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

September 2010

NTP TR 560

NIH Publication No. 10-5901

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

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.

NTP Technical Reports are indexed in the NIH/NLM PubMed database and are available free of charge electronically on the NTP website (*http://ntp.niehs.nih.gov*) or in hardcopy upon request from the NTP Central Data Management group at *cdm@niehs.nih.gov* or (919) 541-3419.

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CONTENTS

SUMMARY

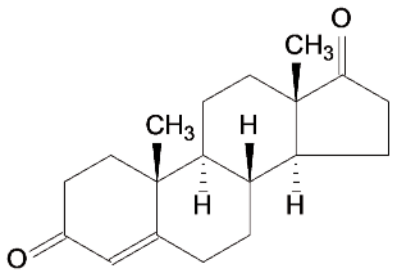
Background
Androstenedione is a natural androgen steroid hormone that is synthesized in men and women. Commercially it is used as an intermediate in the production of other steroids including oral contraceptives and anti-inflammatory products. Until its over-the-counter sales were banned in 2004, androstenedione 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 androstendione 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

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

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 androstene - dione/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.

Incidences 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.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Androstenedione

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Doses in methylcellulose solution by gavage	0, 10, 20, or 50 mg/kg	0, 10, 20, or 50 mg/kg	0, 10, 20, or 50 mg/kg	0, 2, 10, or 50 mg/kg
Body weights	Dosed groups similar to the vehicle control group	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	Dosed groups similar to the vehicle control group	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
Survival rates	21/50, 33/50, 29/50, 27/50	38/50, 37/50, 33/50, 37/50	36/50, 44/50, 34/50, 37/50	35/50, 40/50, 40/50, 40/50
Nonneoplastic effects	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)	Pancreatic islets: hyperplasia (0/50, 4/50, 1/50, 11/50) Exocrine pancreas: atrophy (10/50, 10/50, 16/50, 26/50)	Liver: eosinophilic focus (13/50, 10/50, 11/50, 25/50)	Liver: mixed cell focus (2/50, 5/50, 7/50, 15/50); hepatocyte, cytoplasmic vacuolization (6/50, 3/50, 9/50, 22/50) Clitoral gland: hyperplasia (0/47, 2/47, 13/49, 41/50); duct dilatation (0/47, 2/47, 17/49, 49/50) Kidney: glomerulus, metaplasia (2/50, 1/50, 5/50, 27/50) Submandibular salivary gland: cytoplasmic alteration (0/49, 17/49, 40/49, 45/50)

Summary of the 2-Year Carcinogenesis and Genetic Toxicology Studies of Androstenedione

	Male F344/N Rats	Female F344/N Rats	Male B6C3F1 Mice	Female B6C3F1 Mice
Neoplastic effects	None	None	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) Pancreatic islets: adenoma (includes multiples) (2/50, 2/50, 2/50, 5/49)	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) Pancreatic islets: adenoma (0/49, 2/50, 4/49, 4/48)
Equivocal findings	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)	Mononuclear cell leukemia: (5/50, 11/50, 18/50, 15/50)	None	None
Decreased incidences	Testis: interstitial cell adenoma (42/50, 39/50, 36/50, 26/50)	Mammary gland: fibroadenoma (35/50, 31/50, 22/50, 12/50)	None	Malignant lymphoma: (14/50, 15/50, 11/50, 2/50)
Level of evidence of carcinogenic activity	Equivocal evidence	Equivocal evidence	Clear evidence	Clear evidence
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.

NATIONAL TOXICOLOGY PROGRAM BOARD OF SCIENTIFIC COUNSELORS
TECHNICAL REPORTS REVIEW SUBCOMMITTEE

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.

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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* 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 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.

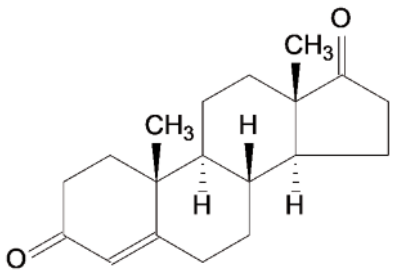
Dr. Eastmond, the second primary reviewer, raised questions about interpreting the statistical significance of tumors with high variability and high background rates. In particular, regarding liver tumors in male mice, he inquired if a rate of 48/50 of treated animals with tumors compared with 41/50 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



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 catalyzation of 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 ^{14}C-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 ^{14}C in rat plasma ranged from 4.4 to 7.1 hours among the three doses, and were highest in the female treatment groups. No ^{14}C-derived androstenedione, estradiol, estrone, or testosterone was detected in the urine of these animals. Although the metabolic profile of the ^{14}C 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 ^{14}C was equally excreted in urine and feces.

In the Green and Catz (2007) studies, the half-lives of the ^{14}C 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 ^{14}C 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 ^{14}C estrone or estradiol was detected in plasma or urine and no ^{14}C androstenedione or testosterone was detected in urine after IV or oral administration. Differences in the ^{14}C 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,

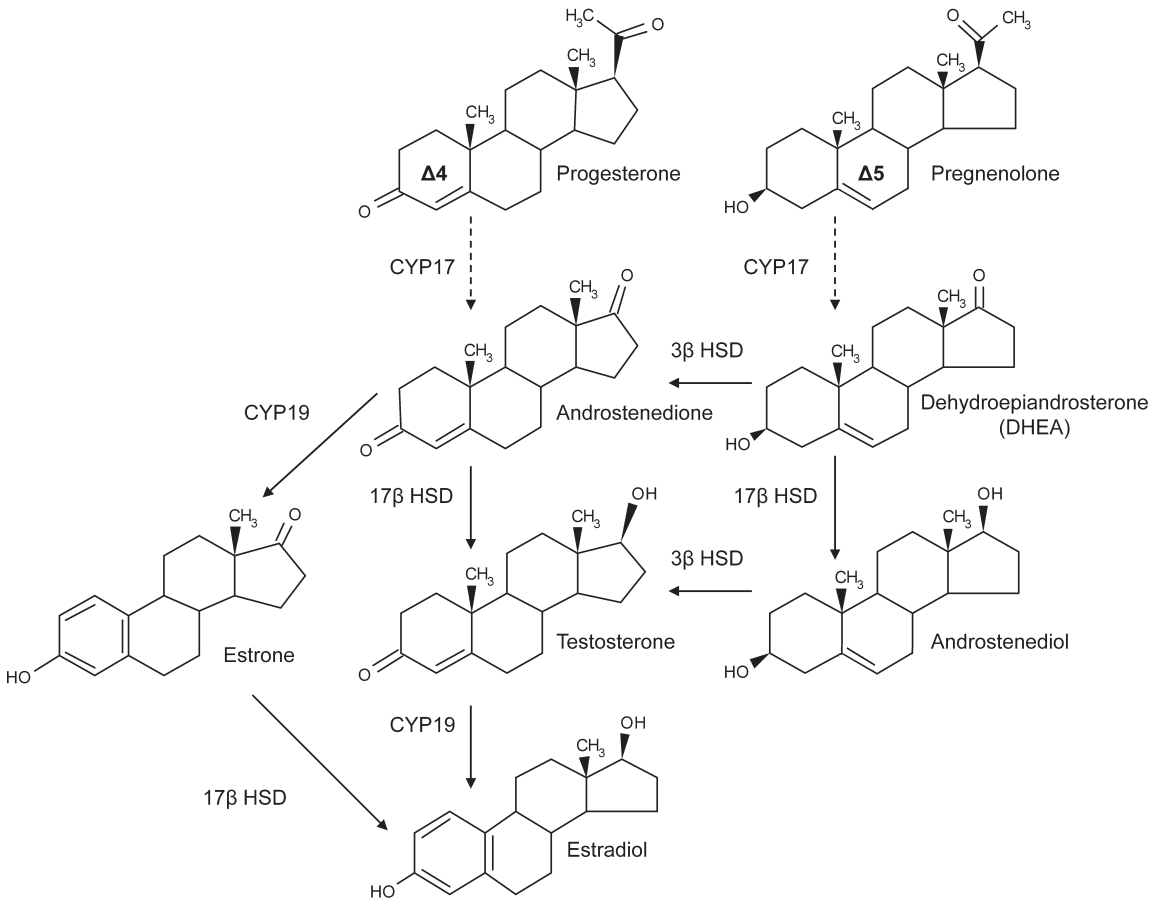


Figure 1
Formation of Endogenous Sex Steroids
(The intermediate from CYP17's two-step reaction is not shown.)

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 exposure via gavage did not produce clear hepatotoxicity in livers of pregnant or nonpregnant rats (Sahu *et al*., 2005; Wiesenfeld *et al*., 2006). 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 F_0 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 promoter 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, anueploidy, 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/kg 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. Vehicle control groups received the methylcellulose solution alone. Feed and water were available *ad libi-*

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

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

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

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

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

2-Week Studies	3-Month Studies	2-Year Studies
Doses		
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		
Observed twice daily; animals were weighed initially, on day 8, and at the end of the studies; clinical findings were recorded daily.	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.	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		
Carbon dioxide asphyxiation	Same as 2-week studies	Same as 2-week studies
Necropsy		
Necropsies were performed on all animals. Organs weighed were heart, right kidney, liver, lung, right testis, and thymus.	Necropsies were performed on core study animals. Organs weighed were heart, right kidney, liver, lung, right testis, thymus, and uterus.	Necropsies were performed on all animals.
Clinical Pathology		
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	None

TABLE 1
Experimental Design and Materials and Methods in the Gavage Studies of Androstenedione

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), harderian 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), harderian 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 spermatozoal 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

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., harderian 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 Bailer, 1989; Piegorsch and Bailer, 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 acentric 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-

nally 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 *in vitro* indicator for rodent carcinogenicity (89% of the *Salmonella* mutagens are rodent carcinogens) (Tennant *et al.,* 1987; Zeiger *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 *in vivo* bone marrow chromosome aberration or micronucleus tests appears to be less than that in the *Salmonella* test (Shelby *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 *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 *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 2
Survival and Body Weights of Rats in the 2-Week Gavage Study of Androstenedione

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	113 ± 5	184 ± 9	71 ± 4	
1	5/5	110 ± 3	181 ± 7	71 ± 6	99
5	5/5	111 ± 3	185 ± 7	74 ± 4	101
10	5/5	110 ± 2	183 ± 3	73 ± 1	100
20	5/5	109 ± 2	176 ± 5	67 ± 4	96
50	5/5	110 ± 3	182 ± 3	71 ± 2	99
Female					
0	5/5	96 ± 2	131 ± 2	36 ± 2	
1	5/5	94 ± 1	127 ± 1	33 ± 1	97
5	5/5	94 ± 2	125 ± 2	30 ± 2	95
10	5/5	96 ± 2	137 ± 3	41 ± 2	104
20	5/5	95 ± 1	132 ± 1	37 ± 0	100
50	5/5	95 ± 2	130 ± 2	35 ± 2	99

[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 gavageability.

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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	101 ± 2	333 ± 7	232 ± 6	
1	10/10	101 ± 2	340 ± 6	239 ± 6	102
5	10/10	101 ± 1	344 ± 4	243 ± 4	103
10	10/10	102 ± 2	343 ± 5	241 ± 4	103
20	10/10	102 ± 1	343 ± 3	241 ± 3	103
50	10/10	101 ± 2	340 ± 5	239 ± 4	102
Female					
0	10/10	92 ± 1	189 ± 2	97 ± 3	
1	10/10	89 ± 1	200 ± 4*	111 ± 4*	106
5	10/10	92 ± 1	198 ± 2*	107 ± 2	105
10	10/10	93 ± 1	198 ± 2*	106 ± 2	105
20	10/10	90 ± 2	206 ± 3**	116 ± 3**	109
50	10/10	89 ± 1	199 ± 4**	111 ± 4**	105

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' test
** $P \leq 0.01$
[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 I1). 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 gavageability.

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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	22	9	17	13
Natural deaths	7	8	4	10
Animals surviving to study termination	21[d]	33	29	27
Percent probability of survival at end of study[a]	42	66	58	54
Mean survival (days)[b]	661	693	684	690
Survival analysis[c]	P=0.658N	P=0.018N	P=0.132N	P=0.255N
Female				
Animals initially in study	50	50	50	50
Moribund	10	7	12	9
Natural deaths	2	6	5	4
Animals surviving to study termination	38	37[d]	33	37
Percent probability of survival at end of study	76	74	66	74
Mean survival (days)	710	703	687	693
Survival analysis	P=0.754	P=0.967	P=0.273	P=0.804

[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] Includes one animal that died during the last week of the study

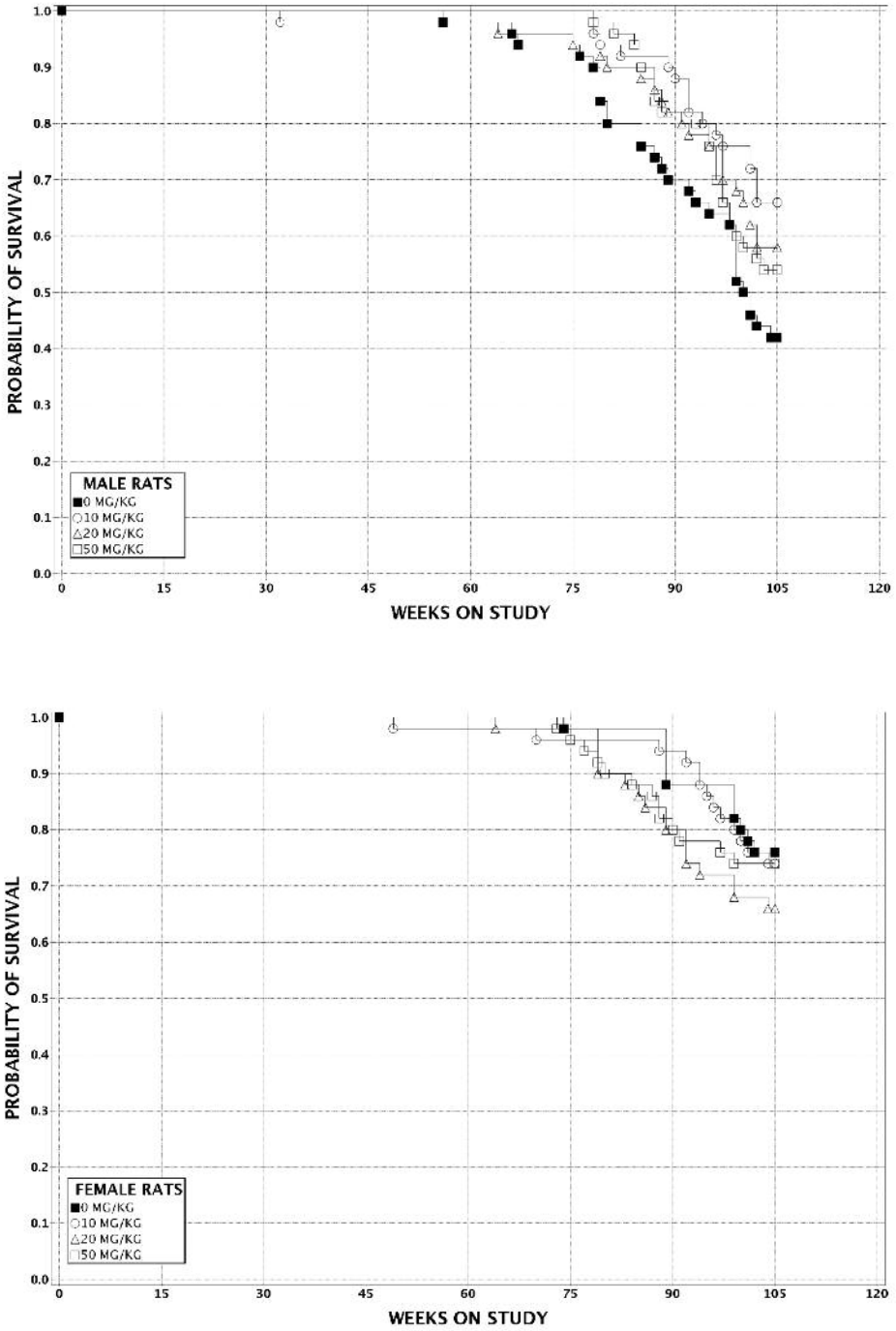


Figure 2
Kaplan-Meier Survival Curves for Male and Female Rats Administered Androstenedione by Gavage for 2 Years

TABLE 5
Mean Body Weights and Survival of Male Rats in the 2-Year Gavage Study of Androstenedione

Weeks on Study	Vehicle Control		10 mg/kg			20 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	121	50	121	100	50	120	99	50	122	100	50
2	158	50	156	98	50	155	98	50	158	100	50
3	197	50	196	99	50	195	99	50	197	100	50
4	228	50	226	99	50	228	100	50	228	100	50
5	253	50	249	99	50	252	100	50	250	99	50
6	270	50	268	99	50	271	100	50	267	99	50
7	287	50	284	99	50	286	100	50	284	99	50
8	304	50	301	99	50	302	100	50	299	99	50
9	319	50	317	99	50	316	99	50	315	99	50
10	331	50	327	99	50	327	99	50	326	99	50
11	341	50	338	99	50	338	99	50	337	99	50
12	351	50	348	99	50	348	99	50	345	98	50
13	359	50	358	100	50	357	99	50	353	98	50
17	390	50	383	98	50	378	97	50	379	97	50
21	413	50	409	99	50	403	98	50	401	97	50
25	431	50	428	99	50	423	98	50	417	97	50
29	445	50	441	99	50	435	98	50	427	96	50
33	459	50	453	99	49	447	97	50	438	96	50
37	470	50	466	99	49	460	98	50	449	96	50
41	473	50	473	100	49	461	98	50	455	96	50
45	485	50	479	99	49	475	98	50	463	96	50
49	491	50	489	100	49	479	98	50	470	96	50
53	497	50	494	100	49	486	98	50	474	95	50
57	502	49	503	100	49	496	99	49	483	96	50
61	510	49	508	100	49	500	98	49	489	96	50
65	512	49	500	98	49	489	96	48	494	97	50
69	513	47	512	100	49	506	99	48	497	97	50
73	515	47	514	100	49	506	98	48	499	97	50
77	508	46	521	103	49	513	101	47	508	100	50
81	518	40	522	101	47	517	100	45	510	99	48
85	519	38	523	101	46	516	99	45	513	99	45
89	509	35	509	100	46	495	97	42	509	100	41
93	505	34	517	102	41	510	101	39	505	100	40
97	502	32	518	103	39	513	102	35	508	101	33
101	493	23	504	102	37	504	102	31	512	104	29
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

Weeks on Study	Vehicle Control		10 mg/kg			20 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	103	50	103	100	50	103	100	50	104	101	50
2	123	50	123	100	50	123	100	50	125	102	50
3	139	50	139	101	50	139	100	50	142	103	50
4	151	50	152	101	50	152	101	50	156	103	50
5	161	50	161	100	50	163	101	50	167	104	50
6	170	50	170	100	50	169	100	50	178	105	50
7	179	50	179	100	50	181	101	50	187	105	50
8	185	50	184	99	50	187	101	50	194	104	50
9	191	50	190	100	50	193	101	50	201	105	50
10	194	50	194	100	50	197	102	50	206	107	50
11	198	50	199	101	50	202	102	50	213	108	50
12	200	50	201	101	50	205	103	50	216	108	50
13	203	50	203	100	50	208	102	50	219	108	50
17	213	50	213	100	50	220	104	50	236	111	50
21	221	50	225	102	50	234	106	50	249	113	50
25	229	50	236	103	50	247	108	50	263	115	50
29	237	50	241	102	50	258	109	50	275	116	50
33	243	50	253	104	50	271	112	50	287	118	50
37	251	50	265	105	50	283	113	50	298	119	50
41	260	50	272	104	50	292	112	50	305	117	50
45	265	50	278	105	50	295	111	50	309	116	50
49	279	50	286	103	49	305	109	50	319	114	50
53	289	50	297	103	49	315	109	50	325	113	50
57	299	50	304	102	49	321	108	50	334	112	50
61	312	50	316	101	49	335	108	50	343	110	50
65	315	50	321	102	49	337	107	49	345	110	50
69	327	50	329	101	49	346	106	49	352	108	50
73	330	50	338	102	48	350	106	49	355	108	50
77	335	49	344	103	48	353	105	49	362	108	47
81	344	49	349	102	48	362	105	45	372	108	45
85	347	49	355	102	48	364	105	43	379	109	44
89	347	44	353	102	47	360	104	40	362	105	41
93	356	44	350	98	46	370	104	37	378	106	39
97	357	44	359	101	42	373	105	36	386	108	38
101	356	39	361	101	38	373	105	34	388	109	37
Mean for weeks											
1-13	169		169	100		171	101		178	105	
14-52	244		252	103		267	109		282	116	
53-101	332		337	101		351	106		360	109	

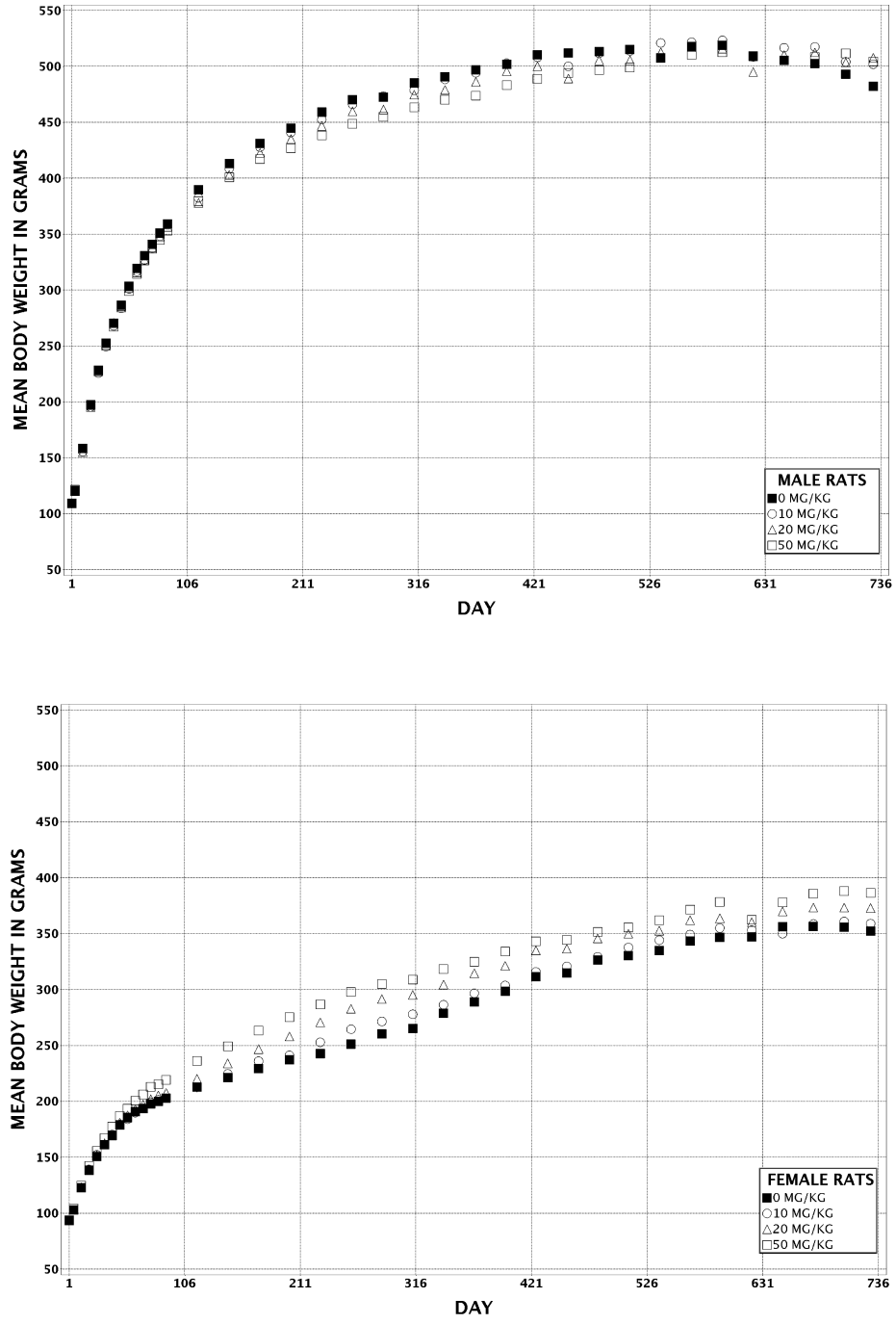


FIGURE 3
Growth Curves for Male and Female Rats Administered Androstenedione by Gavage for 2 Years

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).

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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Male				
Overall rate[a,b]	26/50 (52%)	22/50 (44%)	18/50 (36%)	18/50 (36%)
Adjusted rate[c]	59.0%	47.8%	39.9%	39.2%
Terminal rate[d]	9/21 (43%)	14/33 (42%)	8/29 (28%)	7/27 (26%)
First incidence (days)	526	629	558	563
Poly-3 test[e]	P=0.052N	P=0.191N	P=0.050N	P=0.042N
Female				
Overall rate[f]	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

[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

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

(T) Terminal sacrifice
[a] 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%
[b] Number of animals with neoplasm per number of animals with lung 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.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed
[h] 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%

of clear cell focus 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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Number Examined Microscopically	50	50	50	50
Bilateral Interstitial Cell Adenoma	29	29	28	10**
Interstitial Cell Adenoma (includes bilateral)[a]				
Overall rate[b]	42/50 (84%)	39/50 (78%)	36/50 (72%)	26/50 (52%)
Adjusted rate[c]	91.1%	83.8%	79.2%	58.1%
Terminal rate[d]	20/21 (95%)	30/33 (91%)	24/29 (83%)	19/27 (70%)
First incidence (days)	464	540	558	607
Poly-3 test[e]	P<0.001N	P=0.203N	P=0.070N	P<0.001N

** 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 surrounded and separated by a few layers of collagen with lymphocytes and a few neutrophils at the periphery or as one or more individual 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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Number Examined Microscopically	50	50	50	50
Hyperplasia[a]	48 (2.2)[b]	40** (2.3)	35** (2.0)	23** (1.8)
Cyst	15 (2.1)	3** (1.7)	9 (2.1)	3** (2.7)
Fibroadenoma, Multiple	17	13	6**	3**
Fibroadenoma[c]				
Overall rate[d]	35/50 (70%)	31/50 (62%)	22/50 (44%)	12/50 (24%)
Adjusted rate[e]	72.3%	66.1%	47.6%	26.9%
Terminal rate[f]	28/38 (74%)	26/37 (70%)	13/33 (39%)	11/37 (30%)
First incidence (days)	619	652	547	610
Poly-3 test[g]	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 Carcinoma[h]				
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%

mild if it affected four or more but still had many islets in the section of normal size.

Exocrine Pancreas: The incidence of atrophy was significantly increased in 50 mg/kg females (Tables 11 and B4). Atrophy of the exocrine pancreas was characterized by either focal or lobular atrophy. The atrophied areas had decreased numbers of acini with an increase in small duct-like structures (atrophied acini). Interstitial fibroblasts and collagen (fibrosis) surrounded glands and duct-like structures; macrophages, lymphocytes, and a few plasma cells were noted in the fibrosis. Acini under-

TABLE 11
Incidences of Selected Nonneoplastic Lesions in Rats in the 2-Year Gavage Study of Androstenedione

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

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Poly-3 test
** $P \leq 0.01$
[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

going atrophy 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

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	5/5	24.7 ± 0.5	27.9 ± 1.0	3.2 ± 0.5	
1	5/5	23.0 ± 0.6*	25.6 ± 0.5**	2.6 ± 0.2	92
5	5/5	23.2 ± 0.2*	25.5 ± 0.3**	2.3 ± 0.4	92
10	5/5	23.3 ± 0.2*	24.7 ± 0.3**	1.5 ± 0.2*	89
20	5/5	22.3 ± 0.4**	24.9 ± 0.4**	2.6 ± 0.2	89
50	5/5	22.3 ± 0.4**	25.1 ± 0.5**	2.8 ± 0.7	90
Female					
0	4/5[c]	20.1 ± 0.4	21.1 ± 0.1	1.3 ± 0.2	
1	5/5	19.4 ± 0.4	21.7 ± 0.4	2.3 ± 0.6	103
5	5/5	19.2 ± 0.2	19.9 ± 0.4	0.7 ± 0.4	94
10	5/5	19.6 ± 0.1	21.3 ± 0.3	1.6 ± 0.3	101
20	4/5[c]	19.2 ± 0.3	20.2 ± 0.5	0.8 ± 0.5	96
50	4/5[c]	18.8 ± 0.3*	20.3 ± 0.2	1.4 ± 0.3	96

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[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 13
Survival and Body Weights of Mice in the 3-Month Gavage Study of Androstenedione

Dose (mg/kg)	Survival[a]	Mean Body Weight[b] (g)			Final Weight Relative to Controls (%)
		Initial	Final	Change	
Male					
0	10/10	23.5 ± 0.3	37.5 ± 0.9	14.0 ± 0.7	
1	10/10	23.5 ± 0.4	37.9 ± 1.2	14.4 ± 1.0	101
5	10/10	23.3 ± 0.3	38.7 ± 0.9	15.4 ± 0.8	103
10	10/10	23.4 ± 0.4	39.1 ± 1.3	15.7 ± 1.4	104
20	10/10	23.7 ± 0.3	39.3 ± 0.9	15.5 ± 0.7	105
50	10/10	23.1 ± 0.5	38.2 ± 1.2	15.1 ± 1.0	102
Female					
0	10/10	19.2 ± 0.3	30.9 ± 1.0	11.7 ± 1.0	
1	10/10	19.5 ± 0.3	32.0 ± 1.5	12.6 ± 1.3	104
5	10/10	19.1 ± 0.2	31.6 ± 0.9	12.4 ± 0.8	102
10	9/10[c]	19.1 ± 0.3	30.9 ± 1.0	11.7 ± 0.8	100
20	10/10	19.1 ± 0.2	30.8 ± 0.8	11.8 ± 0.8	100
50	10/10	19.1 ± 0.2	29.3 ± 0.7	10.2 ± 0.6	95

[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
Incidences of Selected Nonneoplastic Lesions in Mice in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
Bone Marrow[a]	10	10	10	10	10	10
Hyperplasia[b]	0	0	4* (1.3)[c]	1 (1.0)	2 (1.5)	5* (1.2)
Female						
Adrenal Cortex	10	10	10	10	10	10
X-Zone Atrophy	0	0	6** (1.7)	9** (1.8)	10** (2.5)	10** (2.8)
X-Zone Cytoplasmic Vacuolization	10 (1.6)	10 (1.7)	10 (1.4)	10 (1.2)	1** (1.0)	0**

* Significantly different ($P \leq 0.05$) from the vehicle control group by the Fisher exact test
** $P \leq 0.01$
[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

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 15
Survival of Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Male				
Animals initially in study	50	50	50	50
Moribund	5	2	5	3
Natural deaths	9	4	11	10
Animals surviving to study termination	36	44	34	37
Percent probability of survival at end of study[a]	72	88	68	74
Mean survival (days)[b]	687	720	689	699
Survival analysis[c]	P=0.797	P=0.059N	P=0.949	P=0.897N
	Vehicle Control	**2 mg/kg**	**10 mg/kg**	**50 mg/kg**
Female				
Animals initially in study	50	50	50	50
Accidental deaths[d]	2	0	1	1
Moribund	6	2	3	3
Natural deaths	7	8	6	6
Animals surviving to study termination	35[e]	40	40[e]	40
Percent probability of survival at end of study	73	80	82	82
Mean survival (days)	688	702	692	697
Survival analysis	P=0.615N	P=0.579N	P=0.493N	P=0.431N

[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

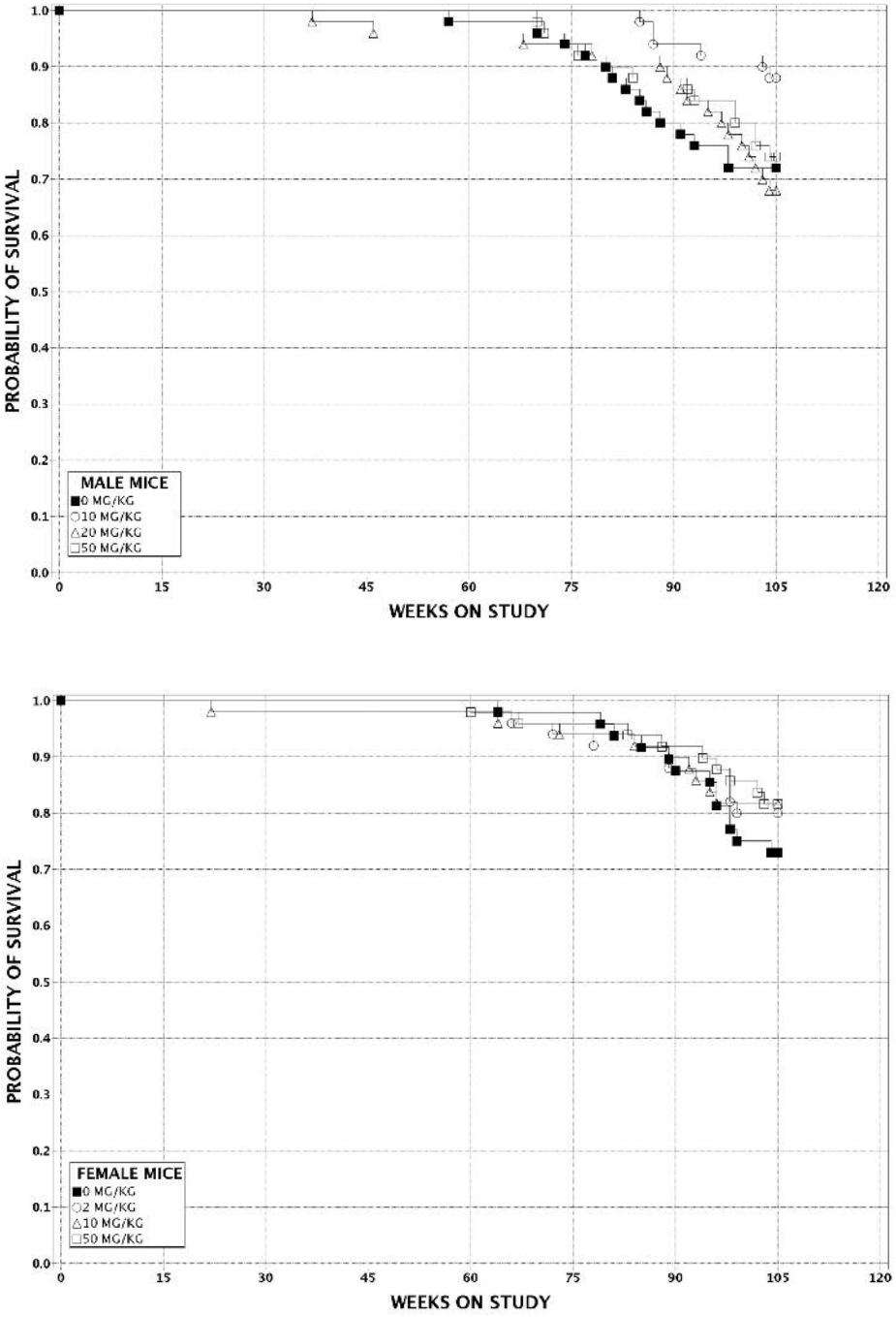


FIGURE 4
Kaplan-Meier Survival Curves for Male and Female Mice Administered Androstenedione by Gavage for 2 Years

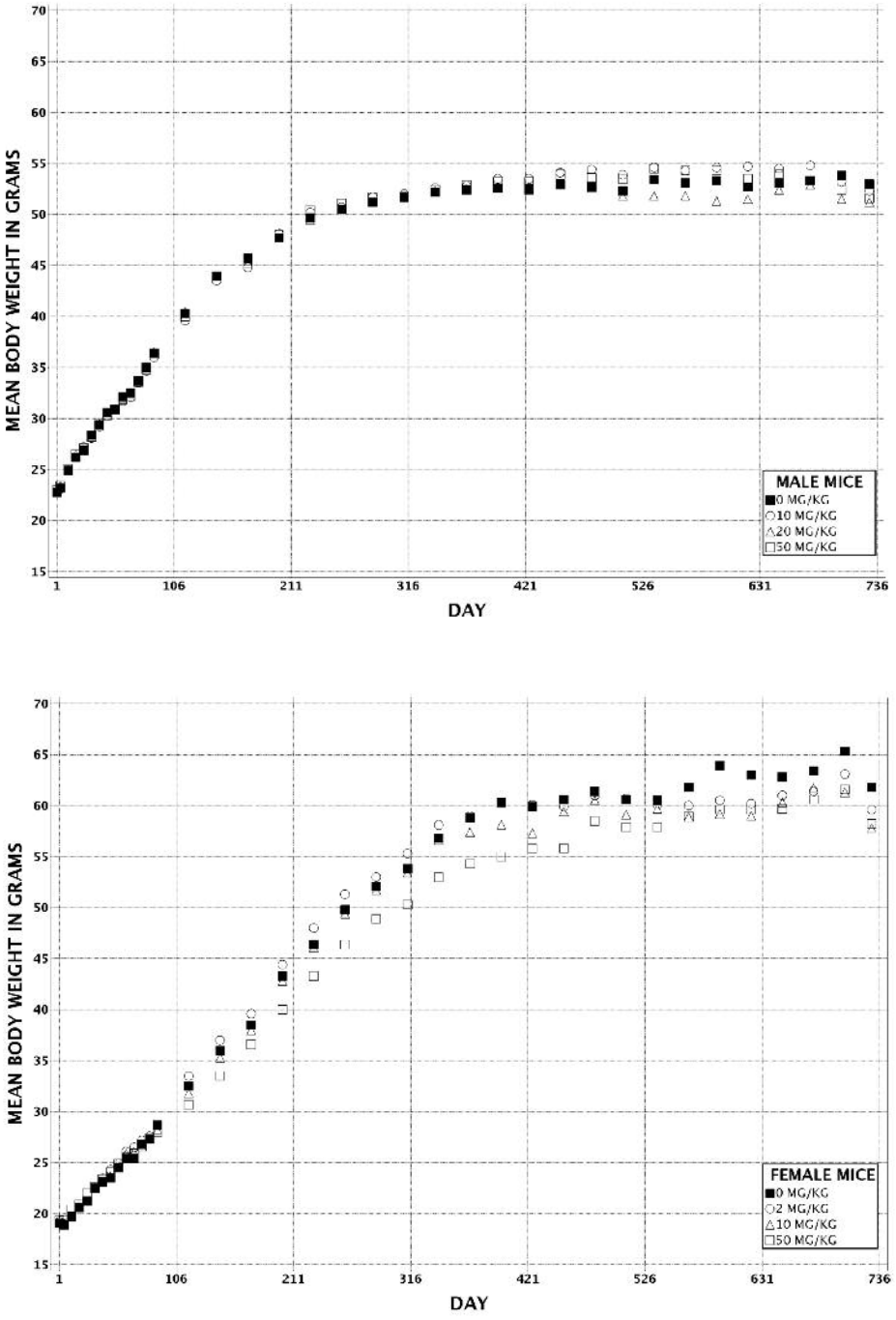


FIGURE 5
Growth Curves for Male and Female Mice Administered Androstenedione by Gavage for 2 Years

TABLE 16
Mean Body Weights and Survival of Male Mice in the 2-Year Gavage Study of Androstenedione

Weeks on Study	Vehicle Control		10 mg/kg			20 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	23.2	50	23.1	100	50	23.4	101	50	23.4	101	50
2	24.9	50	24.9	100	50	25.0	100	50	25.1	101	50
3	26.2	50	26.2	100	50	26.5	101	50	26.5	101	50
4	26.9	50	27.2	101	50	27.0	100	50	27.1	101	50
5	28.4	50	28.1	99	50	28.2	99	50	28.2	99	50
6	29.4	50	29.2	99	50	29.5	100	50	29.3	100	50
7	30.6	50	30.3	99	50	30.6	100	50	30.3	99	50
8	30.9	50	30.9	100	50	31.0	100	50	30.9	100	50
9	32.1	50	31.7	99	50	31.9	99	50	32.0	100	50
10	32.5	50	32.1	99	50	32.4	100	50	32.2	99	50
11	33.7	50	33.5	99	50	33.7	100	50	33.6	100	50
12	35.0	50	34.7	99	50	35.0	100	50	34.8	99	50
13	36.4	50	36.0	99	50	36.5	100	50	36.4	100	50
17	40.3	50	39.6	98	50	40.5	101	50	40.0	99	50
21	44.0	50	43.5	99	50	44.0	100	50	43.7	99	50
25	45.7	50	44.8	98	50	45.4	100	50	45.2	99	50
29	47.7	50	48.1	101	50	47.7	100	50	48.0	101	50
33	49.7	50	50.2	101	50	49.5	99	50	50.4	101	50
37	50.5	50	50.7	101	50	50.5	100	49	51.1	101	50
41	51.2	50	51.7	101	50	51.2	100	49	51.7	101	50
45	51.7	50	52.0	101	50	51.7	100	49	51.8	100	50
49	52.2	50	52.6	101	50	52.3	100	48	52.4	100	50
53	52.4	50	52.8	101	50	52.5	100	48	52.9	101	50
57	52.6	50	53.5	102	50	52.7	100	48	53.2	101	50
61	52.5	49	53.5	102	50	52.4	100	48	53.2	101	50
65	53.0	49	54.1	102	50	52.9	100	48	53.9	102	50
69	52.7	49	54.4	103	50	52.6	100	47	53.6	102	50
73	52.3	48	53.9	103	50	51.8	99	47	53.5	102	48
77	53.4	46	54.6	102	50	51.8	97	47	54.5	102	46
81	53.1	44	54.3	102	50	51.8	98	46	54.3	102	45
85	53.3	42	54.6	103	50	51.3	96	46	54.3	102	44
89	52.7	40	54.7	104	47	51.5	98	44	53.5	102	44
93	53.1	39	54.5	103	47	52.4	99	42	53.9	102	42
97	53.3	38	54.8	103	46	52.9	99	40	53.3	100	42
101	53.8	36	53.2	99	46	51.5	96	37	52.4	98	40
Mean for weeks											
1-13	30.0		29.8	99		30.1	100		30.0	100	
14-52	48.1		48.1	100		48.1	100		48.3	100	
53-101	52.9		54.1	102		52.2	99		53.6	101	

TABLE 17
Mean Body Weights and Survival of Female Mice in the 2-Year Gavage Study of Androstenedione

Weeks on Study	Vehicle Control		2 mg/kg			10 mg/kg			50 mg/kg		
	Av. Wt. (g)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors	Av. Wt. (g)	Wt. (% of controls)	No. of Survivors
1	18.9	50	19.0	101	50	18.9	100	50	19.4	103	50
2	19.8	50	19.8	100	50	19.7	99	50	20.4	103	50
3	20.6	50	20.6	100	50	20.6	100	50	20.9	102	50
4	21.3	50	21.3	100	50	21.2	99	50	22.0	104	50
5	22.5	50	22.7	101	50	22.6	100	50	22.7	101	50
6	23.1	50	23.5	102	50	23.1	100	50	23.4	101	50
7	23.5	50	24.3	104	50	23.7	101	50	24.1	103	50
8	24.5	50	24.9	102	50	24.6	101	50	24.9	102	50
9	25.4	50	26.1	103	50	25.9	102	50	25.6	101	50
10	25.4	50	26.5	105	50	26.1	103	50	25.9	102	50
11	26.8	50	27.2	102	50	26.6	99	50	26.6	99	50
12	27.3	50	27.6	101	50	27.3	100	50	27.4	101	50
13	28.7	50	28.7	100	50	28.2	98	50	28.0	98	50
17	32.5	50	33.5	103	50	31.8	98	50	30.7	95	50
21	36.0	50	37.0	103	50	35.3	98	50	33.5	93	50
25	38.5	50	39.6	103	50	38.0	99	49	36.6	95	49
29	43.3	50	44.4	103	50	42.8	99	49	40.0	92	49
33	46.4	49	48.0	103	50	46.1	99	49	43.3	93	49
37	49.8	49	51.3	103	50	49.4	99	49	46.4	93	49
41	52.1	49	53.0	102	50	51.7	99	49	48.9	94	49
45	53.8	49	55.3	103	50	53.4	99	49	50.3	94	49
49	56.8	49	58.1	102	50	56.6	100	49	53.0	93	49
53	58.8	49	59.0	100	50	57.4	98	49	54.3	92	49
57	60.3	49	60.3	100	50	58.1	96	49	55.0	91	49
61	59.9	49	60.1	100	50	57.3	96	49	55.8	93	48
65	60.6	48	59.9	99	49	59.4	98	48	55.8	92	48
69	61.4	47	61.0	100	48	60.5	99	48	58.5	95	47
73	60.6	47	60.7	100	47	59.1	98	47	57.9	96	47
77	60.5	47	60.1	99	47	59.7	99	46	57.9	96	47
81	61.8	45	60.0	97	46	58.9	95	46	58.9	95	47
85	63.9	44	60.5	95	46	59.2	93	45	59.6	93	46
89	63.0	44	60.2	96	45	59.0	94	44	59.6	95	45
93	62.8	42	61.0	97	44	60.3	96	42	59.7	95	45
97	63.4	39	61.4	97	44	61.7	97	40	60.6	96	43
101	65.3	36	63.1	97	40	61.3	94	40	61.6	94	42
Mean for weeks											
1-13	23.7		24.0	101		23.7	100		23.9	101	
14-52	45.5		46.7	103		45.0	99		42.5	94	
53-101	61.7		60.6	98		59.4	96		58.1	94	

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

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

TABLE 18
Incidences of Neoplasms and Nonneoplastic Lesions of the Liver in Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Female				
Number Examined Microscopically	50	50	50	50
Mixed Cell Focus	2	5	7	15**
Hepatocyte, Vacuolization Cytoplasmic	6 (2.7)[k]	3 (2.3)	9 (2.1)	22** (2.6)
Hepatocellular Adenoma, Multiple	4	7	7	17**
Hepatocellular Adenoma (includes multiple)[l]				
Overall rate	14/50 (28%)	16/50 (32%)	18/50 (36%)	28/50 (56%)
Adjusted rate	31.6%	34.6%	39.1%	61.1%
Terminal rate	13/35 (37%)	14/40 (35%)	16/40 (40%)	27/40 (68%)
First incidence (days)	622	622	520	708
Poly-3 test	P<0.001	P=0.468	P=0.299	P=0.003
Hepatocellular Carcinoma, Multiple	1	2	5	4
Hepatocellular Carcinoma (includes multiple)[m]				
Overall rate	5/50 (10%)	13/50 (26%)	15/50 (30%)	15/50 (30%)
Adjusted rate	11.3%	28.2%	32.0%	32.7%
Terminal rate	3/35 (9%)	11/40 (28%)	11/40 (28%)	13/40 (33%)
First incidence (days)	687	685	442	708
Poly-3 test	P=0.098	P=0.038	P=0.015	P=0.012
Hepatocellular Adenoma or Carcinoma[n]				
Overall rate	17/50 (34%)	23/50 (46%)	27/50 (54%)	32/50 (64%)
Adjusted rate	38.2%	49.5%	57.0%	69.8%
Terminal rate	14/35 (40%)	20/40 (50%)	22/40 (55%)	30/40 (75%)
First incidence (days)	622	622	442	708
Poly-3 test	P=0.004	P=0.188	P=0.052	P<0.001

* 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

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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Female				
Adenoma[h]				
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)	—[i]	729 (T)	729 (T)	654
Poly-3 test	P=0.137	P=0.248	P=0.063	P=0.064

(T) Terminal sacrifice
[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): 2/100 (2.0% ± 2.8%), range 0%-4%; all routes: 17/1,435 (1.2% ± 1.7%), range 0%-6%
[d] Number of animals with neoplasm per number of animals with pancreatic islets examined microscopically
[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 lower incidence in a dosed group is indicated by **N**.
[h] 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%
[i] 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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Clitoral Gland[a]	47	47	49	50
Hyperplasia[b]	0	2 (1.0)[c]	13** (1.9)	41** (2.0)
Duct, Dilatation	0	2 (1.0)	17** (1.4)	49** (2.6)
Kidney	50	50	50	50
Glomerulus, Metaplasia	2 (1.5)	1 (2.0)	5 (1.0)	27** (2.0)
Submandibular Salivary Gland	49	49	49	50
Cytoplasmic Alteration	0	17** (1.2)	40** (1.4)	45** (2.5)

** Significantly different ($P \leq 0.01$) from the vehicle control group by the Poly-3 test
[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

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

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

(T) Terminal sacrifice
[a] Number of animals with malignant lymphoma per number of animals necropsied
[b] 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%
[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**.

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 normochromatic (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.

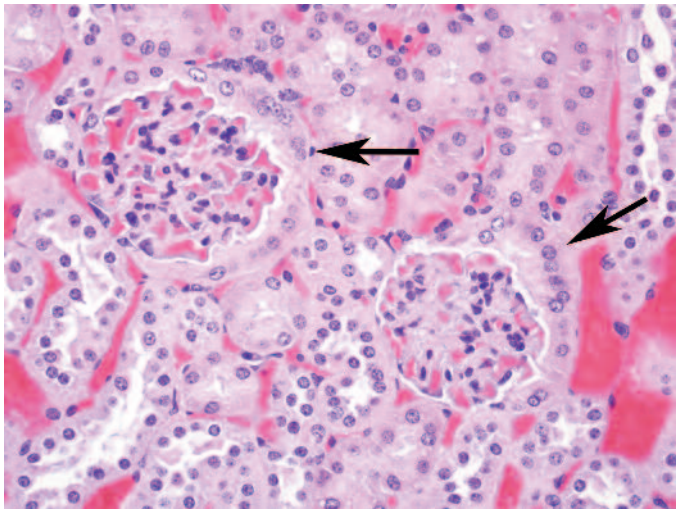


PLATE 1
Glomerular metaplasia in the kidney of a female B6C3F1 mouse administered 50 mg/kg androstenedione by gavage for 2 years. The parietal epithelium lining Bowman's capsule is cuboidal (arrows) rather than flattened. H&E

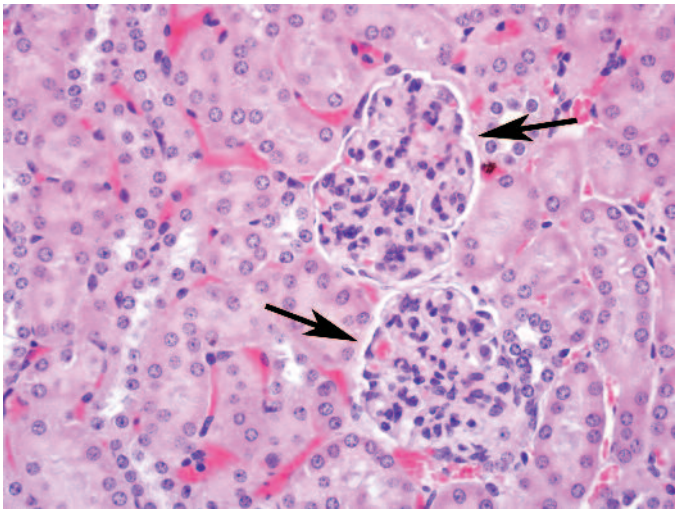


PLATE 2
Glomeruli in the kidney of a female B6C3F1 mouse administered the control vehicle by gavage for 2 years. The parietal epithelium is typically flattened (arrows) representing a normal sexual dimorphism in this species. H&E

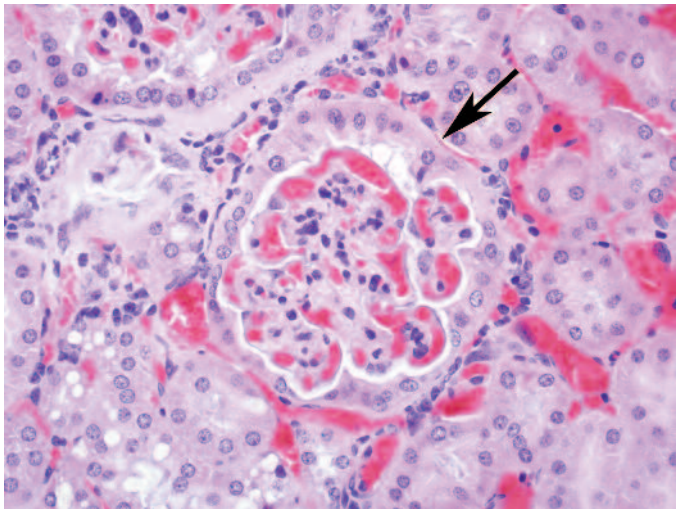


PLATE 3
Glomerulus in the kidney of a male B6C3F1 mouse administered the control vehicle by gavage for 2 years. The parietal epithelium is typically cuboidal (arrow) representing a normal sexual dimorphism in this species. H&E

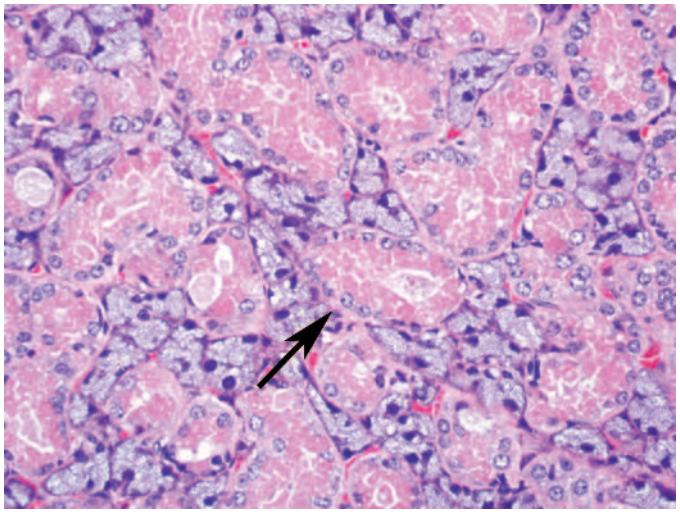


Plate 4
Cytoplasmic alteration in the submandibular salivary gland of a female B6C3F1 mouse administered 50 mg/kg androstenedione by gavage for 2 years. Note the increased prominence and size of the convoluted ducts and increased amount of eosinophilic granular material within the cytoplasm of the duct epithelial cells (arrow). H&E

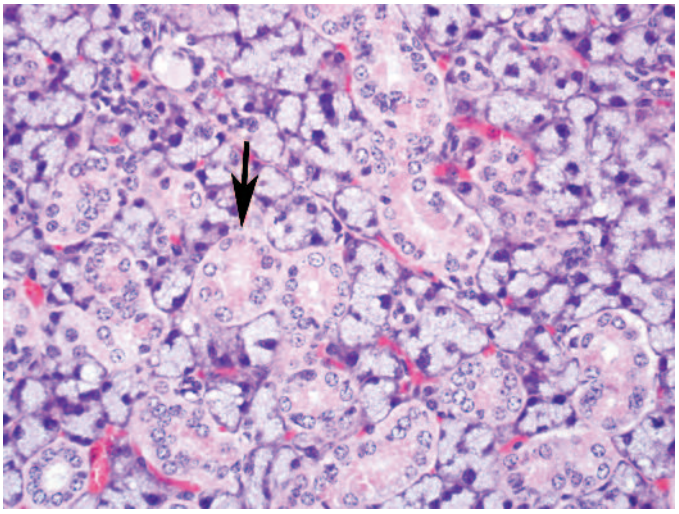


Plate 5
Submandibular salivary gland of a female B6C3F1 mouse administered the control vehicle for 2 years. The ducts are smaller and there is less intracytoplasmic granular material (arrow) compared to male mice (Plate 6), representing a normal sexual dimorphism in this species. H&E

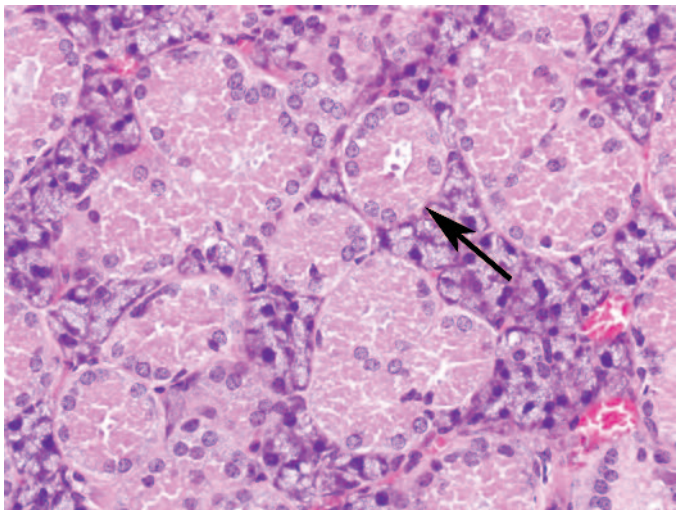


Plate 6
Submandibular salivary gland of a male B6C3F1 mouse administered the control vehicle for 2 years. Compared to a female mouse (Plate 5), the ducts are larger and there is more intracytoplasmic granular material (arrow), representing a normal sexual dimorphism in this species. H&E

DISCUSSION AND CONCLUSIONS

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 testis 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

Tissues with Neoplasms or Neoplasm	Oxymetholone[a]		Androstenedione			
	Male Rats	Female Rats	Male Rats	Female Rats	Male Mice	Female Mice
Testis	↓		↓			
Mammary Gland		↓		↓		
Lung		↑	↑[b]			
Liver		↑			↑	↑
Pituitary Gland		↓				
Skin		↑				
Pancreatic Islet					↑	↑
Mononuclear Cell Leukemia[c]/ Malignant Lymphoma[d]	↓	↓	↓	↑[b]		↓

[a] NTP, 1999
[b] Although a significant increase, this finding was considered to be equivocal evidence of carcinogenicity.
[c] Present in rats
[d] 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.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 12.

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

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	22	9	17	13
Natural deaths	7	8	4	10
Survivors				
Died last week of study	1			
Terminal sacrifice	20	33	29	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Intestine large, cecum	(47)	(46)	(47)	(48)
Intestine large, colon	(44)	(43)	(47)	(44)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Intestine large, rectum	(46)	(46)	(47)	(47)
Intestine small, duodenum	(48)	(47)	(49)	(47)
Intestine small, ileum	(44)	(46)	(47)	(44)
Osteosarcoma			1 (2%)	
Intestine small, jejunum	(44)	(44)	(47)	(42)
Liver	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Carcinoma, metastatic, islets, pancreatic		1 (2%)		
Hepatocellular adenoma	2 (4%)		1 (2%)	3 (6%)
Hepatocellular carcinoma		1 (2%)		1 (2%)
Osteosarcoma, metastatic, uncertain primary site				1 (2%)
Sarcoma, metastatic, skeletal muscle				1 (2%)
Mesentery	(18)	(13)	(12)	(18)
Adenocarcinoma, metastatic, uncertain primary site			1 (8%)	
Pancreas	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Mixed tumor benign				1 (2%)
Acinus, adenoma	3 (6%)		2 (4%)	
Salivary glands	(50)	(50)	(50)	(50)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Sarcoma	1 (2%)			
Schwannoma malignant			1 (2%)	
Stomach, forestomach	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Adenoma, multiple				1 (2%)
Tongue	(1)	(0)	(0)	(2)
Squamous cell papilloma	1 (100%)			
Tooth	(0)	(0)	(1)	(0)
Cardiovascular System				
Blood vessel	(2)	(3)	(1)	(0)
Carcinoma, metastatic, thyroid gland		1 (33%)		
Heart	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, uncertain primary site			1 (2%)	

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Adenoma	3 (6%)			1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	5 (10%)	10 (20%)	3 (6%)	3 (6%)
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma malignant			1 (2%)	
Bilateral, pheochromocytoma benign	2 (4%)	2 (4%)	1 (2%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	4 (8%)	3 (6%)	9 (18%)	1 (2%)
Carcinoma		1 (2%)		1 (2%)
Parathyroid gland	(48)	(50)	(50)	(48)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	19 (38%)	21 (42%)	15 (31%)	24 (48%)
Pars intermedia, carcinoma		1 (2%)		
Thyroid gland	(50)	(50)	(50)	(50)
Schwannoma malignant			1 (2%)	
C-cell, adenoma	3 (6%)	3 (6%)	4 (8%)	4 (8%)
C-cell, carcinoma	1 (2%)	2 (4%)	2 (4%)	2 (4%)
Follicular cell, adenoma	3 (6%)		1 (2%)	
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Paraganglioma			1 (100%)	
Genital System				
Coagulating gland	(0)	(3)	(2)	(0)
Adenocarcinoma, metastatic, uncertain primary site			1 (50%)	
Epididymis	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Penis	(0)	(1)	(0)	(0)
Preputial gland	(50)	(50)	(50)	(50)
Adenoma	3 (6%)	2 (4%)		4 (8%)
Carcinoma			1 (2%)	1 (2%)
Prostate	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Seminal vesicle	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Testes	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Bilateral, interstitial cell, adenoma	29 (58%)	29 (58%)	28 (56%)	10 (20%)
Interstitial cell, adenoma	13 (26%)	10 (20%)	8 (16%)	16 (32%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(25)	(18)	(16)	(13)
Adenocarcinoma, metastatic, uncertain primary site			1 (6%)	
Hemangiosarcoma	1 (4%)			
Deep cervical, carcinoma, metastatic, thyroid gland		1 (6%)		
Lymph node, mandibular	(1)	(0)	(1)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Spleen	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Basal cell carcinoma, metastatic, skin			1 (2%)	
Sarcoma			1 (2%)	
Thymus	(50)	(50)	(50)	(49)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Thymoma malignant			1 (2%)	
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Fibroadenoma	2 (4%)	5 (10%)		6 (12%)
Fibroma	1 (2%)	1 (2%)		
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma	1 (2%)	2 (4%)	4 (8%)	
Keratoacanthoma	3 (6%)	3 (6%)	3 (6%)	1 (2%)
Keratoacanthoma, multiple		1 (2%)		
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Pinna, schwannoma malignant		1 (2%)		
Sebaceous gland, adenoma			2 (4%)	
Sebaceous gland, carcinoma				1 (2%)
Subcutaneous tissue, fibroma	5 (10%)	7 (14%)	9 (18%)	5 (10%)
Subcutaneous tissue, fibrosarcoma	1 (2%)			2 (4%)
Subcutaneous tissue, fibrous histiocytoma				1 (2%)
Subcutaneous tissue, lipoma	1 (2%)			
Subcutaneous tissue, sarcoma		1 (2%)		
Subcutaneous tissue, schwannoma malignant		1 (2%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Carcinoma, metastatic, preputial gland				1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Chordoma	1 (2%)			
Schwannoma malignant			1 (2%)	
Humerus, osteosarcoma			1 (2%)	
Vertebra, osteosarcoma	1 (2%)			
Skeletal muscle	(8)	(1)	(3)	(5)
Adenocarcinoma, metastatic, uncertain primary site			1 (33%)	
Carcinoma, metastatic, thyroid gland		1 (100%)		
Fibrous histiocytoma, metastatic, skin				1 (20%)
Sarcoma				1 (20%)

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Nervous System				
Brain	(50)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)			
Spinal cord	(6)	(1)	(0)	(5)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Alveolar/bronchiolar adenoma			5 (10%)	2 (4%)
Alveolar/bronchiolar carcinoma				1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Chordoma, metastatic, bone	1 (2%)			
Chordoma, metastatic, uncertain primary site			1 (2%)	
Osteosarcoma, metastatic, bone	1 (2%)			
Osteosarcoma, metastatic, uncertain primary site				1 (2%)
Squamous cell carcinoma		1 (2%)		
Mediastinum, alveolar/bronchiolar carcinoma				1 (2%)
Mediastinum, carcinoma, metastatic, uncertain primary site			1 (2%)	
Mediastinum, osteosarcoma, metastatic, uncertain primary site				1 (2%)
Serosa, hemangiosarcoma	1 (2%)			
Nose	(50)	(50)	(50)	(50)
Carcinoma, metastatic, preputial gland				1 (2%)
Special Senses System				
Eye	(48)	(46)	(50)	(49)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(1)	(0)
Adenoma			1 (100%)	
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uncertain primary site			1 (2%)	
Renal tubule, adenoma			2 (4%)	1 (2%)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma	1 (2%)	1 (2%)		
Leukemia mononuclear	26 (52%)	22 (44%)	18 (36%)	18 (36%)
Mesothelioma benign			1 (2%)	
Mesothelioma malignant	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Mesothelioma NOS		1 (2%)		

TABLE A1
Summary of the Incidence of Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	49	48	48	49
Total primary neoplasms	141	135	133	120
Total animals with benign neoplasms	47	47	46	46
Total benign neoplasms	103	98	96	87
Total animals with malignant neoplasms	34	29	25	28
Total malignant neoplasms	38	36	36	33
Total animals with metastatic neoplasms	3	3	5	5
Total metastatic neoplasms	4	9	23	9
Total animals with malignant neoplasms of uncertain primary site		1	4	1
Total animals with uncertain neoplasms-benign or malignant		1		
Total uncertain neoplasms		1		

[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 A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Adrenal Cortex: Adenoma				
Overall rate[a]	3/50 (6%)	0/50 (0%)	0/50 (0%)	1/50 (2%)
Adjusted rate[b]	7.6%	0.0%	0.0%	2.3%
Terminal rate[c]	1/21 (5%)	0/33 (0%)	0/29 (0%)	1/27 (4%)
First incidence (days)	688	—[e]	—	729 (T)
Poly-3 test[d]	P=0.365N	P=0.098N	P=0.105N	P=0.274N
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	7/50 (14%)	12/50 (24%)	4/50 (8%)	5/50 (10%)
Adjusted rate	17.5%	26.9%	9.4%	11.6%
Terminal rate	3/21 (14%)	11/33 (33%)	3/29 (10%)	4/27 (15%)
First incidence (days)	612	708	712	687
Poly-3 test	P=0.117N	P=0.218	P=0.221N	P=0.324N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	8/50 (16%)	12/50 (24%)	5/50 (10%)	5/50 (10%)
Adjusted rate	19.7%	26.9%	11.7%	11.6%
Terminal rate	3/21 (14%)	11/33 (33%)	4/29 (14%)	4/27 (15%)
First incidence (days)	554	708	712	687
Poly-3 test	P=0.089N	P=0.298	P=0.239N	P=0.232N
Liver: Hepatocellular Adenoma				
Overall rate	2/50 (4%)	0/50 (0%)	1/50 (2%)	3/50 (6%)
Adjusted rate	5.1%	0.0%	2.3%	7.0%
Terminal rate	1/21 (5%)	0/33 (0%)	1/29 (3%)	2/27 (7%)
First incidence (days)	701	—	729 (T)	717
Poly-3 test	P=0.217	P=0.210N	P=0.471N	P=0.542
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	2/50 (4%)	1/50 (2%)	1/50 (2%)	4/50 (8%)
Adjusted rate	5.1%	2.3%	2.3%	9.2%
Terminal rate	1/21 (5%)	1/33 (3%)	1/29 (3%)	2/27 (7%)
First incidence (days)	701	729 (T)	729 (T)	676
Poly-3 test	P=0.149	P=0.457N	P=0.471N	P=0.383
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	0/50 (0%)	5/50 (10%)	2/50 (4%)
Adjusted rate	0.0%	0.0%	11.6%	4.6%
Terminal rate	0/21 (0%)	0/33 (0%)	2/29 (7%)	2/27 (7%)
First incidence (days)	—	—	631	729 (T)
Poly-3 test	P=0.195	—[f]	P=0.039	P=0.258
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	0/50 (0%)	0/50 (0%)	5/50 (10%)	3/50 (6%)
Adjusted rate	0.0%	0.0%	11.6%	6.9%
Terminal rate	0/21 (0%)	0/33 (0%)	2/29 (7%)	2/27 (7%)
First incidence (days)	—	—	631	687
Poly-3 test	P=0.083	—	P=0.039	P=0.137
Mammary Gland: Fibroadenoma				
Overall rate	2/50 (4%)	5/50 (10%)	0/50 (0%)	6/50 (12%)
Adjusted rate	5.1%	11.2%	0.0%	13.9%
Terminal rate	1/21 (5%)	3/33 (9%)	0/29 (0%)	6/27 (22%)
First incidence (days)	688	708	—	729 (T)
Poly-3 test	P=0.139	P=0.270	P=0.219N	P=0.162

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Mammary Gland: Fibroma or Fibroadenoma				
Overall rate	3/50 (6%)	6/50 (12%)[g]	0/50 (0%)	6/50 (12%)
Adjusted rate	7.6%	13.4%	0.0%	13.9%
Terminal rate	1/21 (5%)	4/33 (12%)	0/29 (0%)	6/27 (22%)
First incidence (days)	688	708	—	729 (T)
Poly-3 test	P=0.283	P=0.305	P=0.105N	P=0.285
Pancreas: Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	2/50 (4%)	0/50 (0%)
Adjusted rate	7.7%	0.0%	4.7%	0.0%
Terminal rate	3/21 (14%)	0/33 (0%)	2/29 (7%)	0/27 (0%)
First incidence (days)	729 (T)	—	729 (T)	—
Poly-3 test	P=0.141N	P=0.097N	P=0.461N	P=0.102N
Pancreatic Islets: Adenoma				
Overall rate	4/50 (8%)	3/50 (6%)	9/50 (18%)	1/50 (2%)
Adjusted rate	10.1%	6.7%	21.0%	2.3%
Terminal rate	2/21 (10%)	2/33 (6%)	8/29 (28%)	0/27 (0%)
First incidence (days)	686	652	674	717
Poly-3 test	P=0.160N	P=0.432N	P=0.146	P=0.152N
Pancreatic Islets: Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	9/50 (18%)	2/50 (4%)
Adjusted rate	10.1%	8.9%	21.0%	4.6%
Terminal rate	2/21 (10%)	2/33 (6%)	8/29 (28%)	0/27 (0%)
First incidence (days)	686	652	674	697
Poly-3 test	P=0.244N	P=0.570N	P=0.146	P=0.296N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	19/50 (38%)	21/50 (42%)	15/49 (31%)	24/50 (48%)
Adjusted rate	45.5%	44.9%	33.8%	52.3%
Terminal rate	10/21 (48%)	13/33 (39%)	9/29 (31%)	14/27 (52%)
First incidence (days)	547	551	547	563
Poly-3 test	P=0.241	P=0.565N	P=0.184N	P=0.331
Preputial Gland: Adenoma				
Overall rate	3/50 (6%)	2/50 (4%)	0/50 (0%)	4/50 (8%)
Adjusted rate	7.6%	4.5%	0.0%	9.2%
Terminal rate	2/21 (10%)	2/33 (6%)	0/29 (0%)	2/27 (7%)
First incidence (days)	592	729 (T)	—	666
Poly-3 test	P=0.331	P=0.448N	P=0.105N	P=0.550
Preputial Gland: Adenoma or Carcinoma				
Overall rate	3/50 (6%)	2/50 (4%)	1/50 (2%)	5/50 (10%)
Adjusted rate	7.6%	4.5%	2.3%	11.4%
Terminal rate	2/21 (10%)	2/33 (6%)	0/29 (0%)	2/27 (7%)
First incidence (days)	592	729 (T)	547	666
Poly-3 test	P=0.190	P=0.448N	P=0.274N	P=0.411
Skin: Keratoacanthoma				
Overall rate	3/50 (6%)	4/50 (8%)	3/50 (6%)	1/50 (2%)
Adjusted rate	7.5%	9.0%	7.0%	2.3%
Terminal rate	1/21 (5%)	3/33 (9%)	2/29 (7%)	1/27 (4%)
First incidence (days)	589	708	712	729 (T)
Poly-3 test	P=0.165N	P=0.558	P=0.633N	P=0.279N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

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

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Testes: Adenoma				
Overall rate	42/50 (84%)	39/50 (78%)	36/50 (72%)	26/50 (52%)
Adjusted rate	91.1%	83.8%	79.2%	58.1%
Terminal rate	20/21 (95%)	30/33 (91%)	24/29 (83%)	19/27 (70%)
First incidence (days)	464	540	558	607
Poly-3 test	P<0.001N	P=0.203N	P=0.070N	P<0.001N
Thyroid Gland (C-cell): Adenoma				
Overall rate	3/50 (6%)	3/50 (6%)	4/50 (8%)	4/50 (8%)
Adjusted rate	7.6%	6.7%	9.2%	9.2%
Terminal rate	1/21 (5%)	2/33 (6%)	2/29 (7%)	2/27 (7%)
First incidence (days)	699	708	605	666
Poly-3 test	P=0.445	P=0.604N	P=0.552	P=0.553
Thyroid Gland (C-cell): Adenoma or Carcinoma				
Overall rate	4/50 (8%)	4/50 (8%)	6/50 (12%)	6/50 (12%)
Adjusted rate	10.2%	9.0%	13.9%	13.8%
Terminal rate	2/21 (10%)	3/33 (9%)	4/29 (14%)	3/27 (11%)
First incidence (days)	699	708	605	666
Poly-3 test	P=0.322	P=0.574N	P=0.430	P=0.434
Thyroid Gland (Follicular Cell): Adenoma				
Overall rate	3/50 (6%)	0/50 (0%)	1/50 (2%)	0/50 (0%)
Adjusted rate	7.6%	0.0%	2.3%	0.0%
Terminal rate	1/21 (5%)	0/33 (0%)	1/29 (3%)	0/27 (0%)
First incidence (days)	699	—	729 (T)	—
Poly-3 test	P=0.119N	P=0.098N	P=0.276N	P=0.103N
All Organs: Benign, Malignant, or NOS Mesothelioma				
Overall rate	1/50 (2%)	2/50 (4%)	3/50 (6%)	2/50 (4%)
Adjusted rate	2.6%	4.4%	6.8%	4.6%
Terminal rate	1/21 (5%)	0/33 (0%)	0/29 (0%)	1/27 (4%)
First incidence (days)	729 (T)	641	442	684
Poly-3 test	P=0.496	P=0.549	P=0.347	P=0.534
All Organs: Mononuclear Cell Leukemia				
Overall rate	26/50 (52%)	22/50 (44%)	18/50 (36%)	18/50 (36%)
Adjusted rate	59.0%	47.8%	39.9%	39.2%
Terminal rate	9/21 (43%)	14/33 (42%)	8/29 (28%)	7/27 (26%)
First incidence (days)	526	629	558	563
Poly-3 test	P=0.052N	P=0.191N	P=0.050N	P=0.042N
All Organs: Benign Neoplasms				
Overall rate	47/50 (94%)	47/50 (94%)	46/50 (92%)	46/50 (92%)
Adjusted rate	98.3%	97.6%	95.2%	95.4%
Terminal rate	21/21 (100%)	33/33 (100%)	28/29 (97%)	27/27 (100%)
First incidence (days)	464	540	442	563
Poly-3 test	P=0.270N	P=0.746N	P=0.373N	P=0.379N
All Organs: Malignant Neoplasms				
Overall rate	34/50 (68%)	29/50 (58%)	28/50 (56%)	29/50 (58%)
Adjusted rate	72.2%	62.7%	59.4%	60.2%
Terminal rate	10/21 (48%)	20/33 (61%)	13/29 (45%)	11/27 (41%)
First incidence (days)	456	629	387	563
Poly-3 test	P=0.182N	P=0.220N	P=0132N	P=0.150N

TABLE A2
Statistical Analysis of Primary Neoplasms in Male Rats in the 2-Year Gavage Study of Androstenedione

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

(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[a]

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

[a] Data as of November 17, 2008

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

Study (Study Start)	Incidence in Controls		
	Alveolar/Bronchiolar Adenoma	Alveolar/Bronchiolar Carcinoma	Alveolar/Bronchiolar Adenoma or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies			
Androstenedione (February, 2003)	0/50	0/50	0/50
Methylene blue trihydrate (June, 2000)	1/50	0/50	1/50
Total (%)	1/100 (1.0%)	0/100	1/100 (1.0%)
Mean ± standard deviation	1.0% ± 1.4%		1.0% ± 1.4%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	34/1,399 (2.4%)	13/1,399 (0.9%)	47/1,399 (3.4%)
Mean ± standard deviation	2.4% ± 2.8%	0.9% ± 1.0%	3.4% ± 3.0%
Range	0%-8%	0%-2%	0%-10%

[a] Data as of November 17, 2008

TABLE A3c
Historical Incidence of Adenoma of the Testis in Control Male F344/N Rats[a]

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

[a] 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[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	22	9	17	13
Natural deaths	7	8	4	10
Survivors				
Died last week of study	1			
Terminal sacrifice	20	33	29	27
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(50)	(50)
Foreign body	1 (2%)			
Inflammation, suppurative	1 (2%)			
Intestine large, cecum	(47)	(46)	(47)	(48)
Edema			1 (2%)	
Intestine large, colon	(44)	(43)	(47)	(44)
Edema	2 (5%)	1 (2%)	4 (9%)	
Fibrosis	1 (2%)			
Intestine large, rectum	(46)	(46)	(47)	(47)
Hemorrhage			1 (2%)	
Intestine small, duodenum	(48)	(47)	(49)	(47)
Hemorrhage			1 (2%)	
Intestine small, ileum	(44)	(46)	(47)	(44)
Intestine small, jejunum	(44)	(44)	(47)	(42)
Hemorrhage			1 (2%)	
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Basophilic focus	17 (34%)	29 (58%)	29 (58%)	33 (66%)
Clear cell focus	13 (26%)	21 (42%)	23 (46%)	14 (28%)
Clear cell focus, multiple				3 (6%)
Degeneration, cystic	10 (20%)	3 (6%)	12 (24%)	7 (14%)
Eosinophilic focus	3 (6%)	10 (20%)	7 (14%)	13 (26%)
Hematopoietic cell proliferation		1 (2%)		
Hemorrhage			1 (2%)	
Hepatodiaphragmatic nodule	7 (14%)	8 (16%)	7 (14%)	10 (20%)
Infiltration cellular, mixed cell	10 (20%)	3 (6%)	11 (22%)	4 (8%)
Inflammation, chronic	1 (2%)			
Mixed cell focus	1 (2%)	2 (4%)	1 (2%)	4 (8%)
Necrosis, focal	4 (8%)		1 (2%)	1 (2%)
Tension lipidosis	1 (2%)			
Thrombosis			1 (2%)	
Bile duct, hyperplasia	44 (88%)	44 (88%)	40 (80%)	43 (86%)
Hepatocyte, hyperplasia	1 (2%)		2 (4%)	2 (4%)
Hepatocyte, vacuolization cytoplasmic	25 (50%)	18 (36%)	14 (28%)	9 (18%)
Kupffer cell, pigmentation	1 (2%)			

[a] 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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Alimentary System (continued)				
Mesentery	(18)	(13)	(12)	(18)
Accessory spleen	4 (22%)	1 (8%)		3 (17%)
Hemorrhage		1 (8%)		
Inflammation, suppurative	1 (6%)			
Inflammation, granulomatous	1 (6%)			
Fat, necrosis	13 (72%)	10 (77%)	10 (83%)	15 (83%)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	17 (34%)	30 (60%)	19 (38%)	21 (42%)
Cyst	5 (10%)	3 (6%)	7 (14%)	
Infiltration cellular, lymphocyte				1 (2%)
Acinus, cytoplasmic alteration	4 (8%)	3 (6%)	2 (4%)	2 (4%)
Acinus, hyperplasia, focal	1 (2%)	3 (6%)	3 (6%)	2 (4%)
Arteriole, inflammation, chronic		1 (2%)		
Salivary glands	(50)	(50)	(50)	(50)
Atrophy				1 (2%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	4 (8%)	7 (14%)	4 (8%)	4 (8%)
Erosion	1 (2%)			
Hemorrhage			1 (2%)	
Inflammation, chronic active		1 (2%)		
Ulcer	6 (12%)	7 (14%)	4 (8%)	5 (10%)
Epithelium, hyperplasia	5 (10%)	6 (12%)	3 (6%)	5 (10%)
Stomach, glandular	(50)	(50)	(50)	(50)
Edema				1 (2%)
Erosion	6 (12%)	2 (4%)	4 (8%)	5 (10%)
Ulcer	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Epithelium, cyst	1 (2%)			1 (2%)
Epithelium, hyperplasia	1 (2%)		2 (4%)	2 (4%)
Muscularis, hyperplasia				1 (2%)
Tongue	(1)	(0)	(0)	(2)
Hyperplasia, squamous				2 (100%)
Tooth	(0)	(0)	(1)	(0)
Malformation			1 (100%)	
Cardiovascular System				
Blood vessel	(2)	(3)	(1)	(0)
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	47 (94%)	47 (94%)	48 (96%)	48 (96%)
Thrombosis	6 (12%)	3 (6%)	3 (6%)	2 (4%)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Degeneration, fatty	22 (44%)	27 (54%)	25 (50%)	21 (42%)
Hyperplasia, focal	5 (10%)	4 (8%)	3 (6%)	1 (2%)
Hypertrophy, focal	3 (6%)	3 (6%)	2 (4%)	4 (8%)
Hypertrophy, diffuse				1 (2%)
Necrosis	1 (2%)			
Bilateral, necrosis		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	8 (16%)	6 (12%)	10 (20%)
Infiltration cellular, lymphocyte				1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia	5 (10%)	4 (8%)	2 (4%)	9 (18%)
Parathyroid gland	(48)	(50)	(50)	(48)
Cyst		1 (2%)		
Hyperplasia				1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, angiectasis	1 (2%)	1 (2%)		2 (4%)
Pars distalis, cyst	2 (4%)	3 (6%)	1 (2%)	4 (8%)
Pars distalis, hyperplasia, focal	13 (26%)	15 (30%)	21 (43%)	18 (36%)
Pars intermedia, angiectasis				1 (2%)
Pars intermedia, cyst			2 (4%)	
Pars intermedia, hyperplasia, focal			1 (2%)	
Rathke's cleft, cyst			1 (2%)	
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst	1 (2%)	1 (2%)	2 (4%)	
C-cell, hyperplasia	32 (64%)	33 (66%)	26 (52%)	20 (40%)
Follicle, cyst		1 (2%)	5 (10%)	1 (2%)
Follicular cell, hyperplasia		2 (4%)	2 (4%)	1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Genital System				
Coagulating gland	(0)	(3)	(2)	(0)
Inflammation, suppurative		1 (33%)		
Epithelium, hyperplasia		1 (33%)		
Epididymis	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Degeneration	1 (2%)			
Inflammation, chronic				1 (2%)
Penis	(0)	(1)	(0)	(0)
Inflammation, chronic		1 (100%)		
Preputial gland	(50)	(50)	(50)	(50)
Cyst	4 (8%)	2 (4%)		7 (14%)
Fibrosis				1 (2%)
Hyperplasia	1 (2%)	1 (2%)		1 (2%)
Inflammation, chronic	47 (94%)	48 (96%)	41 (82%)	43 (86%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	25 (50%)	24 (48%)	20 (40%)	20 (40%)
Epithelium, hyperplasia	9 (18%)	18 (36%)	9 (18%)	12 (24%)
Seminal vesicle	(50)	(50)	(50)	(50)

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Genital System (continued)				
Testes	(50)	(50)	(50)	(50)
Malformation				1 (2%)
Thrombosis	1 (2%)			
Germinal epithelium, atrophy	9 (18%)	8 (16%)	10 (20%)	11 (22%)
Interstitial cell, hyperplasia	4 (8%)	2 (4%)	2 (4%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	4 (8%)	4 (8%)	7 (14%)
Myelofibrosis	1 (2%)	2 (4%)		
Lymph node	(25)	(18)	(16)	(13)
Mediastinal, angiectasis		1 (6%)		
Mediastinal, ectasia	1 (4%)		2 (13%)	
Mediastinal, fibrosis	1 (4%)			
Mediastinal, hemorrhage		1 (6%)	1 (6%)	
Mediastinal, hyperplasia, histiocytic	1 (4%)			
Mediastinal, hyperplasia, lymphoid	3 (12%)	3 (17%)		
Pancreatic, angiectasis				1 (8%)
Pancreatic, atrophy	1 (4%)			
Pancreatic, ectasia	3 (12%)	1 (6%)	2 (13%)	
Pancreatic, hemorrhage	1 (4%)		1 (6%)	
Pancreatic, hyperplasia, histiocytic	7 (28%)		3 (19%)	2 (15%)
Pancreatic, hyperplasia, lymphoid	1 (4%)			
Pancreatic, necrosis		1 (6%)		
Lymph node, mandibular	(1)	(0)	(1)	(2)
Lymph node, mesenteric	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			
Ectasia	3 (6%)	5 (10%)	7 (14%)	5 (10%)
Fibrosis	1 (2%)			
Hemorrhage			2 (4%)	
Hyperplasia, histiocytic	25 (50%)	13 (26%)	25 (50%)	19 (38%)
Hyperplasia, lymphoid	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Infiltration cellular, eosinophil	1 (2%)			
Infiltration cellular, plasma cell	1 (2%)			
Pigmentation				2 (4%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen		1 (2%)	1 (2%)	
Fibrosis	6 (12%)	1 (2%)	4 (8%)	
Hematopoietic cell proliferation	2 (4%)		4 (8%)	2 (4%)
Hemorrhage	2 (4%)			
Hyperplasia, histiocytic	1 (2%)			1 (2%)
Hyperplasia, lymphoid	1 (2%)			
Necrosis	2 (4%)	1 (2%)	1 (2%)	
Pigmentation	12 (24%)	12 (24%)	8 (16%)	12 (24%)
Lymphoid follicle, hyperplasia		3 (6%)	4 (8%)	3 (6%)
Red pulp, hyperplasia			1 (2%)	
Thymus	(50)	(50)	(50)	(49)
Atrophy			2 (4%)	
Cyst			1 (2%)	
Hemorrhage		1 (2%)	1 (2%)	
Hyperplasia, lymphoid	2 (4%)			

TABLE A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Integumentary System				
Mammary gland	(48)	(50)	(50)	(50)
Cyst	8 (17%)	4 (8%)	4 (8%)	11 (22%)
Hyperplasia	38 (79%)	37 (74%)	33 (66%)	42 (84%)
Inflammation, granulomatous	1 (2%)			
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion	3 (6%)	1 (2%)	1 (2%)	2 (4%)
Fibrosis		2 (4%)	1 (2%)	3 (6%)
Hyperkeratosis	5 (10%)	3 (6%)	6 (12%)	7 (14%)
Inflammation, suppurative				1 (2%)
Inflammation, granulomatous				1 (2%)
Inflammation, chronic			1 (2%)	1 (2%)
Ulcer				1 (2%)
Epidermis, hyperplasia	3 (6%)	1 (2%)	3 (6%)	3 (6%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis	1 (2%)			1 (2%)
Skeletal muscle	(8)	(1)	(3)	(5)
Fibrosis	3 (38%)		2 (67%)	1 (20%)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	13 (26%)	8 (16%)	7 (14%)	9 (18%)
Gliosis	1 (2%)			
Hemorrhage	3 (6%)		2 (4%)	4 (8%)
Necrosis				2 (4%)
Spinal cord	(6)	(1)	(0)	(5)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion			1 (2%)	
Foreign body			1 (2%)	
Hemorrhage	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, histiocyte	17 (34%)	17 (34%)	18 (36%)	18 (36%)
Inflammation, granulomatous		1 (2%)	1 (2%)	1 (2%)
Inflammation, chronic			1 (2%)	3 (6%)
Metaplasia, osseous		1 (2%)	1 (2%)	
Alveolar epithelium, hyperplasia	6 (12%)	7 (14%)	8 (16%)	4 (8%)
Serosa, cyst				1 (2%)
Nose	(50)	(50)	(50)	(50)
Foreign body	5 (10%)	13 (26%)	5 (10%)	
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation, suppurative	3 (6%)	4 (8%)	1 (2%)	1 (2%)
Inflammation, chronic	4 (8%)	3 (6%)	5 (10%)	1 (2%)
Nasolacrimal duct, inflammation, chronic				1 (2%)
Respiratory epithelium, hyperplasia	8 (16%)	11 (22%)	7 (14%)	3 (6%)
Sinus, inflammation, suppurative	1 (2%)			
Vomeronasal organ, atrophy			1 (2%)	

Table A4
Summary of the Incidence of Nonneoplastic Lesions in Male Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Special Senses System				
Eye	(48)	(46)	(50)	(49)
Cataract	1 (2%)		1 (2%)	2 (4%)
Ciliary body, hyperplasia	1 (2%)			
Retina, degeneration	1 (2%)	1 (2%)	1 (2%)	4 (8%)
Retina, edema				1 (2%)
Sclera, metaplasia, osseous		3 (7%)	2 (4%)	1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation, chronic				1 (2%)
Necrosis	1 (2%)			
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(49)	(50)	(50)	(50)
Cyst				1 (2%)
Hydronephrosis				1 (2%)
Infarct	3 (6%)	1 (2%)		
Nephropathy	42 (86%)	48 (96%)	46 (92%)	44 (88%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	1 (2%)		1 (2%)
Renal tubule, necrosis	1 (2%)	1 (2%)	2 (4%)	
Renal tubule, pigmentation	5 (10%)	2 (4%)	6 (12%)	6 (12%)
Urinary bladder	(50)	(50)	(50)	(50)
Calculus microscopic observation only		1 (2%)		
Hemorrhage				1 (2%)
Inflammation, chronic		1 (2%)		
Transitional epithelium, hyperplasia		2 (4%)		

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[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	7	12	9
Natural deaths	2	6	5	4
Survivors				
Died last week of study		1		
Terminal sacrifice	38	36	33	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, rectum	(49)	(47)	(47)	(48)
Intestine small, ileum	(48)	(46)	(45)	(48)
Liver	(50)	(50)	(50)	(50)
Mesentery	(13)	(14)	(15)	(15)
Oral mucosa	(0)	(1)	(0)	(0)
Squamous cell papilloma		1 (100%)		
Pancreas	(50)	(50)	(50)	(50)
Acinus, adenoma				1 (2%)
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Stomach, glandular	(50)	(50)	(50)	(50)
Tongue	(1)	(0)	(1)	(1)
Squamous cell papilloma			1 (100%)	
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Adenoma		1 (2%)	1 (2%)	1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign	3 (6%)	1 (2%)	3 (6%)	1 (2%)
Pheochromocytoma complex	1 (2%)			1 (2%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Adenoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Carcinoma	1 (2%)			
Parathyroid gland	(49)	(48)	(48)	(45)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, adenoma	19 (38%)	19 (38%)	17 (35%)	12 (24%)
Thyroid gland	(50)	(50)	(50)	(50)
C-cell, adenoma	4 (8%)	4 (8%)	5 (10%)	5 (10%)
C-cell, carcinoma			1 (2%)	
Follicular cell, adenoma		1 (2%)		1 (2%)
General Body System				
None				

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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Adenoma	12 (24%)	11 (22%)	11 (22%)	9 (18%)
Adenoma, multiple			1 (2%)	
Carcinoma	1 (2%)	2 (4%)	5 (10%)	2 (4%)
Sarcoma	1 (2%)			
Ovary	(50)	(50)	(50)	(50)
Tubulostromal adenoma	1 (2%)			
Uterus	(50)	(50)	(50)	(50)
Polyp stromal	9 (18%)	12 (24%)	10 (20%)	5 (10%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Lymph node	(5)	(10)	(16)	(10)
Lymph node, mandibular	(0)	(0)	(0)	(1)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Spleen	(50)	(49)	(50)	(50)
Thymus	(50)	(50)	(49)	(50)
Thymoma benign		1 (2%)		1 (2%)
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma	1 (2%)			
Carcinoma	2 (4%)	1 (2%)		
Fibroadenoma	18 (36%)	18 (36%)	16 (32%)	9 (18%)
Fibroadenoma, multiple	17 (34%)	13 (26%)	6 (12%)	3 (6%)
Skin	(50)	(50)	(50)	(50)
Neural crest tumor		1 (2%)		
Squamous cell papilloma			1 (2%)	
Sebaceous gland, carcinoma			1 (2%)	
Subcutaneous tissue, fibroma	3 (6%)	2 (4%)	1 (2%)	
Subcutaneous tissue, fibroma, multiple				1 (2%)
Subcutaneous tissue, fibrosarcoma			2 (4%)	
Subcutaneous tissue, fibrous histiocytoma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma				1 (2%)
Subcutaneous tissue, sarcoma		1 (2%)		
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma			1 (2%)	
Cranium, osteosarcoma		1 (2%)		
Skeletal muscle	(0)	(1)	(0)	(0)
Nervous System				
Brain	(50)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			2 (4%)	1 (2%)
Nose	(50)	(50)	(50)	(50)

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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Special Senses System				
Ear	(0)	(1)	(0)	(0)
Eye	(49)	(48)	(47)	(49)
Harderian gland	(50)	(50)	(50)	(50)
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Urinary bladder	(50)	(50)	(50)	(50)
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Leukemia mononuclear	5 (10%)	11 (22%)	18 (36%)	15 (30%)
Neoplasm Summary				
Total animals with primary neoplasms[c]	46	46	46	40
Total primary neoplasms	99	104	105	70
Total animals with benign neoplasms	44	44	40	32
Total benign neoplasms	88	86	76	51
Total animals with malignant neoplasms	10	16	25	19
Total malignant neoplasms	11	17	29	19
Total animals with metastatic neoplasms			1	
Total metastatic neoplasms			1	
Total animals with uncertain neoplasms-benign or malignant			1	
Total uncertain neoplasms		1		

[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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	3/50 (6%)	1/50 (2%)	3/50 (6%)	1/50 (2%)
Adjusted rate[b]	6.4%	2.2%	6.9%	2.3%
Terminal rate[c]	2/38 (5%)	0/37 (0%)	2/33 (6%)	1/37 (3%)
First incidence (days)	703	704	592	729 (T)
Poly-3 test[d]	P=0.336N	P=0.310N	P=0.630	P=0.326N
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	72.3%	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)	—[e]	—
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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Mammary Gland: Fibroadenoma, Adenoma, or Carcinoma				
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
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	729 (T)	729 (T)
Poly-3 test	P=0.250N	P=0.501N	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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
All Organs: Benign Neoplasms				
Overall rate	44/50 (88%)	44/50 (88%)	40/50 (80%)	32/50 (64%)
Adjusted rate	89.2%	91.4%	82.8%	68.2%
Terminal rate	33/38 (87%)	34/37 (92%)	26/33 (79%)	26/37 (70%)
First incidence (days)	619	610	547	534
Poly-3 test	P<0.001N	P=0.487	P=0.263N	P=0.008N
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
[a] 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.
[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 B3a
Historical Incidence of Mononuclear Cell Leukemia in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Methylcellulose Gavage Studies	
Androstenedione (February, 2003)	5/50
Methylene blue trihydrate (June, 2000)	12/50
Total (%)	17/100 (17.0%)
Mean ± standard deviation	17.0% ± 9.9%
Range	10%-24%
Overall Historical Incidence: All Routes	
Total (%)	297/1,350 (22.0%)
Mean ± standard deviation	22.0% ± 8.8%
Range	8%-40%

[a] Data as of November 17, 2008

TABLE B3b
Historical Incidence of Mammary Gland Neoplasms in Control Female F344/N Rats[a]

Study (Study Start)	Incidence in Controls			
	Fibroadenoma	Adenoma	Carcinoma	Fibroadenoma, Adenoma, or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies				
Androstenedione (February, 2003)	35/50	1/50	2/50	37/50
Methylene blue trihydrate (June, 2000)	28/50	1/50	1/50	30/50
Total (%)	63/100 (63.0%)	2/100 (2.0%)	3/100 (3.0%)	67/100 (67.0%)
Mean ± standard deviation	63.0% ± 9.9%	2.0% ± 0.0%	3.0% ± 1.4%	67.0% ± 9.9%
Range	56%-70%	2%	2%-4%	60%-74%
Overall Historical Incidence: All Routes				
Total (%)	697/1,350 (51.6%)	19/1,350 (1.4%)	67/1,350 (5.0%)	742/1,350 (55.0%)
Mean ± standard deviation	51.6% ± 14.9%	1.4% ± 1.9%	5.0% ± 4.8%	55.0% ± 14.3%
Range	24%-86%	0%-6%	0%-20%	28%-86%

[a] 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[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	10	7	12	9
Natural deaths	2	6	5	4
Survivors				
Died last week of study		1		
Terminal sacrifice	38	36	33	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Intestine large, rectum	(49)	(47)	(47)	(48)
Edema	1 (2%)			
Intestine small, ileum	(48)	(46)	(45)	(48)
Serosa, inflammation, chronic	1 (2%)			
Liver	(50)	(50)	(50)	(50)
Angiectasis		1 (2%)		1 (2%)
Basophilic focus	47 (94%)	46 (92%)	42 (84%)	38 (76%)
Clear cell focus	14 (28%)	10 (20%)	11 (22%)	14 (28%)
Degeneration, cystic				1 (2%)
Eosinophilic focus	5 (10%)		4 (8%)	
Fibrosis	1 (2%)	1 (2%)		
Hematopoietic cell proliferation	1 (2%)			
Hemorrhage				1 (2%)
Hepatodiaphragmatic nodule	10 (20%)	9 (18%)	11 (22%)	9 (18%)
Infiltration cellular, mixed cell	21 (42%)	33 (66%)	32 (64%)	31 (62%)
Mixed cell focus	1 (2%)		2 (4%)	
Necrosis, focal			1 (2%)	1 (2%)
Bile duct, hyperplasia	12 (24%)	16 (32%)	18 (36%)	24 (48%)
Centrilobular, necrosis				1 (2%)
Hepatocyte, vacuolization cytoplasmic	6 (12%)	4 (8%)	7 (14%)	5 (10%)
Kupffer cell, pigmentation	2 (4%)			
Mesentery	(13)	(14)	(15)	(15)
Accessory spleen	2 (15%)		3 (20%)	2 (13%)
Fat, necrosis	12 (92%)	14 (100%)	14 (93%)	12 (80%)
Oral mucosa	(0)	(1)	(0)	(0)
Pancreas	(50)	(50)	(50)	(50)
Atrophy	10 (20%)	10 (20%)	16 (32%)	26 (52%)
Cyst	3 (6%)	7 (14%)	4 (8%)	2 (4%)
Infiltration cellular, lymphocyte			1 (2%)	3 (6%)
Acinus, cytoplasmic alteration		4 (8%)	1 (2%)	2 (4%)
Acinus, hyperplasia, focal			1 (2%)	3 (6%)
Salivary glands	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Vacuolization cytoplasmic		1 (2%)		
Stomach, forestomach	(50)	(50)	(50)	(50)
Edema	1 (2%)	3 (6%)	2 (4%)	3 (6%)
Inflammation, chronic active	1 (2%)			
Ulcer	2 (4%)	2 (4%)	2 (4%)	2 (4%)
Epithelium, hyperplasia	3 (6%)	1 (2%)	2 (4%)	6 (12%)

[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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Alimentary System (continued)				
Stomach, glandular	(50)	(50)	(50)	(50)
Edema				2 (4%)
Erosion				3 (6%)
Ulcer			2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)	2 (4%)		1 (2%)
Tongue	(1)	(0)	(1)	(1)
Hyperkeratosis				1 (100%)
Epithelium, hyperplasia	1 (100%)			
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	46 (92%)	43 (86%)	43 (86%)	48 (96%)
Thrombosis		2 (4%)	1 (2%)	1 (2%)
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	1 (2%)	3 (6%)	1 (2%)	
Angiectasis	1 (2%)			
Degeneration, fatty	15 (30%)	20 (40%)	24 (48%)	13 (26%)
Hematopoietic cell proliferation		2 (4%)		1 (2%)
Hyperplasia, focal	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Hypertrophy, focal	12 (24%)	10 (20%)	9 (18%)	2 (4%)
Hypertrophy, diffuse		1 (2%)		
Necrosis		1 (2%)		
Capsule, developmental malformation		1 (2%)		
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	2 (4%)
Islets, pancreatic	(50)	(50)	(50)	(50)
Hyperplasia		4 (8%)	1 (2%)	11 (22%)
Parathyroid gland	(49)	(48)	(48)	(45)
Hyperplasia				1 (2%)
Pituitary gland	(50)	(50)	(49)	(50)
Pars distalis, angiectasis		1 (2%)	1 (2%)	3 (6%)
Pars distalis, cyst	18 (36%)	12 (24%)	14 (29%)	13 (26%)
Pars distalis, hyperplasia, focal	20 (40%)	20 (40%)	14 (29%)	19 (38%)
Pars intermedia, angiectasis	1 (2%)			
Rathke's cleft, cyst	3 (6%)	1 (2%)	4 (8%)	
Thyroid gland	(50)	(50)	(50)	(50)
Ultimobranchial cyst			2 (4%)	1 (2%)
C-cell, hyperplasia	27 (54%)	33 (66%)	24 (48%)	25 (50%)
Follicle, cyst		1 (2%)	2 (4%)	1 (2%)
Follicular cell, hyperplasia	1 (2%)			
General Body System				
None				

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Genital System				
Clitoral gland	(50)	(50)	(50)	(50)
Cyst	6 (12%)	8 (16%)	8 (16%)	4 (8%)
Hyperplasia	3 (6%)	4 (8%)		3 (6%)
Inflammation, suppurative	1 (2%)		1 (2%)	1 (2%)
Inflammation, chronic	23 (46%)	26 (52%)	18 (36%)	24 (48%)
Ovary	(50)	(50)	(50)	(50)
Angiectasis				1 (2%)
Cyst	3 (6%)	5 (10%)	2 (4%)	1 (2%)
Infiltration cellular, histiocyte				1 (2%)
Bursa, dilatation	6 (12%)	6 (12%)	2 (4%)	6 (12%)
Corpus luteum, hyperplasia		1 (2%)		
Follicle, cyst				1 (2%)
Interstitial cell, cyst				1 (2%)
Uterus	(50)	(50)	(50)	(50)
Decidual reaction			1 (2%)	
Hemorrhage		1 (2%)	1 (2%)	1 (2%)
Hyperplasia, cystic	9 (18%)	8 (16%)	11 (22%)	14 (28%)
Inflammation, suppurative	1 (2%)			
Thrombosis		1 (2%)		
Epithelium, hyperplasia			2 (4%)	
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy		1 (2%)	1 (2%)	
Hyperplasia	3 (6%)	6 (12%)	5 (10%)	1 (2%)
Thrombosis		1 (2%)		
Lymph node	(5)	(10)	(16)	(10)
Congestion			1 (6%)	
Deep cervical, ectasia			1 (6%)	
Deep cervical, necrosis	1 (20%)			
Mediastinal, ectasia		2 (20%)	1 (6%)	
Mediastinal, hemorrhage		3 (30%)	1 (6%)	1 (10%)
Mediastinal, hyperplasia, lymphoid	2 (40%)	2 (20%)	3 (19%)	
Mediastinal, infiltration cellular, mast cell	1 (20%)			
Mediastinal, pigmentation	1 (20%)			
Pancreatic, ectasia			1 (6%)	
Pancreatic, hemorrhage	1 (20%)	2 (20%)	3 (19%)	2 (20%)
Pancreatic, hyperplasia, histiocytic			1 (6%)	
Pancreatic, pigmentation				1 (10%)
Lymph node, mandibular	(0)	(0)	(0)	(1)
Lymph node, mesenteric	(50)	(50)	(49)	(50)
Ectasia	2 (4%)		3 (6%)	4 (8%)
Hemorrhage			1 (2%)	
Hyperplasia, histiocytic	29 (58%)	34 (68%)	24 (49%)	19 (38%)
Hyperplasia, lymphoid		1 (2%)	1 (2%)	1 (2%)
Pigmentation	1 (2%)		2 (4%)	

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Hematopoietic System (continued)				
Spleen	(50)	(49)	(50)	(50)
Fibrosis			1 (2%)	
Hematopoietic cell proliferation	5 (10%)	3 (6%)	6 (12%)	2 (4%)
Hemorrhage		1 (2%)	1 (2%)	1 (2%)
Hyperplasia, lymphoid				1 (2%)
Infiltration cellular, mixed cell				1 (2%)
Pigmentation	43 (86%)	44 (90%)	39 (78%)	36 (72%)
Lymphoid follicle, hyperplasia	1 (2%)	2 (4%)	3 (6%)	11 (22%)
Thymus	(50)	(50)	(49)	(50)
Atrophy				1 (2%)
Hemorrhage		1 (2%)		
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Cyst	15 (30%)	3 (6%)	9 (18%)	3 (6%)
Hyperplasia	48 (96%)	40 (80%)	35 (70%)	23 (46%)
Inflammation, suppurative				1 (2%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion		1 (2%)		1 (2%)
Hyperkeratosis		1 (2%)		2 (4%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Hyperostosis				1 (2%)
Skeletal muscle	(0)	(1)	(0)	(0)
Inflammation, chronic		1 (100%)		
Nervous System				
Brain	(50)	(50)	(50)	(50)
Compression	6 (12%)	10 (20%)	4 (8%)	5 (10%)
Hemorrhage	1 (2%)			1 (2%)
Hydrocephalus	1 (2%)	1 (2%)		
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Congestion		1 (2%)		
Emphysema		1 (2%)		
Hemorrhage		1 (2%)		
Infiltration cellular, histiocyte	31 (62%)	37 (74%)	26 (52%)	27 (54%)
Inflammation, chronic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Pigmentation		1 (2%)		
Alveolar epithelium, hyperplasia	12 (24%)	13 (26%)	11 (22%)	10 (20%)
Artery, hypertrophy		1 (2%)		
Nose	(50)	(50)	(50)	(50)
Foreign body	6 (12%)	4 (8%)	6 (12%)	3 (6%)
Inflammation, suppurative	3 (6%)	2 (4%)	3 (6%)	
Inflammation, chronic	3 (6%)	2 (4%)	1 (2%)	
Respiratory epithelium, hyperplasia	7 (14%)	8 (16%)	1 (2%)	1 (2%)

TABLE B4
Summary of the Incidence of Nonneoplastic Lesions in Female Rats in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Special Senses System				
Ear	(0)	(1)	(0)	(0)
Inflammation, suppurative		1 (100%)		
Eye	(49)	(48)	(47)	(49)
Atrophy	1 (2%)	1 (2%)		1 (2%)
Cataract	3 (6%)			1 (2%)
Cornea, inflammation, chronic		1 (2%)		
Retina, degeneration		1 (2%)	3 (6%)	
Sclera, metaplasia, osseous	1 (2%)		1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte			1 (2%)	
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(50)	(49)	(50)	(50)
Cyst	2 (4%)			1 (2%)
Glomerulosclerosis		1 (2%)	1 (2%)	
Infarct	2 (4%)		1 (2%)	
Nephropathy	48 (96%)	44 (90%)	43 (86%)	43 (86%)
Renal tubule, accumulation, hyaline droplet	3 (6%)			2 (4%)
Renal tubule, necrosis				1 (2%)
Renal tubule, pigmentation	1 (2%)		2 (4%)	3 (6%)
Renal tubule, vacuolization cytoplasmic		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)

APPENDIX C
SUMMARY OF LESIONS IN MALE MICE IN THE 2-YEAR GAVAGE STUDY OF ANDROSTENEDIONE

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	2	5	3
Natural deaths	9	4	11	10
Survivors				
Terminal sacrifice	36	44	34	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(46)	(42)	(44)
Intestine large, cecum	(50)	(49)	(50)	(49)
Intestine small, duodenum	(47)	(49)	(46)	(49)
Carcinoma	1 (2%)			
Intestine small, ileum	(49)	(50)	(49)	(49)
Carcinoma	1 (2%)			
Intestine small, jejunum	(47)	(49)	(47)	(49)
Carcinoma	1 (2%)		2 (4%)	1 (2%)
Liver	(50)	(50)	(50)	(50)
Carcinoma, metastatic, lung		1 (2%)		
Hemangioma	1 (2%)			1 (2%)
Hemangiosarcoma	3 (6%)	1 (2%)	5 (10%)	3 (6%)
Hepatoblastoma	3 (6%)	7 (14%)	6 (12%)	5 (10%)
Hepatoblastoma, multiple		1 (2%)	1 (2%)	3 (6%)
Hepatocellular adenoma	16 (32%)	11 (22%)	6 (12%)	9 (18%)
Hepatocellular adenoma, multiple	16 (32%)	27 (54%)	23 (46%)	34 (68%)
Hepatocellular carcinoma	19 (38%)	21 (42%)	18 (36%)	15 (30%)
Hepatocellular carcinoma, multiple	7 (14%)	12 (24%)	10 (20%)	17 (34%)
Sarcoma, metastatic, skeletal muscle	1 (2%)			
Mesentery	(5)	(8)	(4)	(5)
Hepatoblastoma, metastatic, liver		2 (25%)	1 (25%)	
Hepatocellular carcinoma, metastatic, liver			1 (25%)	
Pancreas	(50)	(50)	(50)	(49)
Acinus, carcinoma			1 (2%)	
Salivary glands	(50)	(50)	(50)	(50)
Stomach, forestomach	(49)	(50)	(50)	(50)
Carcinoma, metastatic, pancreas			1 (2%)	
Squamous cell carcinoma			1 (2%)	
Squamous cell papilloma	2 (4%)		1 (2%)	1 (2%)
Stomach, glandular	(49)	(50)	(50)	(50)
Carcinoma			1 (2%)	
Carcinoma, metastatic, pancreas			1 (2%)	
Tooth	(0)	(1)	(0)	(0)
Odontoma		1 (100%)		
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Adenoma		1 (2%)		
Capsule, adenoma	1 (2%)	4 (8%)	1 (2%)	
Capsule, adenoma, multiple				1 (2%)
Adrenal medulla	(49)	(49)	(50)	(50)
Pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(50)	(50)	(50)	(49)
Adenoma	2 (4%)	2 (4%)	2 (4%)	4 (8%)
Adenoma, multiple				1 (2%)
Parathyroid gland	(48)	(48)	(47)	(46)
Pituitary gland	(50)	(49)	(49)	(50)
Thyroid gland	(50)	(50)	(50)	(50)
Follicular cell, adenoma			1 (2%)	
General Body System				
None				
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Preputial gland	(50)	(50)	(50)	(50)
Prostate	(50)	(50)	(50)	(50)
Seminal vesicle	(50)	(50)	(50)	(50)
Testes	(50)	(50)	(50)	(50)
Interstitial cell, adenoma		1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Lymph node	(2)	(1)	(1)	(4)
Lymph node, mandibular	(47)	(47)	(47)	(47)
Basal cell carcinoma, metastatic, skin			1 (2%)	
Lymph node, mesenteric	(49)	(49)	(49)	(45)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma	1 (2%)	1 (2%)	5 (10%)	1 (2%)
Thymus	(45)	(46)	(42)	(45)
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Subcutaneous tissue, fibroma		1 (2%)		
Subcutaneous tissue, fibrosarcoma			1 (2%)	
Subcutaneous tissue, fibrous histiocytoma	1 (2%)		1 (2%)	
Subcutaneous tissue, hemangiosarcoma	1 (2%)	2 (4%)	1 (2%)	
Subcutaneous tissue, melanoma malignant		1 (2%)		
Subcutaneous tissue, sarcoma			1 (2%)	

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Skeletal muscle	(1)	(1)	(2)	(1)
Hemangiosarcoma		1 (100%)		
Sarcoma	1 (100%)			
Nervous System				
Brain	(50)	(50)	(49)	(50)
Peripheral nerve	(1)	(1)	(4)	(3)
Spinal cord	(1)	(1)	(4)	(3)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma	7 (14%)	6 (12%)	6 (12%)	5 (10%)
Alveolar/bronchiolar adenoma, multiple		1 (2%)	1 (2%)	
Alveolar/bronchiolar carcinoma	8 (16%)	6 (12%)	3 (6%)	8 (16%)
Alveolar/bronchiolar carcinoma, multiple	3 (6%)	2 (4%)	1 (2%)	5 (10%)
Basal cell carcinoma, metastatic, skin			1 (2%)	
Carcinoma		1 (2%)		
Carcinoma, metastatic, harderian gland				1 (2%)
Hepatoblastoma, metastatic, liver		1 (2%)	1 (2%)	1 (2%)
Hepatocellular carcinoma, metastatic, liver	6 (12%)	6 (12%)	11 (22%)	5 (10%)
Sarcoma, metastatic, skeletal muscle	1 (2%)			
Nose	(50)	(49)	(49)	(50)
Carcinoma, metastatic, harderian gland				1 (2%)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Carcinoma, metastatic, harderian gland				1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Adenoma	7 (14%)	9 (18%)	6 (12%)	4 (8%)
Adenoma, multiple			1 (2%)	
Carcinoma	2 (4%)			3 (6%)
Lacrimal gland	(0)	(1)	(0)	(0)
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Carcinoma, metastatic, lung		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Hemangioma			1 (2%)	
Systemic Lesions				
Multiple organs[b]	(50)	(50)	(50)	(50)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant	4 (8%)	6 (12%)	3 (6%)	6 (12%)

TABLE C1
Summary of the Incidence of Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Neoplasm Summary				
Total animals with primary neoplasms[c]	47	50	44	50
Total primary neoplasms	108	128	112	129
Total animals with benign neoplasms	38	43	35	46
Total benign neoplasms	52	65	49	61
Total animals with malignant neoplasms	37	41	38	44
Total malignant neoplasms	56	63	63	68
Total animals with metastatic neoplasms	7	9	14	7
Total metastatic neoplasms	8	11	18	9

[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

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	41/50 (82%)	47/50 (94%)	42/50 (84%)	48/50 (96%)
Adjusted rate	85.2%	94.1%	87.9%	98.5%
Terminal rate	31/36 (86%)	42/44 (96%)	29/34 (85%)	37/37 (100%)
First incidence (days)	398	593	472	485
Poly-3 test	P=0.025	P=0.122	P=0.460	P=0.012
Liver: Hepatoblastoma				
Overall rate	3/50 (6%)	8/50 (16%)	7/50 (14%)	8/50 (16%)
Adjusted rate	6.9%	16.4%	15.7%	17.6%
Terminal rate	3/36 (8%)	7/44 (16%)	6/34 (18%)	6/37 (16%)
First incidence (days)	729 (T)	593	631	648
Poly-3 test	P=0.182	P=0.141	P=0.169	P=0.114
Liver: Hepatocellular Carcinoma or Hepatoblastoma				
Overall rate	27/50 (54%)	35/50 (70%)	30/50 (60%)	36/50 (72%)
Adjusted rate	56.1%	70.1%	62.5%	74.0%
Terminal rate	17/36 (47%)	30/44 (68%)	17/34 (50%)	26/37 (70%)
First incidence (days)	398	593	472	485
Poly-3 test	P=0.077	P=0.108	P=0.333	P=0.047
Liver: Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma				
Overall rate	41/50 (82%)	47/50 (94%)	43/50 (86%)	48/50 (96%)
Adjusted rate	85.2%	94.1%	89.4%	98.5%
Terminal rate	31/36 (86%)	42/44 (96%)	29/34 (85%)	37/37 (100%)
First incidence (days)	398	593	472	485
Poly-3 test	P=0.024	P=0.122	P=0.374	P=0.012
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	7/50 (14%)	7/50 (14%)	7/50 (14%)	5/50 (10%)
Adjusted rate	16.2%	14.4%	15.8%	10.8%
Terminal rate	7/36 (19%)	6/44 (14%)	6/34 (18%)	2/37 (5%)
First incidence (days)	729 (T)	652	708	526
Poly-3 test	P=0.287N	P=0.521N	P=0.594N	P=0.333N
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	11/50 (22%)	9/50 (18%)	4/50 (8%)	13/50 (26%)
Adjusted rate	24.7%	18.4%	9.0%	28.6%
Terminal rate	7/36 (19%)	7/44 (16%)	3/34 (9%)	11/37 (30%)
First incidence (days)	581	606	708	688
Poly-3 test	P=0.244	P=0.316N	P=0.043N	P=0.427
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	16/50 (32%)	15/50 (30%)	10/50 (20%)	17/50 (34%)
Adjusted rate	35.9%	30.6%	22.5%	36.6%
Terminal rate	12/36 (33%)	12/44 (27%)	9/34 (27%)	12/37 (32%)
First incidence (days)	581	606	708	526
Poly-3 test	P=0.422	P=0.371N	P=0.122N	P=0.561
Pancreatic Islets: Adenoma				
Overall rate	2/50 (4%)	2/50 (4%)	2/50 (4%)	5/49 (10%)
Adjusted rate	4.6%	4.1%	4.5%	11.1%
Terminal rate	2/36 (6%)	2/44 (5%)	1/34 (3%)	4/37 (11%)
First incidence (days)	729 (T)	729 (T)	620	493
Poly-3 test	P=0.104	P=0.653N	P=0.683N	P=0.232

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

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

TABLE C2
Statistical Analysis of Primary Neoplasms in Male Mice in the 2-Year Gavage Study of Androstenedione

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

(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[a]

Study (Study Start)	Incidence in Controls				
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma	Hepatoblastoma	Hepatocellular Adenoma, Hepatocellular Carcinoma, or Hepatoblastoma
Historical Incidence: Methylcellulose Gavage Studies					
Androstenedione (December, 2002)	32/50	26/50	41/50	3/50	41/50
Methylene blue trihydrate (July, 2000)	28/50	13/50	37/50	2/50	38/50
Total (%)	60/100 (60.0%)	39/100 (39.0%)	78/100 (78.0%)	5/100 (5.0%)	79/100 (79.0%)
Mean ± standard deviation	60.0% ± 5.7%	39.0% ± 18.4%	78.0% ± 5.7%	5.0% ± 1.4%	79.0% ± 4.2%
Range	56%-64%	26%-52%	74%-82%	4%-6%	76%-82%
Overall Historical Incidence: All Routes					
Total (%)	733/1,447 (50.7%)	415/1,447 (28.7%)	961/1,447 (66.4%)	48/1,447 (3.3%)	972/1,447 (67.2%)
Mean ± standard deviation	50.7% ± 13.9%	28.7% ± 8.8%	66.4% ± 12.6%	3.3% ± 6.4%	67.2% ± 13.1%
Range	22%-72%	16%-52%	36%-84%	0%-34%	36%-92%

[a] Data as of November 19, 2008

TABLE C3b
Historical Incidence of Pancreatic Islet Neoplasms in Control Male B6C3F1 Mice[a]

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies			
Androstenedione (December, 2002)	2/50	0/50	2/50
Methylene blue trihydrate (July, 2000)	0/50	0/50	0/50
Total (%)	2/100 (2.0%)	0/100	2/100 (2.0%)
Mean ± standard deviation	2.0% ± 2.8%		2.0% ± 2.8%
Range	0%-4%		0%-4%
Overall Historical Incidence: All Routes			
Total (%)	17/1,435 (1.2%)	4/1,435 (0.3%)	21/1,435 (1.5%)
Mean ± standard deviation	1.2% ± 1.7%	0.3% ± 0.9%	1.5% ± 1.8%
Range	0%-6%	0%-4%	0%-6%

[a] Data as of November 19, 2008

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Moribund	5	2	5	3
Natural deaths	9	4	11	10
Survivors				
Terminal sacrifice	36	44	34	37
Animals examined microscopically	50	50	50	50
Alimentary System				
Gallbladder	(45)	(46)	(42)	(44)
Epithelium, cytoplasmic alteration		1 (2%)		
Intestine large, cecum	(50)	(49)	(50)	(49)
Edema	1 (2%)	1 (2%)	1 (2%)	
Intestine small, duodenum	(47)	(49)	(46)	(49)
Intestine small, ileum	(49)	(50)	(49)	(49)
Hyperplasia, lymphoid	1 (2%)			
Intestine small, jejunum	(47)	(49)	(47)	(49)
Hyperplasia, lymphoid	1 (2%)		1 (2%)	1 (2%)
Epithelium, hyperplasia		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Basophilic focus	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Clear cell focus	27 (54%)	24 (48%)	18 (36%)	12 (24%)
Cyst	1 (2%)			2 (4%)
Eosinophilic focus	13 (26%)	10 (20%)	11 (22%)	25 (50%)
Hematopoietic cell proliferation		1 (2%)	4 (8%)	
Hemorrhage	1 (2%)	1 (2%)		1 (2%)
Infiltration cellular			2 (4%)	
Infiltration cellular, mixed cell	1 (2%)	1 (2%)	5 (10%)	2 (4%)
Mitotic alteration			1 (2%)	
Mixed cell focus	10 (20%)	10 (20%)	11 (22%)	13 (26%)
Necrosis				1 (2%)
Necrosis, focal	3 (6%)	3 (6%)	5 (10%)	6 (12%)
Tension lipidosis				1 (2%)
Vacuolization cytoplasmic	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Centrilobular, necrosis	2 (4%)		3 (6%)	4 (8%)
Hepatocyte, vacuolization cytoplasmic	17 (34%)	14 (28%)	14 (28%)	12 (24%)
Kupffer cell, hyperplasia	1 (2%)		1 (2%)	
Mesentery	(5)	(8)	(4)	(5)
Hemorrhage	2 (40%)			
Fat, necrosis	4 (80%)	7 (88%)	2 (50%)	4 (80%)
Pancreas	(50)	(50)	(50)	(49)
Atrophy	1 (2%)	2 (4%)		
Thrombosis				1 (2%)
Acinus, cytoplasmic alteration	1 (2%)	3 (6%)	2 (4%)	
Acinus, hyperplasia, focal	1 (2%)			
Salivary glands	(50)	(50)	(50)	(50)
Atrophy	1 (2%)			1 (2%)
Infiltration cellular, lymphocyte	2 (4%)	4 (8%)	3 (6%)	6 (12%)

[a] 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

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Alimentary System (continued)				
Stomach, forestomach	(49)	(50)	(50)	(50)
Edema				1 (2%)
Erosion	1 (2%)			
Inflammation, chronic		1 (2%)		1 (2%)
Ulcer	1 (2%)	1 (2%)	1 (2%)	
Epithelium, hyperplasia	1 (2%)	5 (10%)	1 (2%)	2 (4%)
Stomach, glandular	(49)	(50)	(50)	(50)
Edema			1 (2%)	
Erosion			2 (4%)	1 (2%)
Epithelium, hyperplasia	1 (2%)	2 (4%)		
Epithelium, hyperplasia, focal	1 (2%)			
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	4 (8%)	4 (8%)	5 (10%)	7 (14%)
Hemorrhage				1 (2%)
Inflammation, chronic			1 (2%)	
Mineralization			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(49)	(50)	(50)
Accessory adrenal cortical nodule	6 (12%)	1 (2%)	3 (6%)	2 (4%)
Hyperplasia, focal	4 (8%)	5 (10%)	5 (10%)	3 (6%)
Hypertrophy, focal	10 (20%)	7 (14%)	11 (22%)	9 (18%)
Metaplasia, osseous	1 (2%)			
Capsule, hyperplasia	5 (10%)	4 (8%)	5 (10%)	10 (20%)
Adrenal medulla	(49)	(49)	(50)	(50)
Hyperplasia			1 (2%)	
Islets, pancreatic	(50)	(50)	(50)	(49)
Hyperplasia	12 (24%)	14 (28%)	11 (22%)	6 (12%)
Parathyroid gland	(48)	(48)	(47)	(46)
Cyst	1 (2%)			1 (2%)
Pituitary gland	(50)	(49)	(49)	(50)
Pars distalis, cyst	4 (8%)	2 (4%)	4 (8%)	3 (6%)
Pars distalis, hyperplasia, focal	1 (2%)			1 (2%)
Thyroid gland	(50)	(50)	(50)	(50)
Follicle, cyst				1 (2%)
Follicle, degeneration, focal	12 (24%)	8 (16%)	9 (18%)	9 (18%)
Follicular cell, hyperplasia				2 (4%)
General Body System				
None				

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Genital System				
Epididymis	(50)	(50)	(50)	(50)
Granuloma sperm	1 (2%)			
Spermatocele	1 (2%)			
Preputial gland	(50)	(50)	(50)	(50)
Cyst	1 (2%)	2 (4%)		4 (8%)
Inflammation, chronic	7 (14%)	4 (8%)	3 (6%)	7 (14%)
Prostate	(50)	(50)	(50)	(50)
Inflammation, chronic	3 (6%)			
Seminal vesicle	(50)	(50)	(50)	(50)
Degeneration		1 (2%)		
Inflammation, chronic	1 (2%)			1 (2%)
Testes	(50)	(50)	(50)	(50)
Germinal epithelium, atrophy	2 (4%)	2 (4%)	4 (8%)	2 (4%)
Hematopoietic System				
Bone marrow	(50)	(50)	(50)	(50)
Atrophy			1 (2%)	
Hyperplasia	19 (38%)	30 (60%)	23 (46%)	30 (60%)
Infiltration cellular, histiocyte			1 (2%)	
Myelofibrosis			1 (2%)	
Lymph node	(2)	(1)	(1)	(4)
Iliac, hyperplasia, lymphoid			1 (100%)	
Lymph node, mandibular	(47)	(47)	(47)	(47)
Atrophy			3 (6%)	
Hematopoietic cell proliferation	1 (2%)	1 (2%)		
Hyperplasia, lymphoid	5 (11%)	5 (11%)	3 (6%)	3 (6%)
Pigmentation	1 (2%)			1 (2%)
Lymph node, mesenteric	(49)	(49)	(49)	(45)
Angiectasis			2 (4%)	
Atrophy		4 (8%)	6 (12%)	
Hematopoietic cell proliferation	4 (8%)	4 (8%)	6 (12%)	2 (4%)
Hemorrhage	4 (8%)	4 (8%)	7 (14%)	2 (4%)
Hyperplasia, lymphoid	2 (4%)	5 (10%)	1 (2%)	4 (9%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	1 (2%)	1 (2%)		
Fibrosis	1 (2%)			
Hematopoietic cell proliferation	23 (46%)	25 (50%)	28 (56%)	28 (56%)
Necrosis				1 (2%)
Lymphoid follicle, atrophy		1 (2%)	4 (8%)	
Lymphoid follicle, hyperplasia		1 (2%)	1 (2%)	1 (2%)
Thymus	(45)	(46)	(42)	(45)
Atrophy	4 (9%)	5 (11%)	13 (31%)	8 (18%)
Cyst		2 (4%)		5 (11%)
Hyperplasia, lymphoid		2 (4%)	1 (2%)	

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Integumentary System				
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Edema	1 (2%)	1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			
Hyperkeratosis	1 (2%)			
Ulcer		2 (4%)		1 (2%)
Epidermis, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Cranium, osteopetrosis			1 (2%)	2 (4%)
Skeletal muscle	(1)	(1)	(2)	(1)
Atrophy			1 (50%)	1 (100%)
Nervous System				
Brain	(50)	(50)	(49)	(50)
Cyst epithelial inclusion		1 (2%)		
Hemorrhage	1 (2%)	1 (2%)		
Peripheral nerve	(1)	(1)	(4)	(3)
Atrophy		1 (100%)	2 (50%)	1 (33%)
Spinal cord	(1)	(1)	(4)	(3)
Necrosis			1 (25%)	
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Foreign body			1 (2%)	
Hemorrhage	4 (8%)	1 (2%)	1 (2%)	2 (4%)
Infiltration cellular, histiocyte	10 (20%)	5 (10%)	5 (10%)	11 (22%)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic	1 (2%)			
Metaplasia, osseous		1 (2%)		
Thrombosis	2 (4%)		1 (2%)	
Alveolar epithelium, hyperplasia	4 (8%)	2 (4%)	2 (4%)	3 (6%)
Bronchus, hyperplasia				1 (2%)
Nose	(50)	(49)	(49)	(50)
Foreign body	4 (8%)			
Hemorrhage		1 (2%)		
Inflammation, chronic	5 (10%)	4 (8%)	4 (8%)	3 (6%)

TABLE C4
Summary of the Incidence of Nonneoplastic Lesions in Male Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Atrophy		1 (2%)		
Cataract	1 (2%)	1 (2%)		1 (2%)
Hemorrhage	1 (2%)			
Inflammation, chronic	3 (6%)	1 (2%)	2 (4%)	
Cornea, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Retina, degeneration	1 (2%)			1 (2%)
Harderian gland	(50)	(50)	(50)	(50)
Cyst			1 (2%)	
Lacrimal gland	(0)	(1)	(0)	(0)
Duct, inflammation, suppurative		1 (100%)		
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst	9 (18%)	16 (32%)	9 (18%)	13 (26%)
Hydronephrosis	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Infarct	4 (8%)	2 (4%)	1 (2%)	5 (10%)
Infiltration cellular, lymphocyte	5 (10%)	2 (4%)	4 (8%)	5 (10%)
Inflammation, suppurative			1 (2%)	
Metaplasia, osseous	4 (8%)	2 (4%)	5 (10%)	4 (8%)
Mineralization			2 (4%)	
Nephropathy	38 (76%)	45 (90%)	38 (76%)	44 (88%)
Renal tubule, accumulation, hyaline droplet			1 (2%)	
Renal tubule, dilatation, diffuse			1 (2%)	
Renal tubule, hyperplasia	7 (14%)	6 (12%)	6 (12%)	2 (4%)
Renal tubule, pigmentation	1 (2%)	3 (6%)	6 (12%)	3 (6%)
Transitional epithelium, hyperplasia		1 (2%)		
Urinary bladder	(50)	(50)	(50)	(50)
Edema	1 (2%)	1 (2%)		
Transitional epithelium, hyperplasia		1 (2%)	2 (4%)	

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[a]

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2		1	1
Moribund	6	2	3	3
Natural deaths	7	8	6	6
Survivors				
Died last week of study	1		1	
Terminal sacrifice	34	40	39	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Sarcoma, metastatic, skeletal muscle			1 (2%)	
Gallbladder	(44)	(45)	(42)	(48)
Adenoma				1 (2%)
Intestine large, cecum	(49)	(50)	(50)	(49)
Leiomyoma				1 (2%)
Intestine small, duodenum	(47)	(46)	(46)	(49)
Intestine small, ileum	(46)	(48)	(49)	(50)
Intestine small, jejunum	(46)	(48)	(49)	(50)
Liver	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)	1 (2%)	1 (2%)
Hepatocellular adenoma	10 (20%)	9 (18%)	11 (22%)	11 (22%)
Hepatocellular adenoma, multiple	4 (8%)	7 (14%)	7 (14%)	17 (34%)
Hepatocellular carcinoma	4 (8%)	11 (22%)	10 (20%)	11 (22%)
Hepatocellular carcinoma, multiple	1 (2%)	2 (4%)	5 (10%)	4 (8%)
Mesentery	(7)	(9)	(14)	(8)
Hepatocellular carcinoma, metastatic, liver		1 (11%)		
Oral mucosa	(0)	(0)	(0)	(1)
Squamous cell carcinoma				1 (100%)
Pancreas	(49)	(50)	(50)	(49)
Salivary glands	(49)	(49)	(49)	(50)
Stomach, forestomach	(50)	(50)	(50)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)	
Stomach, glandular	(50)	(50)	(50)	(50)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			

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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Adrenal medulla	(50)	(50)	(50)	(50)
Pheochromocytoma benign			1 (2%)	
Islets, pancreatic	(49)	(50)	(49)	(48)
Adenoma		2 (4%)	4 (8%)	4 (8%)
Parathyroid gland	(48)	(49)	(45)	(50)
Pituitary gland	(49)	(47)	(50)	(50)
Pars distalis, adenoma	1 (2%)	2 (4%)	4 (8%)	4 (8%)
Pars intermedia, adenoma		1 (2%)		
Thyroid gland	(50)	(50)	(48)	(50)
Sarcoma, metastatic, skeletal muscle			1 (2%)	
C-cell, carcinoma			1 (2%)	
Follicular cell, adenoma	1 (2%)			
Follicular cell, carcinoma	1 (2%)			
General Body System				
Tissue NOS	(1)	(0)	(0)	(1)
Hemangioma				1 (100%)
Sarcoma, metastatic, skin	1 (100%)			
Genital System				
Clitoral gland	(47)	(47)	(49)	(50)
Ovary	(49)	(50)	(49)	(48)
Cystadenoma	4 (8%)	3 (6%)	1 (2%)	2 (4%)
Cystadenoma, multiple			1 (2%)	
Hemangioma		1 (2%)		1 (2%)
Uterus	(50)	(50)	(50)	(50)
Hemangiosarcoma			1 (2%)	
Leiomyosarcoma				1 (2%)
Polyp stromal	2 (4%)	1 (2%)		4 (8%)
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hemangiosarcoma				1 (2%)
Lymph node	(10)	(5)	(4)	(1)
Hepatocellular carcinoma, metastatic, liver	1 (10%)			
Iliac, rhabdomyosarcoma, metastatic, skeletal muscle				1 (100%)
Mediastinal, hemangioma	1 (10%)			
Renal, rhabdomyosarcoma, metastatic, skeletal muscle				1 (100%)
Lymph node, mandibular	(46)	(49)	(47)	(49)
Hemangiosarcoma		1 (2%)		
Lymph node, mesenteric	(50)	(50)	(46)	(48)
Spleen	(50)	(50)	(50)	(50)
Hemangiosarcoma		1 (2%)		1 (2%)
Thymus	(47)	(46)	(46)	(46)
Hepatocellular carcinoma, metastatic, liver	1 (2%)			
Sarcoma, metastatic, skin	1 (2%)			

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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Adenoma		1 (2%)		
Carcinoma	1 (2%)	2 (4%)		
Skin	(50)	(50)	(50)	(50)
Basal cell adenoma		1 (2%)		
Squamous cell papilloma			1 (2%)	
Subcutaneous tissue, fibrosarcoma	3 (6%)		2 (4%)	1 (2%)
Subcutaneous tissue, fibrous histiocytoma				1 (2%)
Subcutaneous tissue, hemangioma		1 (2%)		
Subcutaneous tissue, hemangiosarcoma	1 (2%)		1 (2%)	
Subcutaneous tissue, liposarcoma		2 (4%)		
Subcutaneous tissue, myxosarcoma	1 (2%)			
Subcutaneous tissue, sarcoma	1 (2%)	1 (2%)	1 (2%)	
Subcutaneous tissue, schwannoma malignant			1 (2%)	
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Osteosarcoma	1 (2%)		1 (2%)	
Skeletal muscle	(4)	(0)	(4)	(2)
Fibrosarcoma	1 (25%)			
Hepatocellular carcinoma, metastatic, liver	1 (25%)			
Myxosarcoma	1 (25%)			
Rhabdomyosarcoma				1 (50%)
Sarcoma			2 (50%)	
Nervous System				
Brain	(50)	(49)	(50)	(50)
Peripheral nerve	(2)	(1)	(3)	(1)
Spinal cord	(2)	(1)	(4)	(1)
Osteosarcoma, metastatic, bone	1 (50%)			
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma		2 (4%)	3 (6%)	
Alveolar/bronchiolar carcinoma	4 (8%)	3 (6%)		4 (8%)
Alveolar/bronchiolar carcinoma, multiple				1 (2%)
Carcinoma, metastatic, thyroid gland			1 (2%)	
Hepatocellular carcinoma, metastatic, liver	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Liposarcoma, metastatic, skin		1 (2%)		
Osteosarcoma, metastatic, bone	1 (2%)			
Rhabdomyosarcoma, metastatic, skeletal muscle				1 (2%)
Sarcoma, metastatic, skin	1 (2%)			
Nose	(50)	(49)	(50)	(50)
Carcinoma, metastatic, harderian gland	1 (2%)			
Trachea	(50)	(50)	(48)	(50)
Sarcoma, metastatic, skeletal muscle			1 (2%)	

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

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

[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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Harderian Gland: Adenoma				
Overall rate[a]	9/50 (18%)	5/50 (10%)	11/50 (22%)	4/50 (8%)
Adjusted rate[b]	20.3%	10.9%	23.8%	8.8%
Terminal rate[c]	8/35 (23%)	5/40 (13%)	9/40 (23%)	4/40 (10%)
First incidence (days)	622	729 (T)	508	729 (T)
Poly-3 test[d]	P=0.129N	P=0.174N	P=0.442	P=0.103N
Harderian Gland: Adenoma or Carcinoma				
Overall rate	10/50 (20%)	6/50 (12%)	12/50 (24%)	6/50 (12%)
Adjusted rate	22.3%	13.1%	25.9%	13.1%
Terminal rate	8/35 (23%)	6/40 (15%)	10/40 (25%)	5/40 (13%)
First incidence (days)	565	729 (T)	508	718
Poly-3 test	P=0.234N	P=0.192N	P=0.436	P=0.192N
Liver: Hepatocellular Adenoma				
Overall rate	14/50 (28%)	16/50 (32%)	18/50 (36%)	28/50 (56%)
Adjusted rate	31.6%	34.6%	39.1%	61.1%
Terminal rate	13/35 (37%)	14/40 (35%)	16/40 (40%)	27/40 (68%)
First incidence (days)	622	622	520	708
Poly-3 test	P<0.001	P=0.468	P=0.299	P=0.003
Liver: Hepatocellular Carcinoma				
Overall rate	5/50 (10%)	13/50 (26%)	15/50 (30%)	15/50 (30%)
Adjusted rate	11.3%	28.2%	32.0%	32.7%
Terminal rate	3/35 (9%)	11/40 (28%)	11/40 (28%)	13/40 (33%)
First incidence (days)	687	685	442	708
Poly-3 test	P=0.098	P=0.038	P=0.015	P=0.012
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	17/50 (34%)	23/50 (46%)	27/50 (54%)	32/50 (64%)
Adjusted rate	38.2%	49.5%	57.0%	69.8%
Terminal rate	14/35 (40%)	20/40 (50%)	22/40 (55%)	30/40 (75%)
First incidence (days)	622	622	442	708
Poly-3 test	P=0.004	P=0.188	P=0.052	P<0.001
Lung: Alveolar/bronchiolar Adenoma				
Overall rate	0/50 (0%)	2/50 (4%)	3/50 (6%)	0/50 (0%)
Adjusted rate	0.0%	4.4%	6.7%	0.0%
Terminal rate	0/35 (0%)	2/40 (5%)	3/40 (8%)	0/40 (0%)
First incidence (days)	—[e]	729 (T)	729 (T)	—
Poly-3 test	P=0.286N	P=0.246	P=0.123	—[f]
Lung: Alveolar/bronchiolar Carcinoma				
Overall rate	4/50 (8%)	3/50 (6%)	0/50 (0%)	5/50 (10%)
Adjusted rate	9.1%	6.5%	0.0%	10.8%
Terminal rate	3/35 (9%)	2/40 (5%)	0/40 (0%)	4/40 (10%)
First incidence (days)	684	502	—	463
Poly-3 test	P=0.240	P=0.473N	P=0.058N	P=0.532
Lung: Alveolar/bronchiolar Adenoma or Carcinoma				
Overall rate	4/50 (8%)	5/50 (10%)	3/50 (6%)	5/50 (10%)
Adjusted rate	9.1%	10.8%	6.7%	10.8%
Terminal rate	3/35 (9%)	4/40 (10%)	3/40 (8%)	4/40 (10%)
First incidence (days)	684	502	729 (T)	463
Poly-3 test	P=0.490	P=0.531	P=0.490N	P=0.532

TABLE D2
Statistical Analysis of Primary Neoplasms in Female Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Mammary Gland: Adenoma or Carcinoma				
Overall rate	1/50 (2%)	3/50 (6%)	0/50 (0%)	0/50 (0%)
Adjusted rate	2.3%	6.5%	0.0%	0.0%
Terminal rate	1/35 (3%)	2/40 (5%)	0/40 (0%)	0/40 (0%)
First incidence (days)	729 (T)	681	—	—
Poly-3 test	P=0.176N	P=0.321	P=0.495N	P=0.492N
Ovary: Cystadenoma				
Overall rate	4/49 (8%)	3/50 (6%)	2/49 (4%)	2/48 (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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
All Organs: Hemangioma or Hemangiosarcoma				
Overall rate	2/50 (4%)	3/50 (6%)	3/50 (6%)	4/50 (8%)
Adjusted rate	4.5%	6.5%	6.6%	8.6%
Terminal rate	1/35 (3%)	1/40 (3%)	2/40 (5%)	3/40 (8%)
First incidence (days)	672	622	508	420
Poly-3 test	P=0.349	P=0.521	P=0.515	P=0.362
All Organs: Histiocytic Sarcoma				
Overall rate	2/50 (4%)	4/50 (8%)	0/50 (0%)	1/50 (2%)
Adjusted rate	4.5%	8.4%	0.0%	2.2%
Terminal rate	1/35 (3%)	1/40 (3%)	0/40 (0%)	0/40 (0%)
First incidence (days)	672	446	—	654
Poly-3 test	P=0.270N	P=0.374	P=0.233N	P=0.486N
All Organs: Malignant Lymphoma				
Overall rate	14/50 (28%)	15/50 (30%)	11/50 (22%)	2/50 (4%)
Adjusted rate	31.3%	32.5%	24.4%	4.4%
Terminal rate	11/35 (31%)	13/40 (33%)	10/40 (25%)	2/40 (5%)
First incidence (days)	547	680	672	729 (T)
Poly-3 test	P<0.001N	P=0.540	P=0.308N	P<0.001N
All Organs: Benign Neoplasms				
Overall rate	27/50 (54%)	27/50 (54%)	31/50 (62%)	32/50 (64%)
Adjusted rate	59.2%	57.7%	66.0%	69.4%
Terminal rate	23/35 (66%)	22/40 (55%)	27/40 (68%)	30/40 (75%)
First incidence (days)	443	622	508	654
Poly-3 test	P=0.151	P=0.524N	P=0.322	P=0.206
All Organs: Malignant Neoplasms				
Overall rate	27/50 (54%)	36/50 (72%)	31/50 (62%)	25/50 (50%)
Adjusted rate	57.7%	72.6%	63.9%	52.4%
Terminal rate	17/35 (49%)	27/40 (68%)	23/40 (58%)	20/40 (50%)
First incidence (days)	547	446	442	420
Poly-3 test	P=0.085N	P=0.091	P=0.342	P=0.377N
All Organs: Benign or Malignant Neoplasms				
Overall rate	40/50 (80%)	43/50 (86%)	43/50 (86%)	38/50 (76%)
Adjusted rate	83.5%	86.7%	87.7%	79.7%
Terminal rate	28/35 (80%)	34/40 (85%)	34/40 (85%)	33/40 (83%)
First incidence (days)	443	446	442	420
Poly-3 test	P=0.227N	P=0.435	P=0.377	P=0.413N

(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[a]

Study (Study Start)	Incidence in Controls		
	Hepatocellular Adenoma	Hepatocellular Carcinoma	Hepatocellular Adenoma or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies			
Androstenedione (December, 2002)	14/50	5/50	17/50
Methylene blue trihydrate (July, 2000)	15/50	5/50	19/50
Total (%)	29/100 (29.0%)	10/100 (10.0%)	36/100 (36.0%)
Mean ± standard deviation	29.0% ± 1.4%	10.0% ± 0.0%	36.0% ± 2.8%
Range	28%-30%	10%	34%-38%
Overall Historical Incidence: All Routes			
Total (%)	396/1,494 (26.5%)	137/1,494 (9.2%)	481/1,494 (32.2%)
Mean ± standard deviation	26.5% ± 15.2%	9.2% ± 6.7%	32.2% ± 17.3%
Range	2%-62%	0%-28%	6%-64%

[a] Data as of November 19, 2008

TABLE D3b
Historical Incidence of Pancreatic Islet Neoplasms in Control Female B6C3F1 Mice[a]

Study (Study Start)	Incidence in Controls		
	Adenoma	Carcinoma	Adenoma or Carcinoma
Historical Incidence: Methylcellulose Gavage Studies			
Androstenedione (December, 2002)	0/49	0/49	0/49
Methylene blue trihydrate (July, 2000)	1/46	0/46	1/46
Total (%)	1/95 (1.1%)	0/95	1/95 (1.1%)
Mean ± standard deviation	1.1% ± 1.5%		1.1% ± 1.5%
Range	0%-2%		0%-2%
Overall Historical Incidence: All Routes			
Total (%)	11/1,478 (0.7%)	5/1,478 (0.3%)	16/1,478 (1.1%)
Mean ± standard deviation	0.8% ± 1.0%	0.3% ± 0.9%	1.1% ± 1.3%
Range	0%-2%	0%-4%	0%-4%

[a] Data as of November 19, 2008

TABLE D3c
Historical Incidence of Malignant Lymphoma in Control Female B6C3F1 Mice[a]

Study (Study Start)	Incidence in Controls
Historical Incidence: Methylcellulose Gavage Studies	
Androstenedione (December, 2002)	14/50
Methylene blue trihydrate (July, 2000)	6/50
Total (%)	20/100 (20.0%)
Mean ± standard deviation	20.0% ± 11.3%
Range	12%-28%
Overall Historical Incidence: All Routes	
Total (%)	307/1,498 (20.5%)
Mean ± standard deviation	20.5% ± 9.7%
Range	4%-54%

[a] 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[a]

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Disposition Summary				
Animals initially in study	50	50	50	50
Early deaths				
Accidental deaths	2		1	1
Moribund	6	2	3	3
Natural deaths	7	8	6	6
Survivors				
Died last week of study	1		1	
Terminal sacrifice	34	40	39	40
Animals examined microscopically	50	50	50	50
Alimentary System				
Esophagus	(50)	(50)	(49)	(50)
Gallbladder	(44)	(45)	(42)	(48)
Cyst				1 (2%)
Intestine large, cecum	(49)	(50)	(50)	(49)
Edema		1 (2%)		
Intestine small, duodenum	(47)	(46)	(46)	(49)
Metaplasia	1 (2%)			
Epithelium, hyperplasia			1 (2%)	
Intestine small, ileum	(46)	(48)	(49)	(50)
Epithelium, hyperplasia		1 (2%)		
Intestine small, jejunum	(46)	(48)	(49)	(50)
Hyperplasia, lymphoid		1 (2%)		
Liver	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)	1 (2%)	1 (2%)	
Basophilic focus		4 (8%)	3 (6%)	
Clear cell focus	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Eosinophilic focus	9 (18%)	8 (16%)	11 (22%)	11 (22%)
Hematopoietic cell proliferation	6 (12%)	2 (4%)	4 (8%)	3 (6%)
Hemorrhage		1 (2%)		
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)	2 (4%)	
Infiltration cellular, mixed cell	2 (4%)	4 (8%)	2 (4%)	
Mixed cell focus	2 (4%)	5 (10%)	7 (14%)	15 (30%)
Necrosis, focal	2 (4%)	5 (10%)	2 (4%)	2 (4%)
Tension lipidosis	1 (2%)		1 (2%)	2 (4%)
Centrilobular, necrosis	1 (2%)	1 (2%)	1 (2%)	
Hepatocyte, hypertrophy				1 (2%)
Hepatocyte, vacuolization cytoplasmic	6 (12%)	3 (6%)	9 (18%)	22 (44%)
Kupffer cell, hyperplasia		1 (2%)	1 (2%)	
Kupffer cell, pigmentation	2 (4%)			
Oval cell, hyperplasia				1 (2%)
Mesentery	(7)	(9)	(14)	(8)
Fat, necrosis	7 (100%)	6 (67%)	13 (93%)	8 (100%)
Oral mucosa	(0)	(0)	(0)	(1)
Pancreas	(49)	(50)	(50)	(49)
Atrophy	1 (2%)		2 (4%)	4 (8%)
Cyst		1 (2%)	3 (6%)	1 (2%)
Infiltration cellular, lymphocyte	1 (2%)			
Acinus, cytoplasmic alteration	1 (2%)	1 (2%)	3 (6%)	1 (2%)

[a] 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

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Alimentary System (continued)				
Salivary glands	(49)	(49)	(49)	(50)
Atrophy	1 (2%)			
Infiltration cellular, lymphocyte	10 (20%)	12 (24%)	2 (4%)	7 (14%)
Submandibular gland, cytoplasmic alteration		17 (35%)	40 (82%)	45 (90%)
Stomach, forestomach	(50)	(50)	(50)	(50)
Diverticulum		1 (2%)		
Metaplasia, hepatocyte		1 (2%)		
Ulcer	1 (2%)			1 (2%)
Epithelium, hyperplasia		1 (2%)	1 (2%)	3 (6%)
Stomach, glandular	(50)	(50)	(50)	(50)
Cyst	2 (4%)			
Erosion	1 (2%)	1 (2%)		
Metaplasia, hepatocyte				1 (2%)
Mineralization			1 (2%)	
Ulcer	1 (2%)			
Epithelium, hyperplasia	1 (2%)			1 (2%)
Cardiovascular System				
Heart	(50)	(50)	(50)	(50)
Cardiomyopathy	1 (2%)	2 (4%)		2 (4%)
Inflammation, acute	1 (2%)			
Inflammation, chronic				1 (2%)
Mineralization	1 (2%)	1 (2%)		
Thrombosis			1 (2%)	
Endocrine System				
Adrenal cortex	(50)	(50)	(50)	(50)
Accessory adrenal cortical nodule	3 (6%)	7 (14%)	5 (10%)	3 (6%)
Hyperplasia, focal	1 (2%)	1 (2%)		1 (2%)
Hypertrophy, focal		1 (2%)		1 (2%)
Adrenal medulla	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	1 (2%)	1 (2%)	1 (2%)
Islets, pancreatic	(49)	(50)	(49)	(48)
Hyperplasia	1 (2%)	1 (2%)	1 (2%)	
Parathyroid gland	(48)	(49)	(45)	(50)
Cyst		1 (2%)	1 (2%)	2 (4%)
Pituitary gland	(49)	(47)	(50)	(50)
Pars distalis, angiectasis	1 (2%)	1 (2%)	2 (4%)	2 (4%)
Pars distalis, cyst	1 (2%)	1 (2%)		1 (2%)
Pars distalis, hyperplasia		1 (2%)	1 (2%)	
Pars distalis, hyperplasia, focal	5 (10%)	8 (17%)	7 (14%)	7 (14%)
Thyroid gland	(50)	(50)	(48)	(50)
Follicle, cyst			1 (2%)	
Follicle, degeneration, focal	26 (52%)	18 (36%)	20 (42%)	19 (38%)
Follicular cell, hyperplasia		1 (2%)	2 (4%)	1 (2%)
General Body System				
Tissue NOS	(1)	(0)	(0)	(1)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Genital System				
Clitoral gland	(47)	(47)	(49)	(50)
Hyperplasia		2 (4%)	13 (27%)	41 (82%)
Duct, dilatation		2 (4%)	17 (35%)	49 (98%)
Ovary	(49)	(50)	(49)	(48)
Angiectasis		4 (8%)	2 (4%)	1 (2%)
Cyst	11 (22%)	14 (28%)	10 (20%)	15 (31%)
Hemorrhage	4 (8%)		1 (2%)	6 (13%)
Corpus luteum, hyperplasia		1 (2%)		
Interstital cell, hyperplasia	1 (2%)		2 (4%)	
Uterus	(50)	(50)	(50)	(50)
Angiectasis	1 (2%)		2 (4%)	4 (8%)
Hyperplasia, cystic	44 (88%)	42 (84%)	42 (84%)	36 (72%)
Endometrium, dysplasia			1 (2%)	
Hematopoietic System				
Bone marrow	(50)	(49)	(50)	(50)
Hyperplasia	14 (28%)	18 (37%)	23 (46%)	11 (22%)
Infiltraton cellular, histiocyte			1 (2%)	
Lymph node	(10)	(5)	(4)	(1)
Iliac, hematopoietic cell proliferation		2 (40%)		
Iliac, hemorrhage	1 (10%)	1 (20%)		
Iliac, hyperplasia, lymphoid		1 (20%)		
Inguinal, hyperplasia, lymphoid	1 (10%)			
Mediastinal, hyperplasia, lymphoid			1 (25%)	
Renal, hematopoietic cell proliferation		1 (20%)		
Renal, hyperplasia, lymphoid				1 (100%)
Lymph node, mandibular	(46)	(49)	(47)	(49)
Atrophy			1 (2%)	
Hematopoietic cell proliferation		1 (2%)		1 (2%)
Hemorrhage			2 (4%)	
Hyperplasia, lymphoid	4 (9%)	3 (6%)	4 (9%)	2 (4%)
Pigmentation	4 (9%)	4 (8%)	4 (9%)	3 (6%)
Lymph node, mesenteric	(50)	(50)	(46)	(48)
Atrophy	1 (2%)	1 (2%)	2 (4%)	
Hematopoietic cell proliferation			1 (2%)	
Hemorrhage	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Hyperplasia, lymphoid		3 (6%)	5 (11%)	1 (2%)
Spleen	(50)	(50)	(50)	(50)
Accessory spleen	1 (2%)			1 (2%)
Hematopoietic cell proliferation	28 (56%)	29 (58%)	32 (64%)	22 (44%)
Necrosis	1 (2%)	1 (2%)		
Lymphoid follicle, atrophy			1 (2%)	2 (4%)
Lymphoid follicle, hyperplasia	7 (14%)	6 (12%)	15 (30%)	8 (16%)
Red pulp, hyperplasia				1 (2%)
Thymus	(47)	(46)	(46)	(46)
Atrophy	2 (4%)	3 (7%)	4 (9%)	3 (7%)
Cyst	1 (2%)			
Hyperplasia, lymphoid		2 (4%)		

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Integumentary System				
Mammary gland	(50)	(50)	(50)	(50)
Hyperplasia	4 (8%)	3 (6%)	5 (10%)	2 (4%)
Skin	(50)	(50)	(50)	(50)
Cyst epithelial inclusion			1 (2%)	
Edema	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)			
Metaplasia, osseous	1 (2%)			
Ulcer	2 (4%)		2 (4%)	
Epidermis, hyperplasia	1 (2%)			
Musculoskeletal System				
Bone	(50)	(50)	(50)	(50)
Fibrosis	3 (6%)	4 (8%)	4 (8%)	5 (10%)
Hyperostosis	1 (2%)			
Skeletal muscle	(4)	(0)	(4)	(2)
Atrophy			1 (25%)	
Hemorrhage				1 (50%)
Nervous System				
Brain	(50)	(49)	(50)	(50)
Compression			1 (2%)	2 (4%)
Cyst epithelial inclusion	1 (2%)			
Gliosis	1 (2%)			1 (2%)
Hydrocephalus				1 (2%)
Peripheral nerve	(2)	(1)	(3)	(1)
Atrophy	2 (100%)		2 (67%)	1 (100%)
Inflammation, chronic active				1 (100%)
Spinal cord	(2)	(1)	(4)	(1)
Respiratory System				
Lung	(50)	(50)	(50)	(50)
Hemorrhage	2 (4%)	3 (6%)	3 (6%)	7 (14%)
Infiltration cellular, histiocyte	2 (4%)	1 (2%)	1 (2%)	4 (8%)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		
Metaplasia, osseous		1 (2%)		
Mineralization			1 (2%)	
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	1 (2%)		1 (2%)	
Nose	(50)	(49)	(50)	(50)
Inflammation, chronic	1 (2%)	3 (6%)		4 (8%)
Trachea	(50)	(50)	(48)	(50)
Special Senses System				
Eye	(50)	(50)	(50)	(50)
Cataract			2 (4%)	
Inflammation, chronic	2 (4%)		2 (4%)	1 (2%)
Cornea, hyperplasia	2 (4%)		1 (2%)	
Harderian gland	(50)	(50)	(50)	(50)
Hyperplasia, focal	1 (2%)	2 (4%)	1 (2%)	1 (2%)

TABLE D4
Summary of the Incidence of Nonneoplastic Lesions in Female Mice in the 2-Year Gavage Study of Androstenedione

	Vehicle Control	2 mg/kg	10 mg/kg	50 mg/kg
Urinary System				
Kidney	(50)	(50)	(50)	(50)
Cyst		1 (2%)	1 (2%)	1 (2%)
Glomerulosclerosis			3 (6%)	
Hydronephrosis				1 (2%)
Infarct	4 (8%)	3 (6%)	1 (2%)	
Infiltration cellular, lymphocyte	4 (8%)	4 (8%)	2 (4%)	3 (6%)
Inflammation, suppurative	2 (4%)		1 (2%)	
Metaplasia, osseous	1 (2%)	2 (4%)		3 (6%)
Nephropathy	13 (26%)	11 (22%)	13 (26%)	14 (28%)
Glomerulus, metaplasia	2 (4%)	1 (2%)	5 (10%)	27 (54%)
Renal tubule, accumulation, hyaline droplet	1 (2%)	3 (6%)		
Renal tubule, hyperplasia		1 (2%)		1 (2%)
Renal tubule, pigmentation		1 (2%)		1 (2%)
Transitional epithelium, hyperplasia			1 (2%)	
Urinary bladder	(50)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	1 (2%)	4 (8%)	2 (4%)	
Inflammation, chronic	1 (2%)			
Transitional epithelium, hyperplasia	1 (2%)		2 (4%)	1 (2%)

APPENDIX E
GENETIC TOXICOLOGY

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 normochromatic 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]

Strain	Dose (µg/plate)	Revertants/Plate[b] –S9 Trial 1	–S9 Trial 2	+ hamster S9 10%	+ hamster S9 30%	+ rat S9 10%	+ rat S9 30%
Study performed at SRI International							
TA100	0	114 ± 12	108 ± 9	118 ± 7	128 ± 6	126 ± 9	129 ± 2
	100	109 ± 4	120 ± 5	109 ± 11	138 ± 1	129 ± 10	114 ± 12
	333	115 ± 8	105 ± 10	141 ± 11	129 ± 5	135 ± 7	120 ± 3
	1,000	126 ± 8	105 ± 8	98 ± 12	126 ± 6	114 ± 4	116 ± 10
	3,333	115 ± 6[c]	120 ± 4[c]	113 ± 4[c]	108 ± 4[c]	126 ± 6[c]	113 ± 4[c]
	10,000	119 ± 1[c]	113 ± 5[c]	113 ± 1[c]	114 ± 3[c]	109 ± 1[c]	113 ± 5[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control[d]		925 ± 30	890 ± 12	643 ± 28	703 ± 6	623 ± 11	642 ± 26
TA1535	0	22 ± 3	9 ± 3	7 ± 2	18 ± 3	12 ± 4	13 ± 2
	100	17 ± 1	10 ± 0	8 ± 1	15 ± 2	10 ± 2	14 ± 3
	333	18 ± 1	10 ± 3	11 ± 1	14 ± 1	11 ± 3	14 ± 1
	1,000	21 ± 6	9 ± 2	9 ± 2	11 ± 0	12 ± 2	18 ± 1
	3,333	19 ± 5[c]	6 ± 1[c]	7 ± 1[c]	11 ± 1[c]	7 ± 1[c]	14 ± 2[c]
	10,000	16 ± 5[c]	7 ± 2[c]	8 ± 2[c]	10 ± 3[c]	7 ± 2[c]	14 ± 3[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		917 ± 12	909 ± 33	113 ± 9	137 ± 5	113 ± 9	118 ± 5
TA97	0	133 ± 15	159 ± 16	160 ± 10	157 ± 12	191 ± 4	196 ± 6
	100	145 ± 13	165 ± 5	132 ± 7	168 ± 8	163 ± 5	211 ± 4
	333	132 ± 5	160 ± 18	127 ± 3	169 ± 5	157 ± 8	211 ± 5
	1,000	115 ± 8	146 ± 6	164 ± 6	147 ± 1	199 ± 15	177 ± 4
	3,333	113 ± 7[c]	151 ± 12[c]	144 ± 8[c]	131 ± 7[c]	202 ± 15[c]	160 ± 16[c]
	10,000	112 ± 4[c]	125 ± 3[c]	145 ± 1[c]	91 ± 4[c]	204 ± 4[c]	177 ± 12[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		667 ± 16	555 ± 20	619 ± 20	710 ± 5	524 ± 37	660 ± 10
TA98	0	31 ± 1	20 ± 1	24 ± 2	34 ± 2	19 ± 2	30 ± 4
	100	30 ± 3	18 ± 4	27 ± 4	41 ± 2	23 ± 1	35 ± 6
	333	28 ± 2	21 ± 5	22 ± 2	30 ± 1	28 ± 3	29 ± 3
	1,000	30 ± 1	20 ± 5	23 ± 2	28 ± 1	20 ± 3	31 ± 3
	3,333	19 ± 2[c]	15 ± 1[c]	19 ± 1[c]	28 ± 3[c]	21 ± 5[c]	32 ± 4[c]
	10,000	26 ± 3[c]	15 ± 1[c]	17 ± 2[c]	31 ± 2[c]	22 ± 2[c]	33 ± 1[c]
Trial summary		Negative	Negative	Negative	Negative	Negative	Negative
Positive control		439 ± 18	355 ± 20	486 ± 3	446 ± 19	416 ± 20	411 ± 32

TABLE E1
Mutagenicity of Androstenedione in *Salmonella typhimurium*

Strain	Dose (µg/plate)	Revertants/Plate –S9 Trial 1	–S9 Trial 2	+ 10% rat S9 Trial 1	+ 10% rat S9 Trial 2
Study performed at SITEK Research Laboratories					
TA100	0	103 ± 7	64 ± 3	63 ± 4	94 ± 2
	100	97 ± 3	72 ± 3	44 ± 1	70 ± 4
	500	77 ± 6	45 ± 0	42 ± 2	62 ± 2
	1,500	64 ± 3	15 ± 3	34 ± 2	22 ± 4
	2,500	7 ± 2[c]	15 ± 2	24 ± 1	28 ± 4[c]
	3,500	6 ± 1[c]	14 ± 3	24 ± 1	20 ± 5[c]
Trial summary		Negative	Negative	Negative	Negative
Positive control		844 ± 95	337 ± 36	383 ± 10	1,086 ± 2
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 ± 1[c]	7 ± 1	32 ± 2	19 ± 1
	3,500	3 ± 1[c]	3 ± 1	28 ± 3	
	5,000				19 ± 1
	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-*o*-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[a]

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

[a] Study was performed at ILS, Inc. The detailed protocol is presented by Shelby *et al.* (1993). PCE=polychromatic erythrocyte
[b] Mean ± standard error
[c] 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)
[d] Vehicle control
[e] Significance of micronucleated PCEs/1,000 PCEs tested by the one-tailed trend test, significant at P≤0.025 (ILS, 1990).
[f] Positive control

TABLE E3
Frequency of Micronuclei in Peripheral Blood Erythrocytes of Mice Following Gavage Administration of Androstenedione for 3 Months[a]

Compound	Dose (mg/kg)	Number of Mice with Erythrocytes Scored	Micronucleated NCEs/ 1,000 NCEs[b]	P Value[c]	PCEs[b] (%)
Male					
Methylcellulose[d]	0	5	2.60 ± 0.51		2.860 ± 0.19
Androstenedione	1	5	2.90 ± 0.62	0.3427	2.500 ± 0.37
	5	5	2.80 ± 0.30	0.3926	3.020 ± 0.18
	10	5	2.70 ± 0.54	0.4453	3.020 ± 0.20
	20	5	2.40 ± 0.46	0.6115	2.920 ± 0.18
	50	5	2.10 ± 0.10	0.7674	2.540 ± 0.29
			P=0.880[e]		
Female					
Methylcellulose	0	5	1.60 ± 0.43		2.820 ± 0.24
Androstenedione	1	5	1.30 ± 0.20	0.7114	2.860 ± 0.28
	5	5	1.40 ± 0.29	0.6426	3.300 ± 0.29
	10	5	2.00 ± 0.57	0.2523	3.200 ± 0.18
	20	5	1.30 ± 0.12	0.7114	3.260 ± 0.27
	50	5	3.10 ± 0.40	0.0142	2.640 ± 0.14
			P=0.001		

[a] Study was performed at ILS, Inc. The detailed protocol is presented by MacGregor *et al.* (1990).
PCE = polychromatic erythrocyte; NCE = normochromatic erythrocyte.
[b] Mean ± standard error
[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[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
Hematology						
Day 4	10	10	10	10	10	10
Day 24	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 4	43.9 ± 0.9	43.7 ± 0.7	43.7 ± 0.7	42.8 ± 0.9	44.4 ± 0.8	43.6 ± 0.6
Day 24	45.4 ± 0.4	49.1 ± 1.4	45.1 ± 0.6	45.1 ± 0.7	46.3 ± 0.5	50.1 ± 1.7
Week 14	45.3 ± 0.4	44.8 ± 0.5	44.0 ± 0.3	44.4 ± 0.4	44.4 ± 0.3	44.3 ± 0.4
Hematocrit (spun) (%)						
Day 4	43.2 ± 0.9	42.6 ± 0.7	43.1 ± 0.7	42.3 ± 1.0	43.8 ± 0.9	42.6 ± 0.7
Day 24	45.5 ± 0.4	48.0 ± 1.1	45.1 ± 0.6	45.1 ± 0.5	46.3 ± 0.6	49.1 ± 1.3
Week 14	46.3 ± 0.4	46.0 ± 0.5	45.4 ± 0.1	45.2 ± 0.3	45.2 ± 0.1	45.2 ± 0.4
Hemoglobin (g/dL)						
Day 4	14.3 ± 0.3	14.2 ± 0.2	14.0 ± 0.3	13.8 ± 0.3	14.3 ± 0.2	14.1 ± 0.2
Day 24	15.2 ± 0.1	16.2 ± 0.4	14.9 ± 0.2	15.0 ± 0.2	15.3 ± 0.2	16.6 ± 0.5
Week 14	15.1 ± 0.1	14.9 ± 0.2	14.7 ± 0.1	14.8 ± 0.1	14.8 ± 0.1	14.7 ± 0.1
Erythrocytes (10^6/µL)						
Day 4	7.65 ± 0.17	7.61 ± 0.13	7.53 ± 0.12	7.35 ± 0.14	7.62 ± 0.13	7.51 ± 0.10
Day 24	8.00 ± 0.10	8.69 ± 0.23	7.91 ± 0.12	7.95 ± 0.10	8.10 ± 0.10	8.82 ± 0.29
Week 14	9.05 ± 0.08	8.91 ± 0.10	8.77 ± 0.04	8.88 ± 0.09	8.87 ± 0.06	8.83 ± 0.09
Reticulocytes (10^5/µL)						
Day 4	5.60 ± 0.34	5.46 ± 0.39	5.38 ± 0.41	5.73 ± 0.19	5.65 ± 0.33	5.48 ± 0.22
Day 24	2.71 ± 0.17	2.10 ± 0.21	3.07 ± 0.12	1.91 ± 0.21	2.56 ± 0.13	2.00 ± 0.26
Week 14	2.25 ± 0.03	2.03 ± 0.06*	2.20 ± 0.05	2.24 ± 0.04	2.26 ± 0.05	2.24 ± 0.07
Nucleated erythrocytes/100 erythrocytes						
Day 4	0.7 ± 0.3	1.0 ± 0.3	0.3 ± 0.2	0.8 ± 0.2	0.6 ± 0.2	0.6 ± 0.2
Day 24	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.3 ± 0.2
Week 14	0.0 ± 0.0	0.1 ± 0.1	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
Mean cell volume (fL)						
Day 4	57.5 ± 0.3	57.4 ± 0.3	58.0 ± 0.4	58.2 ± 0.3	58.3 ± 0.4	58.1 ± 0.3
Day 24	56.8 ± 0.6	56.5 ± 0.3	57.1 ± 0.3	56.7 ± 0.3	57.2 ± 0.3	56.7 ± 0.2
Week 14	50.1 ± 0.2	50.3 ± 0.1	50.1 ± 0.1	49.9 ± 0.2	50.1 ± 0.2	50.1 ± 0.1
Mean cell hemoglobin (pg)						
Day 4	18.7 ± 0.1	18.6 ± 0.1	18.6 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	18.8 ± 0.1
Day 24	19.0 ± 0.1	18.7 ± 0.1	18.8 ± 0.1	18.8 ± 0.1	18.9 ± 0.1	18.8 ± 0.1
Week 14	16.7 ± 0.1	16.8 ± 0.1	16.7 ± 0.0	16.7 ± 0.1	16.7 ± 0.1	16.7 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	32.5 ± 0.2	32.4 ± 0.1	32.1 ± 0.2	32.3 ± 0.1	32.3 ± 0.3	32.4 ± 0.2
Day 24	33.4 ± 0.2	33.1 ± 0.1	33.0 ± 0.1	33.1 ± 0.2	33.1 ± 0.1	33.1 ± 0.2
Week 14	33.3 ± 0.1	33.4 ± 0.2	33.4 ± 0.1	33.3 ± 0.1	33.2 ± 0.1	33.3 ± 0.1
Platelets (10^3/µL)						
Day 4	1,044.2 ± 43.9	1,078.0 ± 53.0	1,029.4 ± 42.6	977.2 ± 30.8	961.5 ± 37.5	1,081.8 ± 45.6
Day 24	891.4 ± 23.3	938.3 ± 26.6	934.5 ± 26.3	851.2 ± 27.6	873.0 ± 23.2	955.9 ± 11.8
Week 14	617.5 ± 17.9	651.5 ± 13.0	651.2 ± 12.1	661.2 ± 16.7	675.5 ± 10.8*	644.2 ± 11.6
Leukocytes (10^3/µL)						
Day 4	9.78 ± 0.30	9.07 ± 0.31	8.55 ± 0.24*	8.61 ± 0.34*	8.72 ± 0.22*	8.12 ± 0.33**
Day 24	11.16 ± 0.40	9.72 ± 0.47	9.88 ± 0.26	9.62 ± 0.39	9.20 ± 0.43*	10.69 ± 0.32
Week 14	10.79 ± 0.36	10.43 ± 0.30	10.40 ± 0.40	10.49 ± 0.33	9.97 ± 0.30	10.23 ± 0.34

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male (continued)						
Hematology *(continued)*						
Day 4	10	10	10	10	10	10
Day 24	10	10	10	10	10	10
Week 14	10	10	10	10	10	10
Segmented neutrophils (10^3/µL)						
Day 4	1.00 ± 0.04	1.03 ± 0.05	0.90 ± 0.03	0.96 ± 0.07	0.93 ± 0.03	0.86 ± 0.04*
Day 24	1.17 ± 0.07	0.92 ± 0.05*	1.23 ± 0.06	1.17 ± 0.06	1.07 ± 0.05	1.01 ± 0.06
Week 14	1.41 ± 0.03	1.40 ± 0.13	1.36 ± 0.05	1.43 ± 0.05	1.30 ± 0.04	1.21 ± 0.04*
Lymphocytes (10^3/µL)						
Day 4	8.42 ± 0.30	7.72 ± 0.28	7.35 ± 0.22*	7.35 ± 0.28*	7.50 ± 0.23*	7.01 ± 0.30**
Day 24	9.54 ± 0.42	8.50 ± 0.46	8.33 ± 0.22	8.10 ± 0.39	7.82 ± 0.38*	9.34 ± 0.33
Week 14	9.04 ± 0.35	8.69 ± 0.30	8.71 ± 0.36	8.73 ± 0.29	8.35 ± 0.27	8.73 ± 0.32
Monocytes (10^3/µL)						
Day 4	0.16 ± 0.01	0.16 ± 0.01	0.14 ± 0.01	0.15 ± 0.01	0.15 ± 0.01	0.13 ± 0.01**
Day 24	0.19 ± 0.01	0.13 ± 0.01**	0.14 ± 0.01*	0.15 ± 0.01	0.14 ± 0.01*	0.16 ± 0.01
Week 14	0.17 ± 0.02	0.18 ± 0.02	0.15 ± 0.02	0.17 ± 0.02	0.14 ± 0.01	0.15 ± 0.02
Basophils (10^3/µL)						
Day 4	0.065 ± 0.007	0.043 ± 0.004**	0.037 ± 0.003**	0.043 ± 0.007**	0.041 ± 0.003**	0.031 ± 0.003**
Day 24	0.051 ± 0.005	0.043 ± 0.006	0.034 ± 0.003*	0.041 ± 0.003	0.041 ± 0.005	0.043 ± 0.006
Week 14	0.039 ± 0.003	0.051 ± 0.007	0.041 ± 0.006	0.044 ± 0.005	0.046 ± 0.009	0.038 ± 0.007
Eosinophils (10^3/µL)						
Day 4	0.03 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.03 ± 0.00	0.02 ± 0.00
Day 24	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.00*	0.04 ± 0.00
Week 14	0.08 ± 0.01	0.08 ± 0.01	0.09 ± 0.01	0.09 ± 0.00	0.08 ± 0.01	0.06 ± 0.01*
Large unstained cells (10^3/µL)						
Day 4	0.10 ± 0.01	0.10 ± 0.01	0.10 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.08 ± 0.01
Day 24	0.15 ± 0.02	0.09 ± 0.01*	0.11 ± 0.01	0.10 ± 0.01	0.10 ± 0.02	0.11 ± 0.01
Week 14	0.04 ± 0.01	0.04 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.01	0.04 ± 0.00
Clinical Chemistry						
n	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	13.2 ± 0.4	12.7 ± 0.3	13.5 ± 0.4	13.0 ± 0.5	13.1 ± 0.4	13.3 ± 0.4
Day 24	17.0 ± 0.7	17.3 ± 0.5	13.4 ± 0.3**	15.0 ± 0.4	18.9 ± 0.9	16.7 ± 0.6
Week 14	17.9 ± 0.3	18.3 ± 0.5	17.3 ± 0.3	17.1 ± 0.5	18.3 ± 0.6	17.6 ± 0.6
Creatinine (mg/dL)						
Day 4	0.49 ± 0.01	0.50 ± 0.00	0.51 ± 0.01	0.48 ± 0.01	0.49 ± 0.01	0.49 ± 0.01
Day 24	0.54 ± 0.02	0.57 ± 0.02	0.56 ± 0.02	0.57 ± 0.02	0.53 ± 0.02	0.53 ± 0.02
Week 14	0.63 ± 0.02	0.64 ± 0.02	0.62 ± 0.01	0.65 ± 0.02	0.66 ± 0.03	0.63 ± 0.02
Total protein (g/dL)						
Day 4	5.6 ± 0.1	5.5 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	5.6 ± 0.1	5.6 ± 0.1
Day 24	6.0 ± 0.1	6.4 ± 0.2*	5.8 ± 0.1	6.0 ± 0.1	6.1 ± 0.1	6.4 ± 0.2
Week 14	6.8 ± 0.1	6.7 ± 0.1	6.7 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1
Albumin (g/dL)						
Day 4	4.0 ± 0.1	4.1 ± 0.1	4.1 ± 0.1	3.9 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
Day 24	4.3 ± 0.0	4.5 ± 0.1	4.1 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	4.6 ± 0.1
Week 14	4.5 ± 0.0	4.5 ± 0.1	4.5 ± 0.0	4.5 ± 0.0	4.5 ± 0.0	4.4 ± 0.0

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male (continued)						
Clinical Chemistry *(continued)*						
n	10	10	10	10	10	10
Alanine aminotransferase (IU/L)						
Day 4	58 ± 2	62 ± 1	59 ± 2	61 ± 2	59 ± 2	56 ± 1
Day 24	56 ± 2	46 ± 3	50 ± 1	57 ± 3	64 ± 3	43 ± 3
Week 14	74 ± 5	76 ± 9	63 ± 3	64 ± 5	78 ± 5	54 ± 2**
Alkaline phosphatase (IU/L)						
Day 4	633 ± 14	671 ± 20	669 ± 19	653 ± 14	637 ± 17	660 ± 10
Day 24	435 ± 10	413 ± 15	486 ± 8	443 ± 10	410 ± 8	389 ± 12*
Week 14	214 ± 4	215 ± 5	212 ± 3	215 ± 4	220 ± 3	208 ± 4
Creatine kinase (IU/L)						
Day 4	386 ± 26	409 ± 58	430 ± 34	394 ± 41	389 ± 22	373 ± 39
Day 24	351 ± 36	385 ± 44[b]	332 ± 29	263 ± 24	347 ± 26	354 ± 33
Week 14	188 ± 10	179 ± 13	177 ± 14	178 ± 14	178 ± 9	160 ± 15
Sorbitol dehydrogenase (IU/L)						
Day 4	12 ± 1	11 ± 1	12 ± 1	12 ± 1	11 ± 1	13 ± 1
Day 24	11 ± 1	12 ± 2	13 ± 1	14 ± 1	14 ± 1	12 ± 1
Week 14	24 ± 1	25 ± 2	22 ± 1	21 ± 1	24 ± 1	19 ± 1*
Bile salts (µmol/L)						
Day 4	25.9 ± 1.9	28.7 ± 2.1	29.6 ± 2.9	24.1 ± 1.5	25.8 ± 1.9	25.1 ± 2.2
Day 24	20.4 ± 1.8	20.6 ± 1.5	25.0 ± 2.2	16.8 ± 1.0	25.1 ± 2.4	16.5 ± 0.7
Week 14	24.1 ± 2.3	25.6 ± 2.6	25.0 ± 2.1	23.1 ± 2.6	20.9 ± 1.5	23.8 ± 2.0
Female						
Hematology						
Day 4	10	10	10	9	10	10
Day 24	10	9	10	10	9	10
Week 14	10	10	10	10	10	10
Hematocrit (auto) (%)						
Day 4	42.6 ± 0.9	44.6 ± 0.6	45.6 ± 0.6*	45.6 ± 0.9	45.5 ± 0.6	45.3 ± 0.6
Day 24	45.8 ± 0.7	45.2 ± 0.6	45.7 ± 0.6	47.3 ± 1.0	45.7 ± 0.4	46.2 ± 0.6
Week 14	42.7 ± 0.4	42.8 ± 0.4	43.4 ± 0.5	42.7 ± 0.3	43.5 ± 0.4	44.0 ± 0.3
Hematocrit (spun) (%)						
Day 4	42.1 ± 0.9	43.9 ± 0.5	44.7 ± 0.5	44.4 ± 0.7[c]	44.7 ± 0.8	44.4 ± 0.7*
Day 24	44.5 ± 0.5	44.0 ± 0.4	44.0 ± 0.5	46.0 ± 0.9	44.1 ± 0.4	44.7 ± 0.5
Week 14	43.5 ± 0.3	43.4 ± 0.4	44.0 ± 0.5	43.7 ± 0.4	43.7 ± 0.4	44.6 ± 0.3
Hemoglobin (g/dL)						
Day 4	14.4 ± 0.3	15.1 ± 0.2	15.5 ± 0.2*	15.3 ± 0.3	15.4 ± 0.2	15.3 ± 0.2
Day 24	14.9 ± 0.2	14.7 ± 0.2	14.9 ± 0.2	15.4 ± 0.3	14.9 ± 0.1	15.0 ± 0.2
Week 14	14.4 ± 0.1	14.4 ± 0.1	14.5 ± 0.1	14.4 ± 0.1	14.5 ± 0.1	14.8 ± 0.1**
Erythrocytes (10^6/µL)						
Day 4	7.50 ± 0.14	7.86 ± 0.11	8.05 ± 0.10*	7.98 ± 0.18	7.98 ± 0.11	8.01 ± 0.12*
Day 24	8.00 ± 0.10	7.91 ± 0.10	7.99 ± 0.10	8.25 ± 0.15	8.02 ± 0.08	8.06 ± 0.09
Week 14	8.23 ± 0.08	8.22 ± 0.07	8.29 ± 0.08	8.20 ± 0.06	8.32 ± 0.07	8.42 ± 0.06

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Female (continued)						
Hematology *(continued)*						
Day 4	10	10	10	9	10	10
Day 24	10	9	10	10	9	10
Week 14	10	10	10	10	10	10
Reticulocytes (10^5/μL)						
Day 4	4.46 ± 0.18	4.80 ± 0.26	4.91 ± 0.25	4.77 ± 0.13	4.69 ± 0.23	5.02 ± 0.23
Day 24	1.78 ± 0.07	1.76 ± 0.11	1.74 ± 0.07	1.84 ± 0.05	1.82 ± 0.05	1.70 ± 0.05
Week 14	1.71 ± 0.06	1.78 ± 0.08	1.87 ± 0.06	1.75 ± 0.05	1.88 ± 0.04	1.73 ± 0.05
Nucleated erythrocytes/100 leukocytes						
Day 4	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0[c]	0.1 ± 0.1	0.0 ± 0.0
Day 24	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
Week 14	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
Mean cell volume (fL)						
Day 4	56.8 ± 0.2	56.8 ± 0.3	56.8 ± 0.2	57.2 ± 0.3	57.0 ± 0.4	56.6 ± 0.3
Day 24	57.2 ± 0.3	57.2 ± 0.3	57.2 ± 0.3	57.3 ± 0.3	57.0 ± 0.3	57.3 ± 0.4
Week 14	52.0 ± 0.2	52.1 ± 0.2	52.3 ± 0.2	52.1 ± 0.1	52.2 ± 0.2	52.3 ± 0.2
Mean cell hemoglobin (pg)						
Day 4	19.2 ± 0.1	19.3 ± 0.1	19.3 ± 0.1	19.2 ± 0.1	19.4 ± 0.1	19.1 ± 0.2
Day 24	18.6 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.7 ± 0.1	18.6 ± 0.1	18.6 ± 0.1
Week 14	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.1	17.5 ± 0.0	17.6 ± 0.1
Mean cell hemoglobin concentration (g/dL)						
Day 4	33.8 ± 0.2	33.9 ± 0.2	33.9 ± 0.3	33.7 ± 0.1	33.9 ± 0.2	33.8 ± 0.2
Day 24	32.4 ± 0.2	32.6 ± 0.1	32.6 ± 0.1	32.6 ± 0.1	32.6 ± 0.1	32.4 ± 0.1
Week 14	33.7 ± 0.2	33.6 ± 0.2	33.5 ± 0.1	33.7 ± 0.1	33.5 ± 0.2	33.7 ± 0.2
Platelets (10^3/μL)						
Day 4	935.9 ± 35.2	905.7 ± 37.2	1,002.7 ± 76.7	861.2 ± 46.9	959.9 ± 40.8	944.7 ± 32.1
Day 24	846.3 ± 25.6	834.8 ± 30.7	845.2 ± 18.6	818.6 ± 16.3	798.3 ± 21.4	858.1 ± 37.1
Week 14	657.6 ± 21.0	687.8 ± 8.5	672.4 ± 17.9	727.5 ± 11.8*	689.8 ± 15.5	668.4 ± 17.7
Leukocytes (10^3/μL)						
Day 4	9.32 ± 0.71	10.71 ± 0.62	11.81 ± 0.54*	10.17 ± 0.54	10.84 ± 0.59	10.67 ± 0.37
Day 24	9.96 ± 0.36	9.33 ± 0.26	10.02 ± 0.16	9.60 ± 0.34	9.51 ± 0.29	9.76 ± 0.43
Week 14	6.95 ± 0.34	7.08 ± 0.20	7.17 ± 0.30	7.09 ± 0.30	7.17 ± 0.59	8.15 ± 0.21*
Segmented neutrophils (10^3/μL)						
Day 4	0.92 ± 0.07	1.07 ± 0.10	1.12 ± 0.05	1.12 ± 0.08	1.10 ± 0.08	1.09 ± 0.04
Day 24	1.16 ± 0.08	1.14 ± 0.07	1.13 ± 0.06	1.14 ± 0.08	1.15 ± 0.06	1.07 ± 0.04
Week 14	1.07 ± 0.07	1.07 ± 0.05	1.21 ± 0.09	1.24 ± 0.07	1.55 ± 0.18*	1.21 ± 0.08
Lymphocytes (10^3/μL)						
Day 4	8.07 ± 0.63	9.26 ± 0.54	10.22 ± 0.47*	8.67 ± 0.48	9.33 ± 0.52	9.21 ± 0.34
Day 24	8.44 ± 0.35	7.91 ± 0.24	8.56 ± 0.17	8.15 ± 0.27	8.01 ± 0.29	8.34 ± 0.38
Week 14	5.62 ± 0.29	5.73 ± 0.17	5.68 ± 0.23	5.58 ± 0.27	5.35 ± 0.41	6.67 ± 0.23*
Monocytes (10^3/μL)						
Day 4	0.15 ± 0.02	0.18 ± 0.02	0.21 ± 0.02	0.19 ± 0.02	0.20 ± 0.01	0.17 ± 0.01
Day 24	0.17 ± 0.01	0.12 ± 0.01	0.14 ± 0.01	0.14 ± 0.01	0.16 ± 0.01	0.16 ± 0.02
Week 14	0.11 ± 0.01	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.02	0.10 ± 0.01
Basophils (10^3/μL)						
Day 4	0.054 ± 0.007	0.073 ± 0.009	0.092 ± 0.014*	0.059 ± 0.007	0.073 ± 0.006	0.073 ± 0.004
Day 24	0.042 ± 0.004	0.038 ± 0.004	0.041 ± 0.004	0.037 ± 0.003	0.034 ± 0.004	0.047 ± 0.007
Week 14	0.043 ± 0.010	0.056 ± 0.015	0.048 ± 0.012	0.034 ± 0.009	0.042 ± 0.009	0.053 ± 0.009

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Female (continued)						
Hematology *(continued)*						
Day 4	10	10	10	9	10	10
Day 24	10	9	10	10	9	10
Week 14	10	10	10	10	10	10
Eosinophils (10^3/μL)						
Day 4	0.03 ± 0.00	0.04 ± 0.01	0.05 ± 0.00*	0.03 ± 0.00	0.04 ± 0.00	0.03 ± 0.01
Day 24	0.05 ± 0.01	0.04 ± 0.00	0.05 ± 0.01	0.05 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
Week 14	0.07 ± 0.01	0.06 ± 0.00	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Large unstained cells (10^3/μL)						
Day 4	0.09 ± 0.01	0.10 ± 0.01	0.12 ± 0.02	0.09 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
Day 24	0.10 ± 0.01	0.07 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.10 ± 0.01	0.10 ± 0.01
Week 14	0.04 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
Clinical Chemistry						
Day 4	10	10	10	10	10	10
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	10	10
Urea nitrogen (mg/dL)						
Day 4	12.7 ± 0.5	15.2 ± 1.4	20.4 ± 0.5**	12.0 ± 0.3	15.3 ± 0.7	17.6 ± 0.8*
Day 24	14.0 ± 0.5	14.7 ± 0.5	14.3 ± 0.3	15.3 ± 0.4	14.5 ± 0.4	15.0 ± 0.6
Week 14	16.2 ± 0.4	18.8 ± 0.4**	18.3 ± 0.4*	17.8 ± 0.3	17.1 ± 0.4	18.5 ± 0.6*
Creatinine (mg/dL)						
Day 4	0.49 ± 0.01	0.49 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.51 ± 0.01	0.49 ± 0.01
Day 24	0.57 ± 0.02	0.56 ± 0.02	0.58 ± 0.01	0.57 ± 0.02	0.55 ± 0.02	0.57 ± 0.02
Week 14	0.61 ± 0.02	0.59 ± 0.02	0.61 ± 0.01	0.59 ± 0.01	0.59 ± 0.01	0.58 ± 0.01
Total protein (g/dL)						
Day 4	5.5 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Day 24	5.8 ± 0.1	5.8 ± 0.1	5.9 ± 0.1	5.9 ± 0.1	5.7 ± 0.1	5.6 ± 0.1
Week 14	6.6 ± 0.1	6.9 ± 0.1	6.8 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.2 ± 0.1**
Albumin (g/dL)						
Day 4	4.1 ± 0.1	4.2 ± 0.1	4.3 ± 0.1	4.2 ± 0.0	4.3 ± 0.1	4.3 ± 0.1
Day 24	4.3 ± 0.1	4.3 ± 0.0	4.3 ± 0.1	4.4 ± 0.0	4.2 ± 0.0	4.3 ± 0.0
Week 14	4.8 ± 0.1	4.9 ± 0.1	4.9 ± 0.0	4.7 ± 0.1	4.6 ± 0.1	4.5 ± 0.0*
Alanine aminotransferase (IU/L)						
Day 4	52 ± 2	57 ± 2	60 ± 3*	53 ± 1	54 ± 3	59 ± 2
Day 24	41 ± 1	37 ± 1	36 ± 1**	41 ± 1	42 ± 1	36 ± 1
Week 14	57 ± 6	75 ± 11	65 ± 6	65 ± 8	55 ± 5	61 ± 4
Alkaline phosphatase (IU/L)						
Day 4	534 ± 13	487 ± 20	423 ± 14**	527 ± 9	489 ± 17	449 ± 15**
Day 24	334 ± 6	333 ± 6	334 ± 7	340 ± 10	329 ± 6	334 ± 3
Week 14	162 ± 5	174 ± 4	167 ± 6	166 ± 5	181 ± 7*	195 ± 3**
Creatine kinase (IU/L)						
Day 4	410 ± 31	470 ± 26	506 ± 39	431 ± 29	414 ± 21	544 ± 39
Day 24	400 ± 72	331 ± 53	335 ± 29	296 ± 18	435 ± 88	349 ± 53
Week 14	294 ± 50	254 ± 16	232 ± 22	300 ± 32	301 ± 27	261 ± 23

TABLE F1
Hematology and Clinical Chemistry Data for Rats in the 3-Month Gavage Study of Androstenedione

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Female (continued)						
Clinical Chemistry *(continued)*						
Day 4	10	10	10	10	10	10
Day 24	10	9	10	10	10	10
Week 14	10	10	10	10	10	10
Sorbitol dehydrogenase (IU/L)						
Day 4	17 ± 2	22 ± 3	25 ± 3	19 ± 3	28 ± 3*	25 ± 3
Day 24	13 ± 1	13 ± 2	14 ± 1	14 ± 1	13 ± 1	14 ± 1
Week 14	17 ± 2	22 ± 2	19 ± 2	19 ± 2	16 ± 2	19 ± 2
Bile salts (µmol/L)						
Day 4	24.6 ± 1.3	32.9 ± 2.0*	32.7 ± 1.5*	30.6 ± 1.6	30.6 ± 3.0	34.9 ± 1.8**
Day 24	19.8 ± 1.6	19.2 ± 1.6	21.4 ± 1.7	20.9 ± 1.3	18.0 ± 1.0	18.9 ± 1.8
Week 14	23.3 ± 2.3	26.7 ± 3.9	24.4 ± 2.9	22.4 ± 1.6	20.8 ± 2.1	18.0 ± 1.1

* Significantly different ($P \leq 0.05$) from the control group by Dunn's or Shirley's test
** $P \leq 0.01$
[a] Mean ± standard error. Statistical tests were performed on unrounded data.
[b] n = 9
[c] n = 10

TABLE F2
Hematology Data for Mice in the 3-Month Gavage Study of Androstenedione[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
n	10	10	10	10	10	10
Hematocrit (auto) (%)	48.0 ± 0.6	47.8 ± 0.6	47.5 ± 1.0	47.2 ± 0.5	48.0 ± 0.4	47.7 ± 0.4
Hematocrit (spun) (%)	48.2 ± 0.5	48.2 ± 0.6	48.2 ± 0.8	47.8 ± 0.4	48.1 ± 0.4	47.9 ± 0.6
Hemoglobin (g/dL)	16.0 ± 0.2	15.9 ± 0.2	15.8 ± 0.3	15.8 ± 0.1	15.9 ± 0.1	15.9 ± 0.2
Erythrocytes (10^6/µL)	10.62 ± 0.12	10.50 ± 0.14	10.53 ± 0.24	10.47 ± 0.13	10.55 ± 0.10	10.46 ± 0.11
Reticulocytes (10^5/µL)	2.58 ± 0.07	2.58 ± 0.10	2.55 ± 0.07	2.61 ± 0.08	2.55 ± 0.08	2.58 ± 0.04
Nucleated erythrocytes /per 100 erythrocytes	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0
Mean cell volume (fL)	45.2 ± 0.2	45.5 ± 0.1	45.2 ± 0.2	45.1 ± 0.2	45.6 ± 0.1	45.6 ± 0.2
Mean cell hemoglobin (pg)	15.0 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.1	15.1 ± 0.0	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.3 ± 0.1	33.2 ± 0.1	33.3 ± 0.1	33.5 ± 0.1	33.2 ± 0.1	33.4 ± 0.1
Platelets (10^3/µL)	963.2 ± 43.6	931.5 ± 28.7	974.8 ± 56.9	1,009.4 ± 55.1	980.5 ± 51.9	1,023.3 ± 44.6
Leukocytes (10^3/µL)	4.82 ± 0.61	4.17 ± 0.61	5.05 ± 0.40	4.38 ± 0.38	4.81 ± 0.43	4.22 ± 0.33
Segmented neutrophils (10^3/µL)	0.72 ± 0.10	0.51 ± 0.08	0.55 ± 0.04	0.53 ± 0.06	0.56 ± 0.04	0.56 ± 0.05
Lymphocytes (10^3/µL)	3.87 ± 0.50	3.48 ± 0.53	4.27 ± 0.35	3.66 ± 0.33	4.03 ± 0.39	3.46 ± 0.29
Monocytes (10^3/µL)	0.06 ± 0.01	0.05 ± 0.01	0.06 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.06 ± 0.01
Basophils (10^3/µL)	0.012 ± 0.005	0.011 ± 0.002	0.016 ± 0.003	0.011 ± 0.002	0.017 ± 0.003	0.008 ± 0.003
Eosinophils (10^3/µL)	0.14 ± 0.02	0.11 ± 0.01	0.14 ± 0.02	0.11 ± 0.01	0.11 ± 0.01	0.11 ± 0.01
Large unstained cells (10^3/µL)	0.01 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
Female						
n	10	10	10	9	10	10
Hematocrit (auto) (%)	46.4 ± 0.5	46.2 ± 0.5	46.8 ± 0.7	47.7 ± 0.8	46.3 ± 0.5	46.6 ± 0.4
Hematocrit (spun) (%)	47.2 ± 0.6	47.7 ± 0.4	47.7 ± 0.7	48.9 ± 0.9	47.6 ± 0.4	47.8 ± 0.4
Hemoglobin (g/dL)	15.7 ± 0.2	15.6 ± 0.1	15.8 ± 0.2	16.1 ± 0.2	15.7 ± 0.2	15.7 ± 0.2
Erythrocytes (10^6/µL)	10.33 ± 0.12	10.21 ± 0.10	10.37 ± 0.17	10.56 ± 0.18	10.25 ± 0.11	10.31 ± 0.10
Reticulocytes (10^5/µL)	3.09 ± 0.10	2.73 ± 0.16	2.53 ± 0.17*	2.77 ± 0.18	2.60 ± 0.19*	3.05 ± 0.16
Nucleated erythrocytes /per 100 erythrocytes	0.0 ± 0.0	0.2 ± 0.1	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Mean cell volume (fL)	44.9 ± 0.2	45.3 ± 0.2	45.2 ± 0.2	45.2 ± 0.3	45.1 ± 0.3	45.3 ± 0.2
Mean cell hemoglobin (pg)	15.2 ± 0.0	15.3 ± 0.1	15.3 ± 0.1	15.2 ± 0.1	15.3 ± 0.1	15.2 ± 0.1
Mean cell hemoglobin concentration (g/dL)	33.8 ± 0.1	33.7 ± 0.2	33.8 ± 0.1	33.7 ± 0.2	33.9 ± 0.2	33.7 ± 0.2
Platelets (10^3/µL)	749.3 ± 48.2	740.8 ± 51.3	773.7 ± 43.7	732.1 ± 69.4	690.0 ± 35.7	721.5 ± 62.5
Leukocytes (10^3/µL)	4.73 ± 0.23	4.90 ± 0.24	4.50 ± 0.40	4.63 ± 0.31	5.02 ± 0.24	4.62 ± 0.39
Segmented neutrophils (10^3/µL)	0.58 ± 0.07	0.57 ± 0.07	0.52 ± 0.09	0.47 ± 0.05	0.49 ± 0.05	0.54 ± 0.08
Lymphocytes (10^3/µL)	3.91 ± 0.20	4.10 ± 0.18	3.72 ± 0.30	3.94 ± 0.26	4.29 ± 0.20	3.83 ± 0.32
Monocytes (10^3/µL)	0.07 ± 0.01	0.07 ± 0.01	0.06 ± 0.01	0.05 ± 0.01	0.07 ± 0.01	0.07 ± 0.01
Basophils (10^3/µL)	0.013 ± 0.002	0.012 ± 0.001	0.013 ± 0.002	0.013 ± 0.002	0.016 ± 0.003	0.015 ± 0.002
Eosinophils (10^3/µL)	0.14 ± 0.01	0.13 ± 0.02	0.16 ± 0.04	0.14 ± 0.02	0.13 ± 0.01	0.15 ± 0.02
Large unstained cells (10^3/µL)	0.02 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00

* Significantly different ($P \leq 0.05$) from the control group by Dunn's test
[a] 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[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
n	5	5	5	5	5	5
Male						
Acyl CoA oxidase (nmol DCF/minute per mg protein)	1.88 ± 0.06	1.77 ± 0.07	1.84 ± 0.07	1.75 ± 0.07	1.87 ± 0.07	1.79 ± 0.07
Cyclin-dependent kinase (fmol/mg protein)	72.36 ± 9.50	75.75 ± 16.75	73.19 ± 3.46	61.95 ± 4.59	78.88 ± 11.75	63.00 ± 14.08[b]
Proliferating cell nuclear antigen (fmol/mg protein)	27.01 ± 3.10	23.62 ± 1.12	23.55 ± 2.03	24.58 ± 2.52	23.61 ± 0.85	23.36 ± 2.08
Female						
Acyl CoA oxidase (nmol DCF/minute per mg protein)	2.01 ± 0.18	1.91 ± 0.05	1.64 ± 0.10	1.69 ± 0.19	1.71 ± 0.14	1.99 ± 0.16
Cyclin-dependent kinase (fmol/mg protein)	62.09 ± 12.97	70.30 ± 13.61	74.59 ± 9.87	68.18 ± 4.55	54.51 ± 3.66[c]	86.12 ± 12.37
Proliferating cell nuclear antigen (fmol/mg protein)	21.34 ± 0.94	20.42 ± 1.60	21.47 ± 3.80	24.33 ± 4.84	25.68 ± 3.04	18.67 ± 1.41

[a] Data are presented as mean ± standard error. CoA = coenzyme A; DCF = dichlorofluorescein diacetate.
[b] n = 4
[c] n = 3

TABLE G2
Peroxisome and Cell Proliferation Indexes for Mice in the 2-Week Gavage Study of Androstenedione[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
n	5	5	5	5	4	5
Acyl CoA oxidase (nmol DCF/minute per mg protein)	1.37 ± 0.14	1.54 ± 0.16	1.13 ± 0.06	1.36 ± 0.09	1.30 ± 0.13[b]	1.30 ± 0.15
Cyclin-dependent kinase (fmol/mg protein)	86.38 ± 11.06[c]	80.56 ± 5.87	79.95 ± 23.47[c]	63.91 ± 8.92	72.99 ± 3.98	98.07 ± 16.37
Proliferating cell nuclear antigen (fmol/mg protein)	13.77 ± 1.54	16.38 ± 2.71	12.44 ± 0.70	13.01 ± 1.98	15.18 ± 2.42	13.05 ± 1.88
Female						
n	4	4	5	5	4	4
Acyl CoA oxidase (nmol DCF/minute per mg protein)	1.34 ± 0.14	1.28 ± 0.08[b]	1.21 ± 0.09	1.08 ± 0.14	1.19 ± 0.05	1.03 ± 0.09
Cyclin-dependent kinase (fmol/mg protein)	77.74 ± 17.34	86.15 ± 16.36	128.92 ± 3.19[d]	98.45 ± 10.42[c]	149.06 ± 52.27	111.97 ± 30.84
Proliferating cell nuclear antigen (fmol/mg protein)	19.83 ± 3.17	20.57 ± 2.35	18.15 ± 2.09	16.17 ± 2.55	17.31 ± 2.80	17.80 ± 2.02

[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 H1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Rats in the 2-Week Gavage Study of Androstenedione[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
n	5	5	5	5	5	5
Male						
Necropsy body wt	184 ± 9	181 ± 7	185 ± 7	183 ± 3	176 ± 5	182 ± 3
Heart						
Absolute	0.68 ± 0.04	0.67 ± 0.04	0.68 ± 0.03	0.65 ± 0.01	0.64 ± 0.02	0.66 ± 0.01
Relative	3.723 ± 0.111	3.670 ± 0.060	3.648 ± 0.034	3.572 ± 0.051	3.629 ± 0.084	3.655 ± 0.076
R. Kidney						
Absolute	0.76 ± 0.06	0.76 ± 0.02	0.80 ± 0.03	0.75 ± 0.02	0.76 ± 0.02	0.79 ± 0.02
Relative	4.143 ± 0.123	4.186 ± 0.061	4.333 ± 0.051	4.089 ± 0.137	4.345 ± 0.079	4.368 ± 0.083
Liver						
Absolute	8.97 ± 0.70	8.89 ± 0.38	9.35 ± 0.49	9.12 ± 0.36	8.91 ± 0.53	9.15 ± 0.25
Relative	48.602 ± 1.551	49.117 ± 0.655	50.519 ± 1.660	49.867 ± 1.403	50.474 ± 1.622	50.354 ± 0.788
Lung						
Absolute	1.70 ± 0.12	1.55 ± 0.13	1.48 ± 0.15	1.51 ± 0.15	1.32 ± 0.10	1.51 ± 0.18
Relative	9.361 ± 0.820	8.607 ± 0.815	8.044 ± 0.753	8.263 ± 0.879	7.461 ± 0.429	8.315 ± 0.987
R. Testis						
Absolute	1.120 ± 0.052	1.119 ± 0.030	1.107 ± 0.027	1.069 ± 0.021	1.076 ± 0.026	1.086 ± 0.023
Relative	6.109 ± 0.086	6.202 ± 0.137	5.997 ± 0.109	5.851 ± 0.078	6.125 ± 0.113	5.980 ± 0.056
Thymus						
Absolute	0.431 ± 0.028	0.456 ± 0.025	0.435 ± 0.011	0.415 ± 0.010	0.403 ± 0.014	0.427 ± 0.017
Relative	2.362 ± 0.148	2.524 ± 0.107	2.357 ± 0.038	2.272 ± 0.071	2.291 ± 0.038	2.353 ± 0.107
Female						
Necropsy body wt	131 ± 2	127 ± 1	125 ± 2	137 ± 3	132 ± 1	130 ± 2
Heart						
Absolute	0.55 ± 0.02	0.51 ± 0.03	0.48 ± 0.01	0.53 ± 0.02	0.51 ± 0.01	0.56 ± 0.03
Relative	4.167 ± 0.120	4.040 ± 0.204	3.824 ± 0.047	3.855 ± 0.058	3.855 ± 0.069	4.280 ± 0.195
R. Kidney						
Absolute	0.59 ± 0.01	0.57 ± 0.01	0.58 ± 0.02	0.60 ± 0.01	0.58 ± 0.01	0.60 ± 0.01
Relative	4.475 ± 0.072	4.445 ± 0.079	4.635 ± 0.121	4.359 ± 0.063	4.436 ± 0.034	4.630 ± 0.091
Liver						
Absolute	6.17 ± 0.22	5.89 ± 0.26	5.82 ± 0.14	6.49 ± 0.22	6.18 ± 0.10	6.71 ± 0.44
Relative	46.962 ± 1.622	46.313 ± 1.826	46.741 ± 0.844	47.367 ± 0.603	46.900 ± 0.906	51.633 ± 2.965
Lung						
Absolute	1.12 ± 0.04	1.12 ± 0.07	1.15 ± 0.12	1.37 ± 0.18	1.04 ± 0.07	0.98 ± 0.07
Relative	8.524 ± 0.362	8.835 ± 0.567	9.240 ± 0.870	9.965 ± 1.135	7.858 ± 0.546	7.572 ± 0.586
Thymus						
Absolute	0.374 ± 0.014	0.348 ± 0.016	0.327 ± 0.019	0.391 ± 0.015	0.361 ± 0.016	0.358 ± 0.012
Relative	2.849 ± 0.092	2.741 ± 0.108	2.620 ± 0.126	2.862 ± 0.088	2.742 ± 0.128	2.761 ± 0.093

[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[a]

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

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$ 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).

TABLE H3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Mice in the 2-Week Gavage Study of Androstenedione[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
n	5	5	5	5	5	5
Necropsy body wt	27.9 ± 1.0	25.6 ± 0.5**	25.5 ± 0.3**	24.7 ± 0.3**	24.9 ± 0.4**	25.1 ± 0.5**
Heart						
Absolute	0.15 ± 0.01	0.14 ± 0.00	0.13 ± 0.00**	0.12 ± 0.00**	0.12 ± 0.00**	0.12 ± 0.00**
Relative	5.270 ± 0.320	5.265 ± 0.120	4.998 ± 0.119	5.035 ± 0.180	4.970 ± 0.139	4.891 ± 0.126
R. Kidney						
Absolute	0.27 ± 0.01	0.25 ± 0.01	0.24 ± 0.01	0.26 ± 0.01	0.24 ± 0.01	0.25 ± 0.01
Relative	9.581 ± 0.176	9.917 ± 0.161	9.430 ± 0.265	10.369 ± 0.319	9.712 ± 0.202	9.783 ± 0.287
Liver						
Absolute	1.50 ± 0.07	1.40 ± 0.04	1.42 ± 0.02	1.37 ± 0.04	1.41 ± 0.01	1.43 ± 0.08
Relative	53.709 ± 1.387	54.810 ± 1.288	55.716 ± 0.884	55.265 ± 1.505	56.396 ± 1.059	56.749 ± 2.405
Lung						
Absolute	0.22 ± 0.02	0.19 ± 0.01*	0.18 ± 0.01*	0.19 ± 0.01	0.19 ± 0.01*	0.18 ± 0.00**
Relative	8.065 ± 0.643	7.243 ± 0.090	7.218 ± 0.342	7.757 ± 0.216	7.464 ± 0.280	7.250 ± 0.189
R. Testis						
Absolute	0.110 ± 0.003	0.105 ± 0.002	0.106 ± 0.001	0.109 ± 0.001	0.105 ± 0.004	0.106 ± 0.003
Relative	3.991 ± 0.230	4.110 ± 0.073	4.164 ± 0.071	4.422 ± 0.041	4.205 ± 0.161	4.230 ± 0.150
Thymus						
Absolute	0.056 ± 0.006	0.053 ± 0.005	0.043 ± 0.008	0.046 ± 0.007	0.050 ± 0.003	0.050 ± 0.003
Relative	1.980 ± 0.157	2.079 ± 0.190	1.661 ± 0.301	1.884 ± 0.309	2.003 ± 0.158	2.010 ± 0.125
Female						
n	4	5	5	5	4	4
Necropsy body wt	21.1 ± 0.1	21.7 ± 0.4	19.9 ± 0.4	21.3 ± 0.3	20.2 ± 0.5	20.3 ± 0.2
Heart						
Absolute	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01	0.12 ± 0.00	0.12 ± 0.01	0.11 ± 0.00
Relative	5.857 ± 0.258	5.855 ± 0.166	5.874 ± 0.254	5.737 ± 0.083	5.904 ± 0.266	5.533 ± 0.083
R. Kidney						
Absolute	0.17 ± 0.00	0.17 ± 0.00	0.16 ± 0.01	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.00
Relative	7.986 ± 0.159	7.799 ± 0.105	8.162 ± 0.320	8.264 ± 0.119	8.098 ± 0.298	8.442 ± 0.105
Liver						
Absolute	1.10 ± 0.02	1.17 ± 0.03	1.07 ± 0.03	1.19 ± 0.04	1.12 ± 0.05	1.16 ± 0.03
Relative	51.893 ± 0.623	54.219 ± 1.226	53.424 ± 0.822	55.985 ± 1.203*	55.287 ± 1.397*	57.401 ± 1.030**
Lung						
Absolute	0.17 ± 0.01	0.21 ± 0.02	0.19 ± 0.02	0.18 ± 0.01	0.18 ± 0.02	0.25 ± 0.05
Relative	8.204 ± 0.425	9.753 ± 0.634	9.430 ± 1.081	8.378 ± 0.579	8.655 ± 0.594	12.613 ± 2.577
Thymus						
Absolute	0.067 ± 0.003	0.075 ± 0.007	0.072 ± 0.006	0.077 ± 0.003	0.074 ± 0.006	0.081 ± 0.002
Relative	3.161 ± 0.130	3.465 ± 0.308	3.637 ± 0.318	3.628 ± 0.177	3.665 ± 0.242	3.987 ± 0.092

* Significantly different ($P \leq 0.05$) from the vehicle control group by Williams' or Dunnett's test
** $P \leq 0.01$
[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[a]

	Vehicle Control	1 mg/kg	5 mg/kg	10 mg/kg	20 mg/kg	50 mg/kg
Male						
n	10	10	10	10	10	10
Last in-life body wt	37.5 ± 0.9	37.9 ± 1.2	38.7 ± 0.9	39.1 ± 1.3	39.3 ± 0.9	38.2 ± 1.2
Heart						
Absolute	0.17 ± 0.01	0.17 ± 0.01	0.16 ± 0.00	0.16 ± 0.00	0.16 ± 0.01	0.16 ± 0.01
Relative	4.465 ± 0.096	4.558 ± 0.143	4.130 ± 0.153	4.191 ± 0.176	4.172 ± 0.160	4.310 ± 0.128
R. Kidney						
Absolute	0.31 ± 0.01	0.32 ± 0.01	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.01	0.33 ± 0.01
Relative	8.202 ± 0.255	8.368 ± 0.262	8.336 ± 0.216	8.606 ± 0.256	8.490 ± 0.209	8.649 ± 0.244
Liver						
Absolute	1.59 ± 0.04	1.56 ± 0.05	1.59 ± 0.04	1.62 ± 0.05	1.64 ± 0.05	1.63 ± 0.08
Relative	42.452 ± 0.813	41.320 ± 0.677	41.167 ± 1.045	41.491 ± 0.890	41.786 ± 0.612	42.654 ± 0.805
Lung						
Absolute	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.02	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.01
Relative	6.247 ± 0.328	6.294 ± 0.241	5.752 ± 0.403	5.988 ± 0.311	6.111 ± 0.349	5.736 ± 0.267
R. Testis						
Absolute	0.119 ± 0.002	0.122 ± 0.003	0.122 ± 0.003	0.124 ± 0.003	0.124 ± 0.002	0.116 ± 0.003
Relative	3.191 ± 0.060	3.230 ± 0.087	3.173 ± 0.080	3.177 ± 0.077	3.168 ± 0.096	3.046 ± 0.051
Thymus						
Absolute	0.035 ± 0.002	0.033 ± 0.002	0.035 ± 0.002	0.034 ± 0.002	0.034 ± 0.001	0.034 ± 0.001
Relative	0.944 ± 0.054	0.876 ± 0.028	0.900 ± 0.034	0.860 ± 0.036	0.866 ± 0.036	0.882 ± 0.023
Female						
n	10	10	10	9	10	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 ($P \leq 0.01$) 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[a]

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

* Significantly different ($P \leq 0.05$) from the vehicle control group by Shirley's test
** ($P \leq 0.01$)
[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 (spermatid measurements).
[b] n = 9

TABLE I2
Estrous Cycle Characterization for Female Rats in the 3-Month Gavage Study of Androstenedione[a]

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

** Significantly different ($P \leq 0.01$) from the vehicle control group by Dunnett's test
[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 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.
[d] 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[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
n	10	10	10	10
Weights (g)				
Necropsy body wt	37.5 ± 0.9	39.1 ± 1.3	39.3 ± 0.9	38.2 ± 1.2
L. Cauda epididymis	0.0237 ± 0.0023	0.0200 ± 0.0010	0.0222 ± 0.0010	0.0220 ± 0.0015
L. Epididymis	0.0519 ± 0.0023	0.0491 ± 0.0014	0.0522 ± 0.0015	0.0515 ± 0.0019
L. Testis	0.1120 ± 0.0060	0.1204 ± 0.0026	0.1200 ± 0.0014	0.1170 ± 0.0034
Spermatid measurements				
Spermatid heads (10^3/mg testis)	189.53 ± 3.33	208.68 ± 11.00	219.19 ± 9.14*	209.75 ± 11.26
Spermatid heads (10^6/testis)	19.38 ± 0.74	22.20 ± 1.04	23.30 ± 1.05*	20.89 ± 0.82
Epididymal spermatozoal measurements				
Sperm heads (10^3/mg cauda epididymis)	780 ± 110	958 ± 54	840 ± 54	879 ± 77
Sperm heads (10^6/cauda epididymis)	18 ± 2	19 ± 1	18 ± 1	19 ± 1
Sperm motility (%)	67.2 ± 4.4	67.2 ± 2.5	68.8 ± 1.4	56.7 ± 3.9**

* Significantly different ($P \leq 0.05$) from the vehicle control group by Dunn's test
** Significantly different ($P \leq 0.01$) 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[a]

	Vehicle Control	10 mg/kg	20 mg/kg	50 mg/kg
Number weighed at necropsy	10	10	10	10
Necropsy body wt (g)	30.9 ± 1.0	30.5 ± 1.0	30.8 ± 0.8	29.3 ± 0.7
Proportion of regular cycling females[b]	10/10	9/10[c]	10/10	9/10[c]
Estrous cycle length (days)	3.9 ± 0.1	4.1 ± 0.2	3.8 ± 0.1	3.9 ± 0.1
Estrous stages (% of cycle)				
Diestrus	30.8	45.4	32.5	39.8
Proestrus	0.0	0.0	0.8	0.0
Estrus	44.2	35.2	41.7	36.1
Metestrus	25.0	19.4	25.0	24.1

[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

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 forumations 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.

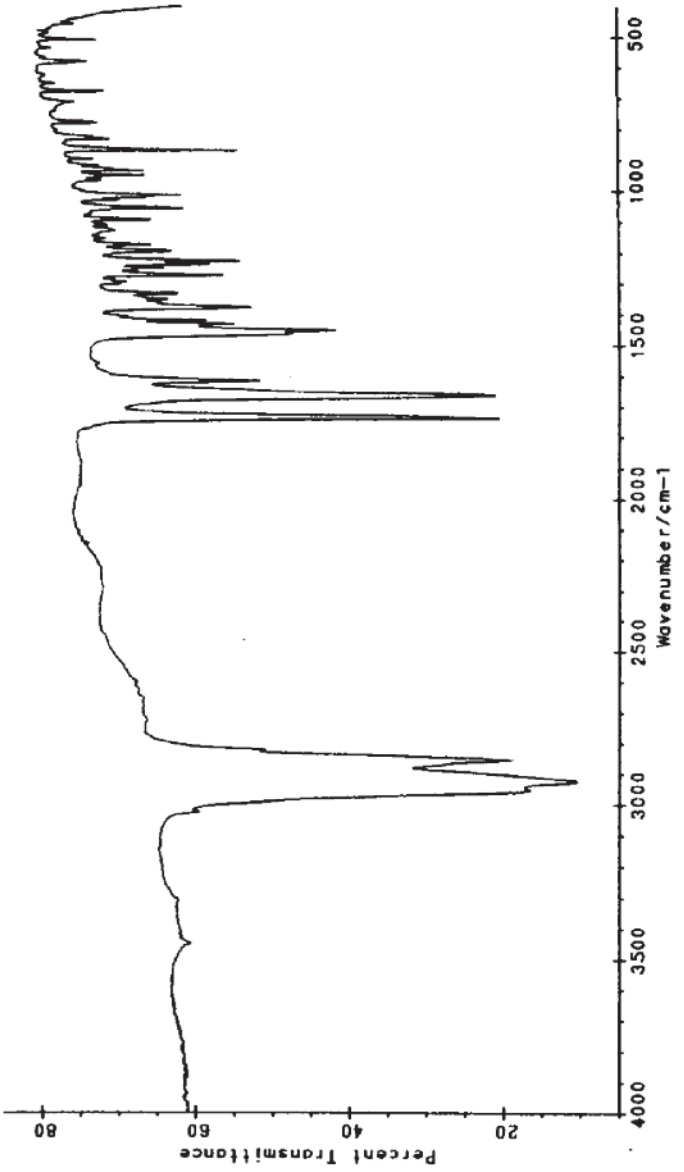

FIGURE J1
Infrared Absorption Spectrum of Androstenedione

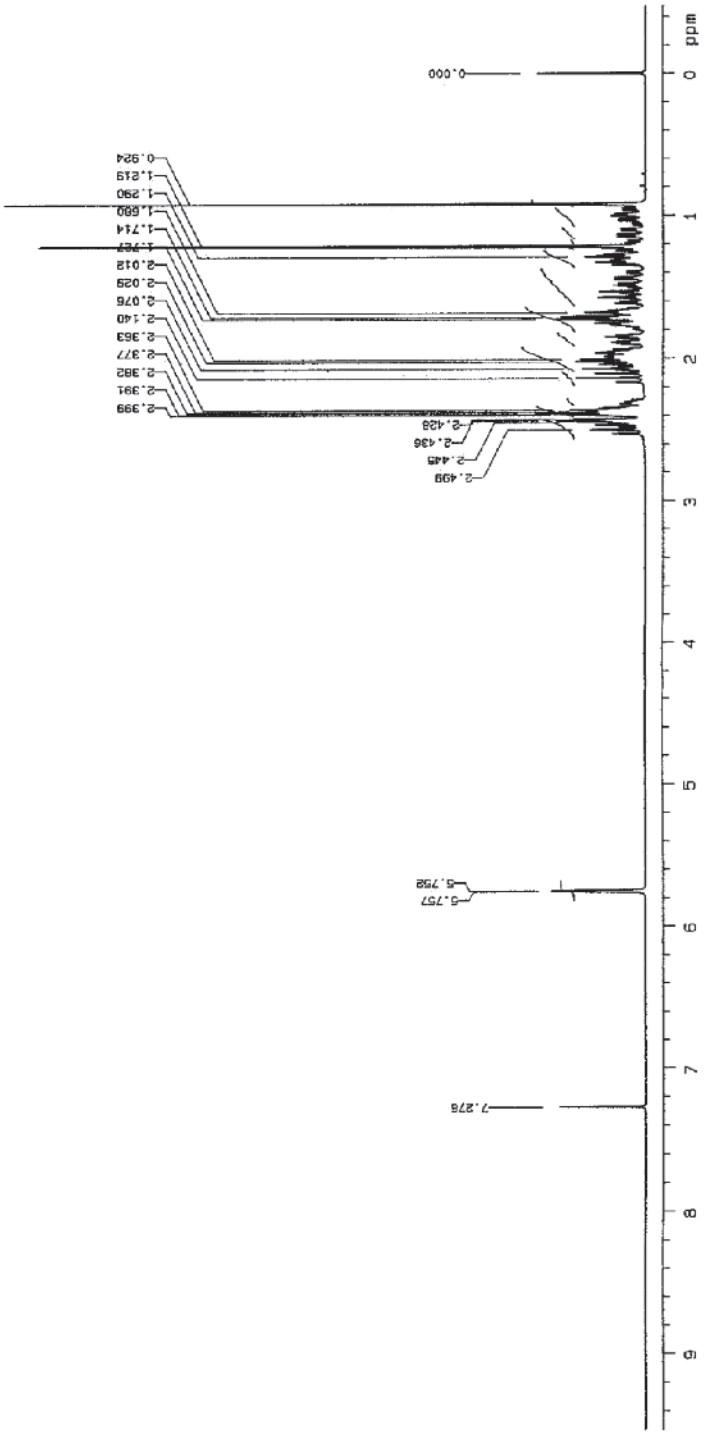

FIGURE J2
Proton Nuclear Magnetic Resonance Spectrum of Androstenedione

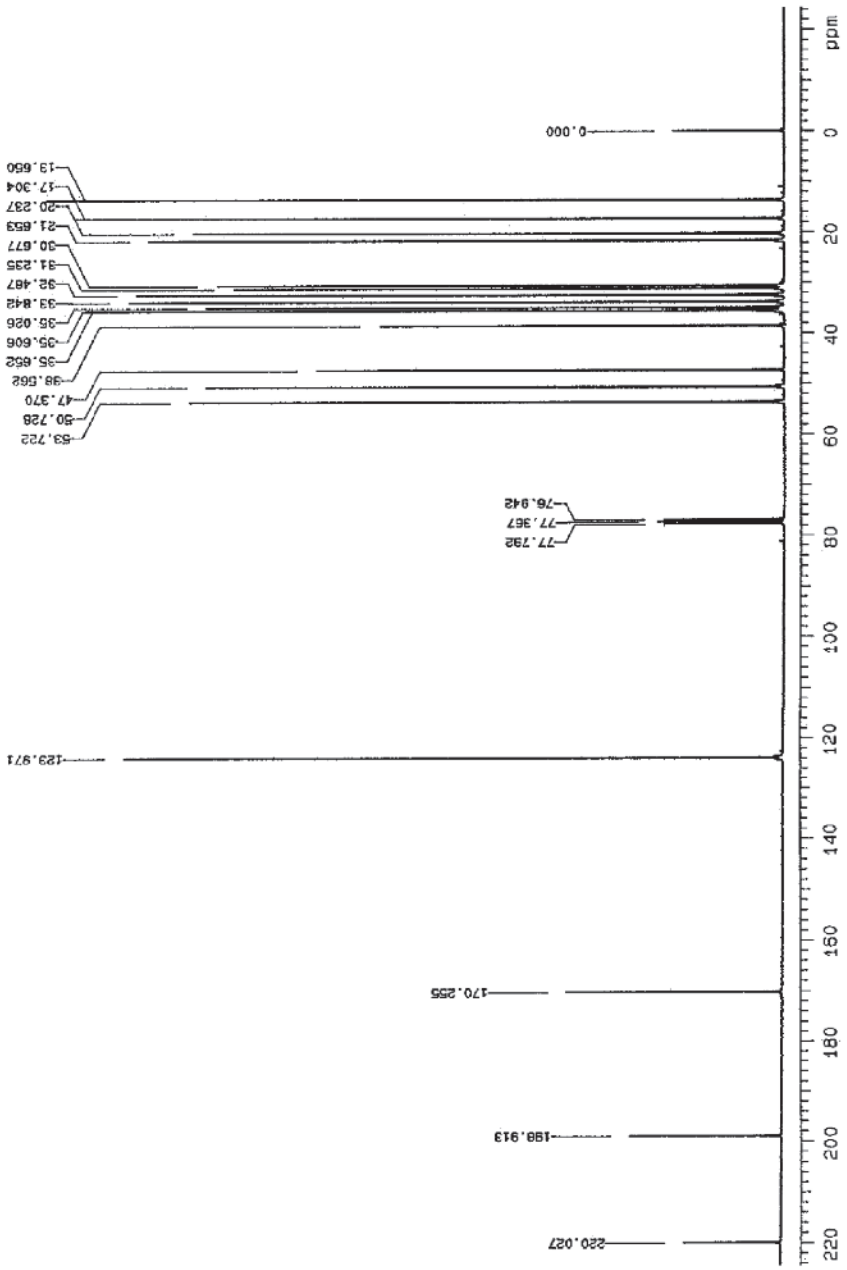

FIGURE J3
Carbon-13 Nuclear Magnetic Resonance Spectrum of Androstenedione

TABLE J1
High-Performance Liquid Chromatography Systems Used in the Gavage Studies of Androstenedione[a]

Detection System	Column	Solvent System
System A Ultraviolet (242 nm) light	Luna C18, 150 mm × 4.6 mm, 5µ (Phenomenex, Inc., Torrance, CA)	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
System B Ultraviolet (242 nm) light	Luna C18, 150 mm × 4.6 mm, 5µ (Phenomenex, Inc.)	60:40 acetonitrile:Milli-Q water; isocratic; flow rate = 0.75 mL/minute

[a] 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

2-Week Studies	3-Month Studies	2-Year Studies
Preparation		
The vehicle was prepared by mixing methylcellulose and heated, deionized water with an overhead 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 1 hour. The dose formulations were prepared once.	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.	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.
Chemical Lot Number		
H408	H408	H408
Maximum Storage Time		
35 days	35 days	35 days
Storage Conditions		
Stored in sealed amber glass bottles, protected from light, refrigerated (5° C)	Stored in sealed amber glass bottles, protected from light, refrigerated (5° C)	Stored in sealed amber glass bottles, protected from light, at room temperature (25° C)
Study Laboratory		
Battelle Columbus Operations (Columbus, OH)	Southern Research Institute (Birmingham, AL)	Southern Research Institute (Birmingham, AL)

TABLE J3
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 2-Week Gavage Studies of Androstenedione[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats				
August 7, 2000	August 3, 2000	0.2	0.1967	–2
		1	1.056	+6
		2	2.003	0
		4	3.804	–5
		10	9.919	–1
	September 15, 2000[b]	0.2	0.1791	–10
		1	1.195	+20
		2	2.016	+1
		4	4.103	+3
		10	10.47	+5
Mice				
August 3, 2000	August 3, 2000	0.1	0.09371	–6
		0.5	0.4921	–2
		1	1.056	+6
		2	2.003	0
		5	4.93	–1
	September 15, 2000[b]	0.1	0.08942	–11
		0.5	0.4936	–1
		1	1.051	+5
		2	1.691	–15
		5	5.035	+1

[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 J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Androstenedione[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats				
April 15, 2002	April 16-17, 2002	0.2	0.199	0
		1	0.970	–3
		2	1.97	–1
		4	3.95	–1
		10	9.83	–2
	May 20, 2002[b]	0.2	0.205	+3
		1	1.20	+20
		2	2.38	+19
		4	4.64	+16
		10	14.5	+45
May 13, 2002	May 14, 2002	0.2	0.187	–7
		1	0.979	–2
		2	1.93	–3
		4	4.00	0
		10	10.0	0
	June 17, 2002[b]	0.2	0.191	–5
		1	0.979	–2
		2	1.94	–3
		4	3.76	–6
		10	8.25	–18
May 31, 2002	June 3, 2002	2	1.96	–2
July 8, 2002	July 9, 2002	1	0.978	–2
		2	2.04	+2
		4	4.07	+2
		10	10.1	+1
	July 29, 2002[b]	0.2	0.217	+9
		1	1.07	+7
		2	2.43	+22
		4	4.37	+9
		10	11.4	+14
July 9, 2002	July 9, 2002	0.2	0.202	+1

TABLE J4
Results of Analyses of Dose Formulations Administered to Rats and Mice in the 3-Month Gavage Studies of Androstenedione

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice				
April 15, 2002	April 16-17, 2002	0.1	0.103	+3
		0.5	0.496	–1
		1	0.970	–3
		2	1.97	–1
		5	5.07	+1
	May 20, 2002[b]	0.1	0.105	+5
		0.5	0.528	+6
		1	1.13	+13
		2	2.31	+16
		5	6.20	+24
May 13, 2002	May 14, 2002	0.1	0.0988	–1
		0.5	0.494	–1
		1	0.979	–2
		2	1.93	–3
		5	4.98	–1
	June 17, 2002[b]	0.1	0.101	+1
		0.5	0.489	–2
		1	0.900	–10
		2	1.93	–4
		5	4.90	–2
July 8, 2002	July 9, 2002	0.1	0.102	+2
		0.5	0.491	–2
		1	0.978	–2
		2	2.04	+2
		5	5.02	0
	July 29, 2002[b]	0.1	0.116	+16
		0.5	0.506	+1
		1	0.966	–3
		2	2.13	+7
		5	5.18	+4

[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[a]

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats				
January 6, 2003	January 8, 2003	2	1.97	–1
		4	3.94	–2
		10	9.98	0
	February 13, 2003[b]	2	2.54	+27
		4	5.56	+39
		10	12.1	+21
March 3, 2003	March 4, 2003	2	1.98	–1
		4	3.99	0
		10	9.99	0
May 27, 2003	May 29, 2003	2	1.90	–5
		4	3.95	–1
		10	9.82	–2
July 21, 2003	July 22, 2003	2	1.98	–1
		4	3.90	–3
		10	10.1	+1
	August 25, 2003[b]	2	2.02	+1
		4	4.17	+4
		10	9.94	–1
September 25, 2003	October 10, 2003	2	1.97	–2
		4	3.92	–2
		10	10.2	+2
	October 10, 2003[b]	2	2.00	0
		4	4.05	+1
		10	10.1	+1
October 13, 2003	October 14, 2003	2	1.96	–2
		4	4.01	0
		10	10.1	+1
	October 24, 2003[b]	2	2.04	+2
		4	4.07	+2
		10	10.1	+1
December 8, 2003	December 9, 2003	2	1.96	–2
		4	3.62	–10
		10	9.77	–2
	January 16, 2004[b]	2	2.01	+1
		4	4.68	+17
		10	8.90	–11

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Rats (continued)				
March 1, 2004	March 2, 2004	2	1.95	–3
		4	3.88	–3
		10	9.88	–1
	April 6, 2004[b]	2	2.00	0
		4	3.93	–2
		10	9.88	–1
April 26, 2004	April 27, 2004	2	2.00	0
		4	4.04	+1
		10	10.1	+1
July 19, 2004	July 23, 2004	2	1.95	–3
		4	3.94	–2
		10	9.92	–1
September 13, 2004	September 15, 2004	2	1.91	–5
		4	3.84	–4
		10	9.97	0
	October 18, 2004[b]	2	2.09	+5
		4	4.11	+3
		10	10.3	+3
December 6, 2004	December 7, 2004	2	1.88	–6
		4	3.97	–1
		10	9.66	–3
Mice				
December 9, 2002	December 10, 2002	0.2	0.193	–3
		1	1.00	0
		2	2.03	+2
		5	5.08	+2
	January 13, 2003[b]	0.2	0.196	–2
		1	0.975	–3
		2	1.88	–6
		5	5.20	+4
January 6, 2003	January 8, 2003	0.2	0.194	–3
		1	0.988	–1
		2	1.97	–1
		5	4.98	0
	February 13, 2003[b]	0.2	0.205	+3
		1	1.08	+8
		2	1.97	–2
		5	5.80	+16

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
March 3, 2003	March 4, 2003	0.2	0.201	+1
		1	0.987	–1
		2	1.98	–1
		5	5.00	0
May 27, 2003	May 29, 2003	0.2	0.200	0
		1	0.965	–4
		2	1.90	–5
		5	4.96	–1
July 21, 2003	July 22, 2003	0.2	0.194	–3
		1	0.985	–2
		2	1.98	–1
		5	4.97	–1
	August 25, 2003[b]	0.2	0.217	+9
		1	0.984	–2
		2	2.36	+18
		5	6.85	+37
September 25, 2003	October 10, 2003	0.2	0.188	–6
		1	0.987	–1
		2	1.97	–2
		5	4.88	–2
	October 10, 2003[b]	0.2	0.188	–6
		1	0.990	–1
		2	2.00	0
		5	5.07	+1
October 13, 2003	October 14, 2003	0.2	0.198	–1
		1	0.985	–2
		2	2.07	+3
		5	5.00	0
	October 24, 2003[b]	0.2	0.198	–1
		1	0.992	–1
		2	2.08	+4
		5	4.94	–1
December 8, 2003	December 9, 2003	0.2	0.198	–1
		1	1.00	0
		2	2.03	+1
		5	5.03	+1
	January 16, 2004[b]	0.2	0.207	+4
		1	1.00	0
		2	1.96	–2
		5	5.00	0

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

Date Prepared	Date Analyzed	Target Concentration (mg/mL)	Determined Concentration (mg/mL)	Difference from Target (%)
Mice (continued)				
March 1, 2004	March 2, 2004	0.2	0.202	+1
		1	0.981	–2
		2	2.02	+1
		5	5.05	+1
	April 6, 2004[b]	0.2	0.196	–2
		1	0.982	–2
		2	1.99	–1
		5	5.12	+2
April 26, 2004	April 27, 2004	0.2	0.199	–1
		1	1.03	+3
		2	2.00	0
		5	5.11	+2
July 19, 2004	July 23, 2004	0.2	0.198	–1
		1	0.961	–4
		2	2.00	0
		5	5.00	0
September 13, 2004	September 15, 2004	0.2	0.198	–1
		1	0.982	–2
		2	1.96	–2
		5	4.94	–1
	October 18, 2004[b]	0.2	0.199	–1
		1	0.980	–2
		2	1.95	–3
		5	4.99	0
December 6, 2004	December 7, 2004	0.2	0.190	–5
		1	0.962	–4
		2	1.97	–1
		5	4.94	–1

[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

TABLE K1
Ingredients of NTP-2000 Rat and Mouse Ration

Ingredients	Percent by Weight
Ground hard winter wheat	22.26
Ground #2 yellow shelled corn	22.18
Wheat middlings	15.0
Oat hulls	8.5
Alfalfa meal (dehydrated, 17% protein)	7.5
Purified cellulose	5.5
Soybean meal (49% protein)	5.0
Fish meal (60% protein)	4.0
Corn oil (without preservatives)	3.0
Soy oil (without preservatives)	3.0
Dried brewer's yeast	1.0
Calcium carbonate (USP)	0.9
Vitamin premix[a]	0.5
Mineral premix[b]	0.5
Calcium phosphate, dibasic (USP)	0.4
Sodium chloride	0.3
Choline chloride (70% choline)	0.26
Methionine	0.2

[a] Wheat middlings as carrier
[b] Calcium carbonate as carrier

TABLE K2
Vitamins and Minerals in NTP-2000 Rat and Mouse Ration[a]

	Amount	Source
Vitamins		
A	4,000 IU	Stabilized vitamin A palmitate or acetate
D	1,000 IU	D-activated animal sterol
K	1.0 mg	Menadione sodium bisulfite complex
α-Tocopheryl acetate	100 IU	
Niacin	23 mg	
Folic acid	1.1 mg	
d-Pantothenic acid	10 mg	*d*-Calcium pantothenate
Riboflavin	3.3 mg	
Thiamine	4 mg	Thiamine mononitrate
B_{12}	52 µg	
Pyridoxine	6.3 mg	Pyridoxine hydrochloride
Biotin	0.2 mg	*d*-Biotin
Minerals		
Magnesium	514 mg	Magnesium oxide
Iron	35 mg	Iron sulfate
Zinc	12 mg	Zinc oxide
Manganese	10 mg	Manganese oxide
Copper	2.0 mg	Copper sulfate
Iodine	0.2 mg	Calcium iodate
Chromium	0.2 mg	Chromium acetate

[a] Per kg of finished product

TABLE K3
Nutrient Composition of NTP-2000 Rat and Mouse Ration

Nutrient	Mean ± Standard Deviation	Range	Number of Samples
Protein (% by weight)	14.9 ± 0.53	13.8 – 16.1	25
Crude fat (% by weight)	8.0 ± 0.37	7.4 – 9.0	25
Crude fiber (% by weight)	9.2 ± 0.45	8.2 – 9.9	25
Ash (% by weight)	5.0 ± 0.21	4.4 – 5.4	25
Amino Acids (% of total diet)			
Arginine	0.770 ± 0.070	0.670 – 0.970	18
Cystine	0.225 ± 0.023	0.150 – 0.250	18
Glycine	0.706 ± 0.043	0.620 – 0.800	18
Histidine	0.362 ± 0.082	0.310 – 0.680	18
Isoleucine	0.542 ± 0.046	0.430 – 0.660	18
Leucine	1.087 ± 0.066	0.960 – 1.240	18
Lysine	0.712 ± 0.118	0.310 – 0.840	18
Methionine	0.407 ± 0.051	0.260 – 0.490	18
Phenylalanine	0.626 ± 0.043	0.540 – 0.720	18
Threonine	0.500 ± 0.046	0.430 – 0.610	18
Tryptophan	0.142 ± 0.024	0.110 – 0.200	18
Tyrosine	0.388 ± 0.058	0.280 – 0.540	18
Valine	0.667 ± 0.045	0.550 – 0.730	18
Essential Fatty Acids (% of total diet)			
Linoleic	3.92 ± 0.243	3.49 – 4.54	18
Linolenic	0.30 ± 0.035	0.21 – 0.35	18
Vitamins			
Vitamin A (IU/kg)	4,920 ± 1,210	3,360 – 8,900	25
Vitamin D (IU/kg)	1,000[a]		
α-Tocopherol (ppm)	84.2 ± 16.60	52.0 - 110.0	15
Thiamine (ppm)[b]	8.5 ± 3.66	5.9 – 25.2	25
Riboflavin (ppm)	6.8 ± 2.11	4.20 – 11.20	15
Niacin (ppm)	79.0 ± 10.50	66.4 – 98.2	15
Pantothenic acid (ppm)	23.9 ± 3.73	17.4 – 29.8	15
Pyridoxine (ppm)[b]	9.21 ± 2.20	6.4 – 13.7	15
Folic acid (ppm)	1.75 ± 0.54	1.20 – 3.27	15
Biotin (ppm)	0.332 ± 0.12	0.225 – 0.704	15
Vitamin B_{12} (ppb)	60.5 ± 46.5	18.3 – 174.0	15
Choline (ppm)[b]	3,064 ± 270	2,700 – 3,790	15
Minerals			
Calcium (%)	0.959 ± 0.046	0.873 – 1.030	25
Phosphorus (%)	0.589 ± 0.028	0.538 – 0.641	25
Potassium (%)	0.665 ± 0.023	0.626 – 0.694	15
Chloride (%)	0.376 ± 0.041	0.300 – 0.474	15
Sodium (%)	0.191 ± 0.017	0.160 – 0.222	15
Magnesium (%)	0.201 ± 0.009	0.185 – 0.217	15
Sulfur (%)	0.170 ± 0.029	0.116 – 0.209	15
Iron (ppm)	182 ± 46.7	135 – 311	15
Manganese (ppm)	54.1 ± 7.89	42.1 – 73.1	15
Zinc (ppm)	55.0 ± 9.55	43.3 – 78.5	15
Copper (ppm)	6.65 ± 1.790	3.21 – 10.50	15
Iodine (ppm)	0.512 ± 0.221	0.233 – 0.972	15
Chromium (ppm)	0.604 ± 0.253	0.330 – 1.380	14
Cobalt (ppm)	0.25 ± 0.074	0.20 – 0.47	14

[a] From formulation
[b] As hydrochloride (thiamine and pyridoxine) or chloride (choline)

TABLE K4
Contaminant Levels in NTP-2000 Rat and Mouse Ration[a]

	Mean ± Standard Deviation[b]	Range	Number of Samples
Contaminants			
Arsenic (ppm)	0.33 ± 0.158	0.14 – 0.50	25
Cadmium (ppm)	0.07 ± 0.021	0.04 – 0.10	25
Lead (ppm)	0.08 ± 0.026	0.05 – 0.13	25
Mercury (ppm)	<0.02		25
Selenium (ppm)	0.20 ± 0.057	0.14 – 0.45	25
Aflatoxins (ppb)	<5.00		25
Nitrate nitrogen (ppm)[c]	14.5 ± 4.33	10.00 – 24.4	25
Nitrite nitrogen (ppm)[c]	<0.61		25
BHA (ppm)[d]	<1.0		25
BHT (ppm)[d]	<1.0		25
Aerobic plate count (CFU/g)	10.0 ± 0.0	10.0 – 10.0	25
Coliform (MPN/g)	3.0 ± 0.0	3.0 – 3.0	25
Escherichia coli (MPN/g)	<10		25
Salmonella (MPN/g)	Negative		25
Total nitrosoamines (ppb)[e]	4.4 ± 2.04	2.3 – 8.5	25
N-Nitrosodimethylamine (ppb)[e]	2.6 ± 1.74	1.1 – 6.9	25
N-Nitrosopyrrolidine (ppb)[e]	1.8 ± 0.79	0.9 – 4.1	25
Pesticides (ppm)			
α-BHC	<0.01		25
β-BHC	<0.02		25
γ-BHC	<0.01		25
δ-BHC	<0.01		25
Heptachlor	<0.01		25
Aldrin	<0.01		25
Heptachlor epoxide	<0.01		25
DDE	<0.01		25
DDD	<0.01		25
DDT	<0.01		25
HCB	<0.01		25
Mirex	<0.01		25
Methoxychlor	<0.05		25
Dieldrin	<0.01		25
Endrin	<0.01		25
Telodrin	<0.01		25
Chlordane	<0.05		25
Toxaphene	<0.10		25
Estimated PCBs	<0.20		25
Ronnel	<0.01		25
Ethion	<0.02		25
Trithion	<0.05		25
Diazinon	<0.10		25
Methyl chlorpyrifos	0.098 ± 0.111	0.020 – 0.416	25
Methyl parathion	<0.02		25
Ethyl parathion	<0.02		25
Malathion	0.189 ± 0.377	0.020 – 1.850	25
Endosulfan I	<0.01		25
Endosulfan II	<0.01		25
Endosulfan sulfate	<0.03		25

[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

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 titers. 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.

Method and Test	Time of Analysis
Rats	
2-Week Study	
ELISA	
PVM (pneumonia virus of mice)	End of quarantine
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	End of quarantine
Sendai	End of quarantine
Immunofluorescence Assay	
Parvovirus	End of quarantine
3-Month Study	
ELISA	
PVM	Study termination
RCV/SDA	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Mycoplasma arthritidis	Study termination
Mycoplasma pulmonis	Study termination
PVM	6, 12, and 18 months, study termination
RCV/SDA	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination
Immunofluorescence Assay	
Parvovirus	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
Mice	
2-Week Study	
ELISA	
Ectromelia virus	End of quarantine
EDIM (epizootic diarrhea of infant mice)	End of quarantine
GDVII (mouse encephalomyelitis virus)	End of quarantine
LCM (lymphocytic choriomeningitis virus)	End of quarantine
Mouse adenoma virus-FL	End of quarantine
MHV (mouse hepatitis virus)	End of quarantine
PVM	End of quarantine
Reovirus 3	End of quarantine
Sendai	End of quarantine
Immunofluorescence Assay	
Parvovirus	End of quarantine
3-Month Study	
ELISA	
Ectromelia virus	Study termination
EDIM	Study termination
GDVII	Study termination
LCM	Study termination
Mouse adenoma virus-FL	Study termination
MHV	Study termination
PVM	Study termination
Reovirus 3	Study termination
Sendai	Study termination
Immunofluorescence Assay	
Parvovirus	Study termination
2-Year Study	
ELISA	
Ectromelia virus	6, 12, and 18 months, study termination
EDIM	6, 12, and 18 months, study termination
GDVII	6, 12, and 18 months, study termination
LCM	6, 12, and 18 months, study termination
MVM (minute virus of mice)	18 months, study termination
Mouse adenoma virus-FL	6, 12, and 18 months, study termination
MHV	6, 12, and 18 months, study termination
MPV (mouse parvovirus)	18 months, study termination
M. arthritidis	18 months, study termination
M. pulmonis	18 months, study termination
PVM	6, 12, and 18 months, study termination
Reovirus 3	6, 12, and 18 months, study termination
Sendai	6, 12, and 18 months, study termination

Method and Test	Time of Analysis
Mice (continued)	
2-Year Study *(continued)*	
Immunofluorescence Assay	
EDIM	18 months
LCM	18 months
MVM	18 months
MCMV (mouse cytomegalovirus)	18 months, study termination
Mouse adenoma virus-FL	6 months, study termination
Parvovirus	6 and 12 months
PVM	12 months
Polymerase Chain Reaction	
Helicobacter species	18 months

RESULTS

All test results were negative.