophil. The cellular infiltrates were usually organized into either a nodular cluster of inflammatory cells with macrophages in the center surrounded by a rim of lymphocytes or as small foci of randomly mixed inflammatory cells.

**Pancreatic Islets:** The incidence of pancreatic islet hyperplasia was significantly increased in 50 mg/kg females (Tables 11 and B4) but generally affected only some of the islets in a section of pancreas. Islets were increased in size and often were elongated rather than round. Hyperplasia tended to be more severe with the occurrence of exocrine atrophy. The islet cells retained normal cytologic appearance. Hyperplasia was graded minimal if it affected one to three islets in a section and
TABLE 10
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Female Rats
in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number Examined Microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Hyperplasiaa</td>
<td>48 (2.2)b</td>
<td>40** (2.3)</td>
<td>35** (2.0)</td>
<td>23** (1.8)</td>
</tr>
<tr>
<td>Cyst</td>
<td>15 (2.1)</td>
<td>3** (1.7)</td>
<td>9 (2.1)</td>
<td>3** (2.7)</td>
</tr>
<tr>
<td>Fibroadenoma, Multiple</td>
<td>17</td>
<td>13</td>
<td>6**</td>
<td>3**</td>
</tr>
</tbody>
</table>

Fibroadenoma
- Overall ratee | 35/50 (70%) | 31/50 (62%) | 22/50 (44%) | 12/50 (24%) |
- Adjusted ratee | 72.3% | 66.1% | 47.6% | 26.9% |
- Terminal ratef | 28/38 (74%) | 26/37 (70%) | 13/33 (39%) | 11/37 (30%) |
- First incidence (days) | 619 | 652 | 547 | 610 |
- Poly-3 testg | P<0.001N | P=0.326N | P=0.009N | P<0.001N |

Adenoma | 1 | 0 | 0 | 0 |
Carcinoma | 2 | 1 | 0 | 0 |

Fibroadenoma, Adenoma, or Carcinomah
- Overall rate | 37/50 (74%) | 32/50 (64%) | 22/50 (44%) | 12/50 (24%) |
- Adjusted rate | 75.7% | 68.2% | 47.6% | 26.9% |
- Terminal rate | 28/38 (74%) | 27/37 (73%) | 13/33 (39%) | 11/37 (30%) |
- First incidence (days) | 619 | 652 | 547 | 610 |
- Poly-3 test | P<0.001N | P=0.272N | P=0.003N | P<0.001N |

** Significantly different (P<0.01) from the vehicle control group by the Poly-3 test
a Number of animals with lesion
b Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
c Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 63/100 (63.0% ± 9.9%), range 56%-70%; all routes: 697/1,350 (51.6% ± 14.9%), range 24%-86%
d Number of animals with neoplasm per number of animals necropsied
e Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
f Observed incidence at terminal kill
g Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.
h Historical incidence for methylcellulose gavage studies: 67/100 (67.0% ± 9.9%), range 60%-74%; all routes: 742/1,350 (55.0% ± 14.3%), range 28%-86%
TABLE 11
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Basophilic Focus</td>
<td>17</td>
<td>29*</td>
<td>29*</td>
<td>33**</td>
</tr>
<tr>
<td>Clear Cell Focus</td>
<td>13</td>
<td>21</td>
<td>23*</td>
<td>17</td>
</tr>
<tr>
<td>Eosinophilic Focus</td>
<td>3</td>
<td>10</td>
<td>7</td>
<td>13**</td>
</tr>
<tr>
<td>Cytoplasmic Vacuolization</td>
<td>25 (1.8)c</td>
<td>18 (1.9)</td>
<td>14* (2.1)</td>
<td>9** (1.4)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Basophilic Focus</td>
<td>47</td>
<td>46</td>
<td>42</td>
<td>38</td>
</tr>
<tr>
<td>Bile Duct Hyperplasia</td>
<td>12 (1.3)</td>
<td>16 (1.3)</td>
<td>18 (1.3)</td>
<td>24** (1.2)</td>
</tr>
<tr>
<td>Mixed Cell Infiltration</td>
<td>21 (1.0)</td>
<td>33** (1.2)</td>
<td>32** (1.2)</td>
<td>31** (1.2)</td>
</tr>
<tr>
<td>Pancreatic Islets</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>0</td>
<td>4 (1.8)</td>
<td>1 (2.0)</td>
<td>11** (1.8)</td>
</tr>
<tr>
<td>Exocrine Pancreas</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Atrophy</td>
<td>10 (1.6)</td>
<td>10 (1.8)</td>
<td>16 (1.8)</td>
<td>26** (1.6)</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05) from the vehicle control group by the Poly-3 test
** P<0.01
a Number of animals with tissue examined microscopically
b Number of animals with lesion
c Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Androstenedione contained fewer exocrine cells and fewer zymogen granules within the cytoplasm of some or all of the exocrine cells in the acini. Transitional structures containing a few normal acinar cells, small atrophic acinar cells, and cuboidal cells similar to those seen in the ducts were also present.

Other Organs: The incidence of lymphoid follicular hyperplasia of the spleen was significantly increased in 50 mg/kg females (Table B4). Lymphoid follicular hyperplasia was characterized by focal to multifocal expansile lesions composed of mature lymphocytes with interspersed aggregates of pale-staining macrophages. Some lesions compressed adjacent splenic tissue and distorted the capsular surface. In the thyroid gland of males, the incidences of C-cell hyperplasia occurred with a negative trend and the incidence in the 50 mg/kg group was significantly decreased (Table A4). In female rats, the incidences of focal hypertrophy of the adrenal cortex occurred with a negative trend and the incidence in the 50 mg/kg group was significantly decreased (Table B4).
MICE

2-WEEK STUDY

One vehicle control female, one 20 mg/kg female, and one 50 mg/kg female died early due to gavage accidents; all other mice survived to the end of the study (Table 12). Initial and final mean body weights of dosed groups of males were significantly less than those of the vehicle controls due to an error in distributing animals among the treatment groups. However, weight change during the treatment period did not differ except for the 10 mg/kg dose group, which suggests treatment did not affect male body weight. Final mean body weights and mean body weight gains of dosed female groups were similar to those of the vehicle controls, except for the initial weight of the 50 mg/kg group. There were no clinical findings attributed to androstenedione administration.

There were no significant differences in acyl coenzyme A oxidase activity, proliferating cell nuclear antigen concentration, or cyclin-dependent kinase concentration between dosed groups and the vehicle control groups (Table G2).

Absolute heart weights of males administered 5 mg/kg or greater and the absolute lung weights of 1, 5, 20, and 50 mg/kg males were significantly less than those of the vehicle controls, which may be due to the error of assigning lower body weight animals to the dosed groups (Table H3). Relative liver weights of 10, 20, and 50 mg/kg females were significantly greater than those of the vehicle controls.

There were no significant chemical-related histopathological changes.

### TABLE 12

Survival and Body Weights of Mice in the 2-Week Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Survivala</th>
<th>Mean Body Weightb (g)</th>
<th>Final Weight Relative to Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>5/5</td>
<td>24.7 ± 0.5</td>
<td>27.9 ± 1.0</td>
</tr>
<tr>
<td>1</td>
<td>5/5</td>
<td>23.0 ± 0.6*</td>
<td>25.6 ± 0.5**</td>
</tr>
<tr>
<td>5</td>
<td>5/5</td>
<td>23.2 ± 0.2*</td>
<td>25.5 ± 0.3**</td>
</tr>
<tr>
<td>10</td>
<td>5/5</td>
<td>23.3 ± 0.2**</td>
<td>24.7 ± 0.3**</td>
</tr>
<tr>
<td>20</td>
<td>5/5</td>
<td>22.3 ± 0.4***</td>
<td>24.9 ± 0.4**</td>
</tr>
<tr>
<td>50</td>
<td>5/5</td>
<td>22.3 ± 0.4**</td>
<td>25.1 ± 0.5**</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4/5c</td>
<td>20.1 ± 0.4</td>
<td>21.1 ± 0.1</td>
</tr>
<tr>
<td>1</td>
<td>5/5</td>
<td>19.4 ± 0.4</td>
<td>21.7 ± 0.4</td>
</tr>
<tr>
<td>5</td>
<td>5/5</td>
<td>19.2 ± 0.2</td>
<td>19.9 ± 0.4</td>
</tr>
<tr>
<td>10</td>
<td>5/5</td>
<td>19.6 ± 0.1</td>
<td>21.3 ± 0.3</td>
</tr>
<tr>
<td>20</td>
<td>4/5c</td>
<td>19.2 ± 0.3</td>
<td>20.2 ± 0.5</td>
</tr>
<tr>
<td>50</td>
<td>4/5c</td>
<td>18.8 ± 0.3*</td>
<td>20.3 ± 0.2</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05) from the vehicle control group by Williams’ or Dunnett’s test
** P<0.01

a Number of animals surviving at 2 weeks/number initially in group
b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
c Gavage accident
Dose Selection Rationale: Because there were no effects of androstenedione in mice on survival, mean body weights, or other dose-limiting factors in the 2-week study, doses selected for the 3-month gavage study in mice were 1, 5, 10, 20, and 50 mg/kg. The top dose of 50 mg/kg was the limit of acceptable gavageability.

3-Month Study
One 10 mg/kg female died early due to a dosing accident; all other mice survived to the end of the study (Table 13). Final mean body weights and mean body weight gains of all dosed groups were similar to those of the vehicle control groups. There were no clinical findings attributed to androstenedione administration.

There were no changes in hematology variables that were considered attributable to androstenedione administration (Table F2).

There were no biologically significant differences in organ weights between the dosed groups and vehicle control groups (Table H4).

The number of spermatids per mg testis and the total number of spermatids per testis in 20 mg/kg males were significantly greater than those of the vehicle controls (Table I3). Sperm motility in 50 mg/kg males was significantly lower than that in the vehicle controls. Androstenedione administration for 3 months elicited changes in the male reproductive system of mice that

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Survivala</th>
<th>Mean Body Weightb (g)</th>
<th>Final Weight Relative to Controls (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10/10</td>
<td>23.5 ± 0.3</td>
<td>37.5 ± 0.9</td>
</tr>
<tr>
<td>1</td>
<td>10/10</td>
<td>23.5 ± 0.4</td>
<td>37.9 ± 1.2</td>
</tr>
<tr>
<td>5</td>
<td>10/10</td>
<td>23.3 ± 0.3</td>
<td>38.7 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>10/10</td>
<td>23.4 ± 0.4</td>
<td>39.1 ± 1.3</td>
</tr>
<tr>
<td>20</td>
<td>10/10</td>
<td>23.7 ± 0.3</td>
<td>39.3 ± 0.9</td>
</tr>
<tr>
<td>50</td>
<td>10/10</td>
<td>23.1 ± 0.5</td>
<td>38.2 ± 1.2</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>10/10</td>
<td>19.2 ± 0.3</td>
<td>30.9 ± 1.0</td>
</tr>
<tr>
<td>1</td>
<td>10/10</td>
<td>19.5 ± 0.3</td>
<td>32.0 ± 1.5</td>
</tr>
<tr>
<td>5</td>
<td>10/10</td>
<td>19.1 ± 0.2</td>
<td>31.6 ± 0.9</td>
</tr>
<tr>
<td>10</td>
<td>9/10c</td>
<td>19.1 ± 0.3</td>
<td>30.9 ± 1.0</td>
</tr>
<tr>
<td>20</td>
<td>10/10</td>
<td>19.1 ± 0.2</td>
<td>30.8 ± 0.8</td>
</tr>
<tr>
<td>50</td>
<td>10/10</td>
<td>19.1 ± 0.2</td>
<td>29.3 ± 0.7</td>
</tr>
</tbody>
</table>

a Number of animals surviving at 3 months/number initially in group
b Weights and weight changes are given as mean ± standard error. Subsequent calculations are based on animals surviving to the end of the study.
c Week of death: 12
would indicate potential to produce adverse effects in studies of fertility and reproductive performance.

The incidences of x-zone atrophy of the adrenal cortex in female mice administered 5 mg/kg or greater were significantly increased, and the severity increased with increasing dose (Table 14). The incidences of x-zone cytoplasmic vacuolization of the adrenal cortex were significantly decreased in 20 and 50 mg/kg females. There were also decreases in the size of the vacuoles in 5, 10, and 20 mg/kg females, as indicated by decreases in severity. The x-zone of the adrenal gland is located at the junction of the cortex and medulla, is unique to the mouse, and is composed of basophilic cells that are typically vacuolated in the female but not in the male (sexual dimorphism). In male mice, this region normally undergoes involution at approximately 5 weeks, but in female mice, this zone reaches a maximum size at approximately 9 weeks and then gradually regresses in virgins and rapidly upon first pregnancy.

There were significantly increased incidences of bone marrow hyperplasia in 5 and 50 mg/kg male mice (Table 14). Bone marrow hyperplasia was characterized by an increase of immature cells in the marrow cavity of the femur.

*Dose Selection Rationale:* Because there were no effects on survival or body weights and there was a lack of significant toxicity in mice administered 50 mg/kg in the 3-

### Table 14
Incidence of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bone Marrow&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Hyperplasia&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>0</td>
<td>4* (1.3)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1 (1.0)</td>
<td>2 (1.5)</td>
<td>5* (1.2)</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal Cortex</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>X-Zone Atrophy</td>
<td>0</td>
<td>0</td>
<td>6** (1.7)</td>
<td>9** (1.8)</td>
<td>10** (2.5)</td>
<td>10** (2.8)</td>
</tr>
<tr>
<td>X-Zone Cytoplasmic Vacuolization</td>
<td>10 (1.6)</td>
<td>10 (1.7)</td>
<td>10 (1.4)</td>
<td>10 (1.2)</td>
<td>1** (1.0)</td>
<td>0**</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of animals with tissue examined microscopically  
<sup>b</sup> Number of animals with lesion  
<sup>c</sup> Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked  
<sup>*</sup> Significantly different (P < 0.05) from the vehicle control group by the Fisher exact test  
<sup>**</sup> P < 0.01
month study, the doses selected for the 2-year gavage study in mice were 10, 20, and 50 mg/kg for males and 2, 10, and 50 mg/kg for females. The lower dose group of 2 mg/kg in females was selected due to suspected ovarian atrophy observed in the 3-month study; however, this finding was not confirmed upon reexamination by the Pathology Working Group.

2-YEAR STUDY

Survival
Estimates of 2-year survival probabilities for male and female mice are shown in Table 15 and in the Kaplan-Meier survival curves (Figure 4). Survival of dosed groups was similar to that of the vehicle control groups.

Body Weights and Clinical Findings
Mean body weights of 10 and 50 mg/kg female mice were generally less than those of the vehicle controls after weeks 81 and 17, respectively; mean body weights of dosed male mice were similar to those of the vehicle controls throughout the study (Figure 5; Tables 16 and 17). There were no clinical findings related to chemical administration.

Pathology and Statistical Analyses
This section describes the statistically significant or biologically noteworthy changes in the incidences of malignant lymphoma and neoplasms and nonneoplastic lesions of the liver, pancreatic islets, clitoral gland, kidney, submandibular salivary gland, bone marrow, and thymus.

<table>
<thead>
<tr>
<th>TABLE 15</th>
<th>Survival of Mice in the 2-Year Gavage Study of Androstenedione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vehicle Control</td>
</tr>
<tr>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>Animals initially in study</td>
<td>50</td>
</tr>
<tr>
<td>Moribund</td>
<td>5</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>9</td>
</tr>
<tr>
<td>Animals surviving to study termination</td>
<td>36</td>
</tr>
<tr>
<td>Percent probability of survival at end of study</td>
<td>72</td>
</tr>
<tr>
<td>Mean survival (days)</td>
<td>687</td>
</tr>
<tr>
<td>Survival analysis</td>
<td>P=0.797</td>
</tr>
<tr>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Animals initially in study</td>
<td>50</td>
</tr>
<tr>
<td>Accidental deaths</td>
<td>2</td>
</tr>
<tr>
<td>Moribund</td>
<td>6</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>7</td>
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<tr>
<td>Animals surviving to study termination</td>
<td>35</td>
</tr>
<tr>
<td>Percent probability of survival at end of study</td>
<td>73</td>
</tr>
<tr>
<td>Mean survival (days)</td>
<td>688</td>
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<tr>
<td>Survival analysis</td>
<td>P=0.615N</td>
</tr>
</tbody>
</table>

\(^a\) Kaplan-Meier determinations
\(^b\) Mean of all deaths (uncensored, censored, and terminal sacrifice).
\(^c\) The result of the life table trend test (Tarone, 1975) is in the vehicle control column, and the results of the life table pairwise comparisons (Cox, 1972) with the vehicle controls are in the dosed group columns. A negative trend or lower mortality in a dose group is indicated by N.
\(^d\) Censored from survival analyses
\(^e\) Includes one animal that died during the last week of the study
FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered Androstenedione by Gavage for 2 Years
FIGURE 5
Growth Curves for Male and Female Mice
Administered Androstenedione by Gavage for 2 Years
Table 16
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Weeks on Study</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Av. Wt. (g)</td>
<td>Av. Wt. (g)</td>
<td>Av. Wt. (g)</td>
<td>Av. Wt. (g)</td>
<td>Av. Wt. (g)</td>
</tr>
<tr>
<td>No. of Survivors</td>
<td>Wt. (% of controls)</td>
<td>No. of Survivors</td>
<td>Wt. (% of controls)</td>
<td>No. of Survivors</td>
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<tr>
<td>1</td>
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<td>23.4</td>
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<td>2</td>
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<td>53.8</td>
<td>53.2</td>
<td>51.5</td>
<td>52.4</td>
</tr>
</tbody>
</table>

Mean for weeks

1-13 30.0 29.8 30.1 30.0
14-52 48.1 48.1 48.1 48.3
53-101 52.9 54.1 52.2 53.6
Table 17
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Weeks on Study</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Av. Wt. (g)</td>
<td>No. of Survivors</td>
<td>Av. Wt. (g)</td>
<td>Wt. (% of controls)</td>
</tr>
<tr>
<td>1</td>
<td>18.9</td>
<td>50</td>
<td>19.0</td>
<td>101</td>
</tr>
<tr>
<td>2</td>
<td>19.8</td>
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<td>19.8</td>
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<td>3</td>
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<td>25.4</td>
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<td>37.0</td>
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<td>21</td>
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<td>23</td>
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<td>59.0</td>
<td>100</td>
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<td>60.6</td>
<td>48</td>
<td>59.9</td>
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<tr>
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<td>61.4</td>
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<td>28</td>
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<td>29</td>
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<td>60.1</td>
<td>99</td>
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<tr>
<td>30</td>
<td>61.8</td>
<td>45</td>
<td>60.0</td>
<td>97</td>
</tr>
<tr>
<td>31</td>
<td>63.9</td>
<td>44</td>
<td>60.5</td>
<td>95</td>
</tr>
<tr>
<td>32</td>
<td>63.0</td>
<td>44</td>
<td>60.2</td>
<td>96</td>
</tr>
<tr>
<td>33</td>
<td>61.8</td>
<td>42</td>
<td>61.0</td>
<td>97</td>
</tr>
<tr>
<td>34</td>
<td>63.4</td>
<td>39</td>
<td>61.4</td>
<td>97</td>
</tr>
<tr>
<td>35</td>
<td>65.3</td>
<td>36</td>
<td>63.1</td>
<td>97</td>
</tr>
</tbody>
</table>

Mean for weeks
1-13 23.7 24.0 97 100 23.7 100 100 23.9 103 50
14-52 45.5 46.7 97 103 45.0 99 99 42.5 94 50
53-101 61.7 60.6 98 96 59.4 96 96 58.1 94 49
Summaries of the incidences of neoplasms and nonneoplastic lesions, statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group, and historical incidences for the neoplasms mentioned in this section are presented in Appendix C for male mice and Appendix D for female mice.

**Liver:** There were positive trends in the incidences of hepatocellular adenoma in male and female mice, and the incidences in the 50 mg/kg groups were significantly increased (Tables 18, C1, C2, D1, and D2). The incidences of hepatocellular carcinoma in all dosed groups of females were significantly increased. In 10 and 50 mg/kg males, there were significantly increased incidences of multiple hepatocellular adenoma and multiple hepatocellular carcinoma. There was a significantly increased incidence of multiple hepatocellular adenoma in 50 mg/kg females. There were positive trends in the incidences of hepatocellular adenoma or carcinoma (combined) in males and females, with significantly increased incidences in 50 mg/kg males and females. The incidences of hepatoblastoma and multiple hepatoblastoma were marginally increased in dosed males. There was a positive trend in the incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma (combined) in males, and the incidence in the 50 mg/kg group was significantly increased.

Hepatocellular adenomas were characterized by well-circumscribed nodular lesions larger than a liver lobule and composed of well-differentiated hepatocytes of variable size and tinctorial properties. The cytoplasm varied among eosinophilic, basophilic, vacuolated, or a mixture. Normal lobular architecture was lost, and hepatic plates at the margins impinged at sharp angles to the surrounding normal liver plates. Central veins and portal tracts were not readily apparent, although some were trapped in the expanding mass at the periphery. Some adenomas were solid and others had plates one to three cell layers thick. Cellular atypia and mitotic figures were occasionally observed. Focal areas of fatty change (lipidosis) within adenomas were occasionally noted.

Hepatocellular carcinomas were not always well demarcated and often had irregular borders as cells infiltrated into the surrounding parenchyma. Cellular atypia and mitotic figures were common. Nucleoli were often enlarged and multiple. Cells had eosinophilic, basophilic, vacuolated, or mixed tinctorial appearances. Some carcinomas had a solid growth pattern, while a trabecular pattern was also common. Necrosis was noted in some tumors.

There were significantly decreased incidences of clear cell focus in 20 and 50 mg/kg males and a significantly increased incidence of eosinophilic focus in 50 mg/kg males (Tables 18 and C4). The incidences of mixed cell focus and cytoplasmic vacuolization were significantly increased in 50 mg/kg females (Tables 18 and D4).

Clear cell foci were composed of hepatocytes in which the cytoplasm was less dense or clear due to loss of glycogen during tissue processing. The hepatocytes were generally of normal size or slightly enlarged and had a centrally located nucleus. Hepatocytes containing discrete vacuoles consistent with lipid were occasionally found within clear cell foci. Although hepatocytes in clear cell foci were somewhat disorganized, they merged gradually with the cords of the surrounding hepatic parenchyma.

Eosinophilic foci were well-circumscribed lesions one to two lobules in diameter and consisted of enlarged hepatocytes with distinct, granular, eosinophilic cytoplasm. Minimal to slight compression of surrounding hepatocytes was noted. Architecture of the liver lobule was retained.

Mixed cell foci generally were round to oval and varied from less than one hepatic lobule to several lobules in diameter. The hepatic plates merged imperceptibly with the surrounding hepatocytes and caused little to no compression of the surrounding parenchyma. The normal architecture was retained with triad areas and central veins found within the focus. Mixed foci were composed of a mixture of hepatocyte cell types as found in basophilic, eosinophilic, clear, or vacuolated cell type foci with generally no predominant cell type, although vacuolated cells were often present.

Hepatocellular vacuolization was characterized by the presence of moderate to fairly large, well-delineated, intracytoplasmic, clear vacuoles. These vacuoles were randomly distributed in the lobules and often occurred in variable numbers in individual lobe sections. Severity grades were determined by assessing the overall number of hepatocytes with vacuoles in all liver lobe sections present.
### TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Examined Microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Clear Cell Focus(^a)</td>
<td>27</td>
<td>24</td>
<td>18*</td>
<td>12**</td>
</tr>
<tr>
<td>Eosinophilic Focus</td>
<td>13</td>
<td>10</td>
<td>11</td>
<td>25*</td>
</tr>
<tr>
<td>Hepatocellular Adenoma, Multiple</td>
<td>16</td>
<td>27*</td>
<td>23</td>
<td>34**</td>
</tr>
<tr>
<td>Hepatocellular Adenoma (includes multiple)(^b)</td>
<td>32/50 (64%)</td>
<td>38/50 (76%)</td>
<td>29/50 (58%)</td>
<td>43/50 (86%)</td>
</tr>
<tr>
<td>Adjusted rate(^d)</td>
<td>71.2%</td>
<td>78.6%</td>
<td>63.9%</td>
<td>91.8%</td>
</tr>
<tr>
<td>Terminal rate(^e)</td>
<td>27/36 (75%)</td>
<td>38/44 (86%)</td>
<td>25/34 (74%)</td>
<td>36/37 (97%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>597</td>
<td>729 (T)</td>
<td>613</td>
<td>554</td>
</tr>
<tr>
<td>Poly-3 test(^f)</td>
<td>P=0.009</td>
<td>P=0.270</td>
<td>P=0.298N</td>
<td>P=0.005</td>
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<tr>
<td>Hepatocellular Carcinoma, Multiple</td>
<td>7</td>
<td>12**</td>
<td>10</td>
<td>17**</td>
</tr>
<tr>
<td>Hepatocellular Carcinoma (includes multiple)(^b)</td>
<td>26</td>
<td>33</td>
<td>28</td>
<td>32</td>
</tr>
<tr>
<td>Hepatocellular Adenoma or Carcinoma(^h)</td>
<td>41/50 (82%)</td>
<td>47/50 (94%)</td>
<td>42/50 (84%)</td>
<td>48/50 (96%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>85.2%</td>
<td>94.1%</td>
<td>87.9%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>31/36 (86%)</td>
<td>42/44 (96%)</td>
<td>29/34 (85%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.025</td>
<td>P=0.122</td>
<td>P=0.460</td>
<td>P=0.012</td>
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<tr>
<td>Hepatoblastoma, Multiple</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Hepatoblastoma (includes multiple)(^j)</td>
<td>3/50 (6%)</td>
<td>8/50 (16%)</td>
<td>7/50 (14%)</td>
<td>8/50 (16%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>6.9%</td>
<td>16.4%</td>
<td>15.7%</td>
<td>17.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/36 (8%)</td>
<td>7/44 (16%)</td>
<td>6/34 (18%)</td>
<td>6/37 (16%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>593</td>
<td>631</td>
<td>648</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.182</td>
<td>P=0.141</td>
<td>P=0.169</td>
<td>P=0.114</td>
</tr>
<tr>
<td>Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma(^i)</td>
<td>41/50 (82%)</td>
<td>47/50 (94%)</td>
<td>43/50 (86%)</td>
<td>48/50 (96%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>85.2%</td>
<td>94.1%</td>
<td>89.4%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>31/36 (86%)</td>
<td>42/44 (96%)</td>
<td>29/34 (85%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.024</td>
<td>P=0.122</td>
<td>P=0.374</td>
<td>P=0.012</td>
</tr>
</tbody>
</table>
### Table 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Number Examined Microscopically</td>
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<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Mixed Cell Focus</td>
<td>2</td>
<td>5</td>
<td>7</td>
<td>15**</td>
</tr>
<tr>
<td>Hepatocyte, Vacuolization Cytoplasmic</td>
<td>6 (2.7)*</td>
<td>3 (2.3)</td>
<td>9 (2.1)</td>
<td>22** (2.6)</td>
</tr>
<tr>
<td>Hepatocellular Adenoma, Multiple</td>
<td>4</td>
<td>7</td>
<td>7</td>
<td>17**</td>
</tr>
<tr>
<td>Overall rate</td>
<td>14/50 (28%)</td>
<td>16/50 (32%)</td>
<td>18/50 (36%)</td>
<td>28/50 (56%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>31.6%</td>
<td>34.6%</td>
<td>39.1%</td>
<td>61.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>13/35 (37%)</td>
<td>14/40 (35%)</td>
<td>16/40 (40%)</td>
<td>27/40 (68%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>622</td>
<td>622</td>
<td>520</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001</td>
<td>P=0.468</td>
<td>P=0.299</td>
<td>P=0.003</td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>13/50 (26%)</td>
<td>15/50 (30%)</td>
<td>15/50 (30%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>11.3%</td>
<td>28.2%</td>
<td>32.0%</td>
<td>32.7%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/35 (9%)</td>
<td>11/40 (28%)</td>
<td>11/40 (28%)</td>
<td>13/40 (33%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>687</td>
<td>685</td>
<td>442</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.098</td>
<td>P=0.038</td>
<td>P=0.015</td>
<td>P=0.012</td>
</tr>
<tr>
<td>Overall rate</td>
<td>17/50 (34%)</td>
<td>23/50 (46%)</td>
<td>27/50 (54%)</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>38.2%</td>
<td>49.5%</td>
<td>57.0%</td>
<td>69.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>14/35 (40%)</td>
<td>20/40 (50%)</td>
<td>22/40 (55%)</td>
<td>30/40 (75%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>622</td>
<td>622</td>
<td>442</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.004</td>
<td>P=0.188</td>
<td>P=0.052</td>
<td>P&lt;0.001</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05) from the vehicle control group by the Poly-3 test
** P<0.01
(T) Terminal sacrifice

- **a** Number of animals with lesion
- **b** Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 60/100 (60.0% ± 5.7%), range 56%-64%; all routes: 733/1,447 (50.7% ± 13.9%), range 22%-72%
- **c** Number of animals with neoplasm per number of animals with liver examined microscopically
- **d** Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
- **e** Observed incidence at terminal kill
- **f** Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by N.
- **g** Historical incidence for methylcellulose gavage studies: 39/100 (39.0% ± 18.4%), range 26%-52%; all routes: 415/1,447 (28.7% ± 8.8%), range 16%-52%
- **h** Historical incidence for methylcellulose gavage studies: 78/100 (78.0% ± 5.7%), range 74%-82%; all routes: 961/1,447 (66.4% ± 12.6%), range 36%-84%
- **i** Historical incidence for methylcellulose gavage studies: 5/100 (5.0% ± 1.4%), range 4%-6%; all routes: 48/1,447 (3.3% ± 6.4%), range 0%-34%
- **j** Historical incidence for methylcellulose gavage studies: 79/100 (79.0% ± 4.2%), range 76%-82%; all routes: 972/1,447 (67.2% ± 13.1%), range 36%-92%
- **k** Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
- **l** Historical incidence for methylcellulose gavage studies: 29/100 (29.0% ± 1.4%), range 28%-30%; all routes: 396/1,494 (26.5% ± 15.2%), range 2%-62%
- **m** Historical incidence for methylcellulose gavage studies: 10/100 (10.0% ± 0.0%), range 10%; all routes: 137/1,494 (9.2% ± 6.7%), range 0%-28%
- **n** Historical incidence for methylcellulose gavage studies: 36/100 (36.0% ± 2.8%), range 34%-38%; all routes: 481/1,494 (32.2% ± 17.3%), range 6%-64%
Pancreatic Islets: There were increased incidences of pancreatic islet adenoma in 50 mg/kg males and 10 and 50 mg/kg females, and a decreased incidence of hyperplasia in 50 mg/kg males, but these differences were not significant (Tables 19, C2, and C4). In 50 mg/kg males, first incidence of pancreatic islet adenoma occurred earlier, and one animal had multiple pancreatic islet adenomas. Pancreatic islet adenomas were characterized by an increased size of a single islet with a more uniform population of cells having a pale pink, lacy cytoplasm and central nuclei with a delicate chromatin pattern.

Clitoral Gland: The incidences and severities of hyperplasia and duct dilatation in female mice increased with increasing dose, and the incidences in the 10 and 50 mg/kg groups were significantly increased (Tables 20 and D4).

Glandular hyperplasia was characterized by increased amounts of sebaceous glands located around the ducts. Individual sebaceous gland cells were of normal size and tinctorial staining. Severity grades for glandular hyperplasia were based on a semiquantitative evaluation of the amount of sebaceous glands in a section: grade 0: none to only one or two small foci of glands; grade 1: three or five small clusters of sebaceous glands; grade 2: several moderately sized clusters or at least one larger cluster; grade 3: several moderate or one large area of sebaceous glands.

Duct dilatation was characterized by an increase in the size and number of duct profiles. The ducts were lined by a keratinizing squamous epithelium, although the lining was often attenuated and consisted of a single layer of flattened squamous epithelial cells. The dilated duct contents were composed of pale, basophilic, amorphous material and keratin debris, although the cyst contents were often lost in processing. Severity of duct dilatation was graded on a semiquantitative estimate of the percentage of a 4× microscopic field that was occupied by the dilated ducts of the clitoral glands: grade 0: neither gland occupied more than 15% of the microscopic field; grade 1 (minimal): at least one gland occupied 16% to 25% of the microscopic field; grade 2 (mild): at least one gland occupied 25% to 31% of the microscopic field; grade 3 (moderate): at least one gland occupied 31% to 75% of the microscopic field; grade 4 (marked): at least one gland occupied 75% to 100% of the microscopic field.

Kidney: The incidence of glomerular metaplasia was significantly increased in 50 mg/kg females (Tables 20 and D4). Glomerular metaplasia was characterized by a cuboidal appearance of the parietal epithelium of Bowman’s capsule from the normally flattened epithelium of vehicle control female mice (Plates 1, 2, and 3). The cuboidal appearance of the epithelial cells involved more than 50% of the surface of the parietal epithelium of a glomerulus. This lesion was considered to be the result of the masculinizing effect of androstenedione. The parietal epithelium of Bowman’s capsule in control male mice is predominantly cuboidal, whereas this epithelium in control female mice has a predominantly flattened or squamoid appearance, a normal sexual dimorphism in this species.

Submandibular Salivary Gland: In females, the incidences and severities of cytoplasmic alteration increased with increasing dose, and the incidences in all dosed groups were significantly increased (Tables 20 and D4). Cytoplasmic alteration was characterized by an increase in prominence and size of the convoluted ducts due to increased amounts of eosinophilic granular material in the cytoplasm of the duct epithelial cells. In addition, the nuclei of cells with cytoplasmic alteration tended to be more basally located within the cytoplasm (Plates 4, 5, and 6). This change was considered to be due to the masculinizing effect of androstenedione, as the granular ducts in male mice normally exhibit a sexual dimorphism with increased amounts of granular eosinophilic material in the cytoplasm and a more basilar location of nucleus beginning at approximately 20 days of age.

Malignant Lymphoma: The incidence of malignant lymphoma (lymphocytic, histiocytic, mixed, or undifferentiated) was significantly decreased in 50 mg/kg female mice (Tables 21 and D2). Malignant lymphoma was characterized by a proliferation of sheets of neoplastic lymphocytes in various organs, particularly the liver and spleen. Variable types of malignant lymphoma were represented.

Other Organs: The incidences of bone marrow hyperplasia were significantly increased in 10 and 50 mg/kg males, and the severities were increased in all dosed male groups (Table C4). The incidence of atrophy of the thymus was significantly increased in 20 mg/kg males (Table C4). The biological significance of these findings is uncertain.
### TABLE 19
Incidences of Neoplasms and Nonneoplastic Lesions of the Pancreatic Islets in Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number Examined</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Hyperplasia&lt;sup&gt;a&lt;/sup&gt;</td>
<td>12 (2.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14 (2.5)</td>
<td>11 (2.3)</td>
<td>6 (2.0)</td>
</tr>
<tr>
<td>Adenoma, Multiple</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Adenoma (includes multiple)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2/50 (4%)</td>
<td>2/50 (4%)</td>
<td>2/50 (4%)</td>
<td>5/49 (10%)</td>
</tr>
<tr>
<td>Overall rate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.6%</td>
<td>4.1%</td>
<td>4.5%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Adjusted rate&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2/36 (6%)</td>
<td>2/44 (5%)</td>
<td>1/34 (3%)</td>
<td>4/37 (11%)</td>
</tr>
<tr>
<td>Terminal rate&lt;sup&gt;f&lt;/sup&gt;</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>620</td>
<td>493</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-3 test&lt;sup&gt;g&lt;/sup&gt;</td>
<td>P=0.104</td>
<td>P=0.653N</td>
<td>P=0.683N</td>
<td>P=0.232</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoma&lt;sup&gt;h&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>0/49 (0%)</td>
<td>2/50 (4%)</td>
<td>4/49 (8%)</td>
<td>4/48 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>0.0%</td>
<td>4.4%</td>
<td>9.0%</td>
<td>8.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/35 (0%)</td>
<td>2/40 (5%)</td>
<td>4/40 (10%)</td>
<td>3/40 (8%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>654</td>
</tr>
<tr>
<td>Poly-3 test&lt;sup&gt;i&lt;/sup&gt;</td>
<td>P=0.137</td>
<td>P=0.248</td>
<td>P=0.063</td>
<td>P=0.064</td>
</tr>
</tbody>
</table>

<sup>(T)</sup> Terminal sacrifice

<sup>a</sup> Number of animals with lesion

<sup>b</sup> Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

<sup>c</sup> Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 2/100 (2.0% ± 2.8%), range 0%-6%; all routes: 17/1,435 (1.2% ± 1.7%), range 0%-6%

<sup>d</sup> Number of animals with neoplasm per number of animals with pancreatic islets examined microscopically

<sup>e</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>f</sup> Observed incidence at terminal kill

<sup>g</sup> Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A lower incidence in a dosed group is indicated by N.

<sup>h</sup> Historical incidence for methylcellulose gavage studies: 1/95 (1.1% ± 1.5%), range 0%-2%; all routes: 11/1,478 (0.8% ± 1.0%), range 0%-2%

<sup>i</sup> Not applicable; no neoplasms in animal group
### Table 20
Incidences of Selected Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Lesion</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clitoral Gland&lt;sup&gt;a&lt;/sup&gt;</td>
<td>47</td>
<td>47</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>Hyperplasia&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0</td>
<td>2 (1.0)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>13** (1.9)</td>
<td>41** (2.0)</td>
</tr>
<tr>
<td>Duct, Dilatation</td>
<td>0</td>
<td>2 (1.0)</td>
<td>17** (1.4)</td>
<td>49** (2.6)</td>
</tr>
<tr>
<td>Kidney</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Glomerulus, Metaplasia</td>
<td>2 (1.5)</td>
<td>1 (2.0)</td>
<td>5 (1.0)</td>
<td>27** (2.0)</td>
</tr>
<tr>
<td>Submandibular Salivary Gland</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>50</td>
</tr>
<tr>
<td>Cytoplasmic Alteration</td>
<td>0</td>
<td>17** (1.2)</td>
<td>40** (1.4)</td>
<td>45** (2.5)</td>
</tr>
</tbody>
</table>

** Significantly different (P<0.01) from the vehicle control group by the Poly-3 test

<sup>a</sup> Number of animals with tissue examined microscopically

<sup>b</sup> Number of animals with lesion

<sup>c</sup> Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

### Table 21
Incidences of Malignant Lymphoma in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate&lt;sup&gt;a,b&lt;/sup&gt;</td>
<td>14/50 (28%)</td>
<td>15/50 (30%)</td>
<td>11/50 (22%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate&lt;sup&gt;c&lt;/sup&gt;</td>
<td>31.3%</td>
<td>32.5%</td>
<td>24.4%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Terminal rate&lt;sup&gt;d&lt;/sup&gt;</td>
<td>11/35 (31%)</td>
<td>13/40 (33%)</td>
<td>10/40 (25%)</td>
<td>2/40 (5%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>547</td>
<td>680</td>
<td>672</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test&lt;sup&gt;e&lt;/sup&gt;</td>
<td>P&lt;0.001N</td>
<td>P=0.540</td>
<td>P=0.308N</td>
<td>P&lt;0.001N</td>
</tr>
</tbody>
</table>

(T) Terminal sacrifice

<sup>a</sup> Number of animals with malignant lymphoma per number of animals necropsied

<sup>b</sup> Historical incidence for 2-year gavage studies with methylcellulose vehicle control groups (mean ± standard deviation): 20/100 (20.0% ± 11.3%), range 12%-28%; all routes: 307/1,498 (20.5% ± 9.7%), range 4%-54%

<sup>c</sup> Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

<sup>d</sup> Observed incidence at terminal kill

<sup>e</sup> Beneath the vehicle control incidence are the P values associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.
GENETIC TOXICOLOGY

Androstenedione was not mutagenic in either of two independent bacterial mutation assays conducted with and without induced rat or hamster liver metabolic activation enzymes (S9) (Table E1). In the first study, concentrations of androstenedione ranged from 100 to 1,000 µg/plate and both 10% and 30% rat and hamster S9 were used with Salmonella typhimurium strains TA97, TA98, TA100, and TA1535. In the second study, Salmonella strains TA98 and TA100 were tested, along with the Escherichia coli strain WP2 uvrA/pKM101; 10% induced rat liver S9 was used to provide metabolic activation.

In vivo, no significant increases in the frequencies of micronucleated polychromatic erythrocytes (PCEs; reticulocytes) were observed in bone marrow of male F344/N rats administered androstenedione (312.5 or 625 mg/kg) by gavage once daily for 3 days (Table E2). Following 3 months of androstenedione administration (1 to 50 mg/kg) by gavage, no increase in the frequency of micronucleated normochromic (mature) erythrocytes (NCEs) was seen in peripheral blood samples from male B6C3F1 mice (Table E3). In female mice, a small increase in the frequency of micronucleated NCEs was observed at the highest dose tested (50 mg/kg); although not significantly elevated above the vehicle control (P=0.0142), this increase resulted in a significant trend (P=0.001) and the test in female mice was judged to be equivocal (Table E3). No significant changes in the percentages of PCEs among total erythrocytes were seen in either the rats or mice, suggesting no androstenedione-associated toxicity in the bone marrow.
PLATE 1
Glomerular metaplasia in the kidney of a female B6C3F1 mouse administered 50 mg/kg androstenedione by gavage for 2 years. The parietal epithelium lining Bowman’s capsule is cuboidal (arrows) rather than flattened. H&E

PLATE 2
Glomeruli in the kidney of a female B6C3F1 mouse administered the control vehicle by gavage for 2 years. The parietal epithelium is typically flattened (arrows) representing a normal sexual dimorphism in this species. H&E

PLATE 3
Glomerulus in the kidney of a male B6C3F1 mouse administered the control vehicle by gavage for 2 years. The parietal epithelium is typically cuboidal (arrow) representing a normal sexual dimorphism in this species. H&E
PLATE 4
Cytoplasmic alteration in the submandibular salivary gland of a female B6C3F1 mouse administered 50 mg/kg androstenedione by gavage for 2 years. Note the increased prominence and size of the convoluted ducts and increased amount of eosinophilic granular material within the cytoplasm of the duct epithelial cells (arrow). H&E

PLATE 5
Submandibular salivary gland of a female B6C3F1 mouse administered the control vehicle for 2 years. The ducts are smaller and there is less intracytoplasmic granular material (arrow) compared to male mice (Plate 6), representing a normal sexual dimorphism in this species. H&E

PLATE 6
Submandibular salivary gland of a male B6C3F1 mouse administered the control vehicle for 2 years. Compared to a female mouse (Plate 5), the ducts are larger and there is more intracytoplasmic granular material (arrow), representing a normal sexual dimorphism in this species. H&E
Prior to the banning of over-the-counter sales, androstenedione was used as a dietary supplement by athletes who believed that it would increase muscle mass during training. Androstenedione was nominated to the NTP for study due to concern about adverse health effects associated with its chronic use. In order to evaluate androstenedione’s toxicity, the NTP conducted 2-week, 3-month, and 2-year studies with male and female rats and mice. The selected doses of androstenedione covered the anticipated range of use by athletes and bodybuilders on a body weight basis. The current studies were limited to a high dose of 50 mg/kg due to parameters of acceptable gavageability to the study animals, but 50 mg/kg did exceed the reported upper range of use (3,500 mg/day for a 70 kg individual). Since androstenedione is an androgenic hormone that can be metabolized to a more potent androgen (e.g., testosterone) or to an estrogen, there may be different hormonal effects depending on the metabolism and presence of steroid receptors within a tissue, which may vary due to the sex of the animal.

The subchronic studies of androstenedione did not demonstrate a dose-limiting toxicity at the doses administered to male and female rats and mice. The increased incidences of adrenal gland x-zone atrophy and x-zone cytoplasmic vacuolization in female mice indicate that androstenedione, or a metabolite, had an androgenic effect. Regression of the adrenal x-zone in female mice normally occurs rapidly during the first pregnancy (McPhail and Read, 1942; Jones, 1952). The regression of the x-zone in female mice can be stimulated by administration of androgens and can be delayed in males via castration (Holmes and Dickson, 1971; Tomooka and Yasui, 1978).

A consistent effect in dosed female rats in both the 3-month and 2-year studies was the increase in body weight compared to the vehicle controls. Depending on the timing of exposure, androgens may affect female body weight. Prenatal exposure to androgens does not increase female pup weight at birth or in adulthood (Wolf et al., 2002; Hotchkiss et al., 2007), which is similar to the results of a postnatal day 30 to 50 administration of testosterone propionate to female rats (Beatty, 1973). Neonatal exposure to testosterone increased female body weights (Beatty et al., 1970), but the current 3-month and 2-year studies started exposure postweaning. However, the increase in female rat body weights due to postnatal androgen exposure may have not been detected in earlier studies, since exposure was far shorter than in the current 3-month and 2-year studies. Oxymetholone, an androgenic anabolic steroid, increased female rat body weights in subchronic and chronic studies (NTP, 1999). Oxymetholone is a potent anabolic steroid, but it displays poor androgen receptor binding, while androstenedione binds the androgen receptor, albeit less potently than dihydrotestosterone, and displays limited evidence of an anabolic effect (Saartok et al., 1984; Jasuja et al., 2005; Kicman, 2008). The increase in female rat body weights may be due to increased muscle mass, but neither muscle mass nor adipose mass were evaluated.

The reduction of sperm concentration in the rat cauda epididymis in the 10 through 50 mg/kg groups in the 3-month study with no effects on spermatid numbers within the rat testes suggests androstenedione treatment may be interfering with sperm maturation through an androgenic or estrogenic mechanism. It is not clear why epididymal and not testicular numbers decreased, but the difference may be related to the treatment’s hormonal action. Administration of testosterone or estrogen via implants decreases sperm concentrations within the testes and results in reduced fertility (Robaire et al., 1979, 1984). Within the epididymis, inhibition of dihydrotestosterone synthesis via 5α-reductase inhibitors adversely affects sperm maturation, and estrogen receptor alpha is important for regulating fluid reabsorption in the efferent ductule (Hess, 2003; Robaire and Henderson, 2006). Sperm motility of male mice was significantly decreased at the top dose, and there was not a decrease in epididymal sperm concentration, but an increase in testicular spermatids at the mid dose. Similar to androstenedione, oxymetholone reduced male mouse sperm motility but did not affect spermatid numbers.
within the testis or spermatozoa numbers with the epididymis (NTP, 1999). Since sperm motility is achieved within the epididymis, androstenedione treatment may have also affected sperm maturation in male mice. These effects indicate a potential for androstenedione to produce adverse effects in studies of fertility and reproductive performance.

The mammary gland is a well known target organ of steroids, which makes it a potential target of androstenedione treatment. There were significant decreases in the incidences of mammary gland hyperplasia, mammary gland cysts, and mammary gland adenomas in female rats in the 2-year study, suggesting that androstenedione ameliorated an endocrine mechanism(s) of these lesions in the rat. The decrease in the incidences of testicular interstitial adenoma in F344/N rats, a neoplasm common to this strain (Haseman et al., 1998), indicates that androstenedione treatment had a similar effect in the 2-year study. The origin of these neoplasms is thought to be through endocrine-mediated mechanisms (Cook et al., 1999), which androstenedione treatment may have alleviated. Oxymetholone treatment had a similar effect on testicular interstitial cell adenomas (NTP, 1999).

There were statistically significant increased incidences of mononuclear cell leukemia, a common neoplasm in F344/N rats (Haseman et al., 1998), in female rats. The increase in mononuclear cell leukemia was considered to be equivocal evidence of carcinogenicity due to the low incidence in vehicle controls (10%) compared to the historical range for all routes (8% to 40%; mean, 22%). In male rats, the incidences of mononuclear cell leukemia were significantly decreased, which was not consistent with the female rat. The decrease in mononuclear cell leukemia in male rats was also observed in male and female rats after chronic exposure to oxymetholone (NTP, 1999). There were decreases in the incidences of malignant lymphoma in female mice, a common type of neoplasm for this strain (Haseman et al., 1998), by androstenedione treatment. Since the immune system is sensitive to steroids (Bouman et al., 2005; Beagley and Gockel, 2008), there may be endocrine-mediated mechanisms for this effect. Male B6C3F1 mice have a considerably lower background rate of malignant lymphoma compared to the female mice (average: 3% versus 21% for all routes), which may be due to higher circulating levels of androgens in males. The decrease in the female malignant lymphoma incidence to levels comparable to that of male historical controls may be due to androstenedione treatment producing higher levels of androgens.

Androstenedione significantly increased the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in 20 mg/kg male rats. Since these neoplasms were increased only in the 20 mg/kg group and there was no reduction in survival or body weight in the 50 mg/kg group, it is unclear if the increased incidence in the 20 mg/kg group was treatment related because of the lack of a dose response. Oxymetholone significantly increased the incidence of alveolar/bronchiolar adenoma or carcinoma (combined) in female rats only in the mid dose group, which was considered treatment related (NTP, 1999). However, the increased incidence of female rat alveolar/bronchiolar adenomas in the oxymetholone mid-dose group was greater than that in male rats in the current androstenedione study. The incidence of adenoma and carcinoma combined did not exceed the historical range of all routes in androstenedione-treated male rats, but did exceed the range in oxymetholone-treated female rats.

The histological changes in the submandibular salivary gland, a sexually dimorphic and androgen-sensitive tissue, of treated female mice indicated masculinization by androstenedione or a metabolite thereof. The withdrawal of androgens in male mice results in demasculinization within this gland and administration of androgens to females induces masculinization (Kronman and Spinale, 1965; Chrétien, 1977; Sawada and Noumura, 1991). In addition to the submandibular gland changes, androstenedione treatment resulted in glomerulus metaplasia in female mice, an appearance similar to that of the male mouse. This effect within the female mouse glomerulus was also noted after oxymetholone treatment (NTP, 1999). The hormonal responsiveness of the clitoral gland to androgens is not well understood (Traish et al., 2002), but the increased incidence of clitoral gland hyperplasia in female mice may be related to the androgenic effects of androstenedione.

The increase in the incidences of pancreatic islet adenoma in female mice was considered to be some evidence of carcinogenic activity. The increase of this rare neoplasm was considered treatment related due to the incidence (8%) in the 50 mg/kg group exceeding concurrent and historical control rates (range 0% to 2%; mean, 1%) from all routes of administration. Male mice had a sim-
ilar increase (10%) at the high dose that was treatment related and exceeded the historical range (0% to 6%; mean, 1%) from all routes of exposure. In addition, the day of first incidence decreased with increasing dose in male mice, which is supportive of a treatment effect. It is not clear by what mechanism androstenedione treatment would induce these neoplasms, but the mouse pancreatic islet β-cells express the androgen receptor, which may play a role in β-cell proliferation (Li et al., 2008). CYP 17, the steroidogenic enzyme responsible for converting progestins to androgens, has been identified in rat pancreatic islets (Ogishima et al., 2008) but has yet to be identified in mouse islet cells. The relation of androgens, pancreatic islet cells, and insulin is not well understood, but it is of interest due to polycystic ovary syndrome, in which individuals have increased levels of circulating androstenedione, and decreased insulin sensitivity (Schüring et al., 2008).

In the 2-year mouse study, the increased incidences of hepatocellular adenoma and carcinoma in female mice were considered to be clear evidence of androstenedione carcinogenicity. There were treatment-related increases in the incidences of multiple hepatocellular adenomas and carcinomas within individual female mice. In male mice, there was an increase in the incidence of hepatocellular adenoma and a marginal increase in the incidence of hepatoblastoma, both of which were considered to be due to androstenedione treatment. Furthermore, there were increased incidences of multiplicity in hepatocellular adenomas, hepatocellular carcinomas, and hepatoblastomas in male mice, which indicates androstenedione is carcinogenic in male mice. The combined incidences of hepatocellular adenoma, hepatocellular carcinoma, or hepatoblastoma were also significantly increased in male mice. Male and female rats displayed no evidence of androstenedione inducing hepatocellular neoplasms. This may be due to species differences in metabolism as evident from the NTP in vitro liver assays (Green and Catz, 2007). In vitro, mouse liver predominately converted androstenedione to testosterone, which was then glucuronidated, while rat liver hydroxylated or reduced androstenedione to different metabolites. There were several nonneoplastic changes (e.g., foci) in the liver of rats and mice that did not appear to correlate with the neoplastic findings, and indications of peroxisome and cell proliferation in rats and mice were not supported by the liver data from the 2-week studies. Androstenedione carcinogenicity within the liver is consistent with other androgens, which are known hepatocellular carcinogens (IARC, 1987). The persistence of the neoplasms in mice might have declined if androstenedione treatment had been stopped. Stopping oxymetholone treatment, a well known androgenic liver carcinogen, results in regression of hepatic tumors (Montgomery et al., 1980; Obeid et al., 1980), and treatment of hepatocellular carcinomas by including androgen blockade is under evaluation (Di Maio et al., 2008; Ma et al., 2008). The androgen receptor also contributes to tumor promotion in the liver. Male mice lacking a functional androgen receptor in the liver have considerably lower hepatocellular tumor prevalence after a N,N-diethylnitrosamine challenge compared to wildtype mice (Kemp et al., 1989).

There were similar findings between oxymetholone and androstenedione in the 2-year rat gavage studies (Table 22), but there were some noted differences. Oxymetholone increased neoplasm incidences in the liver and skin of female rats, while androstenedione did not have a similar effect on these tissues in this sex and species. The differences here may be related to differences in metabolism within the rat. Specifically, the methyl group at the 17α position within oxymetholone decreases the rate of liver metabolism and results in hepatotoxicity (Snyder, 2001), whereas androstenedione may not be as potent since it is an endogenous hormone that may be easily metabolized. The skin neoplasms within oxymetholone-treated female rats may be related to its anabolic activity, as anabolic steroids induce skin lesions, and oxymetholone accumulates within this site (NTP, 1999), whereas there is limited evidence of anabolic activity by androstenedione (Jasuja et al., 2005). The effects of diet, NIH-07 for oxymetholone versus NTP-2000 for androstenedione, on outcome are not known, but could also be a contributing factor for differences between the studies.

In summary, androstenedione was carcinogenic in the mouse liver, which is generally consistent with other androgens. Androstenedione and oxymetholone, the anabolic androgen, had similar sites of increased and decreased incidences of neoplasms. The exceptions may be due to differences in metabolism and anabolic activity. Androstenedione treatment reduced the incidence of neoplasms in several tissues that are well known endocrine targets, suggesting that treatment had an ameliorative effect within these tissues likely due to compensating for adverse endocrine mediated mechanisms that arise during the aging process.
Table 22
Comparison of Chronic Exposure Results Between Oxymetholone and Androstenedione

<table>
<thead>
<tr>
<th>Tissues with Neoplasms or Neoplasm</th>
<th>Oxymetholone&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Androstenedione</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male Rats</td>
<td>Female Rats</td>
</tr>
<tr>
<td>Testis</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Mammary Gland</td>
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<tr>
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<td>Mononuclear Cell Leukemia&lt;sup&gt;c&lt;/sup&gt;</td>
<td>↓</td>
<td>↓</td>
</tr>
<tr>
<td>Malignant Lymphoma&lt;sup&gt;d&lt;/sup&gt;</td>
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<sup>a</sup> NTP, 1999
<sup>b</sup> Although a significant increase, this finding was considered to be equivocal evidence of carcinogenicity.
<sup>c</sup> Present in rats
<sup>d</sup> Present in mice

Conclusions
Under the conditions of these 2-year gavage studies, there was equivocal evidence of carcinogenic activity* of androstenedione in male F344/N rats based on increased incidences of alveolar/bronchiolar adenoma and alveolar/bronchiolar adenoma or carcinoma (combined). There was equivocal evidence of carcinogenic activity of androstenedione in female F344/N rats based on increased incidences of mononuclear cell leukemia. There was clear evidence of carcinogenic activity of androstenedione in male B6C3F1 mice based on increased incidences of multiple hepatocellular adenoma and hepatocellular carcinoma and increased incidence of hepatoblastoma. There was clear evidence of carcinogenic activity of androstenedione in female B6C3F1 mice based on increased incidences of hepatocellular adenoma and hepatocellular carcinoma. Increased incidences of pancreatic islet adenoma in male and female mice were also considered chemical related.

Androstenedione administration caused increased incidences in nonneoplastic lesions of the liver in male and female rats and mice; pancreatic islets and exocrine pancreas of female rats; and clitoral gland, kidney, and submandibular salivary gland of female mice.

Decreases in the incidences of testicular interstitial cell adenoma in male rats, mammary gland fibroadenoma, cysts, and hyperplasia in female rats, and malignant lymphoma in female mice were considered related to androstenedione administration.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.
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United States Code (USC) 21, Chapter 13.


APPENDIX A
SUMMARY OF LESIONS IN MALE RATS
IN THE 2-YEAR GAVAGE STUDY
OF ANDROSTENEDIONE

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<table>
<thead>
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<th>Disposition Summary</th>
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<td>(47)</td>
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<td>(46)</td>
<td>(47)</td>
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<td></td>
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<td>(2%)</td>
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<td>(2%)</td>
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<td>1 (2%)</td>
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<td>(50)</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Schwannoma malignant</td>
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<td></td>
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</tr>
<tr>
<td>C-cell, adenoma</td>
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<td>3 (6%)</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
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<td>2 (4%)</td>
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<td>29 (58%)</td>
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<td>8 (16%)</td>
<td>16 (32%)</td>
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### Table A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

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<th>System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tr>
<td>Lymph node</td>
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<td>(18)</td>
<td>(16)</td>
<td>(13)</td>
</tr>
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<td>Adenocarcinoma, metastatic, uncertain primary site</td>
<td>1 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
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</tr>
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<td>Deep cervical, carcinoma, metastatic, thyroid gland</td>
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<td></td>
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<td>(0)</td>
<td>(1)</td>
<td>(2)</td>
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<td>(50)</td>
<td>(50)</td>
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<td>Spleen</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
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<td></td>
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</tr>
<tr>
<td>Basal cell carcinoma, metastatic, skin</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1 (2%)</td>
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<td></td>
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</tr>
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<td>Thymus</td>
<td>(50)</td>
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<td>(50)</td>
<td>(49)</td>
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<tr>
<td>Alveolar/bronchiolar carcinoma, metastatic, lung</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, thyroid gland</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>(50)</td>
</tr>
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</tr>
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<tr>
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<td>1 (2%)</td>
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</tr>
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<td>Basal cell carcinoma</td>
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<td>Keratoacanthoma</td>
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<td>3 (6%)</td>
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<tr>
<td>Squamous cell carcinoma</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Squamous cell papilloma</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Pinna, schwannoma malignant</td>
<td>1 (2%)</td>
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<tr>
<td>Sebaceous gland, adenoma</td>
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<tr>
<td>Sebaceous gland, carcinoma</td>
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<td></td>
<td>1 (2%)</td>
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<tr>
<td>Subcutaneous tissue, fibroma</td>
<td>5 (10%)</td>
<td>7 (14%)</td>
<td>9 (18%)</td>
<td>5 (10%)</td>
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<td>Bone</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Carcinoma, metastatic, thyroid gland</td>
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<td></td>
<td></td>
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</tr>
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<tr>
<td>Schwannoma malignant</td>
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<td></td>
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<td></td>
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<tr>
<td>Humerus, osteosarcoma</td>
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<td></td>
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<tr>
<td>Vertebra, osteosarcoma</td>
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<td></td>
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<td>Skeletal muscle</td>
<td>(8)</td>
<td>(1)</td>
<td>(3)</td>
<td>(5)</td>
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<td>1 (33%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, thyroid gland</td>
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<td></td>
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<td>Fibrous histiocytoma, metastatic, skin</td>
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<tr>
<td>Sarcoma</td>
<td>1 (20%)</td>
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**Table A1**

**Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione**

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<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<td>Brain</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Astrocystoma malignant</td>
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<tr>
<td>Spinal cord</td>
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<td>(1)</td>
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<td>(5)</td>
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<td><strong>Respiratory System</strong></td>
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<tr>
<td>Lung</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Adenocarcinoma, metastatic, uncertain primary site</td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Alveolar/bronchiolar adenoma</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar/bronchiolar carcinoma</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, thyroid gland</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, uncertain primary site</td>
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<td></td>
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<td></td>
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<td>Chordoma, metastatic, bone</td>
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<td></td>
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<tr>
<td>Chordoma, metastatic, uncertain primary site</td>
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<td>Osteosarcoma, metastatic, bone</td>
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<td>Osteosarcoma, metastatic, uncertain primary site</td>
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</tr>
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<td>Squamous cell carcinoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinum, alveolar/bronchiolar carcinoma</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinum, carcinoma, metastatic, uncertain primary site</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinum, osteosarcoma, metastatic, uncertain primary site</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
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<tr>
<td>Serosa, hemangiosarcoma</td>
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<td></td>
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</tr>
<tr>
<td>Nose</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Carcinoma, metastatic, preputial gland</td>
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<td><strong>Special Senses System</strong></td>
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<td>Eye</td>
<td>(48)</td>
<td>(46)</td>
<td>(50)</td>
<td>(49)</td>
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<td>Harderian gland</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
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<td>Zymbal’s gland</td>
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<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
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<td>Adenoma</td>
<td></td>
<td></td>
<td>1 (100%)</td>
<td></td>
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<tr>
<td><strong>Urinary System</strong></td>
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<td></td>
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<tr>
<td>Kidney</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenocarcinoma, metastatic, uncertain primary site</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Renal tubule, adenoma</td>
<td></td>
<td></td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td><strong>Systemic Lesions</strong></td>
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<td>Multiple organs&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Histiocytic sarcoma</td>
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<tr>
<td>Leukemia mononuclear</td>
<td>26 (52%)</td>
<td>22 (44%)</td>
<td>18 (36%)</td>
<td>18 (36%)</td>
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<tr>
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<td></td>
<td></td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Mesothelioma malignant</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Mesothelioma NOS</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
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<td>Neoplasm Summary</td>
<td>Vehicle Control</td>
<td>10 mg/kg</td>
<td>20 mg/kg</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
<td>----------------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>Total animals with primary neoplasms&lt;sup&gt;c&lt;/sup&gt;</td>
<td>49</td>
<td>48</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Total primary neoplasms</td>
<td>141</td>
<td>135</td>
<td>133</td>
<td>120</td>
</tr>
<tr>
<td>Total animals with benign neoplasms</td>
<td>47</td>
<td>47</td>
<td>46</td>
<td>46</td>
</tr>
<tr>
<td>Total benign neoplasms</td>
<td>103</td>
<td>98</td>
<td>96</td>
<td>87</td>
</tr>
<tr>
<td>Total animals with malignant neoplasms</td>
<td>34</td>
<td>29</td>
<td>25</td>
<td>28</td>
</tr>
<tr>
<td>Total malignant neoplasms</td>
<td>38</td>
<td>36</td>
<td>36</td>
<td>33</td>
</tr>
<tr>
<td>Total animals with metastatic neoplasms</td>
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<td>3</td>
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<td>5</td>
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<td>Total metastatic neoplasms</td>
<td>4</td>
<td>9</td>
<td>23</td>
<td>9</td>
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<tr>
<td>Total animals with malignant neoplasms of uncertain primary site</td>
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<td>4</td>
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<tr>
<td>Total animals with uncertain neoplasms-benign or malignant</td>
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<td>1</td>
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</tr>
<tr>
<td>Total uncertain neoplasms</td>
<td>1</td>
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</tbody>
</table>

<sup>a</sup> Number of animals examined microscopically at the site and the number of animals with neoplasm

<sup>b</sup> Number of animals with any tissue examined microscopically

<sup>c</sup> Primary neoplasms: all neoplasms except metastatic neoplasms
### Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
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<tr>
<th>Tissue</th>
<th>Neoplasm Type</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adrenal Cortex: Adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Overall rate</td>
<td></td>
<td>7.6%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td></td>
<td>1/21 (5%)</td>
<td>0/33 (0%)</td>
<td>0/29 (0%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td>688</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td></td>
<td>P=0.365N</td>
<td>P=0.098N</td>
<td>P=0.105N</td>
<td>P=0.274N</td>
</tr>
<tr>
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<td>Benign</td>
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<td>7/50 (14%)</td>
<td>12/50 (24%)</td>
<td>4/50 (8%)</td>
</tr>
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<td>17.5%</td>
<td>26.9%</td>
<td>9.4%</td>
<td>11.6%</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td></td>
<td>3/21 (14%)</td>
<td>11/33 (33%)</td>
<td>3/29 (10%)</td>
<td>4/27 (15%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td>612</td>
<td>708</td>
<td>712</td>
<td>687</td>
</tr>
<tr>
<td>First incidence (days)</td>
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<td></td>
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<td></td>
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</tr>
<tr>
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<td>P=0.218</td>
<td>P=0.221N</td>
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<td>11.7%</td>
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<td>11/33 (33%)</td>
<td>4/29 (14%)</td>
<td>4/27 (15%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td>554</td>
<td>708</td>
<td>712</td>
<td>687</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
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<td>Poly-3 test</td>
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<tr>
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<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
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<td>0.0%</td>
<td>2.3%</td>
<td>7.0%</td>
</tr>
<tr>
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<td>0/33 (0%)</td>
<td>1/29 (3%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td>701</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First incidence (days)</td>
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<td></td>
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<td>P=0.210N</td>
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<td>2/50 (4%)</td>
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<td>1/50 (2%)</td>
<td>4/50 (8%)</td>
</tr>
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<td>2.3%</td>
<td>2.3%</td>
<td>9.2%</td>
</tr>
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<td>1/33 (3%)</td>
<td>1/29 (3%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
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<td>729 (T)</td>
<td>729 (T)</td>
<td>676</td>
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<td>P=0.457N</td>
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<td>0/50 (0%)</td>
<td>5/50 (10%)</td>
<td>2/50 (4%)</td>
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<tr>
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<td>0.0%</td>
<td>11.6%</td>
<td>4.6%</td>
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<tr>
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<td>0/33 (0%)</td>
<td>2/29 (7%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
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</tr>
<tr>
<td>Poly-3 test</td>
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<td>P=0.195</td>
<td>— f</td>
<td>P=0.039</td>
<td>P=0.258</td>
</tr>
<tr>
<td>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</td>
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<td>0/50 (0%)</td>
<td>5/50 (10%)</td>
<td>3/50 (6%)</td>
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<td>11.6%</td>
<td>6.9%</td>
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<td>0/33 (0%)</td>
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<td>2/27 (7%)</td>
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<td>P=0.039</td>
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</tr>
<tr>
<td>Mammary Gland: Fibroadenoma</td>
<td></td>
<td>2/50 (4%)</td>
<td>5/50 (10%)</td>
<td>0/50 (0%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Overall rate</td>
<td></td>
<td>5.1%</td>
<td>11.2%</td>
<td>0.0%</td>
<td>13.9%</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td></td>
<td>1/21 (5%)</td>
<td>3/33 (9%)</td>
<td>0/29 (0%)</td>
<td>6/27 (22%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td></td>
<td>688</td>
<td>708</td>
<td></td>
<td>729 (T)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-3 test</td>
<td></td>
<td>P=0.139</td>
<td>P=0.270</td>
<td>P=0.219N</td>
<td>P=0.162</td>
</tr>
</tbody>
</table>
TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall rate</td>
<td>Adjusted rate</td>
<td>Terminal rate</td>
<td>First incidence (days)</td>
</tr>
<tr>
<td>Mammary Gland: Fibroma or Fibroadenoma</td>
<td>3/50 (6%)</td>
<td>6/50 (12%)</td>
<td>0/50 (0%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Overall rate</td>
<td>7.6%</td>
<td>13.4%</td>
<td>0.0%</td>
<td>13.9%</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>1/21 (5%)</td>
<td>4/33 (12%)</td>
<td>0/29 (0%)</td>
<td>6/27 (22%)</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>688</td>
<td>708</td>
<td></td>
<td>729 (T)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>P=0.283</td>
<td>P=0.305</td>
<td>P=0.105N</td>
<td>P=0.285</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.141N</td>
<td>P=0.097N</td>
<td>P=0.461N</td>
<td>P=0.102N</td>
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Pancreas: Adenoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.7%</td>
<td>0.0%</td>
<td>4.7%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/21 (14%)</td>
<td>0/33 (0%)</td>
<td>2/29 (7%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.160N</td>
<td>P=0.432N</td>
<td>P=0.146</td>
<td>P=0.152N</td>
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</table>

Pancreatic Islets: Adenoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>3/50 (6%)</td>
<td>9/50 (18%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>10.1%</td>
<td>6.7%</td>
<td>21.0%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>2/33 (6%)</td>
<td>8/29 (28%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>686</td>
<td>652</td>
<td>674</td>
<td>717</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.244N</td>
<td>P=0.570N</td>
<td>P=0.146</td>
<td>P=0.296N</td>
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Pancreatic Islets: Adenoma or Carcinoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>4/50 (8%)</td>
<td>9/50 (18%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>10.1%</td>
<td>8.9%</td>
<td>21.0%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>2/33 (6%)</td>
<td>8/29 (28%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>686</td>
<td>652</td>
<td>674</td>
<td>697</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.241</td>
<td>P=0.565N</td>
<td>P=0.184N</td>
<td>P=0.331</td>
</tr>
</tbody>
</table>

Pituitary Gland (Pars Distalis): Adenoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>19/50 (38%)</td>
<td>21/50 (42%)</td>
<td>15/49 (31%)</td>
<td>24/50 (48%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>45.5%</td>
<td>44.9%</td>
<td>33.8%</td>
<td>52.3%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>10/21 (48%)</td>
<td>13/33 (39%)</td>
<td>9/29 (31%)</td>
<td>14/27 (52%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>547</td>
<td>551</td>
<td>547</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.241</td>
<td>P=0.565N</td>
<td>P=0.184N</td>
<td>P=0.331</td>
</tr>
</tbody>
</table>

Preputial Gland: Adenoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>2/50 (4%)</td>
<td>0/50 (0%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.6%</td>
<td>4.5%</td>
<td>0.0%</td>
<td>9.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>2/33 (6%)</td>
<td>0/29 (0%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>592</td>
<td>729 (T)</td>
<td></td>
<td>666</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.331</td>
<td>P=0.448N</td>
<td>P=0.105N</td>
<td>P=0.550</td>
</tr>
</tbody>
</table>

Preputial Gland: Adenoma or Carcinoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>2/50 (4%)</td>
<td>1/50 (2%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.6%</td>
<td>4.5%</td>
<td>2.3%</td>
<td>11.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>2/33 (6%)</td>
<td>0/29 (0%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>592</td>
<td>729 (T)</td>
<td>547</td>
<td>666</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.190</td>
<td>P=0.448N</td>
<td>P=0.274N</td>
<td>P=0.411</td>
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Skin: Keratoacanthoma
<table>
<thead>
<tr>
<th>Neoplasm Location</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>4/50 (8%)</td>
<td>3/50 (6%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.5%</td>
<td>9.0%</td>
<td>7.0%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>3/33 (9%)</td>
<td>2/29 (7%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>589</td>
<td>708</td>
<td>712</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.165N</td>
<td>P=0.558</td>
<td>P=0.633N</td>
<td>P=0.279N</td>
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</table>
### TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Pathology</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tbody>
<tr>
<td><strong>Skin:</strong> Squamous Cell Papilloma or Keratoacanthoma</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>5/50 (10%)</td>
<td>4/50 (8%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>9.9%</td>
<td>11.2%</td>
<td>9.4%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>4/33 (12%)</td>
<td>3/29 (10%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>589</td>
<td>708</td>
<td>712</td>
<td>589</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.191N</td>
<td>P=0.564</td>
<td>P=0.610N</td>
<td>P=0.301N</td>
</tr>
<tr>
<td><strong>Skin:</strong> Basal Cell Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>4/50 (8%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.5%</td>
<td>4.5%</td>
<td>9.4%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/21 (0%)</td>
<td>1/33 (3%)</td>
<td>4/29 (14%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>699</td>
<td>672</td>
<td>729 (T)</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.272N</td>
<td>P=0.545</td>
<td>P=0.204</td>
<td>P=0.482N</td>
</tr>
<tr>
<td><strong>Skin:</strong> Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>5/50 (10%)</td>
<td>4/50 (8%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>9.9%</td>
<td>11.2%</td>
<td>9.4%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>4/33 (12%)</td>
<td>3/29 (10%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>589</td>
<td>708</td>
<td>712</td>
<td>589</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.191N</td>
<td>P=0.564</td>
<td>P=0.610N</td>
<td>P=0.301N</td>
</tr>
<tr>
<td><strong>Skin:</strong> Basal Cell Carcinoma or Squamous Cell Carcinoma</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>4/50 (8%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.5%</td>
<td>4.5%</td>
<td>9.4%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/21 (0%)</td>
<td>1/33 (3%)</td>
<td>4/29 (14%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>699</td>
<td>672</td>
<td>729 (T)</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.272N</td>
<td>P=0.545</td>
<td>P=0.204</td>
<td>P=0.482N</td>
</tr>
<tr>
<td><strong>Skin:</strong> Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Carcinoma, or Squamous Cell Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>7/50 (14%)</td>
<td>7/50 (14%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>12.4%</td>
<td>15.6%</td>
<td>16.4%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>5/33 (15%)</td>
<td>6/29 (21%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>589</td>
<td>672</td>
<td>712</td>
<td>589</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.098N</td>
<td>P=0.454</td>
<td>P=0.420</td>
<td>P=0.185N</td>
</tr>
<tr>
<td><strong>Skin (Subcutaneous Tissue): Fibroma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>7/50 (14%)</td>
<td>9/50 (18%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>12.5%</td>
<td>15.6%</td>
<td>20.8%</td>
<td>11.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>5/33 (15%)</td>
<td>8/29 (28%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>612</td>
<td>641</td>
<td>558</td>
<td>610</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.424N</td>
<td>P=0.461</td>
<td>P=0.235</td>
<td>P=0.574N</td>
</tr>
<tr>
<td><strong>Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
<td>0/50 (0%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.6%</td>
<td>2.3%</td>
<td>0.0%</td>
<td>6.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>1/33 (3%)</td>
<td>0/29 (0%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>—</td>
<td>589</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.145</td>
<td>P=0.732N</td>
<td>P=0.483N</td>
<td>P=0.344</td>
</tr>
<tr>
<td><strong>Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>6/50 (12%)</td>
<td>8/50 (16%)</td>
<td>9/50 (18%)</td>
<td>8/50 (16%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>15.0%</td>
<td>17.8%</td>
<td>20.8%</td>
<td>18.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/21 (14%)</td>
<td>6/33 (18%)</td>
<td>8/29 (28%)</td>
<td>4/27 (15%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>612</td>
<td>641</td>
<td>558</td>
<td>589</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.462</td>
<td>P=0.477</td>
<td>P=0.343</td>
<td>P=0.465</td>
</tr>
</tbody>
</table>
TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Testes: Adenoma</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>42/50 (84%)</td>
<td>39/50 (78%)</td>
<td>36/50 (72%)</td>
<td>26/50 (52%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>91.1%</td>
<td>83.8%</td>
<td>79.2%</td>
<td>58.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>20/21 (95%)</td>
<td>30/33 (91%)</td>
<td>24/29 (83%)</td>
<td>19/27 (70%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>464</td>
<td>540</td>
<td>558</td>
<td>607</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001N</td>
<td>P=0.203N</td>
<td>P=0.070N</td>
<td>P&lt;0.001N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testes: Thyroid Gland (C-cell): Adenoma</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>3/50 (6%)</td>
<td>4/50 (8%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.6%</td>
<td>6.7%</td>
<td>9.2%</td>
<td>9.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>2/33 (6%)</td>
<td>2/29 (7%)</td>
<td>2/27 (7%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>699</td>
<td>708</td>
<td>605</td>
<td>666</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.445</td>
<td>P=0.604N</td>
<td>P=0.552</td>
<td>P=0.553</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testes: Thyroid Gland (C-cell): Adenoma or Carcinoma</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>4/50 (8%)</td>
<td>6/50 (12%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>10.2%</td>
<td>9.0%</td>
<td>13.9%</td>
<td>13.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/21 (10%)</td>
<td>3/33 (9%)</td>
<td>4/29 (14%)</td>
<td>3/27 (11%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>699</td>
<td>708</td>
<td>605</td>
<td>666</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.322</td>
<td>P=0.574N</td>
<td>P=0.430</td>
<td>P=0.434</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testes: Thyroid Gland (Follicular Cell): Adenoma</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>7.6%</td>
<td>0.0%</td>
<td>2.3%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>0/33 (0%)</td>
<td>1/29 (3%)</td>
<td>0/27 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>699</td>
<td>—</td>
<td>729 (T)</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.119N</td>
<td>P=0.098N</td>
<td>P=0.276N</td>
<td>P=0.103N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Benign, Malignant, or NOS Mesothelioma</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>2/50 (4%)</td>
<td>3/50 (6%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.6%</td>
<td>4.4%</td>
<td>6.8%</td>
<td>4.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/21 (5%)</td>
<td>0/33 (0%)</td>
<td>0/29 (0%)</td>
<td>1/27 (4%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>641</td>
<td>442</td>
<td>684</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.496</td>
<td>P=0.549</td>
<td>P=0.347</td>
<td>P=0.534</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Mononuclear Cell Leukemia</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>26/50 (52%)</td>
<td>22/50 (44%)</td>
<td>18/50 (36%)</td>
<td>18/50 (36%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>59.0%</td>
<td>47.8%</td>
<td>39.9%</td>
<td>39.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>9/21 (43%)</td>
<td>14/33 (42%)</td>
<td>8/29 (28%)</td>
<td>7/27 (26%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>526</td>
<td>629</td>
<td>558</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.052N</td>
<td>P=0.191N</td>
<td>P=0.050N</td>
<td>P=0.042N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Benign Neoplasms</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>47/50 (94%)</td>
<td>47/50 (94%)</td>
<td>46/50 (92%)</td>
<td>46/50 (92%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>98.3%</td>
<td>97.6%</td>
<td>95.2%</td>
<td>95.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>21/21 (100%)</td>
<td>33/33 (100%)</td>
<td>28/29 (97%)</td>
<td>27/27 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>464</td>
<td>540</td>
<td>442</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.270N</td>
<td>P=0.746N</td>
<td>P=0.373N</td>
<td>P=0.379N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Malignant Neoplasms</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>34/50 (68%)</td>
<td>29/50 (58%)</td>
<td>28/50 (56%)</td>
<td>29/50 (58%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>72.2%</td>
<td>62.7%</td>
<td>59.4%</td>
<td>60.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>10/21 (48%)</td>
<td>20/33 (61%)</td>
<td>13/29 (45%)</td>
<td>11/27 (41%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>456</td>
<td>629</td>
<td>387</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.182N</td>
<td>P=0.220N</td>
<td>P=0.012N</td>
<td>P=0.150N</td>
</tr>
</tbody>
</table>
### Table A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>All Organs: Benign or Malignant Neoplasms</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>49/50 (98%)</td>
<td>48/50 (96%)</td>
<td>48/50 (96%)</td>
<td>49/50 (98%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>99.7%</td>
<td>99.0%</td>
<td>97.2%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>21/21 (100%)</td>
<td>33/33 (100%)</td>
<td>28/29 (97%)</td>
<td>27/27 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>456</td>
<td>540</td>
<td>387</td>
<td>563</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.678N</td>
<td>P=0.900N</td>
<td>P=0.422N</td>
<td>P=0.950N</td>
</tr>
</tbody>
</table>

(T) Terminal sacrifice

**a** Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreas, pancreatic islets, pituitary gland, preputial gland, testes, and thyroid gland; for other tissues, denominator is number of animals necropsied.

**b** Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality

**c** Observed incidence at terminal kill

**d** Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.

**e** Not applicable; no neoplasms in animal group

**f** Value of statistic cannot be computed

**g** One carcinoma occurred in an animal that had a fibroadenoma.
TABLE A3a
Historical Incidence of Mononuclear Cell Leukemia in Control Male F344/N Rats

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Historical Incidence: Methylcellulose Gavage Studies</strong></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (February, 2003)</td>
<td>26/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (June, 2000)</td>
<td>23/50</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>49/100 (49.0%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>49.0% ± 4.2%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>46%-52%</td>
</tr>
<tr>
<td><strong>Overall Historical Incidence: All Routes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>553/1,399 (39.5%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>39.5% ± 12.5%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>8%-58%</td>
</tr>
</tbody>
</table>

* Data as of November 17, 2008

TABLE A3b
Historical Incidence of Lung Neoplasms in Control Male F344/N Rats

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Historical Incidence: Methylcellulose Gavage Studies</strong></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (February, 2003)</td>
<td>0/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (June, 2000)</td>
<td>1/50</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>1/100 (1.0%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>1.0% ± 1.4%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0%-2%</td>
</tr>
<tr>
<td><strong>Overall Historical Incidence: All Routes</strong></td>
<td></td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>34/1,399 (2.4%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>2.4% ± 2.8%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0%-8%</td>
</tr>
</tbody>
</table>

* Data as of November 17, 2008
### Table A3c

**Historical Incidence of Adenoma of the Testis in Control Male F344/N Rats**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Historical Incidence: Methylcellulose Gavage Studies</strong></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (February, 2003)</td>
<td>42/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (June, 2000)</td>
<td>41/50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>83/100 (83.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>83.0% ± 1.4%</td>
</tr>
<tr>
<td>Range</td>
<td>82%-84%</td>
</tr>
<tr>
<td><strong>Overall Historical Incidence: All Routes</strong></td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>1,170/1,399 (83.6%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>83.6% ± 11.5%</td>
</tr>
<tr>
<td>Range</td>
<td>58%-98%</td>
</tr>
</tbody>
</table>

* Data as of November 17, 2008
### TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund</td>
<td>22</td>
<td>9</td>
<td>17</td>
<td>13</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>7</td>
<td>8</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died last week of study</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal sacrifice</td>
<td>20</td>
<td>33</td>
<td>29</td>
<td>27</td>
</tr>
<tr>
<td>Animals examined microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alimentary System</th>
<th>(50)</th>
<th>(50)</th>
<th>(50)</th>
<th>(50)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esophagus</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foreign body</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine large, cecum</td>
<td>(47)</td>
<td>(46)</td>
<td>(47)</td>
<td>(48)</td>
</tr>
<tr>
<td>Edema</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine large, colon</td>
<td>(44)</td>
<td>(43)</td>
<td>(47)</td>
<td>(44)</td>
</tr>
<tr>
<td>Edema</td>
<td>2</td>
<td>(5%)</td>
<td>1</td>
<td>(2%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine large, rectum</td>
<td>(46)</td>
<td>(46)</td>
<td>(47)</td>
<td>(47)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, duodenum</td>
<td>(48)</td>
<td>(47)</td>
<td>(49)</td>
<td>(47)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(44)</td>
<td>(46)</td>
<td>(47)</td>
<td>(44)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, jejunum</td>
<td>(44)</td>
<td>(44)</td>
<td>(47)</td>
<td>(42)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Angiectasis</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Basophilic focus</td>
<td>17</td>
<td>(34%)</td>
<td>29</td>
<td>(58%)</td>
</tr>
<tr>
<td>Clear cell focus</td>
<td>13</td>
<td>(26%)</td>
<td>21</td>
<td>(42%)</td>
</tr>
<tr>
<td>Clear cell focus, multiple</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degeneration, cystic</td>
<td>10</td>
<td>(20%)</td>
<td>3</td>
<td>(6%)</td>
</tr>
<tr>
<td>Eosinophilic focus</td>
<td>3</td>
<td>(6%)</td>
<td>10</td>
<td>(20%)</td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatodiaphragmatic nodule</td>
<td>7</td>
<td>(14%)</td>
<td>8</td>
<td>(16%)</td>
</tr>
<tr>
<td>Infiltration cellular, mixed cell</td>
<td>10</td>
<td>(20%)</td>
<td>3</td>
<td>(6%)</td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cell focus</td>
<td>1</td>
<td>(2%)</td>
<td>2</td>
<td>(4%)</td>
</tr>
<tr>
<td>Necrosis, focal</td>
<td>4</td>
<td>(8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tension lipidosis</td>
<td>1</td>
<td>(2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile duct, hyperplasia</td>
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<td>44</td>
<td>(88%)</td>
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<tr>
<td>Hepatocyte, hyperplasia</td>
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<tr>
<td>Hepatocyte, vacuolization cytoplasm</td>
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<td>18</td>
<td>(36%)</td>
</tr>
<tr>
<td>Kupffer cell, pigmentation</td>
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<td>(2%)</td>
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</tr>
</tbody>
</table>

* Number of animals examined microscopically at the site and the number of animals with lesion
### TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tr>
<td>Mesentery</td>
<td>(18)</td>
<td>(13)</td>
<td>(12)</td>
<td>(18)</td>
</tr>
<tr>
<td>Accessory spleen</td>
<td>4 (22%)</td>
<td>1 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (8%)</td>
<td>1 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Inflammation, granulomatous</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, necrosis</td>
<td>13 (72%)</td>
<td>10 (77%)</td>
<td>10 (83%)</td>
<td>15 (83%)</td>
</tr>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>17 (34%)</td>
<td>30 (60%)</td>
<td>19 (38%)</td>
<td>21 (42%)</td>
</tr>
<tr>
<td>Cyst</td>
<td>5 (10%)</td>
<td>3 (6%)</td>
<td>7 (14%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
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<td>Salivary glands</td>
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<td>(50)</td>
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<td>(50)</td>
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<td>Erosion</td>
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<tr>
<td>Hemorrhage</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic active</td>
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</tr>
<tr>
<td>Ulcer</td>
<td>6 (12%)</td>
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<td>4 (8%)</td>
<td>5 (10%)</td>
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<td>(50)</td>
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<td>Edema</td>
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<tr>
<td>Erosion</td>
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<td>2 (4%)</td>
<td>4 (8%)</td>
<td>5 (10%)</td>
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<td>2 (4%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
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<tr>
<td>Epithelium, cyst</td>
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</tr>
<tr>
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<td>2 (4%)</td>
<td>2 (4%)</td>
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<td>(0)</td>
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<td>(0)</td>
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<td>Malformation</td>
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<td>Blood vessel</td>
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<td>(1)</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>47 (94%)</td>
<td>48 (96%)</td>
<td>48 (96%)</td>
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<tr>
<td>Thrombosis</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
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### TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Endocrine System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tbody>
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<td>Adrenal cortex</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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<tr>
<td>Degeneration, fatty</td>
<td>22 (44%)</td>
<td>27 (54%)</td>
<td>25 (50%)</td>
<td>21 (42%)</td>
</tr>
<tr>
<td>Hyperplasia, focal</td>
<td>5 (10%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hypertrophy, focal</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Hypertrophy, diffuse</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>1 (2%)</td>
<td></td>
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</tr>
<tr>
<td>Bilateral, necrosis</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>5 (10%)</td>
<td>8 (16%)</td>
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<td>10 (20%)</td>
</tr>
<tr>
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<td>5 (10%)</td>
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<td>4 (8%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Islets, pancreatic</td>
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<td>4 (8%)</td>
<td>2 (4%)</td>
<td>9 (18%)</td>
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<td>(48)</td>
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<td>(50)</td>
<td>(48)</td>
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<td>(49)</td>
<td>(50)</td>
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<td>1 (2%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
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<tr>
<td>Pars distalis, angiectasis</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Pars distalis, hyperplasia, focal</td>
<td>13 (26%)</td>
<td>15 (30%)</td>
<td>21 (43%)</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>Pars intermedia, angiectasis</td>
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<td></td>
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<td>Pars intermedia, cyst</td>
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<td>Pars intermedia, hyperplasia, focal</td>
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<td>Rathke’s cleft, cyst</td>
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<td>2 (4%)</td>
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<td>C-cell, hyperplasia</td>
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<td>1 (2%)</td>
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<tr>
<td>Follicular cell, hyperplasia</td>
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<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
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| General Body System | Tissue NOS | (0) | (0) | (1) | (0) |

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<th>(3)</th>
<th>(2)</th>
<th>(0)</th>
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<tr>
<td>Inflammation, suppurative</td>
<td>1 (33%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (33%)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Epididymis</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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</tr>
<tr>
<td>Atrophy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degeneration</td>
<td>1 (2%)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
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<tr>
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<td>(0)</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Cyst</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>7 (14%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypermastitis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>47 (94%)</td>
<td>48 (96%)</td>
<td>41 (82%)</td>
<td>43 (86%)</td>
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<td>(50)</td>
<td>(50)</td>
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<td>24 (48%)</td>
<td>20 (40%)</td>
<td>20 (40%)</td>
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<tr>
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<td>9 (18%)</td>
<td>18 (36%)</td>
<td>9 (18%)</td>
<td>12 (24%)</td>
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<td>Seminal vesicle</td>
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<td>(50)</td>
<td>(50)</td>
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</table>
### TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Genital System (continued)</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tbody>
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<td>Testes</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Malformation</td>
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<td></td>
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<td>1 (2%)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>1 (2%)</td>
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<tr>
<td>Germinal epithelium, atrophy</td>
<td>9 (18%)</td>
<td>8 (16%)</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Interstitial cell, hyperplasia</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
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<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
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<td>Bone marrow</td>
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<td>(50)</td>
<td>(50)</td>
</tr>
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<td>4 (8%)</td>
<td>4 (8%)</td>
<td>7 (14%)</td>
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<td>Myelofibrosis</td>
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<td>(18)</td>
<td>(16)</td>
<td>(13)</td>
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<td>Mediastinal, angiectasis</td>
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<td>1 (6%)</td>
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<td>Mediastinal, ecstasia</td>
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<td>2 (13%)</td>
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<td>Pancreatic, atrophy</td>
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<td>Pancreatic, ecstasia</td>
<td>3 (12%)</td>
<td>1 (6%)</td>
<td>2 (13%)</td>
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<tr>
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<td>1 (6%)</td>
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</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Ectasia</td>
<td>3 (6%)</td>
<td>5 (10%)</td>
<td>7 (14%)</td>
<td>5 (10%)</td>
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<tr>
<td>Fibrosis</td>
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<tr>
<td>Hemorrhage</td>
<td></td>
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<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, histiocytic</td>
<td>25 (50%)</td>
<td>13 (26%)</td>
<td>25 (50%)</td>
<td>19 (38%)</td>
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<tr>
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<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Infiltration cellular, eosinophil</td>
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<tr>
<td>Infiltration cellular, plasma cell</td>
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</tr>
<tr>
<td>Pigmentation</td>
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<tr>
<td>Spleen</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Fibrosis</td>
<td>6 (12%)</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, histiocytic</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
<td>12 (24%)</td>
<td>12 (24%)</td>
<td>8 (16%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Lymphoid follicle, hyperplasia</td>
<td>3 (6%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Red pulp, hyperplasia</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Thymus</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Atrophy</td>
<td></td>
<td></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Cyst</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td>2 (4%)</td>
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# TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
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<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tr>
<td><strong>Integumentary System</strong></td>
<td></td>
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</tr>
<tr>
<td>Mammary gland</td>
<td>(48)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cyst</td>
<td>8 (17%)</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>38 (79%)</td>
<td>37 (74%)</td>
<td>33 (66%)</td>
<td>42 (84%)</td>
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<tr>
<td>Inflammation, granulomatous</td>
<td>1 (2%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Skin</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Cyst epithelial inclusion</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td>5 (10%)</td>
<td>3 (6%)</td>
<td>6 (12%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, granulomatous</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Epidermis, hyperplasia</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td><strong>Musculoskeletal System</strong></td>
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</tr>
<tr>
<td>Bone</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cranium, osteopetrosis</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td>(8)</td>
<td>(1)</td>
<td>(3)</td>
<td>(5)</td>
</tr>
<tr>
<td>Fibrosis</td>
<td>3 (38%)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td><strong>Nervous System</strong></td>
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<td></td>
</tr>
<tr>
<td>Brain</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Compression</td>
<td>13 (26%)</td>
<td>8 (16%)</td>
<td>7 (14%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Gliosis</td>
<td>1 (2%)</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>3 (6%)</td>
<td></td>
<td>2 (4%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Necrosis</td>
<td></td>
<td></td>
<td></td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Spinal cord</td>
<td>(6)</td>
<td>(1)</td>
<td>(0)</td>
<td>(5)</td>
</tr>
<tr>
<td><strong>Respiratory System</strong></td>
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<td></td>
</tr>
<tr>
<td>Lung</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Congestion</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Foreign body</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Infiltration cellular, histiocyte</td>
<td>17 (34%)</td>
<td>17 (34%)</td>
<td>18 (36%)</td>
<td>18 (36%)</td>
</tr>
<tr>
<td>Inflammation, granulomatous</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Metaplasia, osseous</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Alveolar epithelium, hyperplasia</td>
<td>6 (12%)</td>
<td>7 (14%)</td>
<td>8 (16%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Serosa, cyst</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Nose</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Foreign body</td>
<td>5 (10%)</td>
<td>13 (26%)</td>
<td>5 (10%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>3 (6%)</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td>5 (10%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Nasolacrimal duct, inflammation, chronic</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Respiratory epithelium, hyperplasia</td>
<td>8 (16%)</td>
<td>11 (22%)</td>
<td>7 (14%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Sinus, inflammation, suppurative</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vomeronasal organ, atrophy</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
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</table>
TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Special Senses System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eye</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cataract</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Ciliary body, hyperplasia</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retina, degeneration</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Retina, edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sclera, metaplasia, osseous</td>
<td>3 (7%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Harderian gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Zymbal’s gland</td>
<td>(0)</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Urinary System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cyst</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Hydronephrosis</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Infarct</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>42 (86%)</td>
<td>48 (96%)</td>
<td>46 (92%)</td>
<td>44 (88%)</td>
</tr>
<tr>
<td>Renal tubule, accumulation, hyaline droplet</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Renal tubule, necrosis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Renal tubule, pigmentation</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td>6 (12%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Calculus microscopic observation only</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transitional epithelium, hyperplasia</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS
IN THE 2-YEAR GAVAGE STUDY
OF ANDROSTENEDIONE

Table B1 Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione ........................................ 94
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### TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund</td>
<td>10</td>
<td>7</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died last week of study</td>
<td>38</td>
<td>36</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Terminal sacrifice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals examined microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
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</table>

### Alimentary System

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<tr>
<th>Structure</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine large, rectum</td>
<td>(49)</td>
<td>(47)</td>
<td>(47)</td>
<td>(48)</td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(48)</td>
<td>(46)</td>
<td>(45)</td>
<td>(48)</td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Mesentery</td>
<td>(13)</td>
<td>(14)</td>
<td>(15)</td>
<td>(15)</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>1</td>
<td>100%</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Acinus, adenoma</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Stomach, forestomach</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Stomach, glandular</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Tongue</td>
<td>(1)</td>
<td>(0)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
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<td></td>
<td>1 (100%)</td>
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</tbody>
</table>

### Cardiovascular System

<table>
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<tr>
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<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
</tbody>
</table>

### Endocrine System

<table>
<thead>
<tr>
<th>Structure</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal cortex</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Pheochromocytoma benign</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Pheochromocytoma complex</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Islets, pancreatic</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Parathyroid gland</td>
<td>(49)</td>
<td>(48)</td>
<td>(48)</td>
<td>(45)</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Pars distalis, adenoma</td>
<td>19 (38%)</td>
<td>19 (38%)</td>
<td>17 (35%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>C-cell, adenoma</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
<td>5 (10%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>C-cell, carcinoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

### General Body System

None
<table>
<thead>
<tr>
<th>Genital System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clitoral gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>12 (24%)</td>
<td>11 (22%)</td>
<td>11 (22%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Adenoma, multiple</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovary</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Tubulostromal adenoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uterus</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Polyp stromal</td>
<td>9 (18%)</td>
<td>12 (24%)</td>
<td>10 (20%)</td>
<td>5 (10%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Hematopoietic System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Lymph node</td>
<td>(5)</td>
<td>(10)</td>
<td>(16)</td>
<td>(10)</td>
</tr>
<tr>
<td>Lymph node, mandibular</td>
<td>0</td>
<td></td>
<td>0</td>
<td>(1)</td>
</tr>
<tr>
<td>Lymph node, mesenteric</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Spleen</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Thymus</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Thymoma benign</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Integumentary System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibroadenoma</td>
<td>18 (36%)</td>
<td>18 (36%)</td>
<td>16 (32%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Fibroadenoma, multiple</td>
<td>17 (34%)</td>
<td>13 (26%)</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Skin</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Neural crest tumor</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Sebaceous gland, carcinoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, fibroma</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, fibroma, multiple</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, fibrosarcoma</td>
<td></td>
<td>2 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, fibrous histiocytoma</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, hemangiosarcoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, sarcoma</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Musculoskeletal System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Cranium, osteosarcoma</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Skeletal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Nervous System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Carcinoma, metastatic, Zymbal’s gland</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Respiratory System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Alveolar/bronchiolar adenoma</td>
<td></td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
</tbody>
</table>
### TABLE B1
Summary of the Incidence of Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Special Senses System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ear</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Eye</td>
<td>(49)</td>
<td>(48)</td>
<td>(47)</td>
<td>(49)</td>
</tr>
<tr>
<td>Harderian gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Zymbal’s gland</td>
<td>(0)</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td><strong>Urinary System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td><strong>Systemic Lesions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple organs$^b$</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Leukemia mononuclear</td>
<td>5 (10%)</td>
<td>11 (22%)</td>
<td>18 (36%)</td>
<td>15 (30%)</td>
</tr>
</tbody>
</table>

**Neoplasm Summary**

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total animals with primary neoplasms$^c$</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>40</td>
</tr>
<tr>
<td>Total primary neoplasms</td>
<td>99</td>
<td>104</td>
<td>105</td>
<td>70</td>
</tr>
<tr>
<td>Total animals with benign neoplasms</td>
<td>44</td>
<td>44</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>Total benign neoplasms</td>
<td>88</td>
<td>86</td>
<td>76</td>
<td>51</td>
</tr>
<tr>
<td>Total animals with malignant neoplasms</td>
<td>10</td>
<td>16</td>
<td>25</td>
<td>19</td>
</tr>
<tr>
<td>Total malignant neoplasms</td>
<td>11</td>
<td>17</td>
<td>29</td>
<td>19</td>
</tr>
<tr>
<td>Total animals with metastatic neoplasms</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total metastatic neoplasms</td>
<td></td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total animals with uncertain neoplasms-</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>benign or malignant</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total uncertain neoplasms</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

---

$^a$ Number of animals examined microscopically at the site and the number of animals with neoplasm

$^b$ Number of animals with any tissue examined microscopically

$^c$ Primary neoplasms: all neoplasms except metastatic neoplasms
TABLE B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrenal Medulla: Benign Pheochromocytoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>6.4%</td>
<td>2.2%</td>
<td>6.9%</td>
<td>2.3%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/38 (5%)</td>
<td>0/37 (0%)</td>
<td>2/33 (6%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>703</td>
<td>704</td>
<td>592</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.336N</td>
<td>P=0.310N</td>
<td>P=0.630</td>
<td>P=0.326N</td>
</tr>
</tbody>
</table>

| Adrenal Medulla: Benign or Complex Pheochromocytoma |                |          |          |          |
| Overall rate            | 4/50 (8%)      | 1/50 (2%)| 3/50 (6%)| 2/50 (4%)|          |
| Adjusted rate           | 8.6%           | 2.2%     | 6.9%     | 4.5%     |          |
| Terminal rate           | 3/38 (8%)      | 0/37 (0%)| 2/33 (6%)| 2/37 (5%)|          |
| First incidence (days)  | 703            | 704      | 592      | 729 (T)  |          |
| Poly-3 test             | P=0.419N       | P=0.183N | P=0.538N | P=0.364N |          |

| Clitoral Gland: Adenoma |                |          |          |          |
| Overall rate            | 12/50 (24%)    | 11/50 (22%)| 12/50 (24%)| 9/50 (18%) |
| Adjusted rate           | 25.4%          | 23.6%    | 27.4%    | 20.2%    |          |
| Terminal rate           | 9/38 (24%)     | 9/37 (24%)| 9/33 (27%)| 8/37 (22%)|          |
| First incidence (days)  | 619            | 610      | 652      | 605      |          |
| Poly-3 test             | P=0.333N       | P=0.514N | P=0.508  | P=0.365N |          |

| Clitoral Gland: Carcinoma |                |          |          |          |
| Overall rate             | 1/50 (2%)      | 2/50 (4%)| 5/50 (10%)| 2/50 (4%) |
| Adjusted rate            | 2.1%           | 4.3%     | 11.4%    | 4.5%     |          |
| Terminal rate            | 0/38 (0%)      | 1/37 (3%)| 3/33 (9%)| 2/37 (5%)|          |
| First incidence (days)   | 689            | 659      | 547      | 729 (T)  |          |
| Poly-3 test              | P=0.424        | P=0.496  | P=0.086  | P=0.480  |          |

| Clitoral Gland: Adenoma or Carcinoma |                |          |          |          |
| Overall rate             | 13/50 (26%)    | 13/50 (26%)| 17/50 (34%)| 11/50 (22%) |
| Adjusted rate            | 27.4%          | 27.7%    | 38.3%    | 24.7%    |          |
| Terminal rate            | 9/38 (24%)     | 10/37 (27%)| 12/33 (36%)| 10/37 (27%)|          |
| First incidence (days)   | 619            | 610      | 547      | 605      |          |
| Poly-3 test              | P=0.432N       | P=0.579  | P=0.185  | P=0.475N |          |

| Mammary Gland: Fibroadenoma |                |          |          |          |
| Overall rate             | 35/50 (70%)    | 31/50 (62%)| 22/50 (44%)| 12/50 (24%) |
| Adjusted rate            | 73.3%          | 66.1%    | 47.6%    | 26.9%    |          |
| Terminal rate            | 28/38 (74%)    | 26/37 (70%)| 13/33 (39%)| 11/37 (30%)|          |
| First incidence (days)   | 619            | 652      | 547      | 610      |          |
| Poly-3 test              | P<0.001N       | P=0.326N | P=0.009N | P<0.001N |          |

| Mammary Gland: Fibroadenoma or Adenoma |                |          |          |          |
| Overall rate             | 36/50 (72%)    | 31/50 (62%)| 22/50 (44%)| 12/50 (24%) |
| Adjusted rate            | 73.8%          | 66.1%    | 47.6%    | 26.9%    |          |
| Terminal rate            | 28/38 (74%)    | 26/37 (70%)| 13/33 (39%)| 11/37 (30%)|          |
| First incidence (days)   | 619            | 652      | 547      | 610      |          |
| Poly-3 test              | P<0.001N       | P=0.269N | P=0.006N | P<0.001N |          |

| Mammary Gland: Adenoma or Carcinoma |                |          |          |          |
| Overall rate             | 3/50 (6%)      | 1/50 (2%)| 0/50 (0%)| 0/50 (0%)|          |
| Adjusted rate            | 6.4%           | 2.2%     | 0.0%     | 0.0%     |          |
| Terminal rate            | 0/38 (0%)      | 1/37 (3%)| 0/33 (0%)| 0/37 (0%)|          |
| First incidence (days)   | 621            | 729 (T)  | g        | g        |
| Poly-3 test              | P=0.069N       | P=0.315N | P=0.136N | P=0.131N |          |
### Table B2
Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mammary Gland</strong>: Fibroadenoma, Adenoma, or Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>37/50 (74%)</td>
<td>32/50 (64%)</td>
<td>22/50 (44%)</td>
<td>12/50 (24%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>75.7%</td>
<td>68.2%</td>
<td>47.6%</td>
<td>26.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>28/38 (74%)</td>
<td>27/37 (73%)</td>
<td>13/33 (39%)</td>
<td>11/37 (30%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>619</td>
<td>652</td>
<td>547</td>
<td>610</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001N</td>
<td>P=0.272N</td>
<td>P=0.003N</td>
<td>P&lt;0.001N</td>
</tr>
</tbody>
</table>

| **Pituitary Gland (Pars Distalis)**: Adenoma |                 |          |          |          |
| Overall rate         | 19/50 (38%)     | 19/50 (38%) | 17/49 (35%) | 12/50 (24%) |
| Adjusted rate        | 39.8%           | 40.4%     | 38.3%    | 26.5%    |
| Terminal rate        | 13/38 (34%)     | 13/37 (35%) | 12/33 (36%) | 10/37 (27%) |
| First incidence (days) | 621             | 610       | 552      | 547      |
| Poly-3 test          | P=0.081N        | P=0.562   | P=0.527N | P=0.125N |

| **Skin (Subcutaneous Tissue)**: Fibroma |                 |          |          |          |
| Overall rate         | 3/50 (6%)       | 2/50 (4%)  | 1/50 (2%)  | 1/50 (2%)  |
| Adjusted rate        | 6.4%            | 4.3%      | 2.3%     | 2.3%     |
| Terminal rate        | 3/38 (8%)       | 0/37 (0%)  | 1/33 (3%)  | 1/37 (3%)  |
| First incidence (days) | 729 (T)         | 610       | 619      | 729 (T)  |
| Poly-3 test          | P=0.250N        | P=0.560N  | P=0.334N | P=0.325N |

| **Skin (Subcutaneous Tissue)**: Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma |                 |          |          |          |
| Overall rate         | 3/50 (6%)       | 3/50 (6%)  | 3/50 (6%)  | 1/50 (2%)  |
| Adjusted rate        | 6.4%            | 6.4%      | 6.9%     | 2.3%     |
| Terminal rate        | 3/38 (8%)       | 0/37 (0%)  | 2/33 (6%)  | 1/37 (3%)  |
| First incidence (days) | 729 (T)         | 610       | 619      | 729 (T)  |
| Poly-3 test          | P=0.241N        | P=0.660N  | P=0.630  | P=0.325N |

| **Thyroid Gland (C-cell)**: Adenoma |                 |          |          |          |
| Overall rate         | 4/50 (8%)       | 4/50 (8%)  | 5/50 (10%) | 5/50 (10%) |
| Adjusted rate        | 8.5%            | 8.6%      | 11.4%    | 11.3%    |
| Terminal rate        | 3/38 (8%)       | 2/37 (5%)  | 4/33 (12%) | 5/37 (14%) |
| First incidence (days) | 619             | 652       | 547      | 729 (T)  |
| Poly-3 test          | P=0.378         | P=0.636   | P=0.454  | P=0.460  |

| **Thyroid Gland (C-cell)**: Adenoma or Carcinoma |                 |          |          |          |
| Overall rate         | 4/50 (8%)       | 4/50 (8%)  | 6/50 (12%) | 5/50 (10%) |
| Adjusted rate        | 8.5%            | 8.6%      | 13.7%    | 11.3%    |
| Terminal rate        | 3/38 (8%)       | 2/37 (5%)  | 5/33 (15%) | 5/37 (14%) |
| First incidence (days) | 619             | 652       | 547      | 729 (T)  |
| Poly-3 test          | P=0.376         | P=0.636   | P=0.324  | P=0.460  |

| **Uterus**: Stromal Polyp |                 |          |          |          |
| Overall rate         | 9/50 (18%)      | 12/50 (24%) | 10/50 (20%) | 5/50 (10%) |
| Adjusted rate        | 19.3%           | 25.7%     | 22.7%    | 11.2%    |
| Terminal rate        | 9/38 (24%)      | 9/37 (24%) | 8/33 (24%) | 4/37 (11%) |
| First incidence (days) | 729 (T)         | 610       | 547      | 534      |
| Poly-3 test          | P=0.110N        | P=0.313   | P=0.444  | P=0.216N |

| **All Organs**: Mononuclear Cell Leukemia |                 |          |          |          |
| Overall rate         | 5/50 (10%)      | 11/50 (22%) | 18/50 (36%) | 15/50 (30%) |
| Adjusted rate        | 10.4%           | 23.3%     | 38.4%    | 30.3%    |
| Terminal rate        | 1/38 (3%)       | 5/37 (14%) | 8/33 (24%) | 3/37 (8%) |
| First incidence (days) | 512             | 610       | 442      | 510      |
| Poly-3 test          | P=0.029         | P=0.079   | P=0.001  | P=0.013  |
## TABLE B2
### Statistical Analysis of Primary Neoplasms in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>All Organs: Benign Neoplasms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>44/50 (88%)</td>
<td>44/50 (88%)</td>
<td>40/50 (80%)</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>89.2%</td>
<td>91.4%</td>
<td>82.8%</td>
<td>68.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>33/38 (87%)</td>
<td>34/37 (92%)</td>
<td>26/33 (79%)</td>
<td>26/37 (70%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>619</td>
<td>610</td>
<td>547</td>
<td>534</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001N</td>
<td>P=0.487</td>
<td>P=0.263N</td>
<td>P=0.008N</td>
</tr>
</tbody>
</table>

| **All Organs: Malignant Neoplasms** |                |          |          |          |
| Overall rate            | 10/50 (20%)    | 16/50 (32%) | 25/50 (50%) | 19/50 (38%) |
| Adjusted rate           | 20.5%          | 32.9%    | 52.3%    | 38.4%    |
| Terminal rate           | 2/38 (5%)      | 6/37 (16%) | 13/33 (39%) | 7/37 (19%) |
| First incidence (days)  | 512            | 485      | 442      | 510      |
| Poly-3 test             | P=0.065        | P=0.125  | P=0.001  | P=0.041  |

| **All Organs: Benign or Malignant Neoplasms** |                |          |          |          |
| Overall rate            | 46/50 (92%)    | 46/50 (92%) | 46/50 (92%) | 40/50 (80%) |
| Adjusted rate           | 92.0%          | 93.7%    | 92.0%    | 80.0%    |
| Terminal rate           | 34/38 (90%)    | 34/37 (92%) | 29/33 (88%) | 27/37 (73%) |
| First incidence (days)  | 512            | 485      | 442      | 510      |
| Poly-3 test             | P=0.016N       | P=0.525  | P=0.642  | P=0.074N |

(T) Terminal sacrifice  

- Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, clitoral gland, pituitary gland, and thyroid gland; for other tissues, denominator is number of animals necropsied.  
- Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality  
- Observed incidence at terminal kill  
- Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.  
- Not applicable; no neoplasms in animal group
### TABLE B3a
**Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5/50</td>
</tr>
</tbody>
</table>

**Historical Incidence: Methylcellulose Gavage Studies**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione (February, 2003)</td>
<td>5/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (June, 2000)</td>
<td>12/50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>17/100 (17.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>17.0% ± 9.9%</td>
</tr>
<tr>
<td>Range</td>
<td>10%-24%</td>
</tr>
</tbody>
</table>

**Overall Historical Incidence: All Routes**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>297/1,350 (22.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>22.0% ± 8.8%</td>
</tr>
<tr>
<td>Range</td>
<td>8%-40%</td>
</tr>
</tbody>
</table>

* Data as of November 17, 2008

### TABLE B3b
**Historical Incidence of Mammary Gland Neoplasms in Control Female F344/N Rats**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Androstenedione (February, 2003)</td>
<td>35/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (June, 2000)</td>
<td>28/50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>63/100 (63.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>63.0% ± 9.9%</td>
</tr>
<tr>
<td>Range</td>
<td>56%-70%</td>
</tr>
</tbody>
</table>

**Overall Historical Incidence: All Routes**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total (%)</td>
<td>697/1,350 (51.6%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>51.6% ± 14.9%</td>
</tr>
<tr>
<td>Range</td>
<td>24%-86%</td>
</tr>
</tbody>
</table>

* Data as of November 17, 2008
### Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund</td>
<td>10</td>
<td>7</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>2</td>
<td>6</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died last week of study</td>
<td>38</td>
<td>36</td>
<td>33</td>
<td>37</td>
</tr>
<tr>
<td>Terminal sacrifice</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals examined microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alimentary System</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Intestine large, rectum</td>
<td>(49)</td>
<td>(47)</td>
<td>(47)</td>
<td>(48)</td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(48)</td>
<td>(46)</td>
<td>(45)</td>
<td>(48)</td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Angiectasis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Basophilic focus</td>
<td>47 (94%)</td>
<td>46 (92%)</td>
<td>42 (84%)</td>
<td>38 (76%)</td>
</tr>
<tr>
<td>Clear cell focus</td>
<td>14 (28%)</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Degeneration, cystic</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Eosinophilic focus</td>
<td>5 (10%)</td>
<td>4 (8%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrosis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatodiaphragmatic nodule</td>
<td>10 (20%)</td>
<td>9 (18%)</td>
<td>11 (22%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Infiltration cellular, mixed cell</td>
<td>21 (42%)</td>
<td>33 (66%)</td>
<td>32 (64%)</td>
<td>31 (62%)</td>
</tr>
<tr>
<td>Mixed cell focus</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Necrosis, focal</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Bile duct, hyperplasia</td>
<td>12 (24%)</td>
<td>16 (32%)</td>
<td>18 (36%)</td>
<td>24 (48%)</td>
</tr>
<tr>
<td>Centrilobular, necrosis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatocyte, vacuolization cytoplasmic</td>
<td>6 (12%)</td>
<td>4 (8%)</td>
<td>7 (14%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Kupffer cell, pigmentation</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mesentery</td>
<td>(13)</td>
<td>(14)</td>
<td>(15)</td>
<td>(15)</td>
</tr>
<tr>
<td>Accessory spleen</td>
<td>2 (15%)</td>
<td></td>
<td>3 (20%)</td>
<td>2 (13%)</td>
</tr>
<tr>
<td>Fat, necrosis</td>
<td>12 (92%)</td>
<td>14 (100%)</td>
<td>14 (93%)</td>
<td>12 (80%)</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>(0)</td>
<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>10 (20%)</td>
<td>10 (20%)</td>
<td>16 (32%)</td>
<td>26 (52%)</td>
</tr>
<tr>
<td>Cyst</td>
<td>3 (6%)</td>
<td>7 (14%)</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Acinus, cytoplasmal alteration</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Acinus, hyperplasia, focal</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Salivary glands</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Atrophy</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Vacuolization cytoplasmic</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Stomach, forestomach</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Edema</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Inflammation, chronic active</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>6 (12%)</td>
</tr>
</tbody>
</table>

a Number of animals examined microscopically at the site and the number of animals with lesion
### Table B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alimentary System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach, glandular</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Erosion</td>
<td></td>
<td></td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Ulcer</td>
<td></td>
<td></td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Tongue</td>
<td>(1)</td>
<td>(0)</td>
<td>(1)</td>
<td>(1)</td>
</tr>
<tr>
<td>Hyperkeratosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cardiovascular System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>46 (92%)</td>
<td>43 (86%)</td>
<td>43 (86%)</td>
<td>48 (96%)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Endocrine System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Accessory adrenal cortical nodule</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Angiectasis</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Degeneration, fatty</td>
<td>15 (30%)</td>
<td>20 (40%)</td>
<td>24 (48%)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, focal</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Hypertrophy, focal</td>
<td>12 (24%)</td>
<td>10 (20%)</td>
<td>9 (18%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Hypertrophy, diffuse</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necrosis</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Capsule, developmental malformation</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Islets, pancreatic</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>4 (8%)</td>
<td>1 (2%)</td>
<td>11 (22%)</td>
<td></td>
</tr>
<tr>
<td>Parathyroid gland</td>
<td>(49)</td>
<td>(48)</td>
<td>(48)</td>
<td>(45)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Pituitary gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Pars distalis, angiectasis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Pars distalis, cyst</td>
<td>18 (36%)</td>
<td>12 (24%)</td>
<td>14 (29%)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Pars distalis, hyperplasia, focal</td>
<td>20 (40%)</td>
<td>20 (40%)</td>
<td>14 (29%)</td>
<td>19 (38%)</td>
</tr>
<tr>
<td>Pars intermedia, angiectasis</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rathke’s cleft, cyst</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Thyroid gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Ultimobranchial cyst</td>
<td></td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>C-cell, hyperplasia</td>
<td>27 (54%)</td>
<td>33 (66%)</td>
<td>24 (48%)</td>
<td>25 (50%)</td>
</tr>
<tr>
<td>Follicle, cyst</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Follicular cell, hyperplasia</td>
<td>1 (2%)</td>
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## TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

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<tr>
<th></th>
<th>Vehicle Control</th>
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<th>20 mg/kg</th>
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<tr>
<td>Clitoral gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cyst</td>
<td>6 (12%)</td>
<td>8 (16%)</td>
<td>8 (16%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>3 (6%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>23 (46%)</td>
<td>26 (52%)</td>
<td>18 (36%)</td>
<td>24 (48%)</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Angiectasis</td>
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</tr>
<tr>
<td>Cyst</td>
<td>3 (6%)</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Infiltration cellular, histiocyte</td>
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<td>Decidual reaction</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hyperplasia, cystic</td>
<td>9 (18%)</td>
<td>8 (16%)</td>
<td>11 (22%)</td>
<td>14 (28%)</td>
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<td>Inflammation, suppurative</td>
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<tr>
<td>Thrombosis</td>
<td>1 (2%)</td>
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<td></td>
<td></td>
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<tr>
<td>Epithelium, hyperplasia</td>
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<td><strong>Hematopoietic System</strong></td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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<tr>
<td>Hyperplasia</td>
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<td>6 (12%)</td>
<td>5 (10%)</td>
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<tr>
<td>Thrombosis</td>
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<td>(16)</td>
<td>(10)</td>
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<td>Congestion</td>
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<tr>
<td>Deep cervical, ectasia</td>
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<tr>
<td>Deep cervical, necrosis</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal, ectasia</td>
<td>2 (20%)</td>
<td>1 (6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal, hemorrhage</td>
<td>3 (30%)</td>
<td>1 (6%)</td>
<td></td>
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</tr>
<tr>
<td>Mediastinal, hyperplasia, lymphoid</td>
<td>2 (40%)</td>
<td>2 (20%)</td>
<td>3 (19%)</td>
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<td>Mediastinal, infiltration cellular, mast cell</td>
<td>1 (20%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal, pigmentation</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pancreatic, ectasia</td>
<td></td>
<td></td>
<td>1 (6%)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic, hemorrhage</td>
<td>1 (20%)</td>
<td>2 (20%)</td>
<td>3 (19%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Pancreatic, hyperplasia, histiocytic</td>
<td></td>
<td></td>
<td>1 (6%)</td>
<td></td>
</tr>
<tr>
<td>Pancreatic, pigmentation</td>
<td></td>
<td></td>
<td>1 (10%)</td>
<td></td>
</tr>
<tr>
<td>Lymph node, mandibular</td>
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<td>(0)</td>
<td>(0)</td>
<td>(1)</td>
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<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
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<tr>
<td>Ectasia</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyperplasia, histiocytic</td>
<td>29 (58%)</td>
<td>34 (68%)</td>
<td>24 (49%)</td>
<td>19 (38%)</td>
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<td>Hyperplasia, lymphoid</td>
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<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Pigmentation</td>
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<td>2 (4%)</td>
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### Hematopoietic System (continued)

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<th>10 mg/kg</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Hematopoietic cell proliferation</td>
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<td>3 (6%)</td>
<td>6 (12%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, mixed cell</td>
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<tr>
<td>Pigmentation</td>
<td>43 (86%)</td>
<td>44 (90%)</td>
<td>39 (78%)</td>
<td>36 (72%)</td>
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<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Atrophy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
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### Integumentary System

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</tr>
</thead>
<tbody>
<tr>
<td>Mammary gland</td>
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<tr>
<td>Cyst</td>
<td>15 (30%)</td>
<td>3 (6%)</td>
<td>9 (18%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Hyperplasia</td>
<td>48 (96%)</td>
<td>40 (80%)</td>
<td>35 (70%)</td>
<td>23 (46%)</td>
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<td>Inflammation, suppurative</td>
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<tr>
<td>Skin</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Cyst epithelial inclusion</td>
<td>1 (2%)</td>
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<td></td>
</tr>
<tr>
<td>Hyperkeratosis</td>
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### Musculoskeletal System

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<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone</td>
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<tr>
<td>Hyperostosis</td>
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<td>Skeletal muscle</td>
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<td>(0)</td>
<td>(0)</td>
</tr>
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<td>Inflammation, chronic</td>
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### Nervous System

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<th>50 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>Brain</td>
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<td></td>
</tr>
<tr>
<td>Compression</td>
<td>6 (12%)</td>
<td>10 (20%)</td>
<td>4 (8%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>1 (2%)</td>
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### Respiratory System

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<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
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</tr>
<tr>
<td>Congestion</td>
<td>1 (2%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Emphysema</td>
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<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, histiocyte</td>
<td>31 (62%)</td>
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<td>Inflammation, chronic</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>1 (2%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Alveolar epithelium, hyperplasia</td>
<td>12 (24%)</td>
<td>13 (26%)</td>
<td>11 (22%)</td>
<td>10 (20%)</td>
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<td>Artery, hypertrophy</td>
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<td>Nose</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Foreign body</td>
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<td>6 (12%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
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<tr>
<td>Respiratory epithelium, hyperplasia</td>
<td>7 (14%)</td>
<td>8 (16%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
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</table>
### TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

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<thead>
<tr>
<th>Condition</th>
<th>Vehicle Control</th>
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<th>50 mg/kg</th>
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<td>Ear</td>
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<td>(0)</td>
<td>(0)</td>
</tr>
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<td>Inflammation, suppurative</td>
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<td>(48)</td>
<td>(47)</td>
<td>(49)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Cataract</td>
<td>3 (6%)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Cornea, inflammation, chronic</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Retina, degeneration</td>
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<td></td>
<td></td>
<td></td>
</tr>
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<td>Sclera, metaplasia, osseous</td>
<td>1 (2%)</td>
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<td></td>
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<td>Harderian gland</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Infiltration cellular, lymphocyte</td>
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<td></td>
<td>(1)</td>
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<tr>
<td>Zymbal’s gland</td>
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<td></td>
<td>(0)</td>
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<tr>
<td><strong>Urinary System</strong></td>
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</tr>
<tr>
<td>Kidney</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cyst</td>
<td>2 (4%)</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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<td>Glomerulosclerosis</td>
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<td>Infarct</td>
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<td>1 (2%)</td>
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<tr>
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<td>43 (86%)</td>
<td>43 (86%)</td>
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<td>Renal tubule, accumulation, hyaline droplet</td>
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<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Renal tubule, necrosis</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Renal tubule, pigmentation</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Renal tubule, vacuolization cytoplasmic</td>
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<td></td>
<td>3 (6%)</td>
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<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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APPENDIX C
SUMMARY OF LESIONS IN MALE MICE
IN THE 2-YEAR GAVAGE STUDY
OF ANDROSTENEDIONE

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
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<td>C1</td>
<td>Summary of the Incidence of Neoplasms in Male Mice</td>
<td>108</td>
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<td></td>
<td>in the 2-Year Gavage Study of Androstenedione</td>
<td></td>
</tr>
<tr>
<td>C2</td>
<td>Statistical Analysis of Primary Neoplasms in Male Mice</td>
<td>112</td>
</tr>
<tr>
<td></td>
<td>in the 2-Year Gavage Study of Androstenedione</td>
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<tr>
<td>C3a</td>
<td>Historical Incidence of Liver Neoplasms</td>
<td>116</td>
</tr>
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<td></td>
<td>in Control Male B6C3F1 Mice</td>
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<tr>
<td>C3b</td>
<td>Historical Incidence of Pancreatic Islet Neoplasms</td>
<td>116</td>
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<td>in Control Male B6C3F1 Mice</td>
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<td>C4</td>
<td>Summary of the Incidence of Nonneoplastic Lesions in Male Mice</td>
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<td>in the 2-Year Gavage Study of Androstenedione</td>
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### Table C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
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<th>50 mg/kg</th>
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<td>Animals initially in study</td>
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<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
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</tr>
<tr>
<td>Moribund</td>
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<td>2</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>9</td>
<td>4</td>
<td>11</td>
<td>10</td>
</tr>
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<td>Survivors</td>
<td></td>
<td></td>
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<td>Terminal sacrifice</td>
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<td>44</td>
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### Alimentary System

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<th>10 mg/kg</th>
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<th>50 mg/kg</th>
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<td>Gallbladder</td>
<td>(45)</td>
<td>(46)</td>
<td>(42)</td>
<td>(44)</td>
</tr>
<tr>
<td>Intestine large, cecum</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Intestine small, duodenum</td>
<td>(47)</td>
<td>(49)</td>
<td>(46)</td>
<td>(49)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(49)</td>
<td>(50)</td>
<td>(49)</td>
<td>(49)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, jejunum</td>
<td>(47)</td>
<td>(49)</td>
<td>(47)</td>
<td>(49)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Carcinoma, metastatic, lung</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hemangioma</td>
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<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td>5 (10%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Hepatoblastoma</td>
<td>3 (6%)</td>
<td>7 (14%)</td>
<td>6 (12%)</td>
<td>5 (10%)</td>
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<tr>
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<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Hepatocellular adenoma</td>
<td>16 (32%)</td>
<td>11 (22%)</td>
<td>6 (12%)</td>
<td>9 (18%)</td>
</tr>
<tr>
<td>Hepatocellular adenoma, multiple</td>
<td>16 (32%)</td>
<td>27 (54%)</td>
<td>23 (46%)</td>
<td>34 (68%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>19 (38%)</td>
<td>21 (42%)</td>
<td>18 (36%)</td>
<td>15 (30%)</td>
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<tr>
<td>Hepatocellular carcinoma, multiple</td>
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<td>12 (24%)</td>
<td>10 (20%)</td>
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<td>Sarcoma, metastatic, skeletal muscle</td>
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<td></td>
<td></td>
</tr>
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<td>Mesentery</td>
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<td>(8)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
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<td>Hepatoblastoma, metastatic, liver</td>
<td>2 (25%)</td>
<td>1 (25%)</td>
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<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
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<td></td>
<td></td>
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<tr>
<td>Pancreas</td>
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<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
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<tr>
<td>Acinus, carcinoma</td>
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<td>Salivary glands</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Stomach, forestomach</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Carcinoma, metastatic, pancreas</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Stomach, glandular</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, pancreas</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Tooth</td>
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<td>(1)</td>
<td>(0)</td>
<td>(0)</td>
</tr>
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<td>Odontoma</td>
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### Cardiovascular System

<table>
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<tbody>
<tr>
<td>Heart</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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### TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tr>
<td><strong>Endocrine System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal cortex</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Capsule, adenoma</td>
<td></td>
<td></td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Capsule, adenoma, multiple</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td>(49)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Pheochromocytoma benign</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
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<tr>
<td>Islets, pancreatic</td>
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<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
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<tr>
<td>Adenoma, multiple</td>
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<td>4 (8%)</td>
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<tr>
<td>Parathyroid gland</td>
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<td>(48)</td>
<td>(47)</td>
<td>(46)</td>
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<td>Pituitary gland</td>
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<td>Thyroid gland</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Follicular cell, adenoma</td>
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</table>

**General Body System**

None

**Genital System**

<table>
<thead>
<tr>
<th>System</th>
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<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epididymis</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Preputial gland</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Prostate</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Seminal vesicle</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Testes</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Interstitial cell, adenoma</td>
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<td></td>
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<td>1 (2%)</td>
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**Hematopoietic System**

<table>
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<tr>
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<th>10 mg/kg</th>
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<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
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<td>Bone marrow</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
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<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>(2)</td>
<td>(1)</td>
<td>(1)</td>
<td>(4)</td>
</tr>
<tr>
<td>Lymph node, mandibular</td>
<td>(47)</td>
<td>(47)</td>
<td>(47)</td>
<td>(47)</td>
</tr>
<tr>
<td>Basal cell carcinoma, metastatic, skin</td>
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<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Lymph node, mesenteric</td>
<td>(49)</td>
<td>(49)</td>
<td>(49)</td>
<td>(45)</td>
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<tr>
<td>Spleen</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
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<td>Hemangiosarcoma</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
<td>5 (10%)</td>
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<td>Thymus</td>
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<td>(45)</td>
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**Integumentary System**

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<td>Skin</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Basal cell carcinoma</td>
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<td>1 (2%)</td>
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</tr>
<tr>
<td>Subcutaneous tissue, fibroma</td>
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</tr>
<tr>
<td>Subcutaneous tissue, fibrosarcoma</td>
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</tr>
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<td>Subcutaneous tissue, fibrous histiocytoma</td>
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<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Subcutaneous tissue, hemangiosarcoma</td>
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<td></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous tissue, melanoma malignant</td>
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</tr>
<tr>
<td>Subcutaneous tissue, sarcoma</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Table C1</td>
<td>Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>-----------</td>
<td>--------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Vehicle Control</td>
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<td>20 mg/kg</td>
<td>50 mg/kg</td>
</tr>
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<td>Bone</td>
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<td>(50)</td>
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<td>(50)</td>
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<td>Skeletal muscle</td>
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<td>(1)</td>
</tr>
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<td>Hemangiosarcoma</td>
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</tr>
<tr>
<td>Sarcoma</td>
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<tr>
<td><strong>Nervous System</strong></td>
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<td>Lung</td>
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<td>(50)</td>
<td>(50)</td>
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<td>Alveolar/bronchiolar adenoma</td>
<td>7 (14%)</td>
<td>6 (12%)</td>
<td>6 (12%)</td>
<td>5 (10%)</td>
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<td>Alveolar/bronchiolar adenoma, multiple</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
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<tr>
<td>Alveolar/bronchiolar carcinoma</td>
<td>8 (16%)</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
<td>8 (16%)</td>
</tr>
<tr>
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<td>3 (6%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Basal cell carcinoma, metastatic, skin</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, hardarian gland</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatoblastoma, metastatic, liver</td>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>6 (12%)</td>
<td>6 (12%)</td>
<td>11 (22%)</td>
<td>5 (10%)</td>
</tr>
<tr>
<td>Sarcoma, metastatic, skeletal muscle</td>
<td>1 (2%)</td>
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<td></td>
</tr>
<tr>
<td>Nose</td>
<td>(50)</td>
<td>(49)</td>
<td>(49)</td>
<td>(50)</td>
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<tr>
<td>Carcinoma, metastatic, hardarian gland</td>
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<td></td>
<td>1 (2%)</td>
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<td><strong>Special Senses System</strong></td>
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<td>Eye</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
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<td>Carcinoma, metastatic, hardarian gland</td>
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<td>1 (2%)</td>
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<tr>
<td>Hardarian gland</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>7 (14%)</td>
<td>9 (18%)</td>
<td>6 (12%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Adenoma, multiple</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>2 (4%)</td>
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<td></td>
<td>3 (6%)</td>
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<td>Lacrimal gland</td>
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<td>(0)</td>
<td>(0)</td>
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<td>Kidney</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Carcinoma, metastatic, lung</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Urinary bladder</td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hemangioma</td>
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<td></td>
<td>1 (2%)</td>
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<td><strong>Systemic Lesions</strong></td>
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<td>Multiple organs</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
<td>Histiocytic sarcoma</td>
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<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Lymphoma malignant</td>
<td>4 (8%)</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
<td>6 (12%)</td>
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</table>
### TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Neoplasm Summary</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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</thead>
<tbody>
<tr>
<td>Total animals with primary neoplasms(^c)</td>
<td>47</td>
<td>50</td>
<td>44</td>
<td>50</td>
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<tr>
<td>Total primary neoplasms</td>
<td>108</td>
<td>128</td>
<td>112</td>
<td>129</td>
</tr>
<tr>
<td>Total animals with benign neoplasms</td>
<td>38</td>
<td>43</td>
<td>35</td>
<td>46</td>
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<tr>
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<td>52</td>
<td>65</td>
<td>49</td>
<td>61</td>
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<tr>
<td>Total animals with malignant neoplasms</td>
<td>37</td>
<td>41</td>
<td>38</td>
<td>44</td>
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<td>63</td>
<td>68</td>
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<tr>
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<td>9</td>
<td>14</td>
<td>7</td>
</tr>
<tr>
<td>Total metastatic neoplasms</td>
<td>8</td>
<td>11</td>
<td>18</td>
<td>9</td>
</tr>
</tbody>
</table>

\(^a\) Number of animals examined microscopically at the site and the number of animals with neoplasm

\(^b\) Number of animals with any tissue examined microscopically

\(^c\) Primary neoplasms: all neoplasms except metastatic neoplasms
### TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Adrenal Cortex: Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate^a</td>
<td>1/50 (2%)</td>
<td>5/49 (10%)</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.3%</td>
<td>10.6%</td>
<td>2.3%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/36 (3%)</td>
<td>5/43 (12%)</td>
<td>1/34 (3%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.289N</td>
<td>P=0.124</td>
<td>P=0.756N</td>
<td>P=0.751N</td>
</tr>
<tr>
<td><strong>Harderian Gland: Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>7/50 (14%)</td>
<td>9/50 (18%)</td>
<td>7/50 (14%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>15.9%</td>
<td>18.6%</td>
<td>15.6%</td>
<td>8.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>6/36 (17%)</td>
<td>8/44 (18%)</td>
<td>5/34 (15%)</td>
<td>3/37 (8%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>489</td>
<td>721</td>
<td>660</td>
<td>712</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.145N</td>
<td>P=0.474</td>
<td>P=0.600N</td>
<td>P=0.244N</td>
</tr>
<tr>
<td><strong>Harderian Gland: Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>2/50 (4%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>4.6%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>6.5%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/36 (6%)</td>
<td>0/44 (0%)</td>
<td>0/34 (0%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>—</td>
<td>—</td>
<td>513</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.188</td>
<td>P=0.214N</td>
<td>P=0.232N</td>
<td>P=0.527</td>
</tr>
<tr>
<td><strong>Harderian Gland: Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>9/50 (18%)</td>
<td>9/50 (18%)</td>
<td>7/50 (14%)</td>
<td>7/50 (14%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>20.5%</td>
<td>18.6%</td>
<td>15.6%</td>
<td>15.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>8/36 (22%)</td>
<td>8/44 (18%)</td>
<td>5/34 (15%)</td>
<td>4/37 (11%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>489</td>
<td>721</td>
<td>660</td>
<td>513</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.311N</td>
<td>P=0.515N</td>
<td>P=0.376N</td>
<td>P=0.354N</td>
</tr>
<tr>
<td><strong>Small Intestine (Duodenum, Jejunum, Ileum): Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>6.9%</td>
<td>0.0%</td>
<td>4.5%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/36 (8%)</td>
<td>0/44 (0%)</td>
<td>2/34 (6%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>—</td>
<td>729 (T)</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.388N</td>
<td>P=0.100N</td>
<td>P=0.489N</td>
<td>P=0.290N</td>
</tr>
<tr>
<td><strong>Liver: Hemangiosarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>1/50 (2%)</td>
<td>5/50 (10%)</td>
<td>3/50 (6%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>6.9%</td>
<td>2.1%</td>
<td>11.3%</td>
<td>6.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/36 (6%)</td>
<td>1/44 (2%)</td>
<td>4/34 (12%)</td>
<td>2/37 (5%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>610</td>
<td>729 (T)</td>
<td>725</td>
<td>690</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.459</td>
<td>P=0.270N</td>
<td>P=0.364</td>
<td>P=0.646N</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>32/50 (64%)</td>
<td>38/50 (76%)</td>
<td>29/50 (58%)</td>
<td>43/50 (86%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>71.2%</td>
<td>78.6%</td>
<td>63.9%</td>
<td>91.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>27/36 (75%)</td>
<td>38/44 (86%)</td>
<td>25/34 (74%)</td>
<td>36/37 (97%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>597</td>
<td>729 (T)</td>
<td>613</td>
<td>554</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.009</td>
<td>P=0.270</td>
<td>P=0.298N</td>
<td>P=0.005</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>26/50 (52%)</td>
<td>33/50 (66%)</td>
<td>28/50 (56%)</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>54.0%</td>
<td>66.0%</td>
<td>58.7%</td>
<td>65.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>16/36 (44%)</td>
<td>28/44 (64%)</td>
<td>16/34 (47%)</td>
<td>22/37 (60%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.225</td>
<td>P=0.155</td>
<td>P=0.397</td>
<td>P=0.163</td>
</tr>
</tbody>
</table>
### TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Neoplasm Type</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Liver: Hepatocellular Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>41/50 (82%)</td>
<td>47/50 (94%)</td>
<td>42/50 (84%)</td>
<td>48/50 (96%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>85.2%</td>
<td>94.1%</td>
<td>87.9%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>31/36 (86%)</td>
<td>42/44 (96%)</td>
<td>29/34 (85%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.025</td>
<td>P=0.122</td>
<td>P=0.460</td>
<td>P=0.012</td>
</tr>
<tr>
<td><strong>Liver: Hepatoblastoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>3/50 (6%)</td>
<td>8/50 (16%)</td>
<td>7/50 (14%)</td>
<td>8/50 (16%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>6.9%</td>
<td>16.4%</td>
<td>17.5%</td>
<td>17.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/36 (8%)</td>
<td>7/44 (16%)</td>
<td>6/34 (18%)</td>
<td>6/37 (16%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>593</td>
<td>631</td>
<td>648</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.182</td>
<td>P=0.141</td>
<td>P=0.169</td>
<td>P=0.114</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Carcinoma or Hepatoblastoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>27/50 (54%)</td>
<td>35/50 (70%)</td>
<td>30/50 (60%)</td>
<td>36/50 (72%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>56.1%</td>
<td>70.1%</td>
<td>62.5%</td>
<td>74.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>17/36 (47%)</td>
<td>30/44 (68%)</td>
<td>17/34 (50%)</td>
<td>26/37 (70%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.077</td>
<td>P=0.108</td>
<td>P=0.333</td>
<td>P=0.047</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>41/50 (82%)</td>
<td>47/50 (94%)</td>
<td>43/50 (86%)</td>
<td>48/50 (96%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>85.2%</td>
<td>94.1%</td>
<td>89.4%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>31/36 (86%)</td>
<td>42/44 (96%)</td>
<td>29/34 (85%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>472</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.024</td>
<td>P=0.122</td>
<td>P=0.374</td>
<td>P=0.012</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>7/50 (14%)</td>
<td>7/50 (14%)</td>
<td>7/50 (14%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>16.2%</td>
<td>14.4%</td>
<td>15.8%</td>
<td>10.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>7/36 (19%)</td>
<td>6/44 (14%)</td>
<td>6/34 (18%)</td>
<td>2/37 (5%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>652</td>
<td>708</td>
<td>526</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.287N</td>
<td>P=0.521N</td>
<td>P=0.594N</td>
<td>P=0.333N</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>11/50 (22%)</td>
<td>9/50 (18%)</td>
<td>4/50 (8%)</td>
<td>13/50 (26%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>24.7%</td>
<td>18.4%</td>
<td>9.0%</td>
<td>28.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>7/36 (19%)</td>
<td>7/44 (16%)</td>
<td>3/34 (9%)</td>
<td>11/37 (30%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>581</td>
<td>606</td>
<td>708</td>
<td>688</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.244</td>
<td>P=0.316N</td>
<td>P=0.043N</td>
<td>P=0.427</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>16/50 (32%)</td>
<td>15/50 (30%)</td>
<td>10/50 (20%)</td>
<td>17/50 (34%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>35.9%</td>
<td>30.6%</td>
<td>22.5%</td>
<td>36.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>12/36 (33%)</td>
<td>12/44 (27%)</td>
<td>9/34 (27%)</td>
<td>12/37 (32%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>581</td>
<td>606</td>
<td>708</td>
<td>526</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.422</td>
<td>P=0.371N</td>
<td>P=0.122N</td>
<td>P=0.561</td>
</tr>
<tr>
<td><strong>Pancreatic Islets: Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>2/50 (4%)</td>
<td>2/50 (4%)</td>
<td>2/50 (4%)</td>
<td>5/49 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>4.6%</td>
<td>4.1%</td>
<td>4.5%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>2/36 (6%)</td>
<td>2/44 (5%)</td>
<td>1/34 (3%)</td>
<td>4/37 (11%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>620</td>
<td>493</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.104</td>
<td>P=0.653N</td>
<td>P=0.683N</td>
<td>P=0.232</td>
</tr>
</tbody>
</table>
TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>0/50 (0%)</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.3%</td>
<td>0.0%</td>
<td>6.6%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/36 (0%)</td>
<td>0/44 (0%)</td>
<td>1/34 (3%)</td>
<td>0/37 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>636</td>
<td>—</td>
<td>256</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.454N</td>
<td>P=0.480N</td>
<td>P=0.322</td>
<td>P=0.493N</td>
</tr>
<tr>
<td><strong>Skin (Subcutaneous Tissue): Fibroma, Fibrous Histiocytoma, Fibrosarcoma, or Sarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.3%</td>
<td>2.1%</td>
<td>6.6%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/36 (0%)</td>
<td>1/44 (2%)</td>
<td>1/34 (3%)</td>
<td>0/37 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>636</td>
<td>729 (T)</td>
<td>256</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.359N</td>
<td>P=0.737N</td>
<td>P=0.322</td>
<td>P=0.493N</td>
</tr>
<tr>
<td><strong>Spleen: Hemangiosarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>1/50 (2%)</td>
<td>5/50 (10%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.3%</td>
<td>2.1%</td>
<td>11.2%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/36 (3%)</td>
<td>1/44 (2%)</td>
<td>3/34 (9%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>683</td>
<td>690</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.614N</td>
<td>P=0.735N</td>
<td>P=0.108</td>
<td>P=0.751N</td>
</tr>
<tr>
<td><strong>All Organs: Hemangiosarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>4/50 (8%)</td>
<td>8/50 (16%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>11.4%</td>
<td>8.3%</td>
<td>17.9%</td>
<td>11.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>4/36 (11%)</td>
<td>4/44 (9%)</td>
<td>5/34 (15%)</td>
<td>4/37 (11%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>610</td>
<td>729 (T)</td>
<td>683</td>
<td>690</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.520</td>
<td>P=0.437N</td>
<td>P=0.288</td>
<td>P=0.608N</td>
</tr>
<tr>
<td><strong>All Organs: Hemangioma or Hemangiosarcoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>4/50 (8%)</td>
<td>9/50 (18%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>11.4%</td>
<td>8.3%</td>
<td>20.2%</td>
<td>13.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>4/36 (11%)</td>
<td>4/44 (9%)</td>
<td>6/34 (18%)</td>
<td>5/37 (14%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>610</td>
<td>729 (T)</td>
<td>683</td>
<td>690</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.381</td>
<td>P=0.437N</td>
<td>P=0.202</td>
<td>P=0.525</td>
</tr>
<tr>
<td><strong>All Organs: Malignant Lymphoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>6/50 (12%)</td>
<td>3/50 (6%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>9.2%</td>
<td>12.4%</td>
<td>6.8%</td>
<td>12.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/36 (8%)</td>
<td>6/44 (14%)</td>
<td>3/34 (9%)</td>
<td>1/37 (3%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>682</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>526</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.399</td>
<td>P=0.438</td>
<td>P=0.490N</td>
<td>P=0.413</td>
</tr>
<tr>
<td><strong>All Organs: Benign Neoplasms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>38/50 (76%)</td>
<td>43/50 (86%)</td>
<td>35/50 (70%)</td>
<td>46/50 (92%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>82.0%</td>
<td>88.3%</td>
<td>75.9%</td>
<td>94.9%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>31/36 (86%)</td>
<td>41/44 (93%)</td>
<td>28/34 (82%)</td>
<td>36/37 (97%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>489</td>
<td>652</td>
<td>613</td>
<td>493</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.048</td>
<td>P=0.269</td>
<td>P=0.312N</td>
<td>P=0.034</td>
</tr>
<tr>
<td><strong>All Organs: Malignant Neoplasms</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>37/50 (74%)</td>
<td>41/50 (82%)</td>
<td>38/50 (76%)</td>
<td>44/50 (88%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>75.8%</td>
<td>82.0%</td>
<td>77.4%</td>
<td>88.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>25/36 (69%)</td>
<td>35/44 (80%)</td>
<td>23/34 (68%)</td>
<td>31/37 (84%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>256</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.095</td>
<td>P=0.306</td>
<td>P=0.521</td>
<td>P=0.092</td>
</tr>
</tbody>
</table>
TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>All Organs: Benign or Malignant Neoplasms</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>47/50 (94%)</td>
<td>50/50 (100%)</td>
<td>44/50 (88%)</td>
<td>50/50 (100%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>95.1%</td>
<td>100.0%</td>
<td>89.6%</td>
<td>100.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>34/36 (94%)</td>
<td>44/44 (100%)</td>
<td>29/34 (85%)</td>
<td>37/37 (100%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>398</td>
<td>593</td>
<td>256</td>
<td>485</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.240</td>
<td>P=0.159</td>
<td>P=0.260N</td>
<td>P=0.159</td>
</tr>
</tbody>
</table>

(T) Terminal sacrifice

a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for adrenal gland, liver, lung, pancreatic islets, and spleen; for other tissues, denominator is number of animals necropsied.
b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
c Observed incidence at terminal kill
d Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.
e Not applicable; no neoplasms in animal group
### Table C3a
**Historical Incidence of Liver Neoplasms in Control Male B6C3F1 Mice**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatocellular Adenoma</td>
</tr>
<tr>
<td>Androstenedione (December, 2002)</td>
<td>32/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (July, 2000)</td>
<td>28/50</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>60/100 (60.0%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>60.0% ± 5.7%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>56%-64%</td>
</tr>
</tbody>
</table>

#### Overall Historical Incidence: All Routes

<table>
<thead>
<tr>
<th></th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatocellular Adenoma</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>733/1,447 (50.7%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>50.7% ± 13.9%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>22%-72%</td>
</tr>
</tbody>
</table>

*a Data as of November 19, 2008*

### Table C3b
**Historical Incidence of Pancreatic Islet Neoplasms in Control Male B6C3F1 Mice**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenoma</td>
</tr>
<tr>
<td>Androstenedione (December, 2002)</td>
<td>2/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (July, 2000)</td>
<td>0/50</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>2/100 (2.0%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>2.0% ± 2.8%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0%-4%</td>
</tr>
</tbody>
</table>

#### Overall Historical Incidence: All Routes

<table>
<thead>
<tr>
<th></th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenoma</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td>17/1,435 (1.2%)</td>
</tr>
<tr>
<td><strong>Mean ± standard deviation</strong></td>
<td>1.2% ± 1.7%</td>
</tr>
<tr>
<td><strong>Range</strong></td>
<td>0%-6%</td>
</tr>
</tbody>
</table>

*a Data as of November 19, 2008*
### Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moribund</td>
<td>5</td>
<td>2</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>9</td>
<td>4</td>
<td>11</td>
<td>10</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal sacrifice</td>
<td>36</td>
<td>44</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>Animals examined microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

### Alimentary System

<table>
<thead>
<tr>
<th>Site</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gallbladder</td>
<td>(45)</td>
<td>(46)</td>
<td>(42)</td>
<td>(44)</td>
</tr>
<tr>
<td>Epithelium, cytoplasmic alteration</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine large, cecum</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Edema</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Intestine small, duodenum</td>
<td>(47)</td>
<td>(49)</td>
<td>(46)</td>
<td>(49)</td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(49)</td>
<td>(50)</td>
<td>(49)</td>
<td>(49)</td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intestine small, jejunum</td>
<td>(47)</td>
<td>(49)</td>
<td>(47)</td>
<td>(49)</td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Angiectasis</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basophilic focus</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Clear cell focus</td>
<td>27 (54%)</td>
<td>24 (48%)</td>
<td>18 (36%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Cyst</td>
<td>1 (2%)</td>
<td></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Eosinophilic focus</td>
<td>13 (26%)</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
<td>25 (50%)</td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td>1 (2%)</td>
<td></td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Infiltration cellular</td>
<td></td>
<td></td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, mixed cell</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Mitotic alteration</td>
<td></td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mixed cell focus</td>
<td>10 (20%)</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
<td>13 (26%)</td>
</tr>
<tr>
<td>Necrosis</td>
<td>3 (6%)</td>
<td>3 (6%)</td>
<td>5 (10%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Necrosis, focal</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Tension lipidosis</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Vacuolization cytoplasmic</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Centrilobular, necrosis</td>
<td>2 (4%)</td>
<td></td>
<td>3 (6%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Hepatocyte, vacuolization cytoplasmic</td>
<td>17 (34%)</td>
<td>14 (28%)</td>
<td>14 (28%)</td>
<td>12 (24%)</td>
</tr>
<tr>
<td>Kupffer cell, hyperplasia</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Mesentery</td>
<td>(5)</td>
<td>(8)</td>
<td>(4)</td>
<td>(5)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>2 (40%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, necrosis</td>
<td>4 (80%)</td>
<td>7 (88%)</td>
<td>2 (50%)</td>
<td>4 (80%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Thrombosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinus, cytoplasmic alteration</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Acinus, hyperplasia, focal</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Salivary glands</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td>6 (12%)</td>
</tr>
</tbody>
</table>

* Number of animals examined microscopically at the site and the number of animals with lesion
### Table C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Alimentary System</strong> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stomach, forestomach</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosion</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (2%)</td>
<td>5 (10%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Stomach, glandular</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosion</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
<td></td>
</tr>
<tr>
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### TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

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<td>4 (8%)</td>
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<tr>
<td>Infiltration cellular, histiocyte</td>
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<td>5 (10%)</td>
<td>11 (22%)</td>
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<tr>
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<td>1 (2%)</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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### Table C4
**Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione**

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<th></th>
<th>Vehicle Control</th>
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<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<td>Cataract</td>
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<tr>
<td>Hemorrhage</td>
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<td>Inflammation, chronic</td>
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<td>2 (4%)</td>
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<td>1 (2%)</td>
<td>1 (2%)</td>
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<td>(50)</td>
<td>(50)</td>
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<tr>
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<td>2 (4%)</td>
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<td>2 (4%)</td>
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</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
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<tr>
<td>Inflammation, suppurative</td>
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<tr>
<td>Metaplasia, osseous</td>
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<td>2 (4%)</td>
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<td>38 (76%)</td>
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<tr>
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<td>6 (12%)</td>
<td>6 (12%)</td>
</tr>
<tr>
<td>Renal tubule, pigmentation</td>
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<td>3 (6%)</td>
<td>6 (12%)</td>
</tr>
<tr>
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<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Transitional epithelium, hyperplasia</td>
<td></td>
<td>1 (2%)</td>
<td>2 (4%)</td>
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</tbody>
</table>
APPENDIX D
SUMMARY OF LESIONS IN FEMALE MICE
IN THE 2-YEAR GAVAGE STUDY
OF ANDROSTENEDIONE

| Table D1 | Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione | 124 |
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### TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Disposition Summary</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
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<tr>
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<td>50</td>
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<tr>
<td>Early deaths</td>
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<td>Accidental deaths</td>
<td>2</td>
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<td>1</td>
<td></td>
</tr>
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<td>Moribund</td>
<td>6</td>
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<td>3</td>
<td>3</td>
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<td>Natural deaths</td>
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<td>6</td>
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<td>Survivors</td>
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</tr>
<tr>
<td>Died last week of study</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
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<td>Terminal sacrifice</td>
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<td>40</td>
<td>39</td>
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<td>Animals examined microscopically</td>
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### Alimentary System

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<th>50 mg/kg</th>
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<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
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<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Gallbladder</td>
<td>(44)</td>
<td>(45)</td>
<td>(42)</td>
<td>(48)</td>
</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Intestine large, cecum</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Intestine small, duodenum</td>
<td>(47)</td>
<td>(46)</td>
<td>(46)</td>
<td>(49)</td>
</tr>
<tr>
<td>Intestine small, ileum</td>
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<td>(49)</td>
<td>(50)</td>
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<td>Intestine small, jejunum</td>
<td>(46)</td>
<td>(48)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
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<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatocellular adenoma</td>
<td>10 (20%)</td>
<td>9 (18%)</td>
<td>11 (22%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Hepatocellular adenoma, multiple</td>
<td>4 (8%)</td>
<td>7 (14%)</td>
<td>7 (14%)</td>
<td>17 (34%)</td>
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<td>4 (8%)</td>
<td>11 (22%)</td>
<td>10 (20%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma, multiple</td>
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<td>2 (4%)</td>
<td>5 (10%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Mesentery</td>
<td>(7)</td>
<td>(9)</td>
<td>(14)</td>
<td>(8)</td>
</tr>
<tr>
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<td>1 (11%)</td>
<td></td>
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<tr>
<td>Oral mucosa</td>
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<td>(0)</td>
<td>(0)</td>
<td>(1)</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td></td>
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<td>1 (100%)</td>
<td></td>
</tr>
<tr>
<td>Pancreas</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Salivary glands</td>
<td>(49)</td>
<td>(49)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Stomach, forestomach</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Squamous cell papilloma</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Stomach, glandular</td>
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### Cardiovascular System

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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Table D1</td>
<td>Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione</td>
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<td></td>
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</tr>
<tr>
<td>----------</td>
<td>-------------------------------------------------------------------------------------------------</td>
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<td><strong>Endocrine System</strong></td>
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<td>Adrenal cortex</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adrenal medulla</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pheochromocytoma benign</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Islets, pancreatic</td>
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</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parathyroid gland</td>
<td></td>
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<td>Pars distalis, adenoma</td>
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<td></td>
</tr>
<tr>
<td>Pars intermedia, adenoma</td>
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<tr>
<td>Thyroid gland</td>
<td></td>
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</tr>
<tr>
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<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-cell, carcinoma</td>
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<td></td>
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<tr>
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<tr>
<td>Follicular cell, carcinoma</td>
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<td><strong>General Body System</strong></td>
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</tr>
<tr>
<td>Hemangioma</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma, metastatic, skin</td>
<td>1 (100%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td><strong>Genital System</strong></td>
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<td>Clitoral gland</td>
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<tr>
<td>Ovary</td>
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</tr>
<tr>
<td>Cystadenoma</td>
<td>4 (8%)</td>
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<td>Cystadenoma, multiple</td>
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<td></td>
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<td></td>
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<tr>
<td>Hemangiosarcoma</td>
<td>1 (2%)</td>
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</tr>
<tr>
<td>Leiomyosarcoma</td>
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<td>Polyp stromal</td>
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<td><strong>Hematopoietic System</strong></td>
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<tr>
<td>Bone marrow</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemangiosarcoma</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>1 (10%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iliac, rhabdomyosarcoma, metastatic, skeletal muscle</td>
<td>1 (100%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mediastinal, hemangioma</td>
<td>1 (100%)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Renal, rhabdomyosarcoma, metastatic, skeletal muscle</td>
<td>1 (100%)</td>
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<tr>
<td>Lymph node, mandibular</td>
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<tr>
<td>Hemangiosarcoma</td>
<td>1 (2%)</td>
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<td>Lymph node, mesenteric</td>
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<tr>
<td>Hemangiosarcoma</td>
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<td>Thymus</td>
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</tr>
<tr>
<td>Sarcoma, metastatic, skin</td>
<td>1 (2%)</td>
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# TABLE D1
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
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<th>50 mg/kg</th>
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<td><strong>Integumentary System</strong></td>
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<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td></td>
<td>1 (2%)</td>
<td></td>
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</tr>
<tr>
<td>Carcinoma</td>
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<td></td>
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</tr>
<tr>
<td>Skin</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Basal cell adenoma</td>
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<td></td>
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</tr>
<tr>
<td>Squamous cell papilloma</td>
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</tr>
<tr>
<td>Subcutaneous tissue, fibrosarcoma</td>
<td>(3) (6%)</td>
<td>(2) (4%)</td>
<td>(1) (2%)</td>
<td>(1) (2%)</td>
</tr>
<tr>
<td>Subcutaneous tissue, fibrous histiocytes</td>
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<tr>
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</tr>
<tr>
<td>Subcutaneous tissue, hemangiosarcoma</td>
<td>(1) (2%)</td>
<td></td>
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<tr>
<td>Subcutaneous tissue, liposarcoma</td>
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<tr>
<td>Subcutaneous tissue, myxosarcoma</td>
<td>(1) (2%)</td>
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</tr>
<tr>
<td>Subcutaneous tissue, sarcoma</td>
<td>(1) (2%)</td>
<td>(1) (2%)</td>
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<td>Subcutaneous tissue, schwannoma malignant</td>
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<td><strong>Musculoskeletal System</strong></td>
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<td>Bone</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Osteosarcoma</td>
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<td>Skeletal muscle</td>
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<tr>
<td>Myxosarcoma</td>
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<td><strong>Nervous System</strong></td>
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<td>Brain</td>
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<td>(50)</td>
<td>(50)</td>
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<td>(1)</td>
<td>(3)</td>
<td>(1)</td>
</tr>
<tr>
<td>Osteosarcoma, metastatic, bone</td>
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<td>1 (50%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Respiratory System</strong></td>
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<td></td>
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</tr>
<tr>
<td>Lung</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Alveolar/bronchiolar adenoma</td>
<td></td>
<td>2 (4%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Alveolar/bronchiolar carcinoma</td>
<td></td>
<td>3 (6%)</td>
<td></td>
<td>(8%)</td>
</tr>
<tr>
<td>Alveolar/bronchiolar carcinoma, multiple</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma, metastatic, thyroid gland</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>(1) (2%)</td>
<td></td>
<td>(1) (2%)</td>
<td>(1) (2%)</td>
</tr>
<tr>
<td>Liposarcoma, metastatic, skin</td>
<td></td>
<td>(1) (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Osteosarcoma, metastatic, bone</td>
<td></td>
<td>(1) (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhabdomyosarcoma, metastatic, skeletal muscle</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sarcoma, metastatic, skin</td>
<td></td>
<td>(1) (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nose</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Carcinoma, metastatic, hardarian gland</td>
<td>(1) (2%)</td>
<td></td>
<td>(1) (2%)</td>
<td>(1) (2%)</td>
</tr>
<tr>
<td>Trachea</td>
<td>(50)</td>
<td>(50)</td>
<td>(48)</td>
<td>(50)</td>
</tr>
<tr>
<td>Sarcoma, metastatic, skeletal muscle</td>
<td>(1) (2%)</td>
<td></td>
<td></td>
<td></td>
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</table>
**TABLE D1**
Summary of the Incidence of Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Special Senses System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eye</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Carcinoma, metastatic, harderian gland</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Harderian gland</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Adenoma</td>
<td>9 (18%)</td>
<td>4 (8%)</td>
<td>11 (22%)</td>
<td>4 (8%)</td>
</tr>
<tr>
<td>Adenoma, multiple</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td><strong>Urinary System</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hepatocellular carcinoma, metastatic, liver</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Renal tubule, carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td><strong>Systemic Lesions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple organs(^b)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Histiocytic sarcoma</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Lymphoma malignant</td>
<td>14 (28%)</td>
<td>15 (30%)</td>
<td>11 (22%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td><strong>Neoplasm Summary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total animals with primary neoplasms(^c)</td>
<td>40</td>
<td>43</td>
<td>43</td>
<td>38</td>
</tr>
<tr>
<td>Total primary neoplasms</td>
<td>70</td>
<td>81</td>
<td>83</td>
<td>83</td>
</tr>
<tr>
<td>Total animals with benign neoplasms</td>
<td>27</td>
<td>27</td>
<td>31</td>
<td>32</td>
</tr>
<tr>
<td>Total benign neoplasms</td>
<td>33</td>
<td>36</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>Total animals with malignant neoplasms</td>
<td>27</td>
<td>36</td>
<td>31</td>
<td>25</td>
</tr>
<tr>
<td>Total malignant neoplasms</td>
<td>37</td>
<td>45</td>
<td>38</td>
<td>33</td>
</tr>
<tr>
<td>Total animals with metastatic neoplasms</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Total metastatic neoplasms</td>
<td>14</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>

\(^a\) Number of animals examined microscopically at the site and the number of animals with neoplasm

\(^b\) Number of animals with any tissue examined microscopically

\(^c\) Primary neoplasms: all neoplasms except metastatic neoplasms
### Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Tissue/Type</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Harderian Gland: Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>9/50 (18%)</td>
<td>5/50 (10%)</td>
<td>11/50 (22%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>20.3%</td>
<td>10.9%</td>
<td>23.8%</td>
<td>8.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>8/35 (23%)</td>
<td>5/40 (13%)</td>
<td>9/40 (23%)</td>
<td>4/40 (10%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>622</td>
<td>729 (T)</td>
<td>508</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.129N</td>
<td>P=0.174N</td>
<td>P=0.442</td>
<td>P=0.103N</td>
</tr>
<tr>
<td><strong>Harderian Gland: Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>10/50 (20%)</td>
<td>6/50 (12%)</td>
<td>12/50 (24%)</td>
<td>6/50 (12%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>22.3%</td>
<td>13.1%</td>
<td>25.9%</td>
<td>13.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>8/35 (23%)</td>
<td>6/40 (15%)</td>
<td>10/40 (25%)</td>
<td>5/40 (13%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>565</td>
<td>729 (T)</td>
<td>508</td>
<td>718</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.234N</td>
<td>P=0.192N</td>
<td>P=0.436</td>
<td>P=0.192N</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>14/50 (28%)</td>
<td>16/50 (32%)</td>
<td>18/50 (36%)</td>
<td>28/50 (56%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>31.6%</td>
<td>34.6%</td>
<td>39.1%</td>
<td>61.1%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>13/35 (37%)</td>
<td>14/40 (35%)</td>
<td>16/40 (40%)</td>
<td>27/40 (68%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>622</td>
<td>622</td>
<td>520</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001</td>
<td>P=0.468</td>
<td>P=0.299</td>
<td>P=0.003</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>5/50 (10%)</td>
<td>13/50 (26%)</td>
<td>15/50 (30%)</td>
<td>15/50 (30%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>11.3%</td>
<td>28.2%</td>
<td>32.0%</td>
<td>32.7%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/35 (9%)</td>
<td>11/40 (28%)</td>
<td>11/40 (28%)</td>
<td>13/40 (33%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>687</td>
<td>685</td>
<td>442</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.098</td>
<td>P=0.038</td>
<td>P=0.015</td>
<td>P=0.012</td>
</tr>
<tr>
<td><strong>Liver: Hepatocellular Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>17/50 (34%)</td>
<td>23/50 (46%)</td>
<td>27/50 (54%)</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>38.2%</td>
<td>49.5%</td>
<td>57.0%</td>
<td>69.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>14/35 (40%)</td>
<td>20/40 (50%)</td>
<td>22/40 (55%)</td>
<td>30/40 (75%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>622</td>
<td>622</td>
<td>442</td>
<td>708</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.004</td>
<td>P=0.188</td>
<td>P=0.052</td>
<td>P&lt;0.001</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Adenoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>0/50 (0%)</td>
<td>2/50 (4%)</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>0.0%</td>
<td>4.4%</td>
<td>6.7%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>0/35 (0%)</td>
<td>2/40 (5%)</td>
<td>3/40 (8%)</td>
<td>0/40 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>—</td>
<td>729 (T)</td>
<td>729 (T)</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.286N</td>
<td>P=0.246</td>
<td>P=0.123</td>
<td>_f</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>9.1%</td>
<td>6.5%</td>
<td>0.0%</td>
<td>10.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/35 (9%)</td>
<td>2/40 (5%)</td>
<td>0/40 (0%)</td>
<td>4/40 (10%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>684</td>
<td>502</td>
<td>—</td>
<td>463</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.240</td>
<td>P=0.473N</td>
<td>P=0.058N</td>
<td>P=0.532</td>
</tr>
<tr>
<td><strong>Lung: Alveolar/bronchiolar Adenoma or Carcinoma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall rate</td>
<td>4/50 (8%)</td>
<td>5/50 (10%)</td>
<td>3/50 (6%)</td>
<td>5/50 (10%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>9.1%</td>
<td>10.8%</td>
<td>6.7%</td>
<td>10.8%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>3/35 (9%)</td>
<td>4/40 (10%)</td>
<td>3/40 (8%)</td>
<td>4/40 (10%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>684</td>
<td>502</td>
<td>729 (T)</td>
<td>463</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.490</td>
<td>P=0.531</td>
<td>P=0.490N</td>
<td>P=0.532</td>
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</table>
### Table D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Mammary Gland: Adenoma or Carcinoma</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>1/50 (2%)</td>
<td>3/50 (6%)</td>
<td>0/50 (0%)</td>
<td>0/50 (0%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>2.3%</td>
<td>6.5%</td>
<td>0.0%</td>
<td>0.0%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/35 (3%)</td>
<td>2/40 (5%)</td>
<td>0/40 (0%)</td>
<td>0/40 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>729 (T)</td>
<td>681</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.176N</td>
<td>P=0.321</td>
<td>P=0.495N</td>
<td>P=0.492N</td>
</tr>
</tbody>
</table>

| Ovary: Cystadenoma                  |                |        |        |        |
| Overall rate                        | 4/49 (8%)      | 3/50 (6%) | 2/49 (4%) | 2/49 (4%) |
| Adjusted rate                       | 9.3%           | 6.6%    | 4.5%   | 4.5%   |
| Terminal rate                       | 4/34 (12%)     | 3/40 (8%) | 1/39 (3%) | 2/39 (5%) |
| First incidence (days)              | 729 (T)        | 729 (T) | 659    | 729 (T) |
| Poly-3 test                         | P=0.355N       | P=0.466N | P=0.323N | P=0.321N |

| Pancreatic Islets: Adenoma          |                |        |        |        |
| Overall rate                        | 0/49 (0%)      | 2/50 (4%) | 4/49 (8%) | 4/48 (8%) |
| Adjusted rate                       | 0.0%           | 4.4%    | 9.0%   | 8.9%   |
| Terminal rate                       | 0/35 (0%)      | 2/40 (5%) | 4/40 (10%) | 3/40 (8%) |
| First incidence (days)              | —              | 729 (T) | 729 (T) | 654    |
| Poly-3 test                         | P=0.137        | P=0.248 | P=0.063 | P=0.064 |

| Pituitary Gland (Pars Distalis): Adenoma |        |        |        |        |
| Overall rate                        | 1/49 (2%)      | 2/47 (4%) | 4/50 (8%) | 4/50 (8%) |
| Adjusted rate                       | 2.3%           | 4.6%    | 8.9%   | 8.8%   |
| Terminal rate                       | 0/35 (0%)      | 2/39 (5%) | 4/40 (10%) | 4/40 (10%) |
| First incidence (days)              | 659            | 729 (T) | 729 (T) | 729 (T) |
| Poly-3 test                         | P=0.228        | P=0.503 | P=0.189 | P=0.195 |

| Skin (Subcutaneous Tissue): Fibrosarcoma |        |        |        |        |
| Overall rate                        | 3/50 (6%)      | 0/50 (0%) | 2/50 (4%) | 1/50 (2%) |
| Adjusted rate                       | 6.7%           | 0.0%    | 4.4%   | 2.2%   |
| Terminal rate                       | 0/35 (0%)      | 0/40 (0%) | 1/40 (3%) | 1/40 (3%) |
| First incidence (days)              | 659            | —       | 646    | 729 (T) |
| Poly-3 test                         | P=0.475N       | P=0.114N | P=0.494N | P=0.297N |

| Skin (Subcutaneous Tissue): Fibrous Histiocytoma, Fibrosarcoma, Myxosarcoma, or Sarcoma |        |        |        |        |
| Overall rate                        | 5/50 (10%)     | 1/50 (2%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate                       | 11.2%          | 2.2%    | 6.6%   | 4.4%   |
| Terminal rate                       | 1/35 (3%)      | 1/40 (3%) | 1/40 (3%) | 2/40 (5%) |
| First incidence (days)              | 659            | 729 (T) | 646    | 729 (T) |
| Poly-3 test                         | P=0.387N       | P=0.097N | P=0.348N | P=0.208N |

| Uterus: Stromal Polyp                |        |        |        |        |
| Overall rate                        | 2/50 (4%)      | 1/50 (2%) | 0/50 (0%) | 4/50 (8%) |
| Adjusted rate                       | 4.5%           | 2.2%    | 0.0%   | 8.8%   |
| Terminal rate                       | 1/35 (3%)      | 1/40 (3%) | 0/40 (0%) | 4/40 (10%) |
| First incidence (days)              | 443            | 729 (T) | —      | 729 (T) |
| Poly-3 test                         | P=0.082        | P=0.492N | P=0.236N | P=0.347 |

| All Organs: Hemangiosarcoma         |        |        |        |        |
| Overall rate                        | 1/50 (2%)      | 2/50 (4%) | 3/50 (6%) | 2/50 (4%) |
| Adjusted rate                       | 2.3%           | 4.3%    | 6.6%   | 4.3%   |
| Terminal rate                       | 1/35 (3%)      | 1/40 (3%) | 2/40 (5%) | 1/40 (3%) |
| First incidence (days)              | 729 (T)        | 622     | 508    | 420    |
| Poly-3 test                         | P=0.591        | P=0.517 | P=0.319 | P=0.520 |
### TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>All Organs: Hemangioma or Hemangiosarcoma</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>2/50 (4%)</td>
<td>3/50 (6%)</td>
<td>3/50 (6%)</td>
<td>4/50 (8%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>4.5%</td>
<td>6.5%</td>
<td>6.6%</td>
<td>8.6%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/35 (3%)</td>
<td>1/40 (3%)</td>
<td>2/40 (5%)</td>
<td>3/40 (8%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>672</td>
<td>622</td>
<td>508</td>
<td>420</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.349</td>
<td>P=0.521</td>
<td>P=0.515</td>
<td>P=0.362</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Histiocytic Sarcoma</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>2/50 (4%)</td>
<td>4/50 (8%)</td>
<td>0/50 (0%)</td>
<td>1/50 (2%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>4.5%</td>
<td>8.4%</td>
<td>0.0%</td>
<td>2.2%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>1/35 (3%)</td>
<td>1/40 (3%)</td>
<td>0/40 (0%)</td>
<td>0/40 (0%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>672</td>
<td>446</td>
<td>—</td>
<td>654</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.270N</td>
<td>P=0.374</td>
<td>P=0.233N</td>
<td>P=0.486N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Malignant Lymphoma</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>14/50 (28%)</td>
<td>15/50 (30%)</td>
<td>11/50 (22%)</td>
<td>2/50 (4%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>31.3%</td>
<td>32.5%</td>
<td>24.4%</td>
<td>4.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>11/35 (31%)</td>
<td>13/40 (33%)</td>
<td>10/40 (25%)</td>
<td>2/40 (5%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>547</td>
<td>680</td>
<td>672</td>
<td>729 (T)</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P&lt;0.001N</td>
<td>P=0.540</td>
<td>P=0.308N</td>
<td>P&lt;0.001N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Benign Neoplasms</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>27/50 (54%)</td>
<td>27/50 (54%)</td>
<td>31/50 (62%)</td>
<td>32/50 (64%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>59.2%</td>
<td>57.7%</td>
<td>66.0%</td>
<td>69.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>23/35 (66%)</td>
<td>22/40 (55%)</td>
<td>27/40 (68%)</td>
<td>30/40 (75%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>443</td>
<td>622</td>
<td>508</td>
<td>654</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.151</td>
<td>P=0.524N</td>
<td>P=0.322</td>
<td>P=0.206</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Malignant Neoplasms</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>27/50 (54%)</td>
<td>36/50 (72%)</td>
<td>31/50 (62%)</td>
<td>25/50 (50%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>57.7%</td>
<td>72.6%</td>
<td>63.9%</td>
<td>52.4%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>17/35 (49%)</td>
<td>27/40 (68%)</td>
<td>23/40 (58%)</td>
<td>20/40 (50%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>547</td>
<td>446</td>
<td>442</td>
<td>420</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.085N</td>
<td>P=0.091</td>
<td>P=0.342</td>
<td>P=0.377N</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>All Organs: Benign or Malignant Neoplasms</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall rate</td>
<td>40/50 (80%)</td>
<td>43/50 (86%)</td>
<td>43/50 (86%)</td>
<td>38/50 (76%)</td>
</tr>
<tr>
<td>Adjusted rate</td>
<td>83.5%</td>
<td>86.7%</td>
<td>87.7%</td>
<td>79.7%</td>
</tr>
<tr>
<td>Terminal rate</td>
<td>28/35 (80%)</td>
<td>34/40 (85%)</td>
<td>34/40 (85%)</td>
<td>33/40 (83%)</td>
</tr>
<tr>
<td>First incidence (days)</td>
<td>443</td>
<td>446</td>
<td>442</td>
<td>420</td>
</tr>
<tr>
<td>Poly-3 test</td>
<td>P=0.227N</td>
<td>P=0.435</td>
<td>P=0.377</td>
<td>P=0.413N</td>
</tr>
</tbody>
</table>

(T) Terminal sacrifice

a Number of neoplasm-bearing animals/number of animals examined. Denominator is number of animals examined microscopically for liver, lung, ovary, pancreatic islets, and pituitary gland; for other tissues, denominator is number of animals necropsied.
b Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
c Observed incidence at terminal kill
d Beneath the vehicle control incidence is the P value associated with the trend test. Beneath the dosed group incidence are the P values corresponding to pairwise comparisons between the vehicle controls and that dosed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice. A negative trend or a lower incidence in a dosed group is indicated by N.
e Not applicable; no neoplasms in animal group
f Value of the statistic cannot be computed
### TABLE D3a
**Historical Incidence of Liver Neoplasms in Control Female B6C3F1 Mice**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hepatocellular Adenoma</td>
</tr>
<tr>
<td>Historical Incidence: Methylcellulose Gavage Studies</td>
<td></td>
</tr>
<tr>
<td>Androstenedione (December, 2002)</td>
<td>14/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (July, 2000)</td>
<td>15/50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>29/100 (29.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>29.0% ± 1.4%</td>
</tr>
<tr>
<td>Range</td>
<td>28%-30%</td>
</tr>
<tr>
<td>Overall Historical Incidence: All Routes</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>396/1,494 (26.5%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>26.5% ± 15.2%</td>
</tr>
<tr>
<td>Range</td>
<td>2%-62%</td>
</tr>
</tbody>
</table>

* Data as of November 19, 2008

### TABLE D3b
**Historical Incidence of Pancreatic Islet Neoplasms in Control Female B6C3F1 Mice**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adenoma</td>
</tr>
<tr>
<td>Historical Incidence: Methylcellulose Gavage Studies</td>
<td></td>
</tr>
<tr>
<td>Androstenedione (December, 2002)</td>
<td>0/49</td>
</tr>
<tr>
<td>Methylene blue trihydrate (July, 2000)</td>
<td>1/46</td>
</tr>
<tr>
<td>Total (%)</td>
<td>1/95 (1.1%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>1.1% ± 1.5%</td>
</tr>
<tr>
<td>Range</td>
<td>0%-2%</td>
</tr>
<tr>
<td>Overall Historical Incidence: All Routes</td>
<td></td>
</tr>
<tr>
<td>Total (%)</td>
<td>11/1,478 (0.7%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>0.8% ± 1.0%</td>
</tr>
<tr>
<td>Range</td>
<td>0%-2%</td>
</tr>
</tbody>
</table>

* Data as of November 19, 2008
### TABLE D3c
**Historical Incidence of Malignant Lymphoma in Control Female B6C3F1 Mice**

<table>
<thead>
<tr>
<th>Study (Study Start)</th>
<th>Incidence in Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Historical Incidence: Methylcellulose Gavage Studies</strong></td>
<td></td>
</tr>
<tr>
<td>Androstenedione (December, 2002)</td>
<td>14/50</td>
</tr>
<tr>
<td>Methylene blue trihydrate (July, 2000)</td>
<td>6/50</td>
</tr>
<tr>
<td>Total (%)</td>
<td>20/100 (20.0%)</td>
</tr>
<tr>
<td>Mean ± standard deviation</td>
<td>20.0% ± 11.3%</td>
</tr>
<tr>
<td>Range</td>
<td>12%-28%</td>
</tr>
</tbody>
</table>

| **Overall Historical Incidence: All Routes** |                       |
| Total (%)                                    | 307/1,498 (20.5%)     |
| Mean ± standard deviation                    | 20.5% ± 9.7%          |
| Range                                        | 4%-54%                |

---

Data as of November 19, 2008; includes data for histiocytic, lymphocytic, mixed, unspecified, or undifferentiated cell types
### TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Disposition Summary</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Animals initially in study</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Early deaths</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accidental deaths</td>
<td>2</td>
<td></td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Moribund</td>
<td>6</td>
<td>2</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Natural deaths</td>
<td>7</td>
<td>8</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Survivors</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Died last week of study</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Terminal sacrifice</td>
<td>34</td>
<td>40</td>
<td>39</td>
<td>40</td>
</tr>
<tr>
<td>Animals examined microscopically</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>50</td>
</tr>
</tbody>
</table>

### Alimentary System

<table>
<thead>
<tr>
<th>Site</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Esophagus</strong></td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Gallbladder</td>
<td>(44)</td>
<td>(45)</td>
<td>(42)</td>
<td>(48)</td>
</tr>
<tr>
<td>Cyst</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Intestine large, cecum</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Edema</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Intestine small, duodenum</td>
<td>(47)</td>
<td>(46)</td>
<td>(46)</td>
<td>(49)</td>
</tr>
<tr>
<td>Metaplasia</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Intestine small, ileum</td>
<td>(46)</td>
<td>(48)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Intestine small, jejunum</td>
<td>(46)</td>
<td>(48)</td>
<td>(49)</td>
<td>(50)</td>
</tr>
<tr>
<td>Hyperplasia, lymphoid</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Liver</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Angiectasis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Basophilic focus</td>
<td></td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Clear cell focus</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Eosinophilic focus</td>
<td>9 (18%)</td>
<td>8 (16%)</td>
<td>11 (22%)</td>
<td>11 (22%)</td>
</tr>
<tr>
<td>Hematopoietic cell proliferation</td>
<td>6 (12%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, mixed cell</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Mixed cell focus</td>
<td>2 (4%)</td>
<td>5 (10%)</td>
<td>7 (14%)</td>
<td>15 (30%)</td>
</tr>
<tr>
<td>Necrosis, focal</td>
<td>2 (4%)</td>
<td>5 (10%)</td>
<td>2 (4%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Tension lipidosis</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td>2 (4%)</td>
</tr>
<tr>
<td>Centrilobular, necrosis</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Hepatocyte, hypertrophy</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Hepatocyte, vacuolization cytoplasmic</td>
<td>6 (12%)</td>
<td>3 (6%)</td>
<td>9 (18%)</td>
<td>22 (44%)</td>
</tr>
<tr>
<td>Kupffer cell, hyperplasia</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Kupffer cell, pigmentation</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Oval cell, hyperplasia</td>
<td></td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Mesentery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat, necrosis</td>
<td>7 (100%)</td>
<td>6 (67%)</td>
<td>13 (93%)</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>Oral mucosa</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Pancreas</td>
<td>(49)</td>
<td>(50)</td>
<td>(50)</td>
<td>(49)</td>
</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td>4 (8%)</td>
<td></td>
</tr>
<tr>
<td>Cyst</td>
<td></td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinus, cytoplasmic alteration</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

* Number of animals examined microscopically at the site and the number of animals with lesion
### TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>System</th>
<th>Vehicle Control</th>
<th>2 mg/kg</th>
<th>10 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Salivary glands</strong> (continued)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrophy</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>10 (20%)</td>
<td>12 (24%)</td>
<td>2 (4%)</td>
<td>7 (14%)</td>
</tr>
<tr>
<td>Submandibular gland, cytoplasmic alteration</td>
<td>17 (35%)</td>
<td>40 (82%)</td>
<td>45 (90%)</td>
<td></td>
</tr>
<tr>
<td><strong>Stomach, forestomach</strong></td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Diverticulum</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaplasia, hepatocyte</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ulcer</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epithelium, hyperplasia</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td><strong>Stomach, glandular</strong></td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Cyst</td>
<td>2 (4%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erosion</td>
<td>1 (2%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaplasia, hepatocyte</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mineralization</td>
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### Table D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

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<td>1 (2%)</td>
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</table>
**TABLE D4**
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

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<td>Infarct</td>
<td>4 (8%)</td>
<td>3 (6%)</td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>4 (8%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Inflammation, suppurative</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Metaplasia, osseous</td>
<td>1 (2%)</td>
<td>2 (4%)</td>
<td></td>
<td>3 (6%)</td>
</tr>
<tr>
<td>Nephropathy</td>
<td>13 (26%)</td>
<td>11 (22%)</td>
<td>13 (26%)</td>
<td>14 (28%)</td>
</tr>
<tr>
<td>Glomerulus, metaplasia</td>
<td>2 (4%)</td>
<td>1 (2%)</td>
<td>5 (10%)</td>
<td>27 (54%)</td>
</tr>
<tr>
<td>Renal tubule, accumulation, hyaline droplet</td>
<td>1 (2%)</td>
<td></td>
<td>3 (6%)</td>
<td></td>
</tr>
<tr>
<td>Renal tubule, hyperplasia</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td>1 (2%)</td>
</tr>
<tr>
<td>Renal tubule, pigmentation</td>
<td>1 (2%)</td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Transitional epithelium, hyperplasia</td>
<td></td>
<td></td>
<td>1 (2%)</td>
<td></td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
<td>(50)</td>
</tr>
<tr>
<td>Infiltration cellular, lymphocyte</td>
<td>1 (2%)</td>
<td>4 (8%)</td>
<td>2 (4%)</td>
<td></td>
</tr>
<tr>
<td>Inflammation, chronic</td>
<td>1 (2%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Transitional epithelium, hyperplasia</td>
<td>1 (2%)</td>
<td></td>
<td>2 (4%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>
APPENDIX E
GENETIC TOXICOLOGY

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Mouse Peripheral Blood Micronucleus Test Protocol .............................................. 141
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GENETIC TOXICOLOGY

Salmonella typhimurium Mutagenicity Test Protocol

Two independent mutagenicity assays were conducted with androstenedione. Testing was first performed as reported by Zeiger et al. (1992). Androstenedione was sent to the laboratory as a coded aliquot from Radian Corporation (Austin, TX). It was incubated with the Salmonella typhimurium tester strains TA97, TA98, TA100, and TA1535 either in buffer or S9 mix (metabolic activation enzymes and cofactors from Aroclor 1254-induced male Sprague-Dawley rat or Syrian hamster liver) for 20 minutes at 37°C. Top agar supplemented with L-histidine and d-biotin was added, and the contents of the tubes were mixed and poured onto the surfaces of minimal glucose agar plates. Histidine-independent mutant colonies arising on these plates were counted following incubation for 2 days at 37°C.

The second assay, conducted with the same lot of androstenedione that was tested in the 2-year studies, used a slightly modified protocol (activation only with Aroclor 1254-induced male Sprague Dawley rat liver S9) and employed Escherichia coli strain WP2 uvrA/pKM101 as a bacterial tester strain in addition to S. typhimurium strains TA100 and TA98. Androstenedione was sent to the testing laboratory as a coded aliquot, and incubation, plating, and colony counting were carried out as described above.

Each trial consisted of triplicate plates of concurrent positive and negative controls and five doses of androstenedione. Because no toxicity was observed in the first study conducted at SRI International, 10,000 µg/plate was selected as the high dose in all strains. The observation of precipitate at the higher concentrations was not dose limiting. For the second study conducted at SITEK Research Laboratories, a high dose of 3,500 µg/plate was used for strain TA100, 7,500 µg/plate for strain TA98, and 10,000 µg/plate for the E. coli tester strain.

In this assay, a positive response is defined as a reproducible, dose-related increase in histidine-independent (revertant) colonies in any one strain/activation combination. An equivocal response is defined as an increase in revertants that is not dose related, is not reproducible, or is not of sufficient magnitude to support a determination of mutagenicity. A negative response is obtained when no increase in revertant colonies is observed following chemical treatment. There is no minimum percentage or fold increase required for a chemical to be judged positive or weakly positive.

Rat Bone Marrow Micronucleus Test Protocol

Preliminary range-finding studies were performed. Factors affecting dose selection included chemical solubility and toxicity and the extent of cell cycle delay induced by androstenedione exposure. The standard three-exposure protocol is described in detail by Shelby et al. (1993). Male F344/N rats received androstenedione dissolved in corn oil by gavage three times at 24-hour intervals; vehicle control animals received corn oil only. The positive control animals received injections of cyclophosphamide (15 or 25 mg/kg). The animals were killed 24 hours after the third dosing, and blood smears were prepared from bone marrow cells obtained from the femurs. Air-dried smears were fixed and stained; 2,000 polychromatic erythrocytes (PCEs; reticulocytes) were scored for the frequency of micronucleated cells in each of up to five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population in the bone marrow was scored for each dose group as a measure of toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among PCEs was analyzed by a statistical software package that tested for increasing trend over dose groups with a one-tailed Cochran-Armitage trend test, followed by pairwise comparisons between each dosed group and the control group (ILS, 1990). In the presence of excess binomial variation, as detected by a binomial dispersion test, the binomial variance of the Cochran-Armitage test was adjusted upward in proportion to the excess variation. In the micronucleus test, an
individual trial is considered positive if the trend test P value is less than or equal to 0.025 or if the P value for any single dosed group is less than or equal to 0.025 divided by the number of dosed groups. A final call of positive for micronucleus induction is preferably based on reproducibly positive trials (as noted above). Ultimately, the final call is determined by the scientific staff after considering the results of statistical analyses, the reproducibility of any effects observed, and the magnitudes of those effects.

**MOUSE PERIPHERAL BLOOD MICRONUCLEUS TEST PROTOCOL**

A detailed discussion of this assay is presented by MacGregor *et al.* (1990). At the end of the 3-month toxicity study, peripheral blood samples were obtained from male and female mice. Smears were immediately prepared and fixed in absolute methanol. The methanol-fixed slides were stained with acridine orange and coded. Slides were scanned to determine the frequency of micronuclei in 2,000 normochromic erythrocytes (NCEs) in each of five animals per dose group. In addition, the percentage of PCEs among the total erythrocyte population was determined as a measure of bone marrow toxicity.

The results were tabulated as the mean of the pooled results from all animals within a treatment group plus or minus the standard error of the mean. The frequency of micronucleated cells among NCEs was analyzed as described for PCEs in the rat bone marrow test.

**EVALUATION PROTOCOL**

These are the basic guidelines for arriving at an overall assay result for assays performed by the National Toxicology Program. Statistical as well as biological factors are considered. For an individual assay, the statistical procedures for data analysis have been described in the preceding protocols. There have been instances, however, in which multiple aliquots of a chemical were tested in the same assay, and different results were obtained among aliquots and/or among laboratories. Results from more than one aliquot or from more than one laboratory are not simply combined into an overall result. Rather, all the data are critically evaluated, particularly with regard to pertinent protocol variations, in determining the weight of evidence for an overall conclusion of chemical activity in an assay. In addition to multiple aliquots, the *in vitro* assays have another variable that must be considered in arriving at an overall test result. *In vitro* assays are conducted with and without exogenous metabolic activation. Results obtained in the absence of activation are not combined with results obtained in the presence of activation; each testing condition is evaluated separately. The summary table in the Abstract of this Technical Report presents a result that represents a scientific judgement of the overall evidence for activity of the chemical in an assay.

**RESULTS**

Androstenedione was not mutagenic in either of two independent bacterial mutation assays conducted with and without induced rat or hamster liver metabolic activation enzymes (S9) (Table E1). In the first study, concentrations of androstenedione ranged from 100 to 10,000 µg/plate and both 10% and 30% rat and hamster S9 were used with *S. typhimurium* strains TA97, TA98, TA100, and TA1535. In the second study, *Salmonella* strains TA98 and TA100 were tested, along with *Escherichia coli* strain WP2 uvrA/pKM101; 10% induced rat liver S9 was used to provide metabolic activation.

*In vivo*, no significant increases in the frequencies of micronucleated PCEs (reticulocytes) were observed in bone marrow of male F344/N rats administered androstenedione (312.5 or 625 mg/kg) by gavage once daily for 3 days (Table E2). Following 3 months of androstenedione administration (1 to 50 mg/kg) by gavage, no increase in the frequency of micronucleated NCEs was seen in peripheral blood samples from male B6C3F1 mice (Table E3). In female mice, a small increase in the frequency of micronucleated NCEs was observed at the highest dose tested (50 mg/kg); although not significantly elevated above the vehicle control (P=0.0142), this increase resulted in a significant trend (P=0.001), and the test in female mice was judged to be equivocal (Table E3). No significant changes in the percentages of PCEs among total erythrocytes were seen in either the rats or mice, suggesting no androstenedione-associated toxicity in the bone marrow.
TABLE E1
Mutagenicity of Androstenedione in *Salmonella typhimurium*\(^a\)

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (µg/plate)</th>
<th>–S9 Revertants/Plate(^b)</th>
<th>+ hamster S9</th>
<th>+ rat S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>TA100</td>
<td>0</td>
<td>114 ± 12</td>
<td>108 ± 9</td>
<td>118 ± 7</td>
</tr>
<tr>
<td>100</td>
<td>109 ± 4</td>
<td>120 ± 5</td>
<td>109 ± 11</td>
<td>138 ± 1</td>
</tr>
<tr>
<td>333</td>
<td>115 ± 8</td>
<td>105 ± 10</td>
<td>141 ± 11</td>
<td>129 ± 5</td>
</tr>
<tr>
<td>1,000</td>
<td>126 ± 8</td>
<td>105 ± 8</td>
<td>98 ± 12</td>
<td>126 ± 6</td>
</tr>
<tr>
<td>3,333</td>
<td>115 ± 6(^c)</td>
<td>120 ± 4(^c)</td>
<td>113 ± 4(^c)</td>
<td>108 ± 4(^c)</td>
</tr>
<tr>
<td>10,000</td>
<td>119 ± 1(^c)</td>
<td>113 ± 5(^c)</td>
<td>113 ± 1(^c)</td>
<td>114 ± 3(^c)</td>
</tr>
<tr>
<td></td>
<td>Trial summary</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control</td>
<td>925 ± 30</td>
<td>890 ± 12</td>
<td>643 ± 28</td>
<td>703 ± 6</td>
</tr>
</tbody>
</table>

TA1535

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (µg/plate)</th>
<th>–S9 Revertants/Plate(^b)</th>
<th>+ hamster S9</th>
<th>+ rat S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>TA1535</td>
<td>0</td>
<td>22 ± 3</td>
<td>9 ± 3</td>
<td>7 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>17 ± 1</td>
<td>10 ± 0</td>
<td>8 ± 1</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>333</td>
<td>18 ± 1</td>
<td>10 ± 3</td>
<td>11 ± 1</td>
<td>14 ± 1</td>
</tr>
<tr>
<td>1,000</td>
<td>21 ± 6</td>
<td>9 ± 2</td>
<td>9 ± 2</td>
<td>11 ± 0</td>
</tr>
<tr>
<td>3,333</td>
<td>19 ± 5(^c)</td>
<td>6 ± 1(^c)</td>
<td>7 ± 1(^c)</td>
<td>11 ± 1(^c)</td>
</tr>
<tr>
<td>10,000</td>
<td>16 ± 5(^c)</td>
<td>7 ± 2(^c)</td>
<td>8 ± 2(^c)</td>
<td>10 ± 3(^c)</td>
</tr>
<tr>
<td></td>
<td>Trial summary</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control</td>
<td>917 ± 12</td>
<td>909 ± 33</td>
<td>113 ± 9</td>
<td>137 ± 5</td>
</tr>
</tbody>
</table>

TA97

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (µg/plate)</th>
<th>–S9 Revertants/Plate(^b)</th>
<th>+ hamster S9</th>
<th>+ rat S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>TA97</td>
<td>0</td>
<td>133 ± 15</td>
<td>159 ± 16</td>
<td>160 ± 10</td>
</tr>
<tr>
<td>100</td>
<td>145 ± 13</td>
<td>165 ± 5</td>
<td>132 ± 7</td>
<td>168 ± 8</td>
</tr>
<tr>
<td>333</td>
<td>132 ± 5</td>
<td>160 ± 18</td>
<td>127 ± 3</td>
<td>169 ± 5</td>
</tr>
<tr>
<td>1,000</td>
<td>115 ± 8</td>
<td>146 ± 6</td>
<td>164 ± 6</td>
<td>147 ± 1</td>
</tr>
<tr>
<td>3,333</td>
<td>113 ± 7(^c)</td>
<td>151 ± 12(^c)</td>
<td>144 ± 8(^c)</td>
<td>131 ± 7(^c)</td>
</tr>
<tr>
<td>10,000</td>
<td>112 ± 4(^c)</td>
<td>125 ± 3(^c)</td>
<td>145 ± 1(^c)</td>
<td>91 ± 4(^c)</td>
</tr>
<tr>
<td></td>
<td>Trial summary</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control</td>
<td>667 ± 16</td>
<td>555 ± 20</td>
<td>619 ± 20</td>
<td>710 ± 5</td>
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</tbody>
</table>

TA98

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (µg/plate)</th>
<th>–S9 Revertants/Plate(^b)</th>
<th>+ hamster S9</th>
<th>+ rat S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
<td>10%</td>
<td>30%</td>
</tr>
<tr>
<td>TA98</td>
<td>0</td>
<td>31 ± 1</td>
<td>20 ± 1</td>
<td>24 ± 2</td>
</tr>
<tr>
<td>100</td>
<td>30 ± 3</td>
<td>18 ± 4</td>
<td>27 ± 4</td>
<td>41 ± 2</td>
</tr>
<tr>
<td>333</td>
<td>28 ± 2</td>
<td>21 ± 5</td>
<td>22 ± 2</td>
<td>30 ± 1</td>
</tr>
<tr>
<td>1,000</td>
<td>30 ± 1</td>
<td>20 ± 5</td>
<td>23 ± 2</td>
<td>28 ± 1</td>
</tr>
<tr>
<td>3,333</td>
<td>19 ± 2(^c)</td>
<td>15 ± 1(^c)</td>
<td>19 ± 1(^c)</td>
<td>28 ± 3(^c)</td>
</tr>
<tr>
<td>10,000</td>
<td>26 ± 3(^c)</td>
<td>15 ± 1(^c)</td>
<td>17 ± 2(^c)</td>
<td>31 ± 2(^c)</td>
</tr>
<tr>
<td></td>
<td>Trial summary</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control</td>
<td>439 ± 18</td>
<td>355 ± 20</td>
<td>486 ± 3</td>
<td>446 ± 19</td>
</tr>
</tbody>
</table>
TABLE E1
Mutagenicity of Androstenedione in *Salmonella typhimurium*

<table>
<thead>
<tr>
<th>Strain</th>
<th>Dose (µg/plate)</th>
<th>Revertants/Plate</th>
<th>–S9</th>
<th>+ 10% rat S9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Trial 1</td>
<td>Trial 2</td>
</tr>
<tr>
<td><strong>TA100</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td>103 ± 7</td>
<td>64 ± 3</td>
</tr>
<tr>
<td>100</td>
<td></td>
<td></td>
<td>97 ± 3</td>
<td>72 ± 3</td>
</tr>
<tr>
<td>500</td>
<td></td>
<td></td>
<td>77 ± 6</td>
<td>45 ± 0</td>
</tr>
<tr>
<td>1,500</td>
<td></td>
<td></td>
<td>64 ± 3</td>
<td>15 ± 3</td>
</tr>
<tr>
<td>2,500</td>
<td></td>
<td></td>
<td>7 ± 26c</td>
<td>15 ± 2</td>
</tr>
<tr>
<td>3,500</td>
<td></td>
<td></td>
<td>6 ± 1c</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>Trial summary</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
<td>Negative</td>
</tr>
<tr>
<td>Positive control</td>
<td>844 ± 95</td>
<td>337 ± 36</td>
<td>383 ± 10</td>
<td>1,086 ± 2</td>
</tr>
</tbody>
</table>

| **TA98** |                  |                  |       |       |       |       |
| 0       |                  |                  | 24 ± 1 | 18 ± 2 | 34 ± 2 | 28 ± 6  |
| 100     |                  |                  | 32 ± 2 | 25 ± 1 | 48 ± 6 |         |
| 500     |                  |                  | 42 ± 5 | 22 ± 2 | 43 ± 4 | 26 ± 1  |
| 1,500   |                  |                  | 36 ± 0 | 12 ± 2 | 26 ± 2 | 22 ± 3  |
| 2,500   |                  |                  | 5 ± 1c | 7 ± 1  | 32 ± 2 | 19 ± 1  |
| 3,500   |                  |                  | 3 ± 1c | 3 ± 1  | 28 ± 3 |         |
| 5,000   |                  |                  | 3 ± 1c |         |         |         |
| 7,500   |                  |                  | 19 ± 1 |         |         |         |
| Trial summary | Negative | Negative | Negative | Negative |
| Positive control | 441 ± 43 | 603 ± 10 | 787 ± 4 | 379 ± 17 |

| **Escherichia coli WP2 uvrA/pKM101** |                  |                  |       |       |       |       |
| 0       |                  |                  | 124 ± 2 | 188 ± 4 | 175 ± 9 | 225 ± 15 |
| 1,000   |                  |                  | 116 ± 4 | 148 ± 3 | 148 ± 13 | 171 ± 6  |
| 2,500   |                  |                  | 120 ± 8 | 132 ± 5 | 150 ± 7 | 168 ± 8  |
| 5,000   |                  |                  | 132 ± 2 | 137 ± 4 | 140 ± 2 | 158 ± 3  |
| 7,500   |                  |                  | 158 ± 2 | 207 ± 2 | 97 ± 1  | 189 ± 8  |
| 10,000  |                  |                  | 168 ± 12 | 164 ± 7 | 96 ± 4  | 186 ± 3  |
| Trial summary | Negative | Negative | Negative | Negative |
| Positive control | 1,572 ± 82 | 1,810 ± 66 | 909 ± 57 | 1,298 ± 51 |

---

a 0 µ/plate was the solvent control. The detailed protocol for the study performed at SRI International is presented by Zeiger et al. (1992).
b Revertants are presented as mean ± standard error from three plates.
c Precipitate on plate.
d The positive controls in the absence of metabolic activation were sodium azide (TA100 and TA1535), 9- aminoacridine (TA97), 4-nitro-α-phenylenediamine (TA98), and methyl methanesulfonate (WP2 uvrA/pKM101). The positive control for metabolic activation with all strains was 2-aminoanthracene.
### Table E2
Induction of Micronuclei in Bone Marrow Polychromatic Erythrocytes of Male Rats Treated with Androstenedione by Gavage

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Number of Male Rats with Erythrocytes Scored</th>
<th>Micronucleated PCEs/1,000 PCEs</th>
<th>Pairwise P Value</th>
<th>Pairwise PCE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corn oil</td>
<td>0</td>
<td>3</td>
<td>0.33 ± 0.17</td>
<td></td>
<td>5.200 ± 0.15</td>
</tr>
<tr>
<td>Androstenedione</td>
<td>312.5</td>
<td>5</td>
<td>0.40 ± 0.10</td>
<td>0.4165</td>
<td>5.120 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>625</td>
<td>5</td>
<td>0.50 ± 0.39</td>
<td>0.3128</td>
<td>4.420 ± 0.60</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>P=0.304e</td>
<td></td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>15</td>
<td>5</td>
<td>24.00 ± 2.47</td>
<td>0.0000</td>
<td>1.180 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>4</td>
<td>19.17 ± 2.37</td>
<td>0.0000</td>
<td>0.700 ± 0.08</td>
</tr>
</tbody>
</table>

---

**Notes:**
- Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al.* (1993).
- PCE=polychromatic erythrocyte
- Mean ± standard error
- Pairwise comparison with the vehicle control; dosed group values are significant at P ≤ 0.013; positive control values are significant at P ≤ 0.05 (ILS, 1990)
- Vehicle control
- Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P ≤ 0.025 (ILS, 1990).
- Positive control
TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Gavage Administration of Androstenedione for 3 Monthsa

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dose (mg/kg)</th>
<th>Number of Mice with Erythrocytes Scored</th>
<th>Micronucleated NCEs/1,000 NCEs b</th>
<th>P Value c</th>
<th>PCEs b (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Male</strong></td>
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<tr>
<td>Methylcellulose d</td>
<td>0</td>
<td>5</td>
<td>2.60 ± 0.51</td>
<td>2.860 ± 0.19</td>
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<td>Androstenedione</td>
<td>1</td>
<td>5</td>
<td>2.90 ± 0.62</td>
<td>0.3427</td>
<td>2.500 ± 0.37</td>
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<tr>
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<td>5</td>
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<td></td>
<td>50</td>
<td>5</td>
<td>2.10 ± 0.10</td>
<td>0.7674</td>
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<td>P=0.880e</td>
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<td>5</td>
<td>1.60 ± 0.43</td>
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<tr>
<td>Androstenedione</td>
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<td>0.6426</td>
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<tr>
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<td>3.260 ± 0.27</td>
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<td></td>
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<td>3.10 ± 0.40</td>
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a Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor et al. (1990).

b PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.

c Pairwise comparison with the vehicle control group; significant at P<=0.005 (ILS, 1990)

d Vehicle control

e Significance of micronucleated NCEs/1,000 NCEs tested by the one-tailed trend test, significant at P<=0.025 (ILS, 1990)
APPENDIX F
CLINICAL PATHOLOGY RESULTS

Table F1  Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione ........................................... 148
Table F2  Hematology Data for Mice in the 3-Month Gavage Study of Androstendione ....................................................... 154
**Table F1**
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
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<tbody>
<tr>
<td>Male</td>
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</table>

**Hematology**

<table>
<thead>
<tr>
<th>Time</th>
<th>Vehicle</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (auto) (%)</td>
<td>43.9 ± 0.9</td>
<td>43.7 ± 0.7</td>
<td>43.7 ± 0.7</td>
<td>42.8 ± 0.9</td>
<td>44.4 ± 0.8</td>
<td>43.6 ± 0.6</td>
</tr>
<tr>
<td>Hematocrit (spun) (%)</td>
<td>42.6 ± 0.7</td>
<td>43.1 ± 0.7</td>
<td>42.3 ± 1.0</td>
<td>43.8 ± 0.9</td>
<td>42.6 ± 0.7</td>
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</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>14.3 ± 0.3</td>
<td>14.2 ± 0.2</td>
<td>14.0 ± 0.3</td>
<td>13.8 ± 0.3</td>
<td>14.3 ± 0.2</td>
<td>14.1 ± 0.2</td>
</tr>
<tr>
<td>Erythrocytes (10^6/µL)</td>
<td>7.65 ± 0.17</td>
<td>7.61 ± 0.13</td>
<td>7.53 ± 0.12</td>
<td>7.35 ± 0.14</td>
<td>7.62 ± 0.13</td>
<td>7.51 ± 0.10</td>
</tr>
<tr>
<td>Mean cell volume (fL)</td>
<td>57.5 ± 0.3</td>
<td>57.4 ± 0.3</td>
<td>58.0 ± 0.4</td>
<td>58.2 ± 0.3</td>
<td>58.3 ± 0.4</td>
<td>58.1 ± 0.3</td>
</tr>
<tr>
<td>Mean cell hemoglobin (pg)</td>
<td>32.5 ± 0.2</td>
<td>32.4 ± 0.1</td>
<td>32.1 ± 0.2</td>
<td>32.3 ± 0.1</td>
<td>32.3 ± 0.3</td>
<td>32.4 ± 0.2</td>
</tr>
<tr>
<td>Leukocytes (10^3/µL)</td>
<td>9.78 ± 0.30</td>
<td>9.07 ± 0.31</td>
<td>8.55 ± 0.24*</td>
<td>8.61 ± 0.24*</td>
<td>8.72 ± 0.22*</td>
<td>8.12 ± 0.33**</td>
</tr>
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</table>

*Statistically significant compared to control group at p < 0.05.
## TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Hematology</th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<tbody>
<tr>
<td>Male (continued)</td>
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<td><strong>Hematology (continued)</strong></td>
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<td>10</td>
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<tr>
<td>Day 24</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Week 14</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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<tr>
<td><strong>Segmented neutrophils (10^3/µL)</strong></td>
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<td>Day 4</td>
<td>1.00 ± 0.04</td>
<td>1.03 ± 0.05</td>
<td>0.90 ± 0.03</td>
<td>0.96 ± 0.07</td>
<td>0.93 ± 0.03</td>
<td>0.86 ± 0.04*</td>
</tr>
<tr>
<td>Day 24</td>
<td>1.17 ± 0.07</td>
<td>0.92 ± 0.05*</td>
<td>1.23 ± 0.06</td>
<td>1.17 ± 0.06</td>
<td>1.07 ± 0.05</td>
<td>1.01 ± 0.06</td>
</tr>
<tr>
<td>Week 14</td>
<td>1.41 ± 0.03</td>
<td>1.40 ± 0.13</td>
<td>1.36 ± 0.05</td>
<td>1.43 ± 0.05</td>
<td>1.30 ± 0.04</td>
<td>1.21 ± 0.04*</td>
</tr>
<tr>
<td><strong>Lymphocytes (10^3/µL)</strong></td>
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<td></td>
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</tr>
<tr>
<td>Day 4</td>
<td>8.42 ± 0.30</td>
<td>7.72 ± 0.28</td>
<td>7.35 ± 0.22*</td>
<td>7.35 ± 0.28*</td>
<td>7.50 ± 0.23*</td>
<td>7.01 ± 0.30**</td>
</tr>
<tr>
<td>Day 24</td>
<td>9.54 ± 0.42</td>
<td>8.50 ± 0.46</td>
<td>8.33 ± 0.22</td>
<td>8.10 ± 0.39</td>
<td>7.82 ± 0.38*</td>
<td>9.34 ± 0.33</td>
</tr>
<tr>
<td>Week 14</td>
<td>9.04 ± 0.35</td>
<td>8.69 ± 0.30</td>
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<td>8.73 ± 0.29</td>
<td>8.35 ± 0.27</td>
<td>8.73 ± 0.32</td>
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<tr>
<td><strong>Monocytes (10^3/µL)</strong></td>
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<tr>
<td>Day 4</td>
<td>0.16 ± 0.01</td>
<td>0.16 ± 0.01</td>
<td>0.14 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.13 ± 0.01**</td>
</tr>
<tr>
<td>Day 24</td>
<td>0.19 ± 0.01</td>
<td>0.13 ± 0.01**</td>
<td>0.14 ± 0.01*</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.01*</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Week 14</td>
<td>0.17 ± 0.02</td>
<td>0.18 ± 0.02</td>
<td>0.15 ± 0.02</td>
<td>0.17 ± 0.02</td>
<td>0.14 ± 0.01</td>
<td>0.15 ± 0.02</td>
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<td><strong>Basophils (10^3/µL)</strong></td>
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<tr>
<td>Day 4</td>
<td>0.065 ± 0.007</td>
<td>0.043 ± 0.004**</td>
<td>0.037 ± 0.003**</td>
<td>0.043 ± 0.007**</td>
<td>0.041 ± 0.003**</td>
<td>0.031 ± 0.003**</td>
</tr>
<tr>
<td>Day 24</td>
<td>0.051 ± 0.005</td>
<td>0.043 ± 0.006</td>
<td>0.034 ± 0.003*</td>
<td>0.041 ± 0.003</td>
<td>0.041 ± 0.005</td>
<td>0.043 ± 0.006</td>
</tr>
<tr>
<td>Week 14</td>
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<td>0.051 ± 0.007</td>
<td>0.041 ± 0.006</td>
<td>0.044 ± 0.005</td>
<td>0.046 ± 0.009</td>
<td>0.038 ± 0.007</td>
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<tr>
<td><strong>Eosinophils (10^3/µL)</strong></td>
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<tr>
<td>Day 4</td>
<td>0.03 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.03 ± 0.00</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>Day 24</td>
<td>0.03 ± 0.00</td>
<td>0.04 ± 0.00</td>
<td>0.04 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.03 ± 0.00</td>
<td>0.04 ± 0.00</td>
</tr>
<tr>
<td>Week 14</td>
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<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.00</td>
<td>0.08 ± 0.01</td>
<td>0.06 ± 0.01*</td>
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<tr>
<td><strong>Large unstained cells (10^3/µL)</strong></td>
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<td>0.10 ± 0.01</td>
<td>0.10 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.08 ± 0.01</td>
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<td>0.15 ± 0.02</td>
<td>0.09 ± 0.01*</td>
<td>0.11 ± 0.01</td>
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<td>0.10 ± 0.02</td>
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<td>Week 14</td>
<td>0.04 ± 0.01</td>
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<td>0.05 ± 0.00</td>
<td>0.04 ± 0.00</td>
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### Clinical Chemistry

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<td><strong>Urea nitrogen (mg/dL)</strong></td>
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<td>Day 4</td>
<td>13.2 ± 0.4</td>
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<td>13.5 ± 0.4</td>
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<td>13.1 ± 0.4</td>
<td>13.3 ± 0.4</td>
</tr>
<tr>
<td>Day 24</td>
<td>17.0 ± 0.7</td>
<td>17.3 ± 0.5</td>
<td>13.4 ± 0.3**</td>
<td>15.0 ± 0.4</td>
<td>18.9 ± 0.9</td>
<td>16.7 ± 0.6</td>
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<tr>
<td>Week 14</td>
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<td>17.1 ± 0.5</td>
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<td><strong>Creatinine (mg/dL)</strong></td>
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<td>Day 4</td>
<td>0.49 ± 0.01</td>
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<td>0.51 ± 0.01</td>
<td>0.48 ± 0.01</td>
<td>0.49 ± 0.01</td>
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</tr>
<tr>
<td>Day 24</td>
<td>0.54 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>0.56 ± 0.02</td>
<td>0.57 ± 0.02</td>
<td>0.53 ± 0.02</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>Week 14</td>
<td>0.63 ± 0.02</td>
<td>0.64 ± 0.02</td>
<td>0.62 ± 0.01</td>
<td>0.65 ± 0.02</td>
<td>0.66 ± 0.03</td>
<td>0.63 ± 0.02</td>
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<td><strong>Total protein (g/dL)</strong></td>
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<td>5.6 ± 0.1</td>
<td>5.4 ± 0.1</td>
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<td>5.6 ± 0.1</td>
</tr>
<tr>
<td>Day 24</td>
<td>6.0 ± 0.1</td>
<td>6.4 ± 0.2*</td>
<td>5.8 ± 0.1</td>
<td>6.0 ± 0.1</td>
<td>6.1 ± 0.1</td>
<td>6.4 ± 0.2</td>
</tr>
<tr>
<td>Week 14</td>
<td>6.8 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>6.7 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.8 ± 0.1</td>
<td>6.7 ± 0.1</td>
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<tr>
<td><strong>Albumin (g/dL)</strong></td>
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</tr>
<tr>
<td>Day 4</td>
<td>4.0 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.1</td>
<td>4.0 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>Day 24</td>
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<td>4.5 ± 0.1</td>
<td>4.1 ± 0.0</td>
<td>4.3 ± 0.1</td>
<td>4.4 ± 0.0</td>
<td>4.6 ± 0.1</td>
</tr>
<tr>
<td>Week 14</td>
<td>4.5 ± 0.0</td>
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<td>4.5 ± 0.0</td>
<td>4.5 ± 0.0</td>
<td>4.5 ± 0.0</td>
<td>4.4 ± 0.0</td>
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</table>
### TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Male</strong> (continued)</td>
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<tr>
<td><strong>Clinical Chemistry (continued)</strong></td>
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<tr>
<td>Alanine aminotransferase (IU/L)</td>
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<tr>
<td>Day 4</td>
<td>58 ± 2</td>
<td>62 ± 1</td>
<td>59 ± 2</td>
<td>61 ± 2</td>
<td>59 ± 2</td>
<td>56 ± 1</td>
</tr>
<tr>
<td>Day 24</td>
<td>56 ± 2</td>
<td>46 ± 3</td>
<td>50 ± 1</td>
<td>57 ± 3</td>
<td>64 ± 3</td>
<td>43 ± 3</td>
</tr>
<tr>
<td>Week 14</td>
<td>74 ± 5</td>
<td>76 ± 9</td>
<td>63 ± 3</td>
<td>64 ± 5</td>
<td>78 ± 5</td>
<td>54 ± 2**</td>
</tr>
<tr>
<td>Alkaline phosphatase (IU/L)</td>
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<td>Day 4</td>
<td>633 ± 14</td>
<td>671 ± 20</td>
<td>669 ± 19</td>
<td>653 ± 14</td>
<td>637 ± 17</td>
<td>660 ± 10</td>
</tr>
<tr>
<td>Day 24</td>
<td>386 ± 26</td>
<td>409 ± 58</td>
<td>430 ± 34</td>
<td>394 ± 41</td>
<td>389 ± 22</td>
<td>373 ± 39</td>
</tr>
<tr>
<td>Week 14</td>
<td>188 ± 10</td>
<td>179 ± 13</td>
<td>177 ± 14</td>
<td>178 ± 14</td>
<td>178 ± 9</td>
<td>160 ± 15</td>
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<tr>
<td>Creatine kinase (IU/L)</td>
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**TABLE F1**
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

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<th>Vehicle Control</th>
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<td>36 ± 1***</td>
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<td>65 ± 8</td>
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<td>527 ± 9</td>
<td>489 ± 17</td>
<td>449 ± 15**</td>
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<td>333 ± 6</td>
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<td>166 ± 5</td>
<td>181 ± 7*</td>
<td>195 ± 3**</td>
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<td>Creatine kinase (IU/L)</td>
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<td>296 ± 18</td>
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<td>254 ± 16</td>
<td>232 ± 22</td>
<td>300 ± 32</td>
<td>301 ± 27</td>
<td>261 ± 23</td>
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### Table F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

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<th>Vehicle Control</th>
<th>1 mg/kg</th>
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<th>10 mg/kg</th>
<th>20 mg/kg</th>
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<td>Sorbitol dehydrogenase (IU/L)</td>
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<td>19 ± 3</td>
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<td>19 ± 2</td>
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<td>19 ± 2</td>
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<td>Bile salts (µmol/L)</td>
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<td>32.9 ± 2.0*</td>
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<td>30.6 ± 1.6</td>
<td>30.6 ± 3.0</td>
<td>34.9 ± 1.8**</td>
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<td>21.4 ± 1.7</td>
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<td>20.8 ± 2.1</td>
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* Significantly different (P<0.05) from the control group by Dunn’s or Shirley’s test
** P<0.01

* Mean ± standard error. Statistical tests were performed on unrounded data.
* n = 9
* n = 10
<table>
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<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
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<td>Hematocrit (auto) (%)</td>
<td>48.0 ± 0.6</td>
<td>47.8 ± 0.6</td>
<td>47.5 ± 1.0</td>
<td>47.2 ± 0.5</td>
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<td>47.7 ± 0.4</td>
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<td>Hematocrit (spun) (%)</td>
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<td>48.2 ± 0.8</td>
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<td>Hemoglobin (g/dL)</td>
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<td>15.9 ± 0.2</td>
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<td>Erythrocytes (10^6/L)</td>
<td>10.62 ± 0.12</td>
<td>10.50 ± 0.14</td>
<td>10.53 ± 0.24</td>
<td>10.47 ± 0.13</td>
<td>10.55 ± 0.10</td>
<td>10.46 ± 0.11</td>
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<td>Reticulocytes (10^5/µL)</td>
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<td>2.58 ± 0.10</td>
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<td>2.61 ± 0.08</td>
<td>2.55 ± 0.06</td>
<td>2.58 ± 0.04</td>
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<td>Neutrophils (per 100 erythrocytes)</td>
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<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
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<td>Mean cell volume (fL)</td>
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<td>45.2 ± 0.2</td>
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<td>33.3 ± 0.1</td>
<td>33.5 ± 0.1</td>
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<td>Platelets (10^9/µL)</td>
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<td>931.5 ± 28.7</td>
<td>974.8 ± 56.9</td>
<td>1,009.4 ± 55.1</td>
<td>980.5 ± 51.9</td>
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<td>Leukocytes (10^9/µL)</td>
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<td>4.17 ± 0.61</td>
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<td>0.016 ± 0.003</td>
<td>0.011 ± 0.002</td>
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<td>Large unstained cells (10^3/µL)</td>
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<td>Hematocrit (auto) (%)</td>
<td>46.4 ± 0.5</td>
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<td>Hematocrit (spun) (%)</td>
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<td>47.7 ± 0.7</td>
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<td>Hemoglobin (g/dL)</td>
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<td>Erythrocytes (10^6/L)</td>
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<td>10.31 ± 0.10</td>
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<td>Reticulocytes (10^5/L)</td>
<td>3.09 ± 0.10</td>
<td>2.73 ± 0.16</td>
<td>2.53 ± 0.17*</td>
<td>2.77 ± 0.18</td>
<td>2.60 ± 0.19*</td>
<td>3.05 ± 0.16</td>
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<td>Neutrophils (per 100 erythrocytes)</td>
<td>0.0 ± 0.0</td>
<td>0.2 ± 0.1</td>
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<td>Mean cell volume (fL)</td>
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<td>45.2 ± 0.3</td>
<td>45.1 ± 0.3</td>
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<td>Mean cell hemoglobin concentration (g/dL)</td>
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<td>33.9 ± 0.2</td>
<td>33.7 ± 0.2</td>
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<td>Platelets (10^9/µL)</td>
<td>749.3 ± 48.2</td>
<td>740.8 ± 51.3</td>
<td>773.7 ± 43.7</td>
<td>732.1 ± 69.4</td>
<td>690.0 ± 53.7</td>
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<td>Leukocytes (10^9/µL)</td>
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<td>4.63 ± 0.31</td>
<td>5.02 ± 0.24</td>
<td>4.62 ± 0.39</td>
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<td>Segmented neutrophils (10^9/µL)</td>
<td>0.58 ± 0.07</td>
<td>0.57 ± 0.07</td>
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<td>0.47 ± 0.05</td>
<td>0.49 ± 0.05</td>
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<td>4.29 ± 0.20</td>
<td>3.83 ± 0.32</td>
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<td>0.05 ± 0.01</td>
<td>0.07 ± 0.01</td>
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<tr>
<td>Basophils (10^9/µL)</td>
<td>0.013 ± 0.002</td>
<td>0.012 ± 0.001</td>
<td>0.013 ± 0.002</td>
<td>0.013 ± 0.002</td>
<td>0.016 ± 0.003</td>
<td>0.015 ± 0.002</td>
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<tr>
<td>Eosinophils (10^9/µL)</td>
<td>0.14 ± 0.01</td>
<td>0.13 ± 0.02</td>
<td>0.16 ± 0.04</td>
<td>0.14 ± 0.02</td>
<td>0.13 ± 0.01</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>Large unstained cells (10^3/µL)</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.01 ± 0.00</td>
<td>0.02 ± 0.00</td>
<td>0.02 ± 0.00</td>
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</tr>
</tbody>
</table>

* Significantly different (P < 0.05) from the control group by Dunn’s test

Mean ± standard error. Statistical tests were performed on unrounded data.
APPENDIX G
HEPATIC BIOMARKERS

TABLE G1  Peroxisome and Cell Proliferation Indexes for Rats in the 2-Week Gavage Study of Androstenedione .......................... 156

TABLE G2  Peroxisome and Cell Proliferation Indexes for Mice in the 2-Week Gavage Study of Androstenedione .......................... 157
### Table G1

**Peroxisome and Cell Proliferation Indexes for Rats in the 2-Week Gavage Study of Androstenedione**

<table>
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<tr>
<th></th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
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<tr>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acyl CoA oxidase</td>
<td>(nmol DCF/minute per mg protein)</td>
<td>1.88 ± 0.06</td>
<td>1.77 ± 0.07</td>
<td>1.84 ± 0.07</td>
<td>1.75 ± 0.07</td>
<td>1.87 ± 0.07</td>
</tr>
<tr>
<td>Cyclin-dependent kinase</td>
<td>(fmol/mg protein)</td>
<td>72.36 ± 9.50</td>
<td>75.75 ± 16.75</td>
<td>73.19 ± 3.46</td>
<td>61.95 ± 4.59</td>
<td>78.88 ± 11.75</td>
</tr>
<tr>
<td>Proliferating cell nuclear antigen</td>
<td>(fmol/mg protein)</td>
<td>27.01 ± 3.10</td>
<td>23.62 ± 1.12</td>
<td>23.55 ± 2.03</td>
<td>24.58 ± 2.52</td>
<td>23.61 ± 0.85</td>
</tr>
<tr>
<td><strong>Female</strong></td>
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<tr>
<td>Acyl CoA oxidase</td>
<td>(nmol DCF/minute per mg protein)</td>
<td>2.01 ± 0.18</td>
<td>1.91 ± 0.05</td>
<td>1.64 ± 0.10</td>
<td>1.69 ± 0.19</td>
<td>1.71 ± 0.14</td>
</tr>
<tr>
<td>Cyclin-dependent kinase</td>
<td>(fmol/mg protein)</td>
<td>62.09 ± 12.97</td>
<td>70.30 ± 13.61</td>
<td>74.59 ± 9.87</td>
<td>68.18 ± 4.55</td>
<td>54.51 ± 3.66^c</td>
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<tr>
<td>Proliferating cell nuclear antigen</td>
<td>(fmol/mg protein)</td>
<td>21.34 ± 0.94</td>
<td>20.42 ± 1.60</td>
<td>21.47 ± 3.80</td>
<td>24.33 ± 4.84</td>
<td>25.68 ± 3.04</td>
</tr>
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</table>

^a Data are presented as mean ± standard error. CoA = coenzyme A; DCF = dichlorofluorescein diacetate.

^b n = 4

^c n = 3
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<th></th>
<th>Vehicle Control</th>
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<td>Acyl CoA oxidase</td>
<td>(nmol DCF/minute per mg protein)</td>
<td>1.37 ± 0.14</td>
<td>1.54 ± 0.16</td>
<td>1.13 ± 0.06</td>
<td>1.36 ± 0.09</td>
<td>1.30 ± 0.13b</td>
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<tr>
<td>Cyclin-dependent kinase</td>
<td>(fmol/mg protein)</td>
<td>86.38 ± 11.06c</td>
<td>80.56 ± 5.87</td>
<td>79.95 ± 23.47c</td>
<td>63.91 ± 8.92</td>
<td>72.99 ± 3.98</td>
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<tr>
<td>Proliferating cell nuclear antigen</td>
<td>(fmol/mg protein)</td>
<td>13.77 ± 1.54</td>
<td>16.38 ± 2.71</td>
<td>12.44 ± 0.70</td>
<td>13.01 ± 1.98</td>
<td>15.18 ± 2.42</td>
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<tr>
<td>Acyl CoA oxidase</td>
<td>(nmol DCF/minute per mg protein)</td>
<td>1.34 ± 0.14</td>
<td>1.28 ± 0.08b</td>
<td>1.21 ± 0.09</td>
<td>1.08 ± 0.14</td>
<td>1.19 ± 0.05</td>
</tr>
<tr>
<td>Cyclin-dependent kinase</td>
<td>(fmol/mg protein)</td>
<td>77.74 ± 17.34</td>
<td>86.15 ± 16.36</td>
<td>128.92 ± 3.19d</td>
<td>98.45 ± 10.42c</td>
<td>149.06 ± 52.27</td>
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<tr>
<td>Proliferating cell nuclear antigen</td>
<td>(fmol/mg protein)</td>
<td>19.83 ± 3.17</td>
<td>20.57 ± 2.35</td>
<td>18.15 ± 2.09</td>
<td>16.17 ± 2.55</td>
<td>17.31 ± 2.80</td>
</tr>
</tbody>
</table>

a Data are presented as mean ± standard error. CoA = coenzyme A; DCF = dichlorofluorescein diacetate.
b n = 5
c n = 4
d n = 2
## APPENDIX H

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

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
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<tbody>
<tr>
<td>Table H1</td>
<td>Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of Androstenedione</td>
<td>160</td>
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<tr>
<td>Table H2</td>
<td>Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Androstenedione</td>
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<td>Table H3</td>
<td>Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Androstenedione</td>
<td>162</td>
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<td>Table H4</td>
<td>Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Androstenedione</td>
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</tr>
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<td>Male</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Necropsy body wt</td>
<td>184 ± 9</td>
<td>181 ± 7</td>
<td>185 ± 7</td>
<td>183 ± 3</td>
<td>176 ± 5</td>
<td>182 ± 3</td>
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</tr>
<tr>
<td>Absolute</td>
<td>0.68 ± 0.04</td>
<td>0.67 ± 0.04</td>
<td>0.68 ± 0.03</td>
<td>0.65 ± 0.01</td>
<td>0.64 ± 0.02</td>
<td>0.66 ± 0.01</td>
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<tr>
<td>Relative</td>
<td>3.723 ± 0.111</td>
<td>3.670 ± 0.060</td>
<td>3.648 ± 0.034</td>
<td>3.572 ± 0.051</td>
<td>3.629 ± 0.084</td>
<td>3.655 ± 0.076</td>
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<td>R. Kidney</td>
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<td>Absolute</td>
<td>0.76 ± 0.06</td>
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<td>0.80 ± 0.03</td>
<td>0.75 ± 0.02</td>
<td>0.76 ± 0.02</td>
<td>0.79 ± 0.02</td>
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<tr>
<td>Relative</td>
<td>4.143 ± 0.123</td>
<td>4.186 ± 0.061</td>
<td>4.333 ± 0.051</td>
<td>4.089 ± 0.137</td>
<td>4.345 ± 0.079</td>
<td>4.368 ± 0.083</td>
</tr>
<tr>
<td>Liver</td>
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<tr>
<td>Absolute</td>
<td>8.97 ± 0.70</td>
<td>8.89 ± 0.38</td>
<td>9.35 ± 0.49</td>
<td>9.12 ± 0.36</td>
<td>8.91 ± 0.53</td>
<td>9.15 ± 0.25</td>
</tr>
<tr>
<td>Relative</td>
<td>48.602 ± 1.551</td>
<td>49.117 ± 0.655</td>
<td>50.519 ± 1.660</td>
<td>49.867 ± 1.403</td>
<td>50.474 ± 1.622</td>
<td>50.354 ± 0.788</td>
</tr>
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<td>Lung</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>1.70 ± 0.12</td>
<td>1.55 ± 0.13</td>
<td>1.48 ± 0.15</td>
<td>1.51 ± 0.15</td>
<td>1.32 ± 0.10</td>
<td>1.51 ± 0.18</td>
</tr>
<tr>
<td>Relative</td>
<td>9.361 ± 0.820</td>
<td>8.607 ± 0.815</td>
<td>8.044 ± 0.753</td>
<td>8.263 ± 0.879</td>
<td>7.461 ± 0.429</td>
<td>8.315 ± 0.987</td>
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<td>R. Testis</td>
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<td></td>
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</tr>
<tr>
<td>Absolute</td>
<td>1.120 ± 0.052</td>
<td>1.119 ± 0.030</td>
<td>1.107 ± 0.027</td>
<td>1.069 ± 0.021</td>
<td>1.076 ± 0.026</td>
<td>1.086 ± 0.023</td>
</tr>
<tr>
<td>Relative</td>
<td>6.109 ± 0.086</td>
<td>6.202 ± 0.137</td>
<td>5.997 ± 0.109</td>
<td>5.851 ± 0.078</td>
<td>6.125 ± 0.113</td>
<td>5.980 ± 0.056</td>
</tr>
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<td>Thymus</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.431 ± 0.028</td>
<td>0.456 ± 0.025</td>
<td>0.435 ± 0.011</td>
<td>0.415 ± 0.010</td>
<td>0.403 ± 0.014</td>
<td>0.427 ± 0.017</td>
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<tr>
<td>Relative</td>
<td>2.362 ± 0.148</td>
<td>2.524 ± 0.107</td>
<td>2.357 ± 0.038</td>
<td>2.272 ± 0.071</td>
<td>2.291 ± 0.038</td>
<td>2.353 ± 0.107</td>
</tr>
<tr>
<td>Female</td>
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<tr>
<td>Necropsy body wt</td>
<td>131 ± 2</td>
<td>127 ± 1</td>
<td>125 ± 2</td>
<td>137 ± 3</td>
<td>132 ± 1</td>
<td>130 ± 2</td>
</tr>
<tr>
<td>Heart</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.55 ± 0.02</td>
<td>0.51 ± 0.03</td>
<td>0.48 ± 0.01</td>
<td>0.53 ± 0.02</td>
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<td>0.56 ± 0.03</td>
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<tr>
<td>Relative</td>
<td>4.167 ± 0.120</td>
<td>4.040 ± 0.204</td>
<td>3.824 ± 0.047</td>
<td>3.855 ± 0.058</td>
<td>3.855 ± 0.069</td>
<td>4.280 ± 0.195</td>
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<tr>
<td>R. Kidney</td>
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<tr>
<td>Absolute</td>
<td>0.59 ± 0.01</td>
<td>0.57 ± 0.01</td>
<td>0.58 ± 0.02</td>
<td>0.60 ± 0.01</td>
<td>0.58 ± 0.01</td>
<td>0.60 ± 0.01</td>
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<tr>
<td>Relative</td>
<td>4.475 ± 0.072</td>
<td>4.445 ± 0.079</td>
<td>4.635 ± 0.121</td>
<td>4.359 ± 0.063</td>
<td>4.436 ± 0.034</td>
<td>4.630 ± 0.091</td>
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<tr>
<td>Absolute</td>
<td>6.17 ± 0.22</td>
<td>5.89 ± 0.26</td>
<td>5.82 ± 0.14</td>
<td>6.49 ± 0.22</td>
<td>6.18 ± 0.10</td>
<td>6.71 ± 0.44</td>
</tr>
<tr>
<td>Relative</td>
<td>46.962 ± 1.622</td>
<td>46.313 ± 1.826</td>
<td>46.741 ± 0.844</td>
<td>47.367 ± 0.603</td>
<td>46.900 ± 0.906</td>
<td>51.633 ± 2.965</td>
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<td>1.12 ± 0.04</td>
<td>1.12 ± 0.07</td>
<td>1.15 ± 0.12</td>
<td>1.37 ± 0.18</td>
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<td>0.98 ± 0.07</td>
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<td>8.524 ± 0.362</td>
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<td>9.240 ± 0.870</td>
<td>9.965 ± 1.135</td>
<td>7.858 ± 0.546</td>
<td>7.572 ± 0.586</td>
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<tr>
<td>Absolute</td>
<td>0.374 ± 0.014</td>
<td>0.348 ± 0.016</td>
<td>0.327 ± 0.019</td>
<td>0.391 ± 0.015</td>
<td>0.361 ± 0.016</td>
<td>0.358 ± 0.012</td>
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<td>Relative</td>
<td>2.849 ± 0.092</td>
<td>2.741 ± 0.108</td>
<td>2.620 ± 0.126</td>
<td>2.862 ± 0.088</td>
<td>2.742 ± 0.128</td>
<td>2.761 ± 0.093</td>
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---

a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
TABLE H2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
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<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
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</tr>
<tr>
<td><strong>Male</strong></td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Last in-life body wt</td>
<td>333 ± 8</td>
<td>340 ± 6</td>
<td>344 ± 4</td>
<td>343 ± 5</td>
<td>343 ± 3</td>
<td>340 ± 5</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Absolute</td>
<td>0.87 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.91 ± 0.03</td>
<td>0.87 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>0.92 ± 0.02</td>
</tr>
<tr>
<td>Relative</td>
<td>2.618 ± 0.021</td>
<td>2.677 ± 0.050</td>
<td>2.652 ± 0.054</td>
<td>2.537 ± 0.036</td>
<td>2.679 ± 0.042</td>
<td>2.697 ± 0.052</td>
</tr>
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<td></td>
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<td></td>
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</tr>
<tr>
<td>Absolute</td>
<td>1.06 ± 0.03</td>
<td>1.05 ± 0.03</td>
<td>1.08 ± 0.02</td>
<td>1.05 ± 0.02</td>
<td>1.08 ± 0.02</td>
<td>1.10 ± 0.02</td>
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<tr>
<td>Relative</td>
<td>3.169 ± 0.043</td>
<td>3.084 ± 0.045</td>
<td>3.140 ± 0.042</td>
<td>3.065 ± 0.038</td>
<td>3.150 ± 0.071</td>
<td>3.227 ± 0.042</td>
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</tr>
<tr>
<td>Absolute</td>
<td>11.08 ± 0.38</td>
<td>11.48 ± 0.36</td>
<td>11.65 ± 0.37</td>
<td>11.78 ± 0.20</td>
<td>11.73 ± 0.36</td>
<td>11.90 ± 0.32</td>
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<tr>
<td>Relative</td>
<td>33.190 ± 0.479</td>
<td>33.712 ± 0.596</td>
<td>33.843 ± 0.847</td>
<td>34.343 ± 0.136</td>
<td>34.201 ± 0.884</td>
<td>34.967 ± 0.601</td>
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</tr>
<tr>
<td>Absolute</td>
<td>1.51 ± 0.08</td>
<td>1.47 ± 0.04</td>
<td>1.48 ± 0.04</td>
<td>1.43 ± 0.03</td>
<td>1.49 ± 0.06</td>
<td>1.47 ± 0.04</td>
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<tr>
<td>Relative</td>
<td>4.509 ± 0.199</td>
<td>4.322 ± 0.106</td>
<td>4.310 ± 0.096</td>
<td>4.172 ± 0.062</td>
<td>4.351 ± 0.179</td>
<td>4.313 ± 0.123</td>
</tr>
<tr>
<td><strong>R. Testis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>1.440 ± 0.024</td>
<td>1.364 ± 0.079</td>
<td>1.450 ± 0.031</td>
<td>1.413 ± 0.022</td>
<td>1.411 ± 0.024</td>
<td>1.386 ± 0.024</td>
</tr>
<tr>
<td>Relative</td>
<td>4.329 ± 0.061</td>
<td>4.023 ± 0.246</td>
<td>4.218 ± 0.073</td>
<td>4.124 ± 0.056</td>
<td>4.122 ± 0.084</td>
<td>4.078 ± 0.043</td>
</tr>
<tr>
<td><strong>Thymus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.276 ± 0.009</td>
<td>0.248 ± 0.007</td>
<td>0.267 ± 0.005</td>
<td>0.279 ± 0.012</td>
<td>0.257 ± 0.009</td>
<td>0.275 ± 0.017</td>
</tr>
<tr>
<td>Relative</td>
<td>0.830 ± 0.023</td>
<td>0.729 ± 0.017*</td>
<td>0.776 ± 0.017</td>
<td>0.811 ± 0.027</td>
<td>0.751 ± 0.024</td>
<td>0.808 ± 0.041</td>
</tr>
<tr>
<td><strong>Female</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Last in-life body wt</td>
<td>189 ± 2</td>
<td>200 ± 4*</td>
<td>198 ± 2*</td>
<td>198 ± 2*</td>
<td>206 ± 4**</td>
<td>199 ± 4**</td>
</tr>
<tr>
<td><strong>Heart</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.60 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.60 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>0.61 ± 0.01</td>
<td>0.59 ± 0.01</td>
</tr>
<tr>
<td>Relative</td>
<td>3.147 ± 0.047</td>
<td>3.023 ± 0.049</td>
<td>3.042 ± 0.041</td>
<td>3.099 ± 0.054</td>
<td>2.949 ± 0.047**</td>
<td>2.941 ± 0.058**</td>
</tr>
<tr>
<td><strong>R. Kidney</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.67 ± 0.02</td>
<td>0.71 ± 0.02</td>
<td>0.70 ± 0.01</td>
<td>0.69 ± 0.01</td>
<td>0.73 ± 0.02*</td>
<td>0.67 ± 0.01</td>
</tr>
<tr>
<td>Relative</td>
<td>3.551 ± 0.073</td>
<td>3.545 ± 0.076</td>
<td>3.546 ± 0.047</td>
<td>3.483 ± 0.074</td>
<td>3.555 ± 0.068</td>
<td>3.382 ± 0.067</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>6.20 ± 0.17</td>
<td>7.05 ± 0.33*</td>
<td>6.57 ± 0.10</td>
<td>6.65 ± 0.12</td>
<td>6.65 ± 0.16</td>
<td>6.34 ± 0.19</td>
</tr>
<tr>
<td>Relative</td>
<td>32.763 ± 0.945</td>
<td>35.221 ± 1.444</td>
<td>33.170 ± 0.448</td>
<td>33.594 ± 0.752</td>
<td>32.294 ± 0.556</td>
<td>31.824 ± 0.645</td>
</tr>
<tr>
<td><strong>Lung</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.97 ± 0.02</td>
<td>1.01 ± 0.02</td>
<td>0.98 ± 0.02</td>
<td>0.97 ± 0.01</td>
<td>0.97 ± 0.03</td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td>Relative</td>
<td>5.122 ± 0.105</td>
<td>5.050 ± 0.090</td>
<td>4.942 ± 0.055</td>
<td>4.908 ± 0.077</td>
<td>4.710 ± 0.128**</td>
<td>4.775 ± 0.101**</td>
</tr>
<tr>
<td><strong>Thymus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.206 ± 0.008</td>
<td>0.220 ± 0.007</td>
<td>0.207 ± 0.007</td>
<td>0.225 ± 0.004</td>
<td>0.245 ± 0.009**</td>
<td>0.230 ± 0.012**</td>
</tr>
<tr>
<td>Relative</td>
<td>1.084 ± 0.034</td>
<td>1.103 ± 0.036</td>
<td>1.042 ± 0.034</td>
<td>1.135 ± 0.025</td>
<td>1.191 ± 0.043</td>
<td>1.159 ± 0.058</td>
</tr>
<tr>
<td><strong>Uterus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.583 ± 0.084</td>
<td>0.581 ± 0.067</td>
<td>0.522 ± 0.053</td>
<td>0.454 ± 0.039</td>
<td>0.429 ± 0.038</td>
<td>0.418 ± 0.050</td>
</tr>
<tr>
<td>Relative</td>
<td>3.078 ± 0.436</td>
<td>2.919 ± 0.341</td>
<td>2.645 ± 0.279</td>
<td>2.295 ± 0.199</td>
<td>2.070 ± 0.158*</td>
<td>2.104 ± 0.252*</td>
</tr>
</tbody>
</table>

* Significantly different (P ≤ 0.05) from the vehicle control group by Williams’ or Dunnett’s test
** P ≤ 0.01 by Williams’ test

Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error)
TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>Necropsy body wt</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>27.9 ± 1.0</td>
<td>25.6 ± 0.5**</td>
<td>25.5 ± 0.3**</td>
<td>24.7 ± 0.3**</td>
<td>24.9 ± 0.4**</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.00</td>
<td>0.13 ± 0.00**</td>
<td>0.12 ± 0.00**</td>
<td>0.12 ± 0.00**</td>
</tr>
<tr>
<td>R. Kidney</td>
<td></td>
<td>5.270 ± 0.320</td>
<td>5.265 ± 0.120</td>
<td>4.998 ± 0.119</td>
<td>5.035 ± 0.180</td>
<td>4.970 ± 0.139</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.581 ± 0.176</td>
<td>9.917 ± 0.161</td>
<td>9.430 ± 0.265</td>
<td>10.369 ± 0.319</td>
<td>9.712 ± 0.202</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>1.50 ± 0.07</td>
<td>1.40 ± 0.04</td>
<td>1.42 ± 0.02</td>
<td>1.37 ± 0.04</td>
<td>1.41 ± 0.01</td>
</tr>
<tr>
<td></td>
<td></td>
<td>53.709 ± 1.387</td>
<td>54.810 ± 1.288</td>
<td>55.716 ± 0.884</td>
<td>55.265 ± 1.505</td>
<td>56.396 ± 1.059</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>0.22 ± 0.02</td>
<td>0.19 ± 0.01*</td>
<td>0.18 ± 0.01*</td>
<td>0.19 ± 0.01</td>
<td>0.19 ± 0.01*</td>
</tr>
<tr>
<td>R. Testis</td>
<td></td>
<td>8.065 ± 0.643</td>
<td>7.243 ± 0.090</td>
<td>7.218 ± 0.342</td>
<td>7.757 ± 0.216</td>
<td>7.464 ± 0.280</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.110 ± 0.003</td>
<td>0.105 ± 0.002</td>
<td>0.106 ± 0.001</td>
<td>0.109 ± 0.001</td>
<td>0.105 ± 0.004</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td>3.991 ± 0.230</td>
<td>4.110 ± 0.073</td>
<td>4.164 ± 0.071</td>
<td>4.422 ± 0.041</td>
<td>4.205 ± 0.161</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.056 ± 0.006</td>
<td>0.053 ± 0.005</td>
<td>0.043 ± 0.008</td>
<td>0.046 ± 0.007</td>
<td>0.050 ± 0.003</td>
</tr>
<tr>
<td>Female</td>
<td></td>
<td>1.980 ± 0.157</td>
<td>2.079 ± 0.190</td>
<td>1.661 ± 0.301</td>
<td>1.884 ± 0.309</td>
<td>2.003 ± 0.158</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Necropsy body wt</td>
<td></td>
<td>21.1 ± 0.1</td>
<td>21.7 ± 0.4</td>
<td>19.9 ± 0.4</td>
<td>21.3 ± 0.3</td>
<td>20.2 ± 0.5</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td>0.12 ± 0.01</td>
<td>0.13 ± 0.01</td>
<td>0.12 ± 0.01</td>
<td>0.12 ± 0.00</td>
<td>0.12 ± 0.01</td>
</tr>
<tr>
<td>R. Kidney</td>
<td></td>
<td>5.857 ± 0.258</td>
<td>5.855 ± 0.166</td>
<td>5.874 ± 0.254</td>
<td>5.737 ± 0.083</td>
<td>5.904 ± 0.266</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7.986 ± 0.159</td>
<td>7.799 ± 0.105</td>
<td>8.162 ± 0.320</td>
<td>8.264 ± 0.119</td>
<td>8.098 ± 0.298</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td>1.10 ± 0.02</td>
<td>1.17 ± 0.03</td>
<td>1.07 ± 0.03</td>
<td>1.19 ± 0.04</td>
<td>1.12 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>51.893 ± 0.623</td>
<td>54.219 ± 1.226</td>
<td>53.424 ± 0.822</td>
<td>55.985 ± 1.203*</td>
<td>55.287 ± 1.397*</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td>0.17 ± 0.01</td>
<td>0.21 ± 0.02</td>
<td>0.19 ± 0.02</td>
<td>0.18 ± 0.01</td>
<td>0.18 ± 0.02</td>
</tr>
<tr>
<td>R. Testis</td>
<td></td>
<td>8.204 ± 0.425</td>
<td>9.753 ± 0.634</td>
<td>9.430 ± 1.081</td>
<td>8.378 ± 0.579</td>
<td>8.655 ± 0.594</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.067 ± 0.003</td>
<td>0.075 ± 0.007</td>
<td>0.072 ± 0.006</td>
<td>0.077 ± 0.003</td>
<td>0.074 ± 0.006</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td>3.161 ± 0.130</td>
<td>3.465 ± 0.308</td>
<td>3.637 ± 0.318</td>
<td>3.628 ± 0.177</td>
<td>3.665 ± 0.242</td>
</tr>
</tbody>
</table>

* Significantly different (P<0.05) from the vehicle control group by Williams’ or Dunnett’s test
** P<0.01
a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
TABLE H4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th>Vehicle Control</th>
<th>1 mg/kg</th>
<th>5 mg/kg</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Last in-life body wt</td>
<td>37.5 ± 0.9</td>
<td>37.9 ± 1.2</td>
<td>38.7 ± 0.9</td>
<td>39.1 ± 1.3</td>
<td>39.3 ± 0.9</td>
</tr>
<tr>
<td>Heart</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.17 ± 0.01</td>
<td>0.17 ± 0.01</td>
<td>0.16 ± 0.00</td>
<td>0.16 ± 0.00</td>
<td>0.16 ± 0.01</td>
</tr>
<tr>
<td>Relative</td>
<td>4.465 ± 0.096</td>
<td>4.558 ± 0.143</td>
<td>4.130 ± 0.153</td>
<td>4.191 ± 0.176</td>
<td>4.172 ± 0.160</td>
</tr>
<tr>
<td>R. Kidney</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.31 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.32 ± 0.01</td>
<td>0.33 ± 0.01</td>
<td>0.33 ± 0.01</td>
</tr>
<tr>
<td>Relative</td>
<td>8.202 ± 0.255</td>
<td>8.368 ± 0.262</td>
<td>8.336 ± 0.216</td>
<td>8.606 ± 0.256</td>
<td>8.490 ± 0.209</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>1.59 ± 0.04</td>
<td>1.56 ± 0.05</td>
<td>1.59 ± 0.04</td>
<td>1.62 ± 0.05</td>
<td>1.64 ± 0.05</td>
</tr>
<tr>
<td>Relative</td>
<td>42.452 ± 0.813</td>
<td>41.320 ± 0.677</td>
<td>41.167 ± 1.045</td>
<td>41.491 ± 0.890</td>
<td>41.786 ± 0.612</td>
</tr>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.01</td>
<td>0.22 ± 0.02</td>
<td>0.23 ± 0.01</td>
<td>0.24 ± 0.01</td>
</tr>
<tr>
<td>Relative</td>
<td>6.247 ± 0.328</td>
<td>6.294 ± 0.241</td>
<td>5.752 ± 0.403</td>
<td>5.988 ± 0.311</td>
<td>6.111 ± 0.349</td>
</tr>
<tr>
<td>R. Testis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.119 ± 0.002</td>
<td>0.122 ± 0.003</td>
<td>0.122 ± 0.003</td>
<td>0.124 ± 0.003</td>
<td>0.124 ± 0.002</td>
</tr>
<tr>
<td>Relative</td>
<td>3.191 ± 0.060</td>
<td>3.230 ± 0.087</td>
<td>3.173 ± 0.080</td>
<td>3.177 ± 0.077</td>
<td>3.168 ± 0.096</td>
</tr>
<tr>
<td>Thymus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absolute</td>
<td>0.035 ± 0.002</td>
<td>0.033 ± 0.002</td>
<td>0.035 ± 0.002</td>
<td>0.034 ± 0.002</td>
<td>0.034 ± 0.001</td>
</tr>
<tr>
<td>Relative</td>
<td>0.944 ± 0.054</td>
<td>0.876 ± 0.028</td>
<td>0.900 ± 0.034</td>
<td>0.860 ± 0.036</td>
<td>0.866 ± 0.036</td>
</tr>
</tbody>
</table>

| Female          |         |         |          |          |         |
| n               | 10      | 10      | 10       | 10       | 9       | 10      |
| Last in-life body wt | 30.9 ± 1.0 | 32.0 ± 1.5 | 31.6 ± 0.9 | 30.9 ± 1.0 | 30.8 ± 0.8 | 29.3 ± 0.7 |
| Heart           |         |         |          |          |         |
| Absolute        | 0.14 ± 0.00 | 0.14 ± 0.01 | 0.13 ± 0.00 | 0.14 ± 0.01 | 0.14 ± 0.00 | 0.14 ± 0.00 |
| Relative        | 4.497 ± 0.173 | 4.489 ± 0.084 | 4.231 ± 0.095 | 4.613 ± 0.130 | 4.641 ± 0.209 | 4.659 ± 0.095 |
| R. Kidney       |         |         |          |          |         |
| Absolute        | 0.19 ± 0.00 | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.01 | 0.20 ± 0.01 |
| Relative        | 6.225 ± 0.220 | 6.433 ± 0.195 | 6.352 ± 0.162 | 6.600 ± 0.145 | 6.598 ± 0.135 | 6.982 ± 0.172** |
| Liver           |         |         |          |          |         |
| Absolute        | 1.32 ± 0.04 | 1.32 ± 0.05 | 1.33 ± 0.05 | 1.32 ± 0.04 | 1.37 ± 0.04 | 1.27 ± 0.04 |
| Relative        | 42.714 ± 0.838 | 41.521 ± 1.551 | 42.253 ± 0.901 | 42.943 ± 0.604 | 44.602 ± 0.500 | 43.392 ± 0.905 |
| Lung            |         |         |          |          |         |
| Absolute        | 0.23 ± 0.02 | 0.23 ± 0.02 | 0.23 ± 0.02 | 0.25 ± 0.02 | 0.24 ± 0.02 | 0.25 ± 0.02 |
| Relative        | 7.434 ± 0.618 | 7.267 ± 0.628 | 7.295 ± 0.569 | 8.076 ± 0.593 | 7.654 ± 0.531 | 8.590 ± 0.609 |
| Thymus          |         |         |          |          |         |
| Absolute        | 0.045 ± 0.002 | 0.040 ± 0.002 | 0.044 ± 0.003 | 0.043 ± 0.002 | 0.042 ± 0.002 | 0.040 ± 0.002 |
| Relative        | 1.468 ± 0.091 | 1.261 ± 0.075 | 1.377 ± 0.079 | 1.395 ± 0.038 | 1.360 ± 0.046 | 1.353 ± 0.043 |
| Uterus          |         |         |          |          |         |
| Absolute        | 0.088 ± 0.005 | 0.111 ± 0.010 | 0.098 ± 0.010 | 0.109 ± 0.013 | 0.096 ± 0.007 | 0.091 ± 0.006 |
| Relative        | 2.846 ± 0.129 | 3.510 ± 0.331 | 3.128 ± 0.331 | 3.592 ± 0.480 | 3.112 ± 0.183 | 3.118 ± 0.217 |

** Significantly different (P < 0.01) from the vehicle control group by Williams’ test

a Organ weights (absolute weights) and body weights are given in grams; organ-weight-to-body-weight ratios (relative weights) are given as mg organ weight/g body weight (mean ± standard error).
APPENDIX I
REPRODUCTIVE TISSUE EVALUATIONS
AND ESTROUS CYCLE CHARACTERIZATION

**TABLE I1**  Summary of Reproductive Tissue Evaluations for Male Rats
in the 3-Month Gavage Study of Androstenedione  ........................................... 166

**TABLE I2**  Estrous Cycle Characterization for Female Rats
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**TABLE I3**  Summary of Reproductive Tissue Evaluations for Male Mice
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**TABLE I4**  Estrous Cycle Characterization for Female Mice
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**Table I1**  
Summary of Reproductive Tissue Evaluations for Male Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Weights (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necropsy body wt</td>
<td>333 ± 8</td>
<td>343 ± 5</td>
<td>343 ± 3</td>
<td>340 ± 5</td>
</tr>
<tr>
<td>L. Cauda epididymis</td>
<td>0.1954 ± 0.0057</td>
<td>0.2082 ± 0.0044</td>
<td>0.2050 ± 0.0034</td>
<td>0.2083 ± 0.0048</td>
</tr>
<tr>
<td>L. Epididymis</td>
<td>0.4562 ± 0.0119</td>
<td>0.4610 ± 0.0075</td>
<td>0.4540 ± 0.0082</td>
<td>0.4303 ± 0.0110</td>
</tr>
<tr>
<td>L. Testis</td>
<td>1.5171 ± 0.0277</td>
<td>1.5272 ± 0.0259</td>
<td>1.5135 ± 0.0175</td>
<td>1.5053 ± 0.0220</td>
</tr>
<tr>
<td><strong>Spermatid measurement</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatid heads (10³/mg testis)</td>
<td>124.86 ± 3.27</td>
<td>127.11 ± 4.83b</td>
<td>131.80 ± 4.53</td>
<td>126.49 ± 3.56</td>
</tr>
<tr>
<td>Spermatid heads (10⁶/testis)</td>
<td>169.38 ± 5.53</td>
<td>172.08 ± 4.07b</td>
<td>178.00 ± 6.85</td>
<td>169.38 ± 5.91</td>
</tr>
<tr>
<td><strong>Epididymal spermatozoal measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm (10⁹/mg cauda epididymis)</td>
<td>575 ± 27</td>
<td>475 ± 28*</td>
<td>463 ± 27*</td>
<td>407 ± 21**</td>
</tr>
<tr>
<td>Sperm (10⁶/cauda epididymis)</td>
<td>112 ± 5</td>
<td>98 ± 5</td>
<td>95 ± 6</td>
<td>85 ± 5**</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>75.7 ± 2.2</td>
<td>76.0 ± 2.1</td>
<td>73.8 ± 2.4</td>
<td>74.7 ± 1.5</td>
</tr>
</tbody>
</table>

* Significantly different (P ≤ 0.05) from the vehicle control group by Shirley’s test  
** (P ≤ 0.01)  
Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett’s test (body and tissue weights) or Dunn’s test (spermatid measurements).

**Table I2**  
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Number weighed at necropsy</strong></td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Necropsy body wt (g)</td>
<td>189 ± 2</td>
<td>198 ± 2</td>
<td>206 ± 3**</td>
<td>199 ± 4</td>
</tr>
<tr>
<td>Proportion of regular cycling females</td>
<td>10/10</td>
<td>10/10</td>
<td>9/10e</td>
<td>7/10d</td>
</tr>
<tr>
<td>Estrous cycle length (days)</td>
<td>4.8 ± 0.2</td>
<td>5.0 ± 0.1</td>
<td>4.9 ± 0.1</td>
<td>5.2 ± 0.2</td>
</tr>
<tr>
<td>Estrous stages (% of cycle)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diestrus</td>
<td>60.0</td>
<td>60.0</td>
<td>60.2</td>
<td>61.1</td>
</tr>
<tr>
<td>Proestrus</td>
<td>10.0</td>
<td>16.7</td>
<td>13.0</td>
<td>7.4</td>
</tr>
<tr>
<td>Estrus</td>
<td>29.2</td>
<td>22.5</td>
<td>21.3</td>
<td>22.2</td>
</tr>
<tr>
<td>Metestrus</td>
<td>0.8</td>
<td>0.8</td>
<td>5.6</td>
<td>7.4</td>
</tr>
<tr>
<td>Uncertain diagnoses</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>1.9</td>
</tr>
</tbody>
</table>

** Significantly different (P ≤ 0.01) from the vehicle control group by Dunnett’s test  
Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunn’s test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.

Number of females with a regular cycle/number of females cycling  
Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.  
Estrous cycle was longer than 12 days or unclear in 3 of 10 animals.
Table I3
Summary of Reproductive Tissue Evaluations for Male Mice in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Weights (g)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necropsy body wt</td>
<td>37.5 ± 0.9</td>
<td>39.1 ± 1.3</td>
<td>39.3 ± 0.9</td>
<td>38.2 ± 1.2</td>
</tr>
<tr>
<td>L. Cauda epididymis</td>
<td>0.0237 ± 0.0023</td>
<td>0.0200 ± 0.0010</td>
<td>0.0222 ± 0.0010</td>
<td>0.0220 ± 0.0015</td>
</tr>
<tr>
<td>L. Epididymis</td>
<td>0.0519 ± 0.0023</td>
<td>0.0491 ± 0.0014</td>
<td>0.0522 ± 0.0015</td>
<td>0.0515 ± 0.0019</td>
</tr>
<tr>
<td>L. Testis</td>
<td>0.1120 ± 0.0060</td>
<td>0.1204 ± 0.0026</td>
<td>0.1200 ± 0.0014</td>
<td>0.1170 ± 0.0034</td>
</tr>
<tr>
<td><strong>Spermatid measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spermatid heads (10^6/mg testis)</td>
<td>189.53 ± 3.33</td>
<td>208.68 ± 11.00</td>
<td>219.19 ± 9.14*</td>
<td>209.75 ± 11.26</td>
</tr>
<tr>
<td>Spermatid heads (10^6/testis)</td>
<td>19.38 ± 0.74</td>
<td>22.20 ± 1.04</td>
<td>23.30 ± 1.05*</td>
<td>20.98 ± 0.82</td>
</tr>
<tr>
<td><strong>Epididymal spermatozoal measurements</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sperm heads (10^6/mg cauda epididymis)</td>
<td>780 ± 110</td>
<td>958 ± 54</td>
<td>840 ± 54</td>
<td>879 ± 77</td>
</tr>
<tr>
<td>Sperm heads (10^6/cauda epididymis)</td>
<td>18 ± 2</td>
<td>19 ± 1</td>
<td>18 ± 1</td>
<td>19 ± 1</td>
</tr>
<tr>
<td>Sperm motility (%)</td>
<td>67.2 ± 4.4</td>
<td>67.2 ± 2.5</td>
<td>68.8 ± 1.4</td>
<td>56.7 ± 3.9**</td>
</tr>
</tbody>
</table>

* Significantly different (P < 0.05) from the vehicle control group by Dunn’s test
** Significantly different (P < 0.01) from the vehicle control group by Shirley’s test

a Data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett’s test (body and tissue weights) or Dunn’s test (sperm heads/mg cauda and sperm heads/cauda).

Table I4
Estrous Cycle Characterization for Female Mice in the 3-Month Gavage Study of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>Vehicle Control</th>
<th>10 mg/kg</th>
<th>20 mg/kg</th>
<th>50 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td><strong>Number weighed at necropsy</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Necropsy body wt</td>
<td>30.9 ± 1.0</td>
<td>30.5 ± 1.0</td>
<td>30.8 ± 0.8</td>
<td>29.3 ± 0.7</td>
</tr>
<tr>
<td><strong>Proportion of regular cycling females</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>10/10</td>
<td>9/10^c</td>
<td>10/10</td>
<td>9/10^c</td>
</tr>
<tr>
<td><strong>Estrous cycle length (days)</strong></td>
<td>3.9 ± 0.1</td>
<td>4.1 ± 0.2</td>
<td>3.8 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td><strong>Estrous stages (% of cycle)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diestrum</td>
<td>30.8</td>
<td>45.4</td>
<td>32.5</td>
<td>39.8</td>
</tr>
<tr>
<td>Proestrum</td>
<td>0.0</td>
<td>0.0</td>
<td>0.8</td>
<td>0.0</td>
</tr>
<tr>
<td>Estrus</td>
<td>44.2</td>
<td>35.2</td>
<td>41.7</td>
<td>36.1</td>
</tr>
<tr>
<td>Metestrum</td>
<td>25.0</td>
<td>19.4</td>
<td>25.0</td>
<td>24.1</td>
</tr>
</tbody>
</table>

a Necropsy body weights and estrous cycle length data are presented as mean ± standard error. Differences from the vehicle control group are not significant by Dunnett’s test (body weight) or Dunn’s test (estrous cycle length). By multivariate analysis of variance, dosed females do not differ significantly from the vehicle control females in the relative length of time spent in the estrous stages.
b Number of females with a regular cycle/number of females cycling
c Estrous cycle was longer than 12 days or unclear in 1 of 10 animals.
APPENDIX J
CHEMICAL CHARACTERIZATION
AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION

Androstenedione

Androstenedione was obtained from Steraloids, Inc. (Newport, RI), in one lot (H408) which was used in the 2-week, 3-month, and 2-year studies. Identity and purity analyses were conducted by the analytical chemistry laboratory (Battelle Memorial Institute, Columbus, OH), Research Triangle Institute (Research Triangle Park, NC), and the study laboratory that conducted the 3-month and 2-year studies (Southern Research Institute, Birmingham, AL). Elemental analyses and Karl Fischer titration were performed by Galbraith Laboratories, Inc. (Knoxville, TN). Reports on the analyses performed in support of the androstenedione studies are on file at the National Institute of Environmental Health Sciences.

Lot H408 of androstenedione, a white, crystalline solid, was identified as androstenedione by infrared (IR) and proton and carbon-13 nuclear magnetic resonance (NMR) spectroscopy. All spectra were consistent with the literature spectra (Sigma, 1986; Simova et al., 1997) of androstenedione. The infrared, proton NMR, and carbon-13 NMR spectra are presented in Figures J1, J2, and J3.

The moisture content of lot H408 was determined by Galbraith Laboratories, Inc., using Karl Fischer titration; this laboratory also performed elemental analyses of lot H408. The purity of lot H408 was determined by gas chromatography (GC) with flame ionization detection (FID) and high-performance liquid chromatography (HPLC) by system A (Table J1). GC/FID was performed with a gas chromatograph (Agilent, Palo Alto, CA) with a helium carrier gas flow rate of 1.5 mL/minute, a RTX-5 column (30 m × 0.25 mm ID, 1.0 µm film thickness (Restek, Bellefonte, PA), with an oven temperature program of 180° C to 325° C at 10° C per minute, then held for 6 minutes. For lot H408, Karl Fischer titration indicated an average water content from two analyses of 0.11% water. Elemental analyses for carbon and hydrogen were in agreement with the theoretical values for androstenedione. GC/FID indicated one major peak and two impurities with a combined area of 2.3% of the total peak area; sample purity was estimated to be 97.7%. HPLC analysis by systems A and B detected one impurity with a relative area of 0.8% of the major peak. The impurity was confirmed to be testosterone using HPLC by system A and mass spectroscopy. The overall purity of lot H408 was determined to be 98% or greater.

The bulk chemical was stored at 25° C protected from light and was reanalyzed by the study laboratory prior to and at the end of the 3-month and 2-year studies and approximately every 23 weeks during the 2-year study using HPLC by system B. No degradation of the bulk chemical was observed.

Methylcellulose

For the 2-week study, methylcellulose was obtained from Fisher Scientific, Inc. (Pittsburgh, PA), in one lot (984735). Identity was confirmed by Research Triangle Institute (Research Triangle Park, NC) using IR. The average methoxyl content determined by Galbraith Laboratories, Inc., was 29.1%. Methylcellulose was obtained from Sigma-Aldrich (St. Louis, MO) in one lot (31K0155) for the 3-month study and in two lots (31K0155 and 113K0078) for the 2-year studies. Identity was confirmed using IR; spectra were consistent with the structure of methylcellulose. The results of 12 analyses of lot 31K0155 and two analyses of lot 113K0078 by Galbraith Laboratories, Inc., found an average methoxyl content of 31.0% and 32.1%, respectively.
**Preparation and Analysis of Dose Formulations**

The vehicle was prepared by mixing methylcellulose with heated, deionized water, stirring with an overhead stirrer (2-week study) or with a magnetic stirrer (3-month and 2-year studies) to form a 0.5% solution, then cooled. Formulations of androstenedione were prepared by mixing the required amount of test article and 0.5% methylcellulose vehicle in a mortar with a pestle to form a smooth paste. The paste was transferred to a glass beaker; the mortar was rinsed three times with the vehicle, and the rinsings were transferred to the glass beaker containing the test article/vehicle paste; the contents of the glass beaker were diluted to final volume with the vehicle. The glass beaker was placed on a stir plate and stirred with a stir bar for 1 hour (2-week study) or at least 2 hours (3-month and 2-year studies) (Table J2). The dose formulations were prepared once for the 2-week studies, four times during the 3-month studies, and every 4 weeks during the 2-year studies. The dose formulations were stored in sealed amber glass bottles at room temperature (25°C) during the 2-week studies and refrigerated during the 3-month and 2-year studies for up to 35 days.

The analytical chemistry laboratory conducted homogeneity, gavageability, resuspendability, and stability studies. Homogeneity studies of 0.5 and 20 mg/mL dose formulations were performed using HPLC by system B (Table J1). Gavageability of a 20 mg/mL dose formulation was tested using a 20-gauge gavage needle. Resuspendability of a 20 mg/mL dose formulation was tested after stirring for approximately 10 minutes with a magnetic stirrer which caused a visible vortex using HPLC by system B. Stability studies of 0.05 and 0.5 mg/mL dose formulations were performed using HPLC by system B. Homogeneity, gavageability, and resuspendability were confirmed. Stability was confirmed for up to 35 days for dose formulations stored in sealed amber glass bottles protected from light at room temperature or 5°C.

Prior to the 2-week studies, the study laboratory (Battelle Columbus Operations, Columbus, OH) performed homogeneity and gavageability studies. Homogeneity studies of 0.1 and 10 mg/mL dose formulations were conducted using HPLC by system B. Gavageability of a 10 mg/mL dose formulation was tested using a 20-gauge gavage needle. Homogeneity was confirmed with the recommendation that dose formulations be stirred continuously while sampling and during administration; gavageability was confirmed.

Prior to the 3-month studies, the study laboratory conducted homogeneity studies of 0.1 and 10.0 mg/mL dose formulations using HPLC by system B. Homogeneity was confirmed.

Prior to the 2-year studies, the study laboratory tested the homogeneity of 0.2, 2, 5, and 10 mg/mL dose formulations using HPLC by system B. Homogeneity was confirmed.

Periodic analyses of the dose formulations of androstenedione in 0.5% methylcellulose were conducted at the study laboratories. Dose formulations were analyzed once for the 2-week studies using HPLC by system B; animal room samples were also analyzed. All dose formulations were within 10% of the target concentrations; three of five rat animal room samples and three of five mouse animal room samples were within 10% of target concentrations (Table J3). Dose formulations were analyzed three times during the 3-month studies using HPLC by system B; animal room samples were also analyzed. All 31 dose formulations analyzed were within 10% of the target concentrations; 8 of 15 rat animal room samples and 11 of 15 mouse animal room samples were within 10% of the target concentrations (Table J4). Dose formulations were analyzed every 2 to 3 months during the 2-year studies using HPLC by system B; animal room samples were also analyzed. Of the dose formulations analyzed, all 81 were within 10% of the target concentrations; 16 of 21 rat animal room samples and 29 of 32 mouse animal room samples were within 10% of target concentrations (Table J5). Difficulties in resuspending the formulations from the animal rooms caused some results to be further from target values than expected based on the original analyses. Improvements in handling the samples minimized this problem in the 2-year studies.
FIGURE J1
Infrared Absorption Spectrum of Androstenedione
FIGURE J2
Proton Nuclear Magnetic Resonance Spectrum of Androstenedione
FIGURE J3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Androstenedione
TABLE J1
High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Androstenedione

<table>
<thead>
<tr>
<th>Detection System</th>
<th>Column</th>
<th>Solvent System</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>System A</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
| Ultraviolet (242 nm) light | Luna C18, 150 mm × 4.6 mm, 5µ | A) 10:90 acetonitrile:0.1% phosphoric acid  
B) 90:10 acetonitrile:0.1% phosphoric acid;  
50% A:50% B for 15 minutes, changed to  
0% A:100% B over 15 minutes, then held for  
10 minutes, changed to 50% A:50% B over  
0.1 minute, then held for 9.9 minutes; flow  
rate = 0.75 mL/minute |
|                  | (Phenomenex, Inc., Torrance, CA) |                |
| **System B**     |        |                |
| Ultraviolet (242 nm) light | Luna C18, 150 mm × 4.6 mm, 5µ | 60:40 acetonitrile:Milli-Q water; isocratic;  
flow rate = 0.75 mL/minute |
|                  | (Phenomenex, Inc.) |                |

* The high-performance liquid chromatographs were manufactured by Waters (Milford, MA) (System A) and PerkinElmer (Waltham, MA) (System B).
**TABLE J2**
Preparation and Storage of Dose Formulations in the Gavage Studies of Androstenedione

<table>
<thead>
<tr>
<th></th>
<th>2-Week Studies</th>
<th>3-Month Studies</th>
<th>2-Year Studies</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preparation</strong></td>
<td>The vehicle was prepared by mixing methylcellulose and heated, deionized water with a magnetic stirrer to form a 0.5% solution, then cooled. Dose formulations were prepared by mixing the required amount of test article and 0.5% methylcellulose vehicle in a mortar with a pestle to form a smooth paste; the paste was transferred to a glass beaker; the mortar was rinsed 3 times with vehicle, and the washings transferred to the glass beaker containing the test article/vehicle paste and diluted to final volume with vehicle. The glass beaker was placed on a stir plate and stirred with a stir bar for at least 2 hours. The dose formulations were prepared four times.</td>
<td>The vehicle was prepared by mixing methylcellulose and heated, deionized water with a magnetic stirrer to form a 0.5% solution, then cooled. Dose formulations were prepared by mixing the required amount of test article and 0.5% methylcellulose vehicle in a mortar with a pestle to form a smooth paste; the paste was transferred to a glass beaker; the mortar was rinsed 3 times with vehicle, and the washings transferred to the glass beaker containing the test article/vehicle paste and diluted to final volume with vehicle. The glass beaker was placed on a stir plate and stirred with a stir bar for at least 2 hours. The dose formulations were prepared every 4 weeks.</td>
<td>The vehicle was prepared by mixing methylcellulose and heated, deionized water with a magnetic stirrer to form a 0.5% solution, then cooled. Dose formulations were prepared by mixing the required amount of test article and 0.5% methylcellulose vehicle in a mortar with a pestle to form a smooth paste; the paste was transferred to a glass beaker; the mortar was rinsed 3 times with vehicle, and the washings transferred to the glass beaker containing the test article/vehicle paste and diluted to final volume with vehicle. The glass beaker was placed on a stir plate and stirred with a stir bar for at least 2 hours. The dose formulations were prepared every 4 weeks.</td>
</tr>
<tr>
<td><strong>Chemical Lot Number</strong></td>
<td>H408</td>
<td>H408</td>
<td>H408</td>
</tr>
<tr>
<td><strong>Maximum Storage Time</strong></td>
<td>35 days</td>
<td>35 days</td>
<td>35 days</td>
</tr>
<tr>
<td><strong>Storage Conditions</strong></td>
<td>Stored in sealed amber glass bottles, protected from light, refrigerated (5° C)</td>
<td>Stored in sealed amber glass bottles, protected from light, refrigerated (5° C)</td>
<td>Stored in sealed amber glass bottles, protected from light, at room temperature (25° C)</td>
</tr>
<tr>
<td><strong>Study Laboratory</strong></td>
<td>Battelle Columbus Operations (Columbus, OH)</td>
<td>Southern Research Institute (Birmingham, AL)</td>
<td>Southern Research Institute (Birmingham, AL)</td>
</tr>
</tbody>
</table>
## TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of Androstenedione<sup>a</sup>

<table>
<thead>
<tr>
<th>Date Prepared</th>
<th>Date Analyzed</th>
<th>Target Concentration (mg/mL)</th>
<th>Determined Concentration (mg/mL)</th>
<th>Difference from Target (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>August 7, 2000</td>
<td>August 3, 2000</td>
<td>0.2</td>
<td>0.1967</td>
<td>–2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
<td>1.056</td>
<td>+6</td>
</tr>
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### Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Androstenedione

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a Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 0.2 mg/mL = 1 mg/kg, 1 mg/mL = 5 mg/kg, 2 mg/mL = 10 mg/kg, 4 mg/mL = 20 mg/kg, 10 mg/mL = 50 mg/kg. For mice, dosing volume = 10 mL/kg; 0.1 mg/mL = 1 mg/kg, 0.5 mg/mL = 5 mg/kg, 1 mg/mL = 10 mg/kg, 2 mg/mL = 20 mg/kg, 5 mg/mL = 50 mg/kg.

b Animal room samples
### TABLE J5
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Androstenedione

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### Table J5

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Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Year Gavage Studies of Androstenedione

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a Results of duplicate analyses. For rats, dosing volume = 5 mL/kg; 2 mg/mL = 10 mg/kg, 4 mg/mL = 20 mg/kg, 10 mg/mL = 50 mg/kg. For mice, dosing volume = 10 mL/kg; 0.2 mg/mL = 2 mg/kg, 1 mg/mL = 10 mg/kg, 2 mg/mL = 20 mg/kg, 5 mg/mL = 50 mg/kg.
b Animal room samples
APPENDIX K
INGREDIENTS, NUTRIENT COMPOSITION,
AND CONTAMINANT LEVELS
IN NTP-2000 RAT AND MOUSE RATION

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<td>Contaminant Levels in NTP-2000 Rat and Mouse Ration</td>
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<td>Soy oil (without preservatives)</td>
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<sup>a</sup> Wheat middlings as carrier  
<sup>b</sup> Calcium carbonate as carrier

## TABLE K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration<sup>a</sup>

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<tr>
<td>Chromium</td>
<td>0.2 mg</td>
<td>Chromium acetate</td>
</tr>
</tbody>
</table>

<sup>a</sup> Per kg of finished product
TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Mean ± Standard Deviation</th>
<th>Range</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein (% by weight)</td>
<td>14.9 ± 0.53</td>
<td>13.8 – 16.1</td>
<td>25</td>
</tr>
<tr>
<td>Crude fat (% by weight)</td>
<td>8.0 ± 0.37</td>
<td>7.4 – 9.0</td>
<td>25</td>
</tr>
<tr>
<td>Crude fiber (% by weight)</td>
<td>9.2 ± 0.45</td>
<td>8.2 – 9.9</td>
<td>25</td>
</tr>
<tr>
<td>Ash (% by weight)</td>
<td>5.0 ± 0.21</td>
<td>4.4 – 5.4</td>
<td>25</td>
</tr>
<tr>
<td>Amino Acids (% of total diet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arginine</td>
<td>0.770 ± 0.070</td>
<td>0.670 – 0.970</td>
<td>18</td>
</tr>
<tr>
<td>Cystine</td>
<td>0.225 ± 0.023</td>
<td>0.150 – 0.250</td>
<td>18</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.706 ± 0.043</td>
<td>0.620 – 0.800</td>
<td>18</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.362 ± 0.082</td>
<td>0.310 – 0.680</td>
<td>18</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.542 ± 0.046</td>
<td>0.430 – 0.660</td>
<td>18</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.087 ± 0.066</td>
<td>0.960 – 1.240</td>
<td>18</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.712 ± 0.118</td>
<td>0.310 – 0.840</td>
<td>18</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.407 ± 0.051</td>
<td>0.260 – 0.490</td>
<td>18</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.626 ± 0.043</td>
<td>0.540 – 0.720</td>
<td>18</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.500 ± 0.046</td>
<td>0.430 – 0.610</td>
<td>18</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.142 ± 0.024</td>
<td>0.110 – 0.200</td>
<td>18</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.388 ± 0.058</td>
<td>0.280 – 0.540</td>
<td>18</td>
</tr>
<tr>
<td>Valine</td>
<td>0.667 ± 0.045</td>
<td>0.550 – 0.730</td>
<td>18</td>
</tr>
<tr>
<td>Essential Fatty Acids (% of total diet)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Linoleic</td>
<td>3.92 ± 0.243</td>
<td>3.49 – 4.54</td>
<td>18</td>
</tr>
<tr>
<td>Linolenic</td>
<td>0.30 ± 0.035</td>
<td>0.21 – 0.35</td>
<td>18</td>
</tr>
<tr>
<td>Vitamins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A (IU/kg)</td>
<td>4,920 ± 1,210</td>
<td>3,360 – 8,900</td>
<td>25</td>
</tr>
<tr>
<td>Vitamin D (IU/kg)</td>
<td>1,000a</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Tocopherol (ppm)</td>
<td>84.2 ± 16.60</td>
<td>52.0 – 110.0</td>
<td>15</td>
</tr>
<tr>
<td>Thiamine (ppm)</td>
<td>8.5 ± 3.66</td>
<td>5.9 – 25.2</td>
<td>25</td>
</tr>
<tr>
<td>Riboflavin (ppm)</td>
<td>6.8 ± 2.11</td>
<td>4.20 – 11.20</td>
<td>15</td>
</tr>
<tr>
<td>Niacin (ppm)</td>
<td>79.0 ± 10.50</td>
<td>66.4 – 98.2</td>
<td>15</td>
</tr>
<tr>
<td>Pantothenic acid (ppm)</td>
<td>23.9 ± 3.73</td>
<td>17.4 – 29.8</td>
<td>15</td>
</tr>
<tr>
<td>Pyridoxine (ppm)</td>
<td>9.21 ± 2.20</td>
<td>6.4 – 13.7</td>
<td>15</td>
</tr>
<tr>
<td>Folic acid (ppm)</td>
<td>1.75 ± 0.54</td>
<td>1.20 – 3.27</td>
<td>15</td>
</tr>
<tr>
<td>Biotin (ppm)</td>
<td>0.332 ± 0.12</td>
<td>0.225 – 0.704</td>
<td>15</td>
</tr>
<tr>
<td>Vitamin B12 (ppb)</td>
<td>60.5 ± 46.5</td>
<td>18.3 – 174.0</td>
<td>15</td>
</tr>
<tr>
<td>Choline (ppm)</td>
<td>3,064 ± 270</td>
<td>2,700 – 3,790</td>
<td>15</td>
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<tr>
<td>Minerals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium (%)</td>
<td>0.959 ± 0.046</td>
<td>0.873 – 1.030</td>
<td>25</td>
</tr>
<tr>
<td>Phosphorus (%)</td>
<td>0.589 ± 0.028</td>
<td>0.538 – 0.641</td>
<td>25</td>
</tr>
<tr>
<td>Potassium (%)</td>
<td>0.665 ± 0.023</td>
<td>0.626 – 0.694</td>
<td>15</td>
</tr>
<tr>
<td>Chloride (%)</td>
<td>0.376 ± 0.041</td>
<td>0.300 – 0.474</td>
<td>15</td>
</tr>
<tr>
<td>Sodium (%)</td>
<td>0.191 ± 0.017</td>
<td>0.160 – 0.222</td>
<td>15</td>
</tr>
<tr>
<td>Magnesium (%)</td>
<td>0.201 ± 0.009</td>
<td>0.185 – 0.217</td>
<td>15</td>
</tr>
<tr>
<td>Sulfur (%)</td>
<td>0.170 ± 0.029</td>
<td>0.116 – 0.209</td>
<td>15</td>
</tr>
<tr>
<td>Iron (ppm)</td>
<td>182 ± 46.7</td>
<td>135 – 311</td>
<td>15</td>
</tr>
<tr>
<td>Manganese (ppm)</td>
<td>54.1 ± 7.89</td>
<td>42.1 – 73.1</td>
<td>15</td>
</tr>
<tr>
<td>Zinc (ppm)</td>
<td>55.0 ± 9.55</td>
<td>43.3 – 78.5</td>
<td>15</td>
</tr>
<tr>
<td>Copper (ppm)</td>
<td>6.65 ± 1.790</td>
<td>3.21 – 10.50</td>
<td>15</td>
</tr>
<tr>
<td>Iodine (ppm)</td>
<td>0.512 ± 0.221</td>
<td>0.233 – 0.972</td>
<td>15</td>
</tr>
<tr>
<td>Chromium (ppm)</td>
<td>0.604 ± 0.253</td>
<td>0.330 – 1.380</td>
<td>14</td>
</tr>
<tr>
<td>Cobalt (ppm)</td>
<td>0.25 ± 0.074</td>
<td>0.20 – 0.47</td>
<td>14</td>
</tr>
</tbody>
</table>

a From formulation
b As hydrochloride (thiamine and pyridoxine) or chloride (choline)
### TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration

<table>
<thead>
<tr>
<th>Contaminants</th>
<th>Mean ± Standard Deviation</th>
<th>Range</th>
<th>Number of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic (ppm)</td>
<td>0.33 ± 0.158</td>
<td>0.14 – 0.50</td>
<td>25</td>
</tr>
<tr>
<td>Cadmium (ppm)</td>
<td>0.07 ± 0.021</td>
<td>0.04 – 0.10</td>
<td>25</td>
</tr>
<tr>
<td>Lead (ppm)</td>
<td>0.08 ± 0.026</td>
<td>0.05 – 0.13</td>
<td>25</td>
</tr>
<tr>
<td>Mercury (ppm)</td>
<td>&lt;0.02</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Selenium (ppm)</td>
<td>0.20 ± 0.057</td>
<td>0.14 – 0.45</td>
<td>25</td>
</tr>
<tr>
<td>Aflatoxins (ppb)</td>
<td>&lt;5.00</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Nitrate nitrogen (ppm)</td>
<td>14.5 ± 4.33</td>
<td>10.00 – 24.4</td>
<td>25</td>
</tr>
<tr>
<td>Nitrite nitrogen (ppm)</td>
<td>&lt;0.61</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>BHA (ppm)d</td>
<td>&lt;1.0</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>BHT (ppm)d</td>
<td>&lt;1.0</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Aerobic plate count (CFU/g)</td>
<td>10.0 ± 10.0</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Coliform (MPN/g)</td>
<td>3.0 ± 3.0</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (MPN/g)</td>
<td>&lt;10</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td><em>Salmonella</em></td>
<td>Negative</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Total nitrosamines (ppb)</td>
<td>4.4 ± 2.04</td>
<td>2.3 – 8.5</td>
<td>25</td>
</tr>
<tr>
<td><em>N</em>-Nitrosodimethylamine (ppb)</td>
<td>2.6 ± 1.74</td>
<td>1.1 – 6.9</td>
<td>25</td>
</tr>
<tr>
<td><em>N</em>-Nitrosopyrrolidine (ppb)</td>
<td>1.8 ± 0.79</td>
<td>0.9 – 4.1</td>
<td>25</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pesticides (ppm)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>α-BHC</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>β-BHC</td>
<td>&lt;0.02</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>γ-BHC</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>δ-BHC</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Aldrin</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>DDE</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>DDD</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>DDT</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>HCB</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Mirex</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Methoxychlor</td>
<td>&lt;0.05</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Endrin</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Telodrin</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Chlor dane</td>
<td>&lt;0.05</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Toxaphene</td>
<td>&lt;0.10</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Estimated PCBs</td>
<td>&lt;0.20</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Ronnel</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Ethion</td>
<td>&lt;0.02</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Trithion</td>
<td>&lt;0.05</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Diazinon</td>
<td>&lt;0.10</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Methyl chlorpyrifos</td>
<td>0.098 ± 0.111</td>
<td>0.020 – 0.416</td>
<td>25</td>
</tr>
<tr>
<td>Methyl parathion</td>
<td>&lt;0.02</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Ethyl parathion</td>
<td>&lt;0.02</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Malathion</td>
<td>0.189 ± 0.377</td>
<td>0.020 – 1.850</td>
<td>25</td>
</tr>
<tr>
<td>Endosulfan I</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Endosulfan II</td>
<td>&lt;0.01</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>&lt;0.03</td>
<td></td>
<td>25</td>
</tr>
</tbody>
</table>

---

*a* All samples were irradiated. CFU = colony-forming units; MPN = most probable number; BHC = hexachlorocyclohexane or benzene hexachloride.

*b* For values less than the limit of detection, the detection limit is given as the mean.

*c* Sources of contamination: alfalfa, grains, and fish meal

*d* Sources of contamination: soy oil and fish meal

*e* All values were corrected for percent recovery.
APPENDIX L
SENTINEL ANIMAL PROGRAM

METHODS ................................................................. 190
RESULTS ................................................................. 192
SENTINEL ANIMAL PROGRAM

METHODS

Rodents used in the Carcinogenesis Program of the National Toxicology Program are produced in optimally clean facilities to eliminate potential pathogens that may affect study results. The Sentinel Animal Program is part of the periodic monitoring of animal health that occurs during the toxicologic evaluation of chemical compounds. Under this program, the disease state of the rodents is monitored via serology on sera from extra (sentinel) animals in the study rooms. These animals and the study animals are subject to identical environmental conditions. The sentinel animals come from the same production source and weanling groups as the animals used for the studies of chemical compounds.

Serum samples were collected from five male and five female rats and mice at the start of the 2-week studies, from five male and five female control rats and mice at the end of the 3-month studies, from five male and five female sentinel rats and mice at 6, 12, and 18 months during the 2-year studies, and from five male and five female 50 mg/kg rats and mice at the end of the 2-year studies. Blood from each animal was collected and allowed to clot, and the serum was separated. The samples were processed appropriately and sent to BioReliance (Rockville, MD) for determination of antibody titer.

Fecal samples were taken from sentinel mice at 18 months in the 2-year study. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the studies are also listed.

<table>
<thead>
<tr>
<th>Method and Test</th>
<th>Time of Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Rats</strong></td>
<td></td>
</tr>
<tr>
<td><strong>2-Week Study</strong></td>
<td></td>
</tr>
<tr>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>PVM (pneumonia virus of mice)</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>RCV/SDA (rat coronavirus/sialodacryoadenitis virus)</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>Sendai</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>Immunofluorescence Assay</td>
<td></td>
</tr>
<tr>
<td>Parvovirus</td>
<td>End of quarantine</td>
</tr>
<tr>
<td><strong>3-Month Study</strong></td>
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<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>PVM</td>
<td>Study termination</td>
</tr>
<tr>
<td>RCV/SDA</td>
<td>Study termination</td>
</tr>
<tr>
<td>Sendai</td>
<td>Study termination</td>
</tr>
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<td>Immunofluorescence Assay</td>
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<tr>
<td>Parvovirus</td>
<td>Study termination</td>
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<td><strong>2-Year Study</strong></td>
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<tr>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td><em>Mycoplasma arthritidis</em></td>
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</tr>
<tr>
<td><em>Mycoplasma pulmonis</em></td>
<td>Study termination</td>
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<td>PVM</td>
<td>6, 12, and 18 months, study termination</td>
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<td>RCV/SDA</td>
<td>6, 12, and 18 months, study termination</td>
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<tr>
<td>Sendai</td>
<td>6, 12, and 18 months, study termination</td>
</tr>
<tr>
<td>Immunofluorescence Assay</td>
<td>6, 12, and 18 months, study termination</td>
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<td>Parvovirus</td>
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<td>Method and Test</td>
<td>Time of Analysis</td>
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<td>------------------</td>
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<tr>
<td><strong>Mice</strong></td>
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<td><strong>2-Week Study</strong></td>
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<tr>
<td>ELISA</td>
<td></td>
</tr>
<tr>
<td>Ectromelia virus</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>EDIM (epizootic diarrhea of infant mice)</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>GDVII (mouse encephalomyelitis virus)</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>LCM (lymphocytic choriomeningitis virus)</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>Mouse adenoma virus-FL</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>MHV (mouse hepatitis virus)</td>
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</tr>
<tr>
<td>PVM</td>
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</tr>
<tr>
<td>Reovirus 3</td>
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</tr>
<tr>
<td>Sendai</td>
<td>End of quarantine</td>
</tr>
<tr>
<td>Immunofluorescence Assay</td>
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</tr>
<tr>
<td>Parovirus</td>
<td>End of quarantine</td>
</tr>
<tr>
<td><strong>3-Month Study</strong></td>
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<td>ELISA</td>
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</tr>
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<td>Ectromelia virus</td>
<td>Study termination</td>
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<td>EDIM</td>
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<td>GDVII</td>
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<td>LCM</td>
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<tr>
<td>Mouse adenoma virus-FL</td>
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<tr>
<td>MHV</td>
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<td>PVM</td>
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<td>Reovirus 3</td>
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<td>Sendai</td>
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<tr>
<td>Immunofluorescence Assay</td>
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<tr>
<td>Parovirus</td>
<td>Study termination</td>
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</tr>
<tr>
<td>Ectromelia virus</td>
<td>6, 12, and 18 months, study termination</td>
</tr>
<tr>
<td>EDIM</td>
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<tr>
<td>GDVII</td>
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<tr>
<td>LCM</td>
<td>6, 12, and 18 months, study termination</td>
</tr>
<tr>
<td>MVM (minute virus of mice)</td>
<td>18 months, study termination</td>
</tr>
<tr>
<td>Mouse adenoma virus-FL</td>
<td>6, 12, and 18 months, study termination</td>
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<tr>
<td>MHV</td>
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<tr>
<td>MPV (mouse parvovirus)</td>
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<tr>
<td>M. arthritidis</td>
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</tr>
<tr>
<td>M. pulmonis</td>
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</tr>
<tr>
<td>PVM</td>
<td>6, 12, and 18 months, study termination</td>
</tr>
<tr>
<td>Reovirus 3</td>
<td>6, 12, and 18 months, study termination</td>
</tr>
<tr>
<td>Sendai</td>
<td>6, 12, and 18 months, study termination</td>
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### Method and Test

<table>
<thead>
<tr>
<th>Mice (continued)</th>
<th>Time of Analysis</th>
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<tr>
<td>2-Year Study (continued)</td>
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<tr>
<td>Immunofluorescence Assay</td>
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<td>18 months</td>
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<tr>
<td>MVM</td>
<td>18 months</td>
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<tr>
<td>MCMV (mouse cytomegalovirus)</td>
<td>18 months, study termination</td>
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<tr>
<td>Mouse adenoma virus-FL</td>
<td>6 months, study termination</td>
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<tr>
<td>Parvovirus</td>
<td>6 and 12 months</td>
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<tr>
<td>PVM</td>
<td>12 months</td>
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#### Helicobacter species

#### RESULTS

All test results were negative.