NTP TECHNICAL REPORT

ON THE

TOXICOLOGY AND CARCINOGENESIS

STUDY OF ETHINYL ESTRADIOL

(CAS NO. 57-63-6)

IN SPRAGUE-DAWLEY RATS

(FEED STUDY)

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

July 2010

NTP TR 548

NIH Publication No. 10-5889

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.

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The study on ethinyl estradiol was conducted at the FDA's National Center for Toxicological Research under an interagency agreement between the FDA and the NIEHS. The study was designed and monitored by a Toxicology Study Selection and Review Committee composed of representatives from the NCTR and other FDA product centers, NIEHS, and other *ad hoc* members from other government agencies and academia. The interagency agreement was designed to use the staff and facilities of the NCTR in the testing of FDA priority chemicals and to provide FDA scientists and regulatory policymakers information for hazard identification and risk assessment.

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SUMMARY

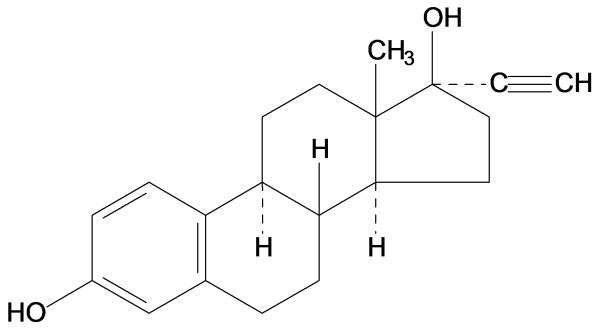
Background
Ethinyl estradiol is a potent synthetic estrogen that is widely prescribed in oral contraceptives and is also used in the treatment of breast and prostate cancer. Ethinyl estradiol is one of a class of chemicals known as "environmental estrogens" that can affect the hormone activities and possibly reproductive function of wildlife and humans through exposure. The NTP conducted a series of studies on three such chemicals to detect if exposure over the course of multiple generations could have any cumulative effect on animals' reproductive systems or development of cancers. This report describes the results of a set of studies in which rats were exposed to ethinyl estradiol for part or all of the study period and examined at the end of two years.

Methods
The study consisted of three separate study components; in each, animals were exposed to ethinyl estradiol from the time of conception and through weaning through their mothers, who were given ethinyl estradiol in their feed. In one study we gave feed containing 2, 10, or 50 parts per billion (ppb) of ethinyl estradiol to groups of 50 male and female rats from conception through two years. In the second study, groups of 50 male and female rats were given the same feed concentrations up to 20 weeks following birth, followed by untreated feed for the remainder of the two years. In the third study groups of 50 male and female rats were exposed from conception through weaning, and then given untreated feed for the duration of the study. Control animals received the same feed with no ethinyl estradiol added. Enthinyl estradiol is known to cause cancer at higher dose levels; the concentrations given in this study were below the levels of detection by chemical analysis, to determine the possible effects of trace amounts in the environment. At the end of the study tissues from more than 40 sites were examined for every animal.

Results
In all three study sets effects were seen in the uterus of female rats. The rates of squamous metaplasia increased in females exposed for two years and in females exposed from conception through weaning; endometrial hyperplasia and atypical focal hyperplasia of the uterus also were increased in females exposed for two years. Uterine stromal polyps were increased in female rats exposed from conception through 20 weeks after birth or from conception through weaning. Male rats exposed from conception through weaning had small increases in the rates of preputial gland tumors and three male rats in that study had rare mammary gland adenomas or carcinomas.

Conclusions
We conclude that exposure to trace amounts of ethinyl estradiol during the period from conception through weaning may have been related to development of uterine stromal polyps in female rats and to preputial gland tumors and mammary gland tumors in male rats.

ABSTRACT



ETHINYL ESTRADIOL

CAS No. 57-63-6

Chemical Formula: $C_{20}H_{24}O_2$ Molecular Weight: 296.40

Synonyms: 17-ethinylestradiol; ethynylestradiol; 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol
Trade Names: Amenoron, Amenorone, Anovlar, Diogyn-E, Diprol, Dyloform, EE, EE_2, EE2, Ertonyl, Esteed, Estigyn, Estinyl, Eston-E, Estopherol, Estoral, Eticyclin, Eticyclol, Eticylol, Etinestrol, Etinestryl, Etinoestryl, Etistradiol, Feminone, Follicoral, Ginestrene, Halodrin, Inestra, Linoral, Loestrin, Lynoral, Menolyn, Microfollin, Neo-Estrone, Nogest-S, Nordette, Novestrol, Oradiol, Orestralyn, Orestrayln, Palonyl, Perovex, Primogyn, Primogyn C, Primogyn M, Progynon C, Spanestrin, Ylestrol

Ethinyl estradiol is a potent synthetic estrogen widely used in pharmaceutical preparations. Its high potency and widespread use led to its selection by the National Toxicology Program for inclusion in studies to examine endocrine disrupting compounds with estrogenic activity both because of its utility as a positive control to which weaker estrogens can be compared and because of potential human developmental exposures resulting from unintentional continuation of the use of oral contraceptives containing ethinyl estradiol during early pregnancy. The study protocol utilized Sprague-Dawley rats and was designed to evaluate the effects of short-term, multigenerational, and long-term exposures to doses of estrogenic agents that produce subtle reproductive tract lesions in developmentally exposed Sprague-Dawley rat pups.

Results of the 2-year study are reported in this Technical Report, and results of short-term reproductive dose range-finding and multigenerational reproductive toxicology studies are reported separately (NTP TR 547). Data from the short-term reproductive dose range-finding study were used to select dietary exposure concentrations of 0, 2, 10, and 50 ppb for the current study. The multigenerational reproductive toxicology study examined F_0 through F_4 generations with F_5 litters terminated at weaning and focused on reproductive endpoints. Animals were exposed from the time that the F_0 generation was 6 weeks old through weaning of the F_3 generation, and animals of the F_0 through F_4 generations were necropsied at 20 weeks of age.

The current study was a 2-year dietary study utilizing three exposure arms: continuous exposure from conception through 2 years (designated F_1 continuous, or F_1C), exposure from conception through 20 weeks followed by control diet to 2 years (designated F_1 truncated at

postnatal day (PND) 140, or F_1T140), and exposure from conception through weaning followed by control diet to 2 years (designated F_3 truncated at PND 21, or F_3T21). The "F_3" designation for the F_3T21 arm indicates that these animals were siblings of the F_3 animals from the multigenerational reproductive toxicology study. The F_1C and F_1T140 animals were also siblings, but were derived from a separate breeding that was identical to the procedure used to produce the F_1 generation of the multigenerational reproductive toxicology study. The animals in this study were exposed to ethinyl estradiol during various phases of their lives from conception until termination at 2 years, and the ingested doses varied over the course of the study. During pregnancy, the ingested doses of the dams were approximately 0, 0.2, 0.9, or 5.8 µg/kg per day. During lactation, the dams' ingested doses were 0, 0.3, 2.0, or 10.3 µg/kg per day. The mean directly ingested ethinyl estradiol doses during the period prior to PND 140 were approximately 0.2, 0.9, or 4.9 µg/kg per day for females and 0.2, 0.8, or 4.5 µg/kg per day for males. For the period between PND 140 and the end of the study, mean ingested doses were approximately 0.1, 0.6, or 3.3 µg/kg per day for females and 0.1, 0.4, or 2.1 µg/kg per day for males. Under these dosing conditions, serum levels in the high dose group (50 ppb) were below the limit of detection (10 pg/mL) of a sensitive liquid chromatography-mass spectrometry method (Twaddle *et al.*, 2003).

For the current study, 50 animals per sex were initially assigned to each exposure group in each arm of the study. In control groups, histopathology data from one or two additional animals that had been assigned as sentinels but that became moribund or died early were also included in the analysis and presentation. Survival was similar in all control and exposed groups and ranged from 55% to 70% for males and 32% to 58% for females. The mean body weights of the 2 and 50 ppb F_1C males were less than those of the controls throughout the study, and the mean body weights of the 2 and 50 ppb F_1T140 males were less than those of the controls early in the study. The mean body weights of all exposed groups of F_1C and F_1T140 females were less than those of the controls throughout the study.

In males, the only neoplastic effects observed were positive trends in the incidences of preputial gland epithelial neoplasms and mammary gland adenoma or adenocarcinoma (combined) in F_3T21 animals. There were increased incidences of mammary gland alveolar hyperplasia in the 10 and 50 ppb F_1C and F_1T140 groups and in the 50 ppb F_3T21 group. There were increased incidences of ductal hyperplasia in the mammary gland of 50 ppb F_1C and F_1T140 males. In the liver, there were increased incidences of basophilic and eosinophilic foci in 50 ppb F_1C and F_1T140 males.

In females, there was a marginally positive dose trend in the incidences of uterine stromal polyps in F_1T140 animals and a significantly increased incidence of uterine stromal polyps in 2 ppb F_3T21 animals. In the F_1C females, increased incidences of uterine nonneoplastic lesions included endometrial hyperplasia in the 50 ppb group, squamous metaplasia in the 10 and 50 ppb groups, and atypical focal hyperplasia in all exposed groups. In the uterus of F_3T21 females, there were increased incidences of atypical focal hyperplasia in all exposed groups and an increased incidence of squamous metaplasia in the 50 ppb group. There was an increased incidence of eosinophilic focus in the liver of 50 ppb F_1C females and an increased incidence of basophilic focus of the liver in the 50 ppb group of F_1T140 females. There was no discernible effect of ethinyl estradiol on the time of onset of aberrant estrous cycles in any arm of the study.

CONCLUSIONS

Under the conditions of this 2-year feed study with continuous exposure to the test compound from conception through termination (F_1C), there was *no evidence of carcinogenic activity** of ethinyl estradiol in male or female Sprague-Dawley rats exposed to 2, 10, or 50 ppb. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the uterus and liver of females.

Under the conditions of this 2-year feed study with exposure to the test compound from conception through 20 weeks followed by control feed until termination (F_1T140), there was *no evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats exposed to 2, 10, or 50 ppb. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the liver and clitoral gland of females.

Under the conditions of this study where offspring of two prior generations of animals exposed to ethinyl estradiol in feed were exposed from conception through weaning (PND 21), followed by control feed through termination (F_3T21), there was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats based on increased incidences of preputial gland epithelial neoplasms and a marginal increased incidence of mammary gland adenoma or adenocarcinoma (combined). A significantly increased incidence of male mammary gland alveolar hyperplasia was also observed. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Increased incidences of squamous metaplasia and atypical hyperplasia in the uterus and hyperplasia in the clitoral gland were also observed.

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* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

Summary of the 2-Year Carcinogenesis Study of Ethinyl Estradiol in Sprague-Dawley rats

	F_1C		F_1T140		F_3T21	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**
Concentrations in feed	0, 2, 10, or 50 ppb	0, 2, 10, or 50 ppb	0, 2, 10, or 50 ppb	0, 2, 10, or 50 ppb	0, 2, 10, or 50 ppb	0, 2, 10, or 50 ppb
Body weights	2 and 50 ppb group less than the control group	Exposed groups less than the control group	Exposed groups similar to the control group after week 32	Exposed groups less than the control group	Exposed groups similar to the control group	Exposed groups similar to the control group
Survival rates	34/51, 31/50, 30/50, 32/50	26/51, 23/50, 19/50, 25/50	34/51, 31/50, 33/50, 33/50	26/51, 16/50, 22/50, 22/50	30/50, 27/49, 31/50, 35/50	27/52, 29/50, 21/50, 24/50
Early onset of aberrant estrous cycles	N/A	No effect of exposure	N/A	No effect of exposure	N/A	No effect of exposure
Nonneoplastic effects	Mammary gland: alveolar hyperplasia (1/44, 4/45, 6/47, 18/44); ductal hyperplasia (0/44, 0/45, 2/47, 3/44) Liver: basophilic focus (1/49, 3/49, 3/50, 17/49); eosinophilic focus (3/49, 5/49, 8/50, 15/49)	Uterus: endometrial hyperplasia (17/51, 18/50, 22/49, 25/50); squamous metaplasia (2/51, 6/50, 8/49, 13/50); atypical focal hyperplasia (6/51, 14/50, 16/49, 20/50) Liver: eosinophilic focus (1/51, 1/50, 1/49, 5/50)	Mammary gland: alveolar hyperplasia (1/44, 2/45, 6/47, 14/48); ductal hyperplasia (0/44, 0/45, 1/47, 3/48) Liver: basophilic focus (1/49, 3/50, 11/48, 6/49); eosinophilic focus (3/49, 11/50, 5/48, 10/49)	Liver: basophilic focus (1/51, 1/50, 1/50, 6/50) Clitoral gland: hyperplasia (2/50, 1/50, 2/49, 8/49)	Mammary gland: alveolar hyperplasia (2/42, 6/42, 4/40, 9/45)	Uterus: squamous metaplasia (1/52, 4/50, 3/50, 11/50); atypical focal hyperplasia (6/52, 16/50, 15/50, 21/50) Clitoral gland: hyperplasia (0/50, 1/50, 2/49, 3/48)
Neoplastic effects	None	None	None	None	None	None
Equivocal findings	None	None	None	Uterus: stromal polyp (2/51, 5/50, 6/50, 7/50)	Preputial gland: epithelial neoplasms (2/49, 4/49, 4/50, 8/49) Mammary gland: adenoma or adenocarcinoma (0/42, 0/42, 0/40, 3/45)	Uterus : stromal polyp (1/52, 7/50, 2/50, 5/50)
Level of evidence of carcinogenic activity	No evidence	No evidence	No evidence	Equivocal evidence	Equivocal evidence	Equivocal evidence

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 ethinyl estradiol on May 16, 2007, 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 May 16, 2007, the draft Technical Report on the toxicology and carcinogenesis studies of ethinyl estradiol received public review by the National Toxicology Program's Board of Scientific Counselors' Technical Reports Review Subcommittee. The review meeting was held at the National Institute of Environmental Health Sciences (NIEHS), Research Triangle Park, NC.

Dr. K.B. Delclos, National Center for Toxicological Research (NCTR), introduced the toxicology and carcinogenesis studies of ethinyl estradiol by noting that the chemical was already a known carcinogen. The purpose of the present study was to evaluate effects from chronic exposure to lower doses, reversibility of effects, and potential generational carryover effects. Dr. Delclos described the three exposure arms of the study and the body weights, survival, reproductive system effects, and nonneoplastic and neoplastic lesions observed in exposed rats. The proposed conclusions were:

Under the conditions of the 2-year feed study with continuous exposure to ethinyl estradiol from conception through termination (F_1C), there was *no evidence of carcinogenic activity* in male or female Sprague-Dawley rats.

Under the conditions of the 2-year feed study with continuous exposure to ethinyl estradiol from conception through 20 weeks followed by control feed until termination (F_1T140), there was *no evidence of carcinogenic activity* in male Sprague-Dawley rats and *equivocal evidence of carcinogenic activity* in female Sprague-Dawley rats.

Under the conditions of the 2-year feed study with continuous exposure to ethinyl estradiol from conception through weaning (PND 21), followed by control feed through termination (F_3T21), there was *some evidence of carcinogenic activity* in male Sprague-Dawley rats and *equivocal evidence of carcinogenic activity* in female Sprague-Dawley rats.

Dr. Soper, the first principal reviewer, felt the complicated study was well performed and reported. He asked if there should be some statement in the conclusions about the question of whether there was some magnification of effect or not.

Dr. Bradfield, the second principal reviewer, inquired if differences in the serum levels of the test chemical were known. Dr. Delclos replied that the serum specimens were available, but measurement usually involved more concentrated samples. Dr. Bradfield also inquired about the ultimate use of these studies; Dr. Delclos replied that a comparative overview of the various environmental estrogen studies would be performed to look for commonalities. Dr. Bradfield also asked what might be learned about the mechanism of action of these compounds. Dr. J.R. Bucher, NIEHS, replied that at the time these studies were designed, less was known about the possible mechanisms and the focus was on detecting common effects at low doses.

Dr. Pino, the third principal reviewer, suggested that the conclusion regarding the preputial gland tumors might better be classified as *equivocal evidence* rather than *some evidence*. Dr. Walker also supported classifying the preputial gland tumors as *equivocal evidence*. Dr. Crump concurred. By a unanimous vote, the conclusion for the preputial gland tumors in male rats in the F_3 generation was changed from *some* to *equivocal evidence*. Subsequently it was noticed that the mammary gland adenomas for these male rats, designated as may also have been related, fit the same category, and the panel unanimously approved combining the two lesions under the category of *equivocal evidence*. The amended conclusions were then approved unanimously with seven votes.

OVERVIEW

Study Rationale and General Design

Following a 1994 meeting sponsored by the National Institute for Environmental Health Sciences (NIEHS) entitled "Estrogens in the Environment III," the NIEHS (1995) proposed to expand and develop mammalian animal models to determine if environmentally relevant doses of endocrine-disrupting chemicals and mixtures of these chemicals during exposure windows that included development could cause reproductive problems or influence the incidence of reproductive tract cancers. Investigation of the potential for magnification of subtle reproductive effects over multiple generations, the importance of exposure windows, and whether effects are reversible or are imprinted to carry over across generations were also deemed to be important. The utility of such a program was agreed to by the National Toxicology Program (NTP) Board of Scientific Counselors at their meeting on October 18, 1994. The series of studies related to this initiative were conducted under an Interagency Agreement between the NIEHS/NTP and the Food and Drug Administration/National Center for Toxicological Research (FDA/NCTR). Study protocols were generated and reproductive dose range-finding studies were initiated at NCTR in 1997.

The overall goal of this series of studies was to evaluate the long-term consequences of exposure to endocrine-active agents that produce subtle short-term effects in exposed animals. The idea behind the studies was to evaluate aspects of the "endocrine disruptor hypothesis," which is the hypothesis that environmental exposure to endocrine-active chemicals contributes to a variety of adverse effects in wildlife and humans (NRC, 1999). As originally conceived, the plan was to evaluate neurobiological, behavioral, immunological, reproductive, and chronic toxicities in the main studies. This plan was modified to assess all of these endpoints in short-term studies conducted prior to the main studies that focused on reproductive and chronic toxicity. The compounds selected for multigenerational reproductive toxicology studies were three agents that vary in estrogenic potency: the soy isoflavone, genistein; the industrial intermediate, *p*-nonylphenol; and the potent and widely used synthetic estrogen, ethinyl estradiol.

A short-term reproductive dose range-finding study was conducted for each compound to assess general and reproductive toxicity, behavioral toxicity, neurotoxicity, and immunotoxicity. The test compounds were administered in a soy- and alfalfa-free rodent diet. Pregnant females were given dosed feed from gestation day 7 (GD 7) until the pups were weaned, and the pups were continued on the same diet as their dams until termination. Separate sets of animals were bred for the reproductive, behavioral, and immunological studies. One pup per sex per litter from the reproductive toxicity study was used for the neurotoxicity study. Data from the reproductive dose range-finding study were the primary data used for selection of exposure concentrations for the subsequent multigenerational reproductive toxicology and chronic studies, although data from the other studies were considered in choosing the range of exposure concentrations to be tested. All of these studies utilized outbred CD (Sprague-Dawley) rats from the NCTR breeding colony. The Sprague-Dawley rat was selected because of its widespread use in reproductive toxicology studies, including those conducted by the NTP, its robust breeding performance, and its relatively low background incidences of testicular Leydig cell tumors and large granular lymphocyte leukemia relative to the F344/N rat commonly used in NTP carcinogenesis studies. The relatively high background incidences of pituitary gland and female mammary gland tumors in Sprague-Dawley rats were recognized as a possible concern. The relatively poor breeding performance of the F344/N rat would have presented a considerable challenge to the conduct of the studies described here, as it would for any evaluation of reproductive toxicity. Reproductive toxicity testing guidelines, for example, those of the EPA, FDA, and The Organization for Economic Cooperation and Development generally indicate that animals with low fecundity not be used. The NCTR breeding colony was established in 1972 using Sprague-Dawley rats from the Charles River Laboratories. The NCTR colony at present is a distinct substrain of Sprague-Dawley rat and has been previously shown to differ substantially from the Charles River and other strains of Sprague-Dawley rat in terms of body weight, which is lower than

that reported for other substrains, and survival, which is longer than that reported for other substrains (Duffy *et al*., 2001).

It was intended that exposure concentrations that were within the range of human exposures and/or below previously reported no-observed-adverse-effect levels be incorporated in the main studies. The experimental design was intended to determine if subtle effects would be magnified in subsequent generations and if observed effects were reversible. In standard reproductive toxicity studies conducted for regulatory purposes, high doses are chosen to produce some maternal toxicity while the low dose is selected with the goal of not producing parental effects (OECD, 2004; CFSAN, 2006). The high dose for chronic studies is set as the maximum tolerated dose. In the present series of studies, the goal was to select a high dose, based on the results of the reproductive dose range-finding study, that did not produce significant maternal toxicity but did produce reproductive tract lesions in the offspring of a degree that would not severely affect reproductive capacity in the first generation. The questions addressed in the chronic studies were whether exposures producing subtle modifications of the reproductive tract could produce chronic toxicity and whether any observed chronic toxicity was induced by early developmental exposure or rather required continuous long-term exposure.

The need to maintain consistent dietary composition was taken into account in the design of this series of studies. A soy- and alfalfa-free diet (PMI 5K96, Appendix H) with consistently low concentrations of the phytoestrogens genistein and daidzein was utilized in all studies. A preliminary study indicated that rats fed this diet had reproductive capacity equivalent to rats fed NIH-31 diet, the standard soy- and alfalfa-containing diet used at the test facility (NCTR), although feed consumption in both sexes and the body weights of males fed PMI 5K96 were significantly lower than in rats fed NIH-31.

Design of the Multigenerational Reproductive Toxicology and Chronic Studies Conducted Subsequent to the Reproductive Dose Range-Finding Studies

As in the short-term studies, the multigenerational reproductive toxicology and chronic studies were conducted with the NCTR CD Sprague-Dawley rat and test compounds were administered in the soy- and alfalfa-free 5K96 diet. The design of the multigenerational reproductive toxicology and chronic studies is outlined in Figure 1. For the multigenerational reproductive toxicology studies, males and females of the original parental generation (F_0) were placed on 5K96 diet at weaning, and dosed feed was administered starting on postnatal day (PND) 42, 4 to 6 weeks before breeding. The F_0 generation was maintained on dosed feed until termination at PND 140. For breeding, one male was cohabited with one female for 14 days or until a vaginal plug (*in situ* or in pan below cage) was detected. Subsequent generations (F_1 through F_4) were bred similarly. The F_1 and F_2 generations were exposed to the test compound administered in the diet continuously from conception through termination at PND 140; the F_3 generation was removed from exposure at weaning (PND 21) and continued on control feed until PND 140, while the F_4 generation received no dietary exposure to the test compound. The F_4 generation was bred to produce an unexposed F_5 generation. The F_5 litters were terminated at weaning following collection of basic litter information. Thus, this design incorporated an evaluation of the magnification (or reduction) of effects across exposed generations, an evaluation of the reversibility of effects, and an evaluation of the carry-over of effects into subsequent unexposed generations. Standard toxicological data and reproductive development and performance data were collected for all generations, and organ weights and histopathology data were collected for 25 randomly selected animals per sex per exposure concentration for each generation at necropsy.

Chronic toxicity was examined for two test compounds (ethinyl estradiol and genistein). Three exposure windows were examined in the chronic studies (Figure 1): 1) Continuous exposure from conception through 2 years (designated F_1 continuous, or F_1C) to evaluate the effects of lifelong exposure, 2) Exposure from conception through PND 140 followed by control diet to 2 years (designated F_1 truncated at PND 140, or F_1T140) to determine if effects observed in the multigenerational study led to long-term adverse effects, and 3) Exposure from conception through weaning followed by control diet to 2 years (designated F_3 truncated at PND 21, or F_3T21) to evaluate the long-term effects of developmental exposure. The F_3 designation for the F_3T21 exposure indicates that these animals were siblings of the F_3 animals from the multigenerational reproductive toxicology study. Because of the

number of animals required for the chronic study of each test chemical, separate sets of animals were used for the multigenerational reproductive toxicology study and the F_1 generation chronic study. The assessment of chronic toxicity resulting from dietary exposure from conception through weaning was conducted with animals from the F_3 generation of the multigenerational reproductive toxicology study.

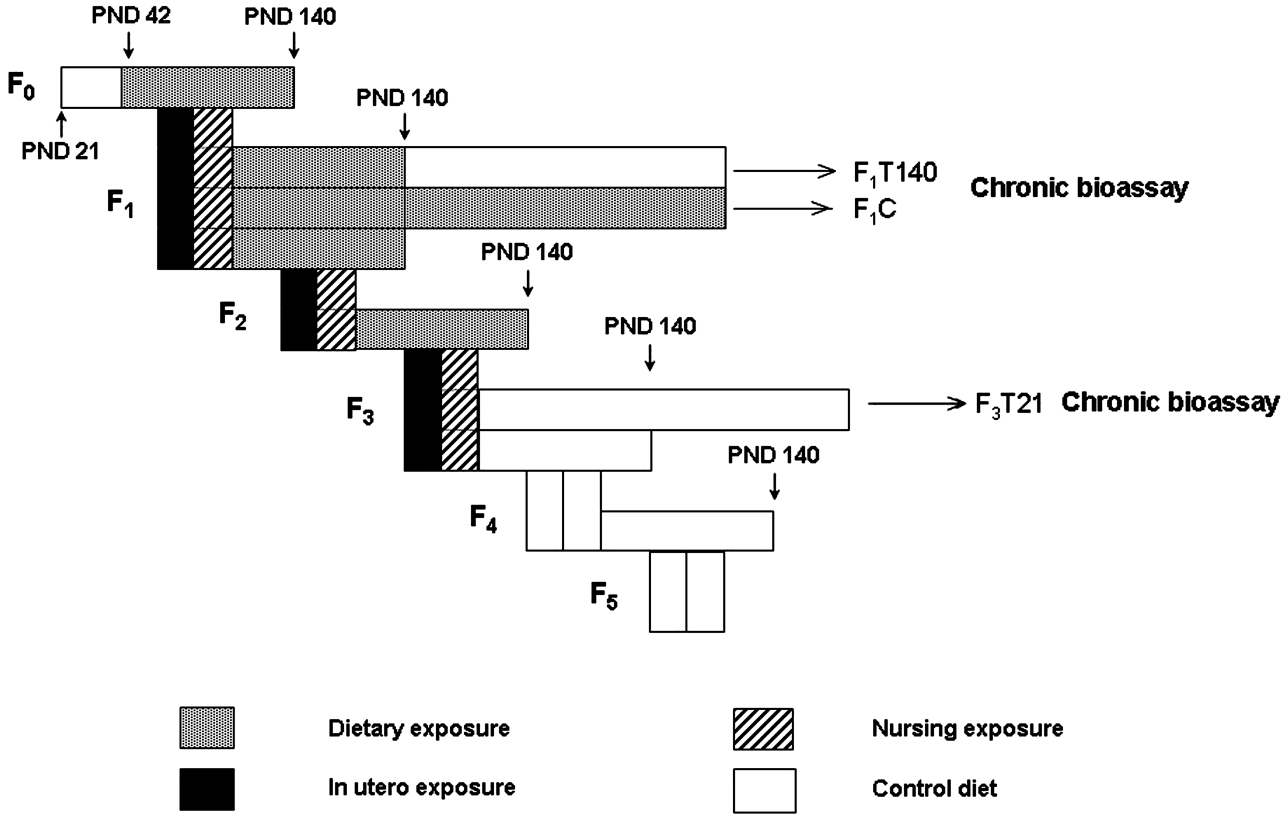
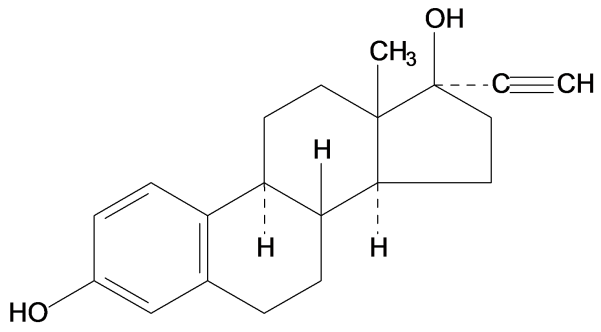


FIGURE 1
Dosing Schedule for the Multigenerational Reproductive Toxicology and Chronic Studies

INTRODUCTION



ETHINYL ESTRADIOL

CAS No. 57-63-6

Chemical Formula: $C_{20}H_{24}O_2$ Molecular Weight: 296.40

Synonyms: 17-ethinylestradiol; ethynylestradiol; 17α-ethynyl-1,3,5(10)-estratriene-3,17β-diol
Trade Names: Amenoron, Amenorone, Anovlar, Diogyn-E, Diprol, Dyloform, EE, EE_2, EE2, Ertonyl, Esteed, Estigyn, Estinyl, Eston-E, Estopherol, Estoral, Eticyclin, Eticyclol, Eticylol, Etinestrol, Etinestryl, Etinoestryl, Etistradiol, Feminone, Follicoral, Ginestrene, Halodrin, Inestra, Linoral, Loestrin, Lynoral, Menolyn, Microfollin, Neo-Estrone, Nogest-S, Nordette, Novestrol, Oradiol, Orestralyn, Orestrayln, Palonyl, Perovex, Primogyn, Primogyn C, Primogyn M, Progynon C, Spanestrin, Ylestrol

Physical Properties, Production, Use, and Exposure

Ethinyl estradiol is a white crystalline powder that is water insoluble but soluble in various nonaqueous solvents such as ethanol, ether, acetone, dioxane, chloroform, and vegetable oil (*Merck*, 2006). It is a potent synthetic estrogen first reported by Inhoffen and Hohlweg (1938) that is a widely prescribed drug, primarily as the estrogenic component of oral contraceptives, but it has also been used in the treatment of breast and prostate gland cancers, menopausal symptoms, and female hypogonadism (Loose and Stancel, 2006). Oral contraceptive formulations containing greater than 50 µg ethinyl estradiol were removed from the United States market in 1989 and currently marketed formulations generally contain between 20 and 35 µg ethinyl estradiol, which result in doses of approximately 0.3 to 0.6 µg/kg assuming an average body weight of 60 kg. Ethinyl estradiol is also used as the estrogenic component of contraceptives administered vaginally or transdermally, which are used to a lesser extent than oral contraceptives. As a result of its widespread use in humans, ethinyl estradiol has also been detected as an environmental contaminant at low levels and is a potential concern for aquatic organisms (Nash *et al.*, 2004).

Metabolism and Pharmacokinetics

Estradiol itself has poor bioavailability after oral administration due to extensive metabolism, and the addition of the 17α-ethinyl group to estradiol greatly enhances oral activity in humans due to inhibition of hepatic

metabolism at the C_{16} and C_{17} positions, particularly 16α-hydroxylation (Bolt, 1979). In addition, as is the case with other acetylenic compounds, ethinyl estradiol is a mechanism-based inactivator of several cytochromes P450 [3A4, 2B1, and 2B6] (Guengerich, 1988; Kent *et al.*, 2002; Lin *et al.*, 2002). Ethinyl estradiol has low affinity for sex steroid binding proteins in humans and rodents (sex hormone binding globulin and alphafetoprotein) but is extensively bound to serum albumin (Raynaud, 1973; Fotherby, 1996). There is a large body of data on the pharmacokinetic behavior of ethinyl estradiol in women, and wide individual differences in the metabolism and elimination of ethinyl estradiol have been shown to exist such that the systemic bioavailability of ethinyl estradiol following oral ingestion has been reported to range from about 20% to greater than 80% (Goldzieher, 1990; Baumann *et al.*, 1996; Fotherby, 1996). In several animal species, including rats, first pass metabolism of ethinyl estradiol is higher than that in humans, and the bioavailability of ethinyl estradiol is substantially lower than that in humans. Dusterberg *et al.* (1986), for example, reported the bioavailabilities of oral ethinyl estradiol to be 3%, 0.3%, 9%, 0.6%, and 2% in rats, rabbits, beagles, rhesus monkeys, and baboons, respectively, and discussed the differences in the pharmacokinetics of ethinyl estradiol between these laboratory species and humans. Hirai *et al.* (1981) reported extensive metabolism of ethinyl estradiol by the gut wall (40%) and by the liver (79% of the compound in portal blood) after oral administration to rats. The major metabolites of ethinyl estradiol in the rat result from hydroxylation at the C_2 position and subsequent methylation, glucuronidation, and sulfation of the hydroxy metabolite (Maggs *et al.*, 1982, 1983). The predominant route of metabolism in humans is also 2-hydroxylation (Guengerich, 1990), and in both rats and humans, the predominant forms of cytochromes P450 responsible for the metabolism of ethinyl estradiol differ from those responsible for the metabolism of endogenous estradiol (Ball *et al.*, 1990). In keeping with the literature results on the low bioavailability of ethinyl estradiol in rats, attempts to measure serum ethinyl estradiol levels in studies at the National Center for Toxicological Research (NCTR) indicated that serum levels of ethinyl estradiol could not be detected at the highest exposure concentration, 50 ppb in feed, using a liquid chromatography–mass spectrometry assay with a limit of detection of 10 pg/mL (30 pM) (Twaddle *et al.*, 2003). Administration of single doses of ethinyl estradiol ranging from 0.125 to 1 mg/kg by gavage showed a linear increase in C_{max} (maximal concentration). Following an oral gavage dose of 1 mg/kg in the same study, 57% of the serum ethinyl estradiol was present in conjugated forms (glucuronides and sulfates) and elimination was slower in females than in males (half-life of 2.8 hours for males and 6.1 hours for females). The areas under the curves (AUCs) were 2,910 and 2,570 pg • hour/mL for males and females, respectively, and the C_{max} values were 800 and 1,100 pg/mL for males and females, respectively. There was high variability among animals, and there were no significant differences between the sexes for AUC or C_{max}. These results can be contrasted to the pharmacokinetic parameters reported in women after single oral doses of ethinyl estradiol or an oral contraceptive containing ethinyl estradiol. Baumann *et al.* (1996) administered a single oral dose of 120 µg ethinyl estradiol (approximately 2 µg/kg) to 16 postmenopausal women and determined a C_{max} of 340 pg/mL, an AUC of 2,621 pg • hour/mL and a half-life of 16.8 hours. Scheffler *et al*. (1999) administered a single dose of two oral contraceptive tablets containing a total of 70 µg ethinyl estradiol (approximately 1.1 µg/kg) to 12 healthy premenopausal women and determined a C_{max} of 245 pg/mL, an AUC of 2,365 pg • hour/mL, and a half-life of 16.6 hours. The substantial difference in bioavailability between the rat and humans needs to be considered when comparing the relative responsiveness of the species to ethinyl estradiol.

CARCINOGENICITY

The carcinogenic activity of estrogens has been extensively studied and reviewed. Steroidal estrogens, a class of compounds that includes ethinyl estradiol, have been found to be human carcinogens with clear increases in uterine endometrial cancer and some evidence for increased breast cancers under certain exposure conditions, particularly postmenopausal hormone replacement therapy (IARC 1979, 1987, 1999; NTP, 2004; Reeves *et al*., 2006). In addition, oral contraceptives containing ethinyl estradiol have been implicated in increasing liver, cervical, and breast tumors, although the results on breast cancer are mixed, and in decreasing the risk for developing ovarian and endometrial cancers (IARC, 1999; Moreno *et al*., 2002; NTP, 2004). Many of the experimental animal studies considered by the International Agency for Research on Cancer (IARC) and the National Toxicology Program (NTP) that

involved ethinyl estradiol utilized mixtures of compounds as used in oral contraceptives, but it was concluded from studies in which ethinyl estradiol was administered alone that treatment increased pituitary gland and mammary gland tumors in male and female mice as well as endometrial and cervical tumors in female mice, and mammary gland and pituitary gland tumors and neoplastic liver nodules in rats (IARC, 1979, 1987; NTP, 2004). In addition, ethinyl estradiol has been shown to be a kidney carcinogen in hamsters (Yager and Liehr, 1996; Yager, 2000), a uterine carcinogen after developmental exposure in mice (Newbold and Liehr, 2000), and a potent liver tumor promoter in rats (Campen *et al*., 1990; Yager and Liehr, 1996). Many of these carcinogenicity studies utilized subcutaneous implants or injections for dosing or expressed doses in terms of multiples of the human contraceptive dose in use at the time and are thus somewhat difficult to relate directly to exposures used in the present study. In a more directly comparable study, Schardein (1980) administered ethinyl estradiol mixed in an unspecified diet ("standard powdered ration") at 0.15 and 1.5 ppm resulting in delivered doses of 6 to 8 and 60 to 80 µg/kg body weight/day, respectively, to male and female Sprague-Dawley rats starting at 7 or 8 weeks of age and continuing for 2 years. Liver and pituitary gland neoplasms were reported to be elevated at the high exposure concentration in both sexes. In addition, male rats in the high exposure concentration group had a 6% (3 of 50) incidence of mammary gland fibroepithelial tumors compared to 0% in the control (0 of 100) and low exposure concentration (0 of 50) groups.

While carcinogenic effects resulting from *in utero* exposures to diethylstilbestrol, an orally available synthetic estrogen with potency similar to ethinyl estradiol, are well documented in humans and in animal models (Mittendorf, 1995; Newbold, 1995; Newbold *et al*., 2006), the available data relating to ethinyl estradiol-induced carcinogenesis following developmental exposures are limited. Walker *et al*. (1990) studied the effects of prenatal administration of ethinyl estradiol (0.02 or 0.2 mg/kg body weight/day on gestational days 11 and 12) in a strain of mice (129 Sv-S1 CP) in which the males are susceptible to teratomas. Ethinyl estradiol exposure caused a dose-related increase in cryptorchid testes, but the observed increased incidence of teratomas was not statistically significant.

Genotoxicity

While the exact mechanisms whereby ethinyl estradiol and other estrogens act as carcinogens and tumor promoters have not been established definitively, both genotoxic mechanisms and estrogen receptor-mediated stimulation of hyperplasia have been implicated (Yager and Liehr, 1996; Yager, 2000; Yager and Davidson, 2006). Ethinyl estradiol has generally proved to be negative in bacterial or mammalian gene mutation assays, but has been reported to induce chromosomal damage (Siddique *et al*., 2005). Evidence of indirect genotoxicity through oxidative stress and direct DNA binding of catechol metabolites of estrogens has been demonstrated (Dwivedy *et al*., 1992; Ogawa *et al*., 1995; Stack *et al*., 1996; Yager and Liehr, 1996; Bhat *et al*., 2003). Despite its high estrogenic potency, ethinyl estradiol has been found to be a weak carcinogen in hamster kidney relative to many other estrogens (Li *et al*., 1983), and this has been attributed to the low generation of catechol metabolites and oxidative stress relative to more potent carcinogenic estrogens in this system (Zhu *et al*., 1993; Yager, 2000).

Dose Selection for the 2-Year Feed Study of Ethinyl Estradiol

Results from the reproductive dose range-finding feed study of ethinyl estradiol and the rationale for exposure concentration selection for the multigenerational reproductive toxicology and 2-year studies are presented in NTP Technical Report 547 (NTP, 2010). The questions to be addressed in the chronic study were whether exposures producing subtle modifications of the reproductive tract could produce chronic toxicity and whether any observed chronic toxicity was induced by early developmental exposure or rather required continuous long-term exposure. Dietary exposures of 0, 0.1, 1, 5, 25, 100, and 200 ppb were evaluated in the reproductive dose range-finding study. Daily body weight gain and feed consumption of the treated dams prior to parturition showed a decreasing trend with the 100 and 200 ppb groups significantly different from the controls on gestation days (GD) 12 to 21 and 10 to 21, respectively. Daily feed consumption was also depressed in the 100 and 200 ppb groups on multiple days in the early period of treatment (within the period from GD 8 to 14). Overall body weight gain and feed consumption during preg-

nancy also showed significant decreasing trends and were significantly less than controls in the 100 and 200 ppb groups. Mean live pup birth weight was significantly less than controls in the 100 and 200 ppb groups. Other pregnancy (gestation duration, proportion of vaginal plug-positive dams producing litters) or litter (total pups per litter, proportion of stillborn pups, sex ratio, anogenital distance) parameters measured did not show significant treatment-related effects. Preputial separation, a marker of male puberty, was accelerated at 5 and 25 ppb and the proportion of male pups showing preputial separation in the 200 ppb group by the time of the scheduled sacrifice at PND 50 was significantly lower than in the control group. Vaginal opening, a marker of female puberty, was accelerated in the 25, 100, and 200 ppb groups. Female and male pups showed significant decreasing trends in body weights beginning on PND 34 and mean body weights in the 200 ppb group in both sexes were significantly lower than controls in pairwise comparisons from PND 41 onward. Total body weight gain and feed consumption after weaning were not significantly altered by treatment for either sex. Organ weights were analyzed by three statistical models, one utilizing the absolute organ weight and the others incorporating a body weight adjustment by utilizing a ratio of organ to body weight or by using body weight as a covariable in an analysis of covariance. For 200 ppb males, ventral prostate gland (absolute and relative) and testes (all statistical models) weights were reduced relative to controls while the pituitary gland weight, adjusted for body weight, was increased. Regardless of the statistical model used, the dorsolateral prostate gland weight was increased relative to controls at 5 ppb. In 200 ppb females, absolute and relative ovary weights were decreased while liver weight, adjusted for body weight, was increased.

Microscopic evaluation indicated ethinyl estradiol-induced changes in multiple organs of both sexes. Male mammary gland hyperplasia was observed at 25 ppb or greater. In the testes, degeneration of pachytene spermatocytes and depletion of elongated spermatids (100 and 200 ppb) and round spermatids (200 ppb) were observed. Testicular spermatid head counts were also significantly lower than controls in the 200 ppb group. Seminal vesicles showed depletion of secretory material (100 and 200 ppb) and atrophy (200 ppb). Mild mineralization of renal tubules was observed in males at 100 and 200 ppb. In females, significant disturbances of the estrous cycle were evident in animals in the 200 ppb group, with the ovaries of 14 of 15 animals examined diagnosed as anestrus. Two of 15 animals examined in the 100 ppb group were diagnosed as anestrus. In the 200 ppb group, a significant incidence of uterine (atrophy) and vaginal (mucocyte metaplasia and dystrophy) abnormalities were also observed.

A subset of animals from the reproductive dose range-finding study (sacrificed on PND 50) was utilized for assessment of the sexually dimorphic central nucleus of the medial preoptic area of the hypothalamus (SDN). The results indicated no significant differences from controls in any exposed group, although for males the 1, 25, 100, and 200 ppb groups were significantly smaller than the 0.1 ppb group (NTP, 2010).

In behavioral assessments, a separate set of pregnant rats were fed soy-free diets containing 0, 1, 5, or 200 ppb ethinyl estradiol beginning on GD 7, and offspring continued on these diets through PND 77. Male and female offspring were assessed for levels of sexually dimorphic behaviors: open field activity, play behavior, running wheel activity, and consumption of saccharin- and sodium chloride-flavored solutions. Increased intake of sodium-flavored solution and regular water were seen in both sexes at 200 ppb as the only treatment-related behavioral effects. As in the reproductive dose range-finding study summarized earlier, treatment-related reduction of body weight gain and feed consumption were observed in dams and mean pup birth weight was depressed in the 200 ppb group. No effects on gestation duration, sex ratio, or number of live or dead pups per litter were observed. Body weight and feed consumption were significantly depressed in offspring of both sexes after weaning (Ferguson *et al.*, 2003).

The immunotoxicologic study was conducted under identical exposure conditions to the reproductive and behavioral studies (doses: 0, 5, 25, and 200 ppb) except that F_1 animals were sacrificed on PND 63. Terminal body weights for the F_1 pups of both sexes were depressed at 200 ppb. The activity of natural killer (NK) cells was enhanced in F_0 and F_1 females at 25 and 200 ppb. Splenocyte proliferation induced by anti-CD3 antibodies, a marker of cell-mediated immunity, was increased in F_1 males and females at 200 ppb. Spleen cell numbers were decreased at 200 ppb in F_1 males (B, T, and NK cells) and females (B cells). A significant decrease in bone marrow DNA synthesis was observed in F_1 males at 5 ppb, but not at 25 and 200 ppb, and decreased erythrocyte progenitors were observed in F_1 females at 5 and 25 ppb, but not at 200 ppb (Guo *et al.*, 2005).

In summary, the reproductive dose range-finding study results indicated that, under the conditions of these experiments, ethinyl estradiol altered body weight gain and feed consumption and affected multiple reproductive and nonreproductive organs. The severity of reproductive tract effects in both sexes of the F_1 generation at 200 ppb clearly eliminated that exposure concentration from consideration for the 2-year feed study, while the effects of 100 ppb on dam body weight and feed consumption, litter weight, and reproductive tract effects in pups (anestrus ovaries, degeneration of spermatocytes, depletion of secretory material in seminal vesicles) were primary reasons for concern for the use of that exposure concentration in the 2-year feed study. The high exposure concentration for the 2-year feed study was thus set at 50 ppb. Intermediate exposure concentrations of 2 and 10 ppb were selected to bracket the 5 ppb exposure concentration used in the reproductive dose range-finding study where apparent increased prostate gland weight and acceleration of preputial separation were observed as described above. The calculated ingested doses of ethinyl estradiol by animals consuming these dietary levels of ethinyl estradiol in the 2-year feed study are given in Table 1.

Table 1
Approximate Ingested Doses of Ethinyl Estradiol in Rats in the 2 Year Feed Study of Ethinyl Estradiol[a]

	2 ppb	10 ppb	50 ppb
F_0 Dams, nonlactating period	0.2 ± 0.0 (7)	0.9 ± 0.1 (7)	5.8 ± 0.7 (7)
F_0 Dams, lactation	0.3 ± 0.0 (3)	2.0 ± 0.3 (3)	10.3 ± 1.4 (3)
F_1 Female pups, continuous dosing, before PND 140	0.2 ± 0.0 (17)	0.9 ± 0.1 (17)	5.0 ± 0.5 (17)
F_1 Female pups, truncated dosing, before PND 140	0.2 ± 0.0 (17)	1.0 ± 0.1 (17)	4.8 ± 0.4 (17)
F_1 Female pups, continuous dosing, after PND 140	0.1 ± 0.0 (21)	0.6 ± 0.0 (21)	3.3 ± 0.1 (17)
F_1 Male pups, continuous dosing, before PND 140	0.2 ± 0.0 (17)	0.7 ± 0.1 (17)	4.7 ± 0.9 (17)
F_1 Male pups, truncated dosing, before PND 140	0.2 ± 0.0 (17)	0.8 ± 0.1 (17)	4.2 ± 0.6 (17)
F_1 Male pups, continuous dosing, after PND 140	0.1 ± 0.0 (21)	0.4 ± 0.0 (21)	2.1 ± 0.0 (21)

[a] Data are presented as µg ethinyl estradiol/kg body weight per day [mean ± standard error (number of weeks measured)]. PND=postnatal day. The mean ingested dose was calculated for each available week by multiplying the dietary concentrations of ethinyl estradiol (ppb) by the mean measured amount of feed ingested weekly and dividing the result by the mean body weight for the week. These values were divided by 7 to give the mean daily dose given in the table. Weekly body weight and feed consumption data were used for the F_0 calculations and for the F_1 animals prior to PND 140; monthly data (one week per month) were used for the F_1 animals after PND 140. For the F_0 dams, data are reported separately for the nonlactating period and the lactating period. The values presented for the lactating females include the period, primarily during the last week of nursing, during which the pups were beginning to directly consume food. For F_1 animals, data are reported separately for the time before PND 140 and from PND 140 to termination at 2 years for the subset of animals that were continuously dosed over this time period. F_3T21 animals are not included in this table. They were exposed only through gestation and lactation, and the relevant information on the exposure of the F_2 dams during the pregnancy and lactation is presented in Technical Report 547 (NTP, 2010). Ingested doses were similar to those presented here for the F_0 dams.

MATERIALS AND METHODS

Procurement and Characterization of Ethinyl Estradiol

Ethinyl estradiol was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in one lot (57H1178). Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR) (Appendix C). Reports on analyses performed in support of the ethinyl estradiol study are on file at the NCTR.

The chemical, a white crystalline solid, was identified as ethinyl estradiol by ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy and by gas chromatography-electron impact mass spectrometry (GC-EI MS). A nuclear Overhauser effect experiment was performed to distinguish between the α and β isomers of ethinyl estradiol; results confirmed that the chemical was the α isomer. Carbon-13 chemical shift data were in agreement with those that have been reported for 17α-derivatives of estradiol (Dionne and Poirier, 1995).

Before, during, and after the study, the purity of lot 57H1178 was determined using ^{1}H-NMR (based on –CH groups), GC-EI MS, and GC with flame ionization detection (FID). ^{1}H-NMR consistently indicated a purity of 98.5%. GC-EI MS gave somewhat inconsistent values for purity ranging from 95.3% to greater than 99% due to thermal and solvent decomposition of the test material, but measurements at the end of the study indicated a purity of 99%. GC-FID indicated a purity of 99.7%. The overall purity of lot 57H1178 was determined to be greater than 98.5%, and no identifiable impurities were detected.

To ensure stability, the bulk chemical was stored in amber glass bottles at room temperature. The stability of the bulk chemical was monitored during the study using ^{1}H-NMR and GC-EI MS; no degradation of the bulk chemical was detected.

Background Isoflavone Content of Base Diet

The base diet used for the current study was an irradiated soy- and alfalfa-free rodent feed, designated 5K96, obtained from Purina Mills, Inc. (Richmond, IN), in an attempt to maintain consistently low background exposure to phytoestrogens. This feed maintains the nutritional specifications of the NIH-31 feed and contains casein in place of soy and alfalfa (NTP, 2008a). The control feed was routinely assayed for total isoflavone content (that is, genistein and daidzein) after acid hydrolysis by the study laboratory. Prior to the current study, native isoflavone content was determined for several lots of 5K96 feed using HPLC-electrospray MS methods; methodological details and the data from these studies have been published elsewhere (Doerge *et al*., 2000). During and following the current study, an additional 27 consecutive lots of 5K96 feed were analyzed by HPLC MS. The results for analyses of 5K96 feed showed the concentrations of genistein and daidzein (mean ± standard error) to be 0.32 ± 0.26 ppm and 0.19 ± 0.15 ppm, respectively.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared at least every 9 weeks by mixing ethinyl estradiol with feed (Table C2). The study laboratory performed a series of homogeneity studies: the 1 and 5 ppb dose formulations were analyzed using GC-EI MS, the 10 ppb dose formulation was analyzed using GC with electron capture (EC) detection, and a 200 ppb dose formulation was analyzed by HPLC-fluorescence. Stability studies of a 5 ppb dose formulation were also performed by the study laboratory using GC-EI MS. Homogeneity was confirmed, and stability was confirmed for at least 24 weeks for dose formulations stored in stainless steel cans at 2° to 8° C and for up to 16 days under simulated animal room conditions.

Periodic analyses of the dose formulations of ethinyl estradiol were performed by the study laboratory using GC-EC. Because of the very low exposure concentrations used in this study, the technical difficulties associated with measurements of such concentrations in the complex diet matrix were recognized, and a somewhat higher degree of variability than would be seen in studies with higher exposure concentrations was anticipated and accepted prior to the start of the study. Specifications for the dose formulations for the 2-year feed study were set as being within 30% of the target concentrations with a coefficient of variation of ± 20%. All 82 of the dose formulations analyzed met the study specifications (Table C3).

2-Year Study

Study Design

Three exposure windows were examined in the 2-year study: continuous exposure from conception through 2 years (F_1C); exposure from conception through postnatal day 140 (PND 140), followed by control diet until termination (F_1T140); and exposure from conception through weaning at PND 21, followed by control diet until termination (F_3T21).

Groups of 35 (F_0) or approximately 50 (F_1 and F_3) male and female rats were exposed to diets containing 0, 2, 10, or 50 ppb ethinyl estradiol for 77 (F_0 generation), 775 (F_1C), 161 (F_1T140), or 42 (F_3T21) days. One F_3 group had 49 males and most control groups included one or two sentinel animals that died and were necropsied early. The same sets of dams produced F_1 offspring for the F_1C and F_1T140 exposure groups. The F_0 ancestral generation of the F_3T21 animals was that used in the separate multigenerational reproductive toxicology study (NTP, 2010). Exposure schedules for the three treatment arms of the study are shown in Figure 1.

Source and Specification of Animals

The Multigeneration Support System, which was developed by ROW Sciences at the NCTR, was used to track the genealogy of all animals in the current study and to collect animal data. For the parental (F_0) generation, 140 male and 140 female weanling NCTR CD rats (Strain Code 23) were obtained from the NCTR breeding colony and placed on irradiated control 5K96 feed. Until weaning, these rats and their dams had been maintained on NIH-31 pellets. The NIH-31 diet has been reported to contain approximately 30 ppm each of the soy-derived isoflavones genistein and daidzein, which are present predominantly as the glucosides genistin and daidzin (Thigpen *et al.,* 1999).

The NCTR CD rat strain was founded in 1972 from Sprague-Dawley rats from Charles River Laboratories and has been maintained in the NCTR breeding facility since that time. Rats of the F_0 generation were acclimated to the Purina 5K96 diet for 3 weeks from PND 21 to PND 42 and were 6 weeks old at the beginning of the study. Animals in the F_1 and F_3 generations were on study from conception. The health of the animals in all generations was monitored during the study according to the protocols of the Study Laboratory's Sentinel Animal Program (Appendix I).

Animal Breeding and Maintenance

Animals in the F_0 generation were identified by tail tattoos and housed in pairs until assignment to exposure groups. On PND 42, animals in the F_0 generation were weighed and allocated to one of four exposure groups by a stratified randomization procedure based on body weight to give 35 males and 35 females in each exposure group. Animals were reidentified with a unique tail tattoo after assignment to exposure groups. Males were housed individually in wire breeding cages between PND 56 to PND 60 for acclimation. Pairings within exposure groups were randomly generated by the Multigeneration Support System, and females were introduced into breeding cages with the males. The F_0 animals were no younger than PND 70 and no older than PND 84 at the time they were paired.

The date of vaginal plug detection (*in situ* or in pan below the cage) was designated as the day of conception or gestation day 0 (GD 0). In order to maximize mating success and thus the number of litters and pups available for the study, breeders used to generate the F_1C and F_1T140 animals were kept together in the breeding cage for an additional 5 days (the length of one estrous cycle) if a vaginal plug was detected within the first 3 days of the mating period. The 2-year study animals that had exposure truncated at weaning (F_3T21) were from the F_3 generation of the previous multigenerational reproductive toxicology study (NTP, 2010). Briefly, F_0 animals were exposed to 0, 2, 10, or 50 ppb ethinyl estradiol from PND 42, and they and their descendants were exposed continuously to the same dosed feed through the F_2 generation. All groups in the F_3 generation were placed on control 5K96 feed at weaning. In all generations, on postconception day 23, corresponding to

PND 2, litters were randomly standardized to four males and four females per litter. Animals were occasionally fostered within exposure groups to maintain constant litter size. After standardization, excess pups were sacrificed. Pups were marked on the day of standardization by paw tattoos so that a unique animal identification was provided by cage number, sex, and tattoo pattern. Animals were identified with a unique identification number by tail tattoo at weaning.

At weaning of the F_1 generation, 50 control animals of each sex and 100 animals of each sex from the three other exposure groups were selected for continuation on the study and were housed individually until termination. Additional control animals were designated as sentinel animals, housed with the study animals, and removed for microbiological assessment periodically during the study. After weaning, animals were maintained on the same feed as their dams. At PND 140, one half of the animals in the three exposed groups (2, 10, and 50 ppb) were placed on control feed until termination of the study. Fifty animals from the 0, 2, 10, and 50 ppm groups in the F_3 generation were placed on control feed at weaning until termination of the study. In all cases, study animals were selected so that the maximum number of litters was represented and no more than two animals of the same sex were taken from a single litter. The number of litters from which the animals were derived in each exposure group were as follows: F_1 0 ppb, 29 litters; F_1 2 ppb, 32 litters; F_1 10 ppb, 31 litters; F_1 50 ppb, 34 litters; F_3 0 ppb, 29 litters; F_3 2 ppb, 31 litters; F_3 10 ppb, 31 litters; F_3 50 ppb, 35 litters.

Animals were maintained on soy- and alfalfa-free 5K96 meal available *ad libitum* until the day before necropsy. Millipore-filtered tap water, which was analyzed routinely by the Division of Microbiology and Chemistry, NCTR, was provided *ad libitum*. The 5K96 meal underwent routine analyses as well as periodic analyses for isoflavone levels. Feeders were gently agitated daily with a vibrating tool (Dremel, Racine, WI) to prevent caking and were changed once per week. Cages were changed weekly. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix H.

In-life Examinations and Pathology

The data collected during the in-life phase of the study and at necropsy are detailed in Table 2. Animals were observed twice daily and clinical findings were recorded weekly. Animals in the F_1 generation were weighed weekly until postnatal week 21, then approximately every 4 weeks for the remainder of the study and at termination of the study. Animals in the F_3 generation were weighed weekly until postnatal week 15, then approximately every 4 weeks for the remainder of the study and at termination of the study. Feed consumption was recorded every 4 weeks after weaning for the F_1 and F_3 generations. Weekly body weight and feed consumption data were also monitored for the parental generation (F_0) for the F_1 animals in order to assess ingested doses during pregnancy and lactation (Table 1). Body weight and feed consumption data for the ancestral generations of the F_3T21 animals were collected as part of the multigenerational reproductive toxicology study and are reported elsewhere (NTP, 2010).

One half of the females in each exposure group were subjected to vaginal smears for 5 consecutive days once per month. These smears were then evaluated for stage of the estrous cycle. If there was evidence that the animals were not cycling normally (i.e., 3 consecutive days of estrus, 4 consecutive days of diestrus) for 2 consecutive months, the animal was considered to have begun to show aberrant cycles during the first month in which abnormal cycling was observed.

Complete necropsies and microscopic evaluations were performed on all F_1C, F_1T140, and F_3T21 rats. From terminal sacrifice animals, the following organs were weighed prior to fixation: adrenal gland, brain, epididymis, kidney (left and right), liver, ovaries/oviducts, seminal vesicle with coagulating gland, spleen, testis (left and right), thymus, and uterus. The following organs were weighed after fixation: dorsal, lateral, and ventral prostate gland (lobes were separated after fixation), pituitary gland, and thyroid gland. All organs and tissues were examined for grossly visible lesions and lesion descriptions were recorded on the Individual Animal Necropsy Record. All major tissues except the testis were fixed and preserved in 10% neutral buffered formalin, processed and trimmed, embedded in Tissue Prep II, sectioned to a thickness of 4 to 6 µm, and stained with hematoxylin and eosin for microscopic examination. The testis was similarly treated, except that it was fixed in Bouin's fixative and stained with periodic acid-Schiff stain to better aid in the characterization of sperm maturation. When applicable, nonneoplastic lesions were graded for severity as 1 (minimal), 2 (mild), 3 (moderate), or 4 (marked). All tissues examined microscopically are listed in Table 2.

Microscopic evaluations were completed by two NCTR pathologists, one for males and one for females. Pathology data were entered into NCTR's Micropath Data Management System. The slides, paraffin blocks, and residual wet tissues were sent to NCTR's Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit. The slides, individual animal data records, pathology tables, and study pathologists' narrative were evaluated by an independent quality assessment group. The individual animal records were compared for accuracy, the slide and tissue counts were verified, and the histotechnique was evaluated. Quality assessment pathologists evaluated all lesions diagnosed by the study pathologists.

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 chairperson to the PWG for review. The slides reviewed by the PWG included the uterus of female rats and the mammary gland, liver, kidney, and oral mucosa of male and female rats. 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 pathologists, 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).

Table 2
Experimental Design and Materials and Methods in the 2-Year Feed Study of Ethinyl Estradiol

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

Strain and Species
Sprague-Dawley/CD23/NCTR BR rats

Animal Source
National Center for Toxicological Research (Jefferson, AR)

Acclimation Time
3 weeks: F_0 animals were allocated to the study at weaning and placed on a soy- and alfalfa-free meal diet (Purina 5K96).

Average Age When Exposure Began
F_0: 6 weeks (PND 42)
F_1 and F_3: 0 weeks (on study from conception)

Date of First Exposure (Conception Date for F_1 and F_3)
F_0: September 19, 2000 (for F_3 ancestors)
F_1: December 10, 2000
F_3: May 13, 2001

Duration of Exposure
F_0: From PND 42 until F_1 weaning (77 days)
F_1 (F_1C): From conception to 2 years (775 days)
F_1 (F_1T140): From conception to PND 140 (161 days), then fed control diet to 2 years
F_3 (F_3T21): From conception to PND 21 (42 days), then fed control diet to 2 years

Date of Last Exposure
F_1C: January 24, 2003
F_1T140: May 26, 2001
F_3T21: June 13, 2001

Average Age at Necropsy
F_1: 111 weeks
F_3: 113 weeks

Date of Necropsy
F_1: January 24, 2003
F_3: July 13, 2003

Size of Study Groups
35 males and 35 females (F_0 generation), 50 males (except F_3 2 ppb = 49) and 50 females (F_1 and F_3 exposed groups); control groups had 50, 51, or 52 animals (some control groups included one or two sentinels that died early)

Method of Distribution
F_0 animals were allocated to exposure groups by a stratified randomization procedure to give groups of approximately the same initial mean body weight; litters of subsequent generations were randomly culled to eight pups on PND 2. At weaning, 50 male and 50 female F_1 pups were allocated to the control groups to serve as controls for both the F_1C and F_1T140 study arms and 100 male and 100 female F_1 pups were allocated to each exposed group. All available litters were represented as equally as possible, with no more than two animals of the same sex from a single litter.

For the F_3 animals, which were exposed to dosed feed only through weaning, 50 male and 50 female pups per exposure group were allocated from the litters produced in the previous multigenerational reproductive toxicology study (NTP, 2010). All available litters were represented as equally as possible and no more than two animals of the same sex from a single litter were used.

Table 2
Experimental Design and Materials and Methods in the 2-Year Feed Study of Ethinyl Estradiol

Animals per Cage
F_0 animals were held two per cage from weaning until allocation to exposure groups, then single housed. F_1 and F_3 animals were single housed from the time of weaning.

Method of Animal Identification
Tail tattoo; newborns were identified by paw tattoo until tail tattoo identification at weaning.

Diet
Rodent chow 5K96 (Test Diets, Purina Mills, Inc., Richmond, IN), available *ad libitum* until the day before sacrifice

Water
Millipore-filtered tap water (Jefferson, AR municipal supply) via water bottles, available *ad libitum*

Cages
Polycarbonate cages (Allentown Caging Equipment Co., Allentown, NJ), changed weekly

Bedding
Hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly

Cage Bonnets
Microisolator tops (Lab Products, Inc., Maywood, NJ)

Racks
Metal animal cage racks (Allentown Caging Equipment Co., Allentown, NJ), changed every 28 days

Animal Room Environment
Temperature: 23° ± 3° C
Relative humidity: 50% ± 20%
Room fluorescent light: 12 hours/day
Room air changes: at least 10/hour

Exposure Concentrations
0, 2, 10, or 50 ppb in feed, available *ad libitum*

Type and Frequency of Observation
Observed twice daily and clinical findings were recorded weekly. Animals in the F_1 generation were weighed weekly until postnatal week 21, then approximately every 4 weeks for the remainder of the study and at termination of the study. Animals in the F_3 generation were weighed weekly until postnatal week 15, then approximately every 4 weeks for the remainder of the study and at termination of the study. Feed consumption was recorded every 4 weeks after weaning for the F_1 and F_3 generations.

Weekly body weight and feed consumption data were also monitored for the parental generation (F_0) for the F_1 animals in order to assess ingested doses during pregnancy and lactation. Body weight and feed consumption data for the ancestral generations of the F_3T21 animals were collected as part of the previous multigenerational reproductive toxicology study (NTP, 2010).

Method of Sacrifice
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all F_1C, F_1T140, and F_3T21 animals. From terminal sacrifice animals, the following organs were weighed prior to fixation: adrenal gland, brain, epididymis, kidney (left and right), liver, ovaries/oviducts, seminal vesicle with coagulating gland, spleen, testis (left and right), thymus, and uterus. The following organs were weighed after fixation: dorsal, lateral, and ventral prostate gland (lobes were separated after fixation); pituitary gland; and thyroid gland.

Table 2
Experimental Design and Materials and Methods in the 2-Year Feed Study of Ethinyl Estradiol

Histopathology
Complete histopathology was performed on all rats. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone marrow (femur and sternum), brain (cerebellum, cerebrum, stem), clitoral gland, coagulating gland, epididymis, esophagus, eye, harderian gland, heart with aorta, 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 (dorsal and ventral lobes), salivary gland, seminal vesicle, skin, spleen, stomach (forestomach and glandular), testis, thymus, thyroid gland, trachea, urinary bladder, uterus, vagina, and Zymbal's gland.

Onset of Aberrant Estrous Cycles
Starting at 5 months of age, vaginal smears were obtained from 25 females in each exposure group to evaluate the stage of the estrous cycle for 5 consecutive days every 4 weeks until they were determined not to be cycling for 2 consecutive months.

Statistical Methods

Survival Analyses and Time of Onset of Aberrant Estrous Cycles

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented graphically. The data were analyzed within each of the three arms of the study using a log-rank test for homogeneity and Tarone's test (Tarone, 1975) for overall trend. For pairwise comparisons, Tarone's and the log-rank test are equivalent, but Tarone's was preferred because the trend direction may be noted. P values reported are right sided unless the trend was negative. In this case, the trend was left sided and an "N" was appended to the P value.

Vaginal cytology data collected to evaluate whether exposure to ethinyl estradiol affected the time that female rats began to show aberrant cycles prior to reproductive senescence were analyzed by an accelerated failure time model. The data for this endpoint contained all three classical types of censoring, that is left, right, and interval censoring. Left censoring occurred because some animals had begun to show aberrant cycles prior to the time that observations were begun at 5 months of age. Right censoring occurred because some animals died or reached the end of the study without showing evidence of aberrant cycles. Finally, the intermittent nature of the data collection (one 5-day period every month) made it impossible to determine the exact time when aberrant cycles began, so the data exhibited interval censoring. An accelerated failure time Kaplan-Meier model that accommodated all three types of censoring was used to analyze these data. A generalized gamma model (Weibull, 1951) was used as the distributional model.

Analysis of Continuous Variables

A mixed models approach to repeated measures ANOVA was used to analyze body weights and feed consumption. Testing for linear and quadratic exposure concentration trends was conducted at each time interval. Because of the spacing of the exposure concentrations, a standard linear trend analysis would cause the 0, 2, and 10 ppb groups to basically be averaged, or treated as a single point, and compared to the 50 ppb group. For this reason, trend analyses for many endpoints were also conducted using a natural log-transformed dose scale [ln(dose + 1)] that resulted in a more evenly weighted scale (0, 1.1, 2.4, and 3.9). Organ weights, terminal body weights, and the ratios of organ weight to terminal body weight for terminally sacrificed animals were analyzed using ANOVA procedures. Terminal body weights were also used as a covariate in an ANCOVA procedure for organ weight analyses. For each endpoint analyzed, Dunnett's two-sided test (Dunnett, 1955) was used to compare the control group mean to each exposed group mean, either overall or at each point of time, whichever was appropriate. Results of one-way tests of exposure concentration effects within each of the three arms of the study are reported.

The separate F_0 generations used to generate the F_1 and F_3 animals used in this 2-year study were derived from breeders in the NCTR colony. The breeders used to produce the F_0 generations did cross breed, that is, sires were mated with multiple dams to produce litters from which the F_0 animals were derived. If a litter or family line effect was causing differences between exposure groups, then isolating and measuring the family line

variation and removing it would increase confidence in significant exposure effects. Thus, variations associated with the parents of the F_0 animals were incorporated as random effects into the covariance structure of the model when any of these effects were significant via a log-likelihood ratio test at an α of 0.50 and their inclusion was computationally feasible. The high α value of 0.50 was selected to guard against Type II error. In this case, Type II error occurs when one falsely assumes no random effect. It was deemed to be a more serious error to incorrectly assume no random "litter" effect was present than to incorrectly assume a random "litter" effect was present. Therefore, α was chosen to be high in order to err on the side of inclusion of the effect rather than exclusion. Nesting of original sires and dams that produced the F_0 generation within exposure groups could not be done because there were instances of progeny in more than one exposure group arising from the same original sire or dam.

Statistical Analysis of Histopathology Data

Analyses of the incidences of neoplastic lesions were conducted separately for each of the three arms of the study (F_1C, F_1T140, and F_3T21).

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1a,b,c, A3a,b,c, B1a,b,c, and B3a,b,c 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 A2a,b,c and B2a,b,c) and all nonneoplastic lesions are given as the numbers of animals affected at each site examined microscopically. Tables A2a,b,c and B2a,b,c 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.

The Poly-k test (Bailer and Portier, 1988; Portier and Bailer, 1989; Piegorsch and Bailer, 1997) was used to assess neoplasm 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). 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 B6C3F_1 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 exposed group with controls and tests for overall exposure concentration-related trends. Because the exposure concentrations within this study were logarithmic, two P values are given for the overall linear exposure concentration trend test. The first is for the exposure concentrations as given, while the second uses roughly logarithmic coded doses (0, 1, 2, 3), which treat the exposure concentrations as evenly spaced. 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).

Statistical analysis of the following nonneoplastic lesions are presented in this report: alveolar hyperplasia and ductal hyperplasia of the male mammary gland; atypical focus and galactocele of the female mammary gland; endometrium hyperplasia, squamous metaplasia, and atypical hyperplasia of the uterus; clitoral gland hyperplasia; preputial gland atrophy; and basophilic focus and eosinophilic focus of the liver. The analysis presented is a Jonckheere-Terpstra test (Jonckheere,

1954) for increasing trend coupled with Shirley's test (Shirley, 1977) for pairwise comparisons. This method presumes a monotonic dose-response relationship and allows both incidence and severity information to be used.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. Only two control groups are included in the present study: a single control group for both the F_1C and F_1T140 arms of the study and a separate control group for the F_3T21 arm. 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. There are sparse directly relevant historical data from 2-year studies on the NCTR CD rat (males only, receiving NIH-31 diet *ad libitum* or with three levels of dietary restriction, or AIN-93M diet *ad libitum* or with one level of dietary restriction evaluated at approximately 1 and 2 years of age: Duffy *et al.,* 2001, 2002, 2004). Control data from a study of identical design (genistein administered in 5K96 diet to NCTR CD rats) are also available (NTP, 2008a). Historical data on spontaneous neoplastic lesions in Sprague-Dawley rats of various origins have been published (Chandra *et al.,* 1992; McMartin *et al.,* 1992; Pettersen *et al.,* 1996; Kaspareit and Rittinghausen, 1999; Giknis and Clifford, 2004; Tennekes *et al.,* 2004; Baldrick, 2005; Brix *et al.,* 2005), although these data are of limited utility given the differences in genetic background, diet, and other study conditions.

Quality Assurance Methods

This study was conducted in compliance with Food and Drug Administration Good Laboratory Practice Regulations (21 CFR, Part 58). The Quality Assurance Unit of the NCTR performed audits and inspections of protocols, procedures, data, and reports throughout the course of the study. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, final pathology tables, and a draft of this NTP Technical Report were conducted. Audit procedures and findings are presented in the reports and are on file at the NCTR. The audit findings were reviewed and assessed by NCTR staff, and all comments were resolved or otherwise addressed during the preparation of this Technical Report.

RESULTS

RATS

Survival

Survival data for males and females are shown in Tables 3 and 4. Kaplan-Meier survival curves for males and females under the three exposure regimens are shown in Figures 2 and 3. The mean percentage of animals that survived to terminal sacrifice was 63% for males (range 55% to 70%) and 46% for females (range 32% to 58%). The only statistically significant effect (P=0.016) was a lower rate of survival relative to controls in the F_1T140 2 ppb females (32% versus 51%), though this was deemed a chance observation rather than a true treatment effect.

TABLE 3
Survival of Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
F_1C[a]				
Animals initially in study	51	50	50	50
Moribund	9	13	17	14
Natural deaths	8	6	3	4
Animals surviving to study termination	34	31	30	32
Percent survival at end of study	67	62	60	64
Survival analysis[b]	P=0.449N/0.407	P=0.294	P=0.264	P=0.395
F_1T140[a]				
Animals initially in study	51	50	50	50
Moribund	9	15	7	14
Natural deaths	8	4	10	3
Animals surviving to study termination	34	31	33	33
Percent survival at end of study	67	62	66	66
Survival analysis	P=0.395N/0.433N	P=0.326	P=0.481	P=0.486N
F_3T21				
Animals initially in study	50	49	50	50
Moribund	13	16	13	12
Natural deaths	7	6	6	3
Animals surviving to study termination	30	27	31	35
Percent survival at end of study	60	55	62	70
Survival analysis	P=0.079N/0.105N	P=0.353	P=0.386N	P=0.134N

[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] The results of life table trend tests (Tarone, 1975) are in the control column (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). The results of the pairwise comparisons (Tarone, 1975) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

Table 4
Survival of Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
F_1C[a]				
Animals initially in study	51	50	50	50
Moribund	25	22	28	17
Natural deaths	0	5	3	8
Animals surviving to study termination	26	23	19	25
Percent survival at end of study	51	46	38	50
Survival analysis[b]	P=0.370N/0.358	P=0.252N	P=0.245	P=0.343
F_1T140[a]				
Animals initially in study	51	50	50	50
Moribund	25	27	27	22
Natural deaths	0	7	1	6
Animals surviving to study termination	26	16	22	22
Percent survival at end of study	51	32	44	44
Survival analysis	P=0.375N/0.419	P=0.016	P=0.232	P=0.257
F_3T21				
Animals initially in study	52	50	50	50
Moribund	24	19	24	23
Natural deaths	1	2	5	3
Animals surviving to study termination	27	29	21	24
Percent survival at end of study	52	58	42	48
Survival analysis	P=0.253/0.206	P=0.252N	P=0.245	P=0.343

[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] The results of life table trend tests (Tarone, 1975) are in the control column (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). The results of the pairwise comparisons (Tarone, 1975) with the controls are in the exposed group columns. A negative trend or lower mortality in an exposed group is indicated by **N**.

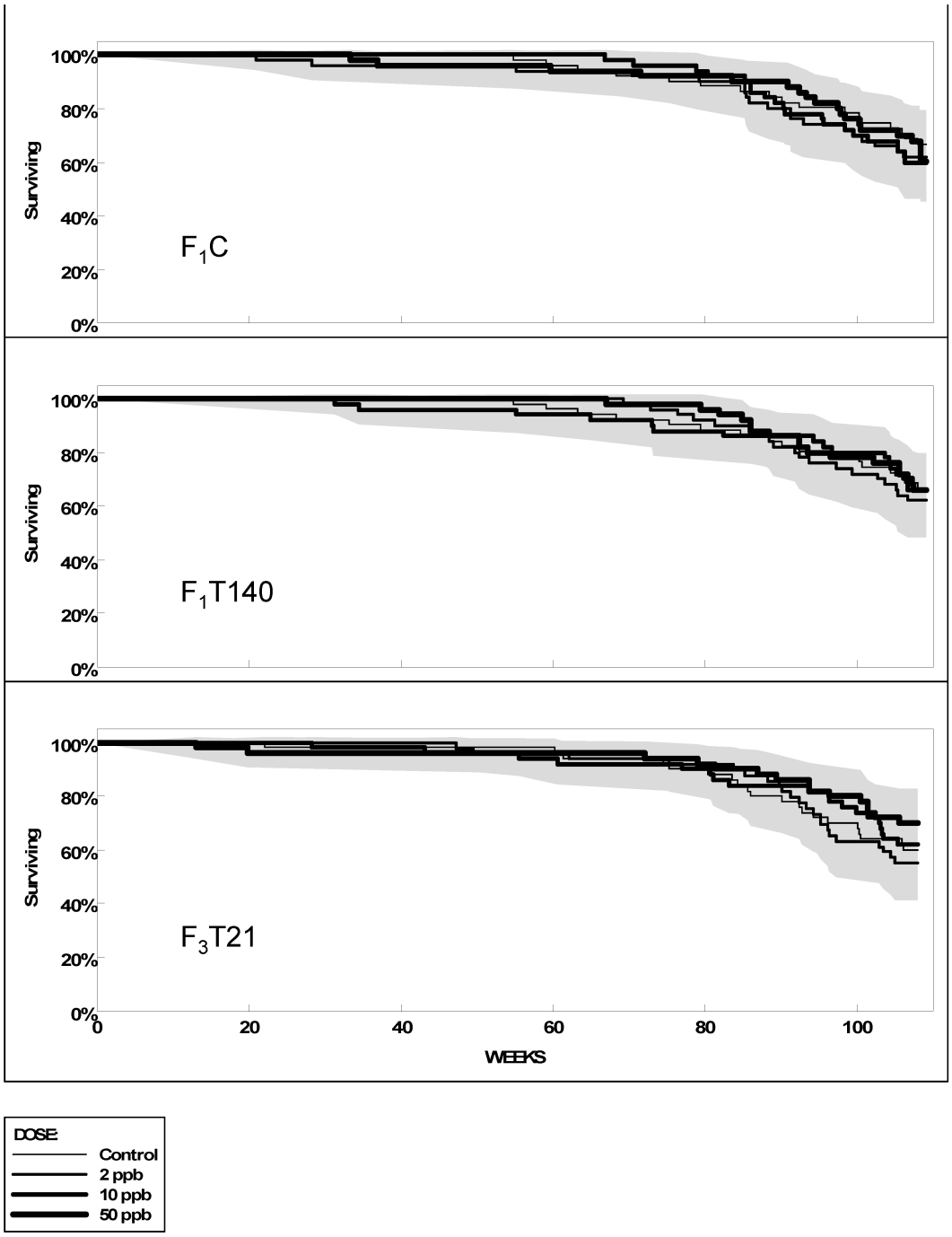


FIGURE 2
Kaplan-Meier Survival Curves for Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

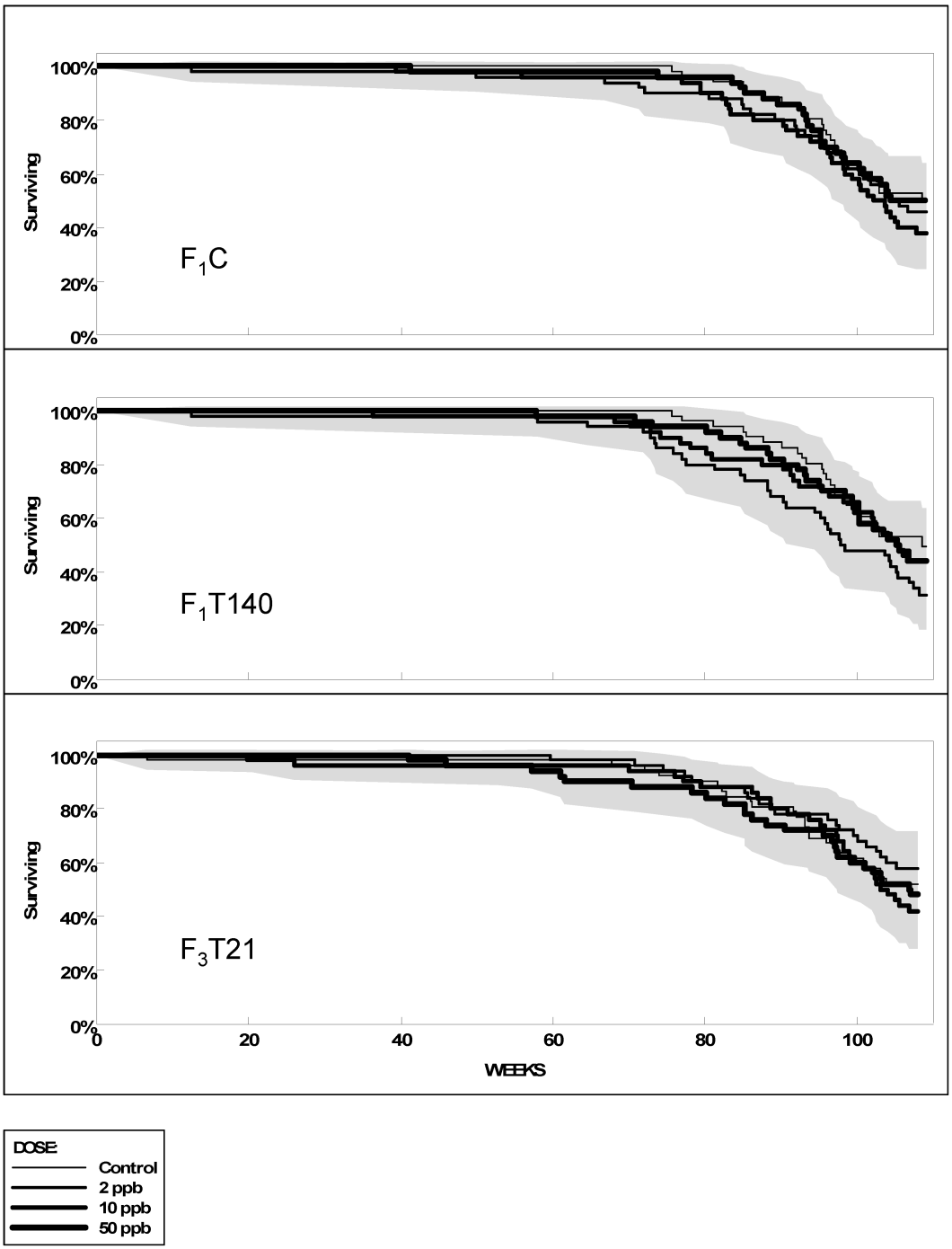


FIGURE 3
Kaplan-Meier Survival Curves for Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Body Weights, Feed Consumption, and Clinical Findings

Mean body weights of male and female rats in the F_1C, F_1T140, and F_3T21 treatment arms of the study are shown in Figures 4, 5, and 6 and Tables D1 through D6. Mean body weights of the 2 and 50 ppb F_1C males and all exposed groups of F_1C females were significantly less than those of the controls throughout the study. Mean body weights of the 2 and 50 ppb F_1T140 males were less than those of the controls early in the study while the mean body weights of all exposed groups of F_1T140 females were less than those of the controls throughout the study. Mean body weights of the exposed groups of F_3T21 rats were generally similar to those of the controls throughout the study.

Other than significantly decreased feed consumption in F_1C rats late in the study, ethinyl estradiol did not show any consistent effects on feed consumption in this study (Appendix E). Analysis of the metabolic efficiency (ratio of body weight gain to food consumed in a given time period) data in the present study indicated a clear significant reduction in metabolic efficiency for both sexes in F_1C rats, with females affected at 10 and 50 ppb and males at 50 ppb. There was an attenuated effect in F_1T140 rats and little to no effect in F_3T21 rats, consistent with the effects of treatment on body weight. While there were cases of inflammation of the foot in both sexes that sometimes became severe enough to require early removal from the study, these were not related to exposure. Foot inflammation is included in the incidences of skin inflammation in Tables A3a, A3b, A3c, B3a, B3b, and B3c. There were no exposure-related clinical findings.

Onset of Aberrant Estrous Cycles

Statistical analyses of estrous cycle data are found in Appendix F. There were no statistically significant effects of exposure to ethinyl estradiol on the time to onset of aberrant estrous cycles in any arm of the study.

Organ Weights

The absolute and relative weights of the seminal vesicle with coagulating gland were significantly increased in 10 and 50 ppb F_3T21 males (Table G5). In females, the relative uterus weight was significantly increased in the 50 ppb F_1C group; the relative ovary weight was also increased in this group, but the increase was not statistically significant (Table G2). The absolute spleen weight of 2 and 10 ppb F_3T21 females and the relative spleen weight of 2 ppb F_3T21 females were significantly increased (Table G6).

Most organ weight differences were associated with the decreased body weights, and only the increased uterus weight was associated with an adverse histopathologic effect.

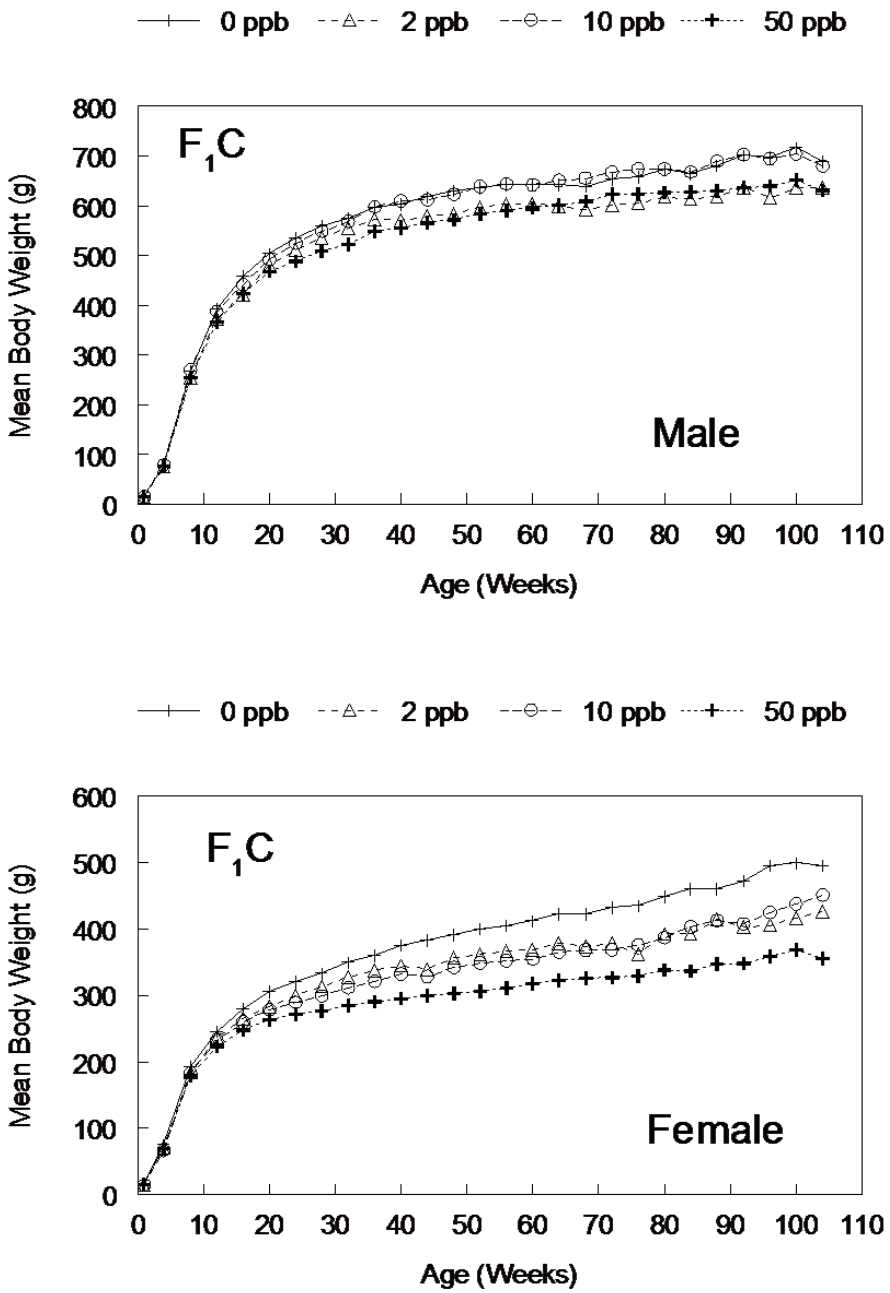


FIGURE 4
Growth Curves for F_1C Rats in the 2-Year Feed Study of Ethinyl Estradiol

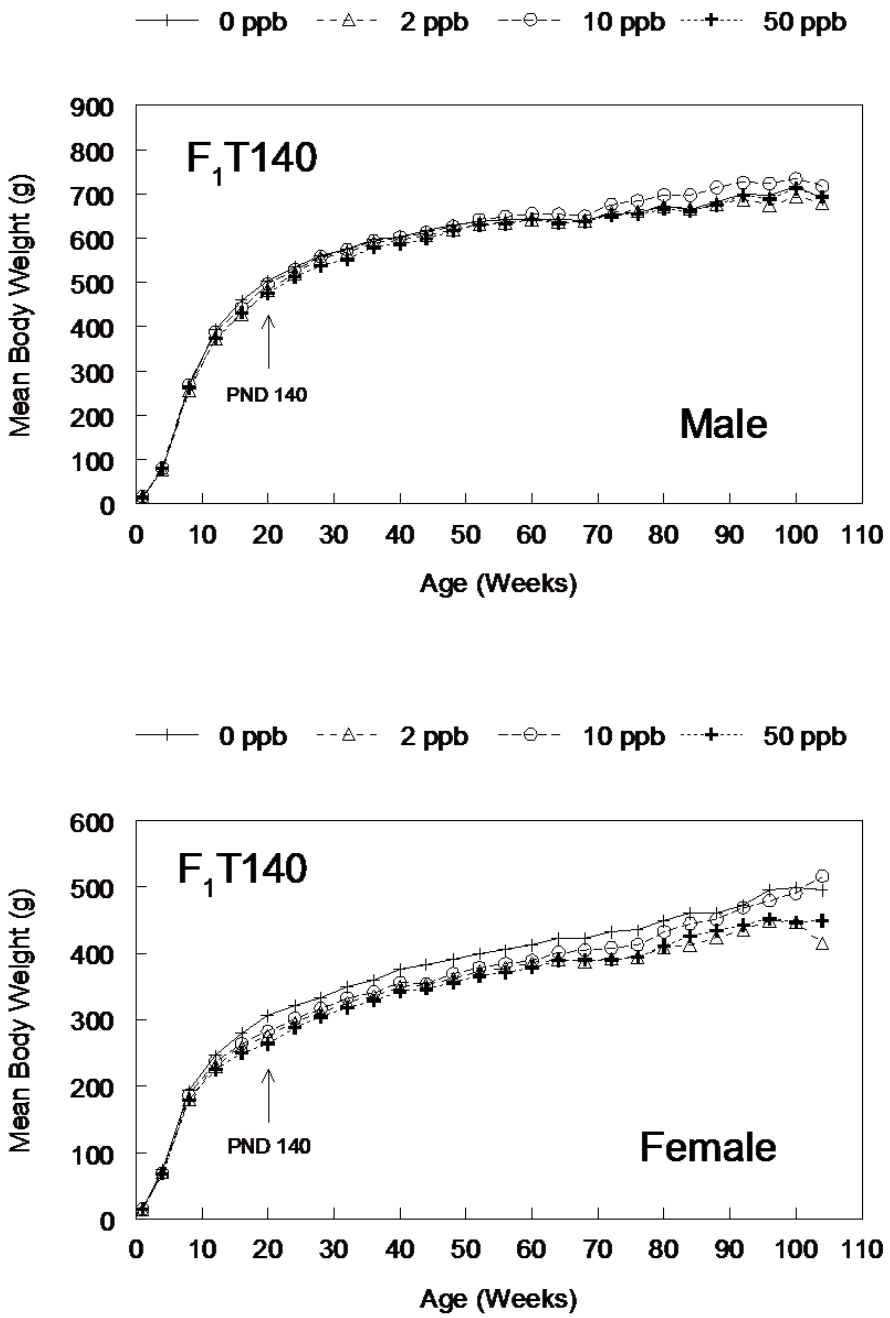


FIGURE 5
Growth Curves for F_1T140 Rats in the 2-Year Feed Study of Ethinyl Estradiol

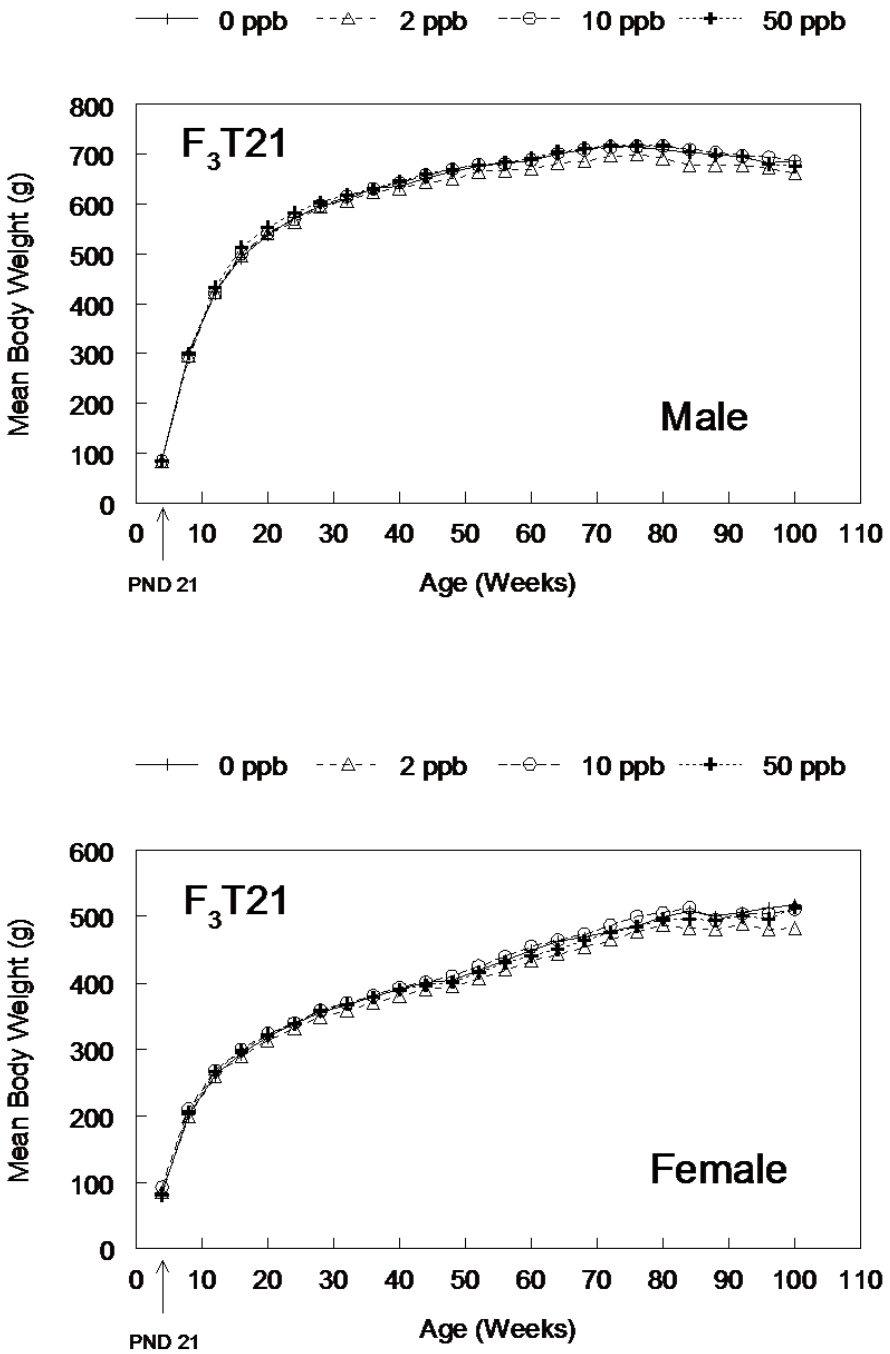


Figure 6
Growth Curves for F_3T21 Rats in the 2-Year Feed Study of Ethinyl Estradiol

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of neoplasms and/or nonneoplastic lesions of the uterus, mammary gland, liver, clitoral gland, preputial gland, kidney, and spleen. Summaries of the incidences of neoplasms and nonneoplastic lesions and statistical analyses of primary neoplasms that occurred with an incidence of at least 5% in at least one animal group are presented in Appendix A for male rats and Appendix B for female rats.

Uterus: Uterine stromal polyps were more prevalent in exposed groups, but the only statistically significant increased incidence occurred in the 2 ppb F_3T21 group (Tables 5 and B2c). There was also a marginally positive coded dose trend (P=0.052) in the F_1T140 groups (Tables 5 and B2b). The background incidences of uterine stromal polyps in the control groups of this study were lower than the incidence found in the 2-year feed study of genistein (9/107 in two control groups; NTP, 2008a). There was a decrease in the combined incidence of adenoma and adenocarcinomas in F_3T21 uteri (Table B2c). There were four adenocarcinomas in the F_3T21 control group as compared to 0 in F_1 controls for this study and in the two control groups for the 2-year feed study of genistein (NTP, 2008a).

In females, the uterus showed the most evident exposure-related nonneoplastic effects (Tables 5, B3a, B3b, and B3c). The nonneoplastic lesions included endometrial hyperplasia, atypical focal hyperplasia, and squamous metaplasia. There was an increased incidence of endometrial hyperplasia in the 50 ppb F_1C group. There were exposure concentration-related increased incidences of atypical focal hyperplasia and squamous metaplasia in all exposed groups of F_1C females. There were increased incidences of atypical hyperplasia in all exposed groups of F_3T21 females and an increased incidence of squamous metaplasia in the 50 ppb F_3T21 group.

Endometrial hyperplasia was characterized by proliferation of endometrial cells. These cells were increased in number and were situated either in the submucosa or were piled up on the surface of the uterine lumen. No cyst formation was present. In atypical focal hyperplasia, foci of affected glands were lined by several layers of hyperplastic cells characterized by increased size and increased basophilic staining intensity. Nuclei of the hyperplastic cells were usually larger than normal and somewhat pleomorphic although a few nuclei were small and pyknotic. Cytoplasm of hyperplastic cells was often vacuolated. Squamous metaplasia was characterized by the proliferation of squamous cells in endometrial glands. There appeared to be a morphological continuum between the atypical focal hyperplasia and squamous metaplasia.

Table 5
Incidences of Neoplasms and Nonneoplastic Lesions of the Uterus in Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
F_1C[a]				
Number Examined Microscopically	51	50	49	50
Endometrium, Hyperplasia[b,c]	17 (1.9)[d]	18 (2.0)	22 (2.0)	25 (2.2)*
Hyperplasia, Atypical, Focal[c]	6 (1.0)	14 (1.4)*	16 (1.4)**	20 (1.6)***
Metaplasia, Squamous[c]	2 (1.0)	6 (2.0)	8 (1.4)*	13 (1.6)***
Stromal Polyp				
Overall rate[e]	2/51 (3.9%)	4/50 (8.0%)	5/49 (10.2%)	5/50 (10.0%)
Adjusted rate[f]	2/42.4 (4.7%)	4/39.8 (10.1%)	5/38.7 (12.9%)	5/42.3 (11.8%)
Terminal rate[g]	2/26 (7.7%)	3/23 (13.0%)	2/19 (10.5%)	0/25 (0.0%)
First incidence (days)	756 (T)	563	687	626
Poly-3 test[h]	P=0.321/0.143	P=0.306	P=0.178	P=0.212
F_1T140[a]				
Number Examined Microscopically	51	50	50	50
Endometrium, Hyperplasia	17 (1.9)	24 (1.9)	14 (1.9)	24 (2.1)
Hyperplasia, Atypical, Focal	6 (1.0)	10 (1.3)	7 (1.6)	9 (1.7)
Metaplasia, Squamous	2 (1.0)	6 (1.5)	6 (1.0)	1 (1.0)
Stromal Polyp				
Overall rate	2/51 (3.9%)	5/50 (10.0%)	6/50 (12.0%)	7/50 (14.0%)
Adjusted rate	2/42.4 (4.7%)	5/36.6 (13.7%)	6/40.0 (15.0%)	7/41.2 (17.0%)
Terminal rate	2/26 (7.7%)	2/16 (12.5%)	3/22 (13.6%)	4/22 (18.2%)
First incidence (days)	756 (T)	618	641	561
Poly-3 test	P=0.152/0.052	P=0.158	P=0.112	P=0.069
F_3T21				
Number Examined Microscopically	52	50	50	50
Endometrium, Hyperplasia	20 (2.3)	13 (2.1)	16 (2.2)	22 (2.4)
Hyperplasia, Atypical, Focal[c]	6 (1.7)	16 (1.4)**	15 (1.6)*	21 (1.5)***
Metaplasia, Squamous[c]	1 (2.0)	4 (1.5)	3 (1.7)	11 (1.7)***
Stromal Polyp				
Overall rate	1/52 (1.9%)	7/50 (14.0%)	2/50 (4.0%)	5/50 (10.0%)
Adjusted rate	1/41.8 (2.4%)	7/41.3 (16.9%)	2/39.1 (5.1%)	5/39.5 (12.7%)
Terminal rate	0/27 (0.0%)	7/29 (24.1%)	2/21 (9.5%)	2/24 (8.3%)
First incidence (days)	548	747 (T)	749 (T)	561
Poly-3 test	P=0.315/0.202	P=0.027	P=0.476	P=0.087

(T) Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** ($P \leq 0.01$)
***($P \leq 0.001$)
[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] Number of animals with lesion
[c] Significant trend ($P \leq 0.05$) by the Jonckheere-Terpstra test
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Number of animals with neoplasm per number of animals with uterus examined microscopically
[f] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the control group incidence are P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed group. The Poly-3 test accounts for the differential mortality in animals that do not reach terminal sacrifice.

Mammary gland: There was a significant positive trend in the incidences of mammary gland adenoma or adenocarcinoma (combined) in the F_3T21 males, but no increased incidences of these neoplasms in F_1C or F_1T140 males where the incidences of mammary gland hyperplasia were significantly increased (Tables 6, A1a, A1b, A1c, and A2c). The control incidences in the current study are similar to the control incidences (0/41 and 0/39, respectively, in the two control groups) observed in a feed study of genistein conducted under conditions identical to the current study (NTP, 2008a). In males, there were also increased incidences of mammary gland alveolar and ductal hyperplasia (Tables 6, A3a, A3b, and A3c). In F_1C and F_1T140 males, there were significantly increased incidences of alveolar hyperplasia of the mammary gland in the 10 and 50 ppb groups. In F_3T21 males, the incidence of mammary gland alveolar hyperplasia was significantly increased in the 50 ppb group.

The incidences of mammary gland adenocarcinoma occurred with marginally increased trends in F_1T140 (dose, P=0.096) and F_3T21 (coded dose, P=0.080) females; however, there were no statistically significant differences between exposed and control groups (Tables 6, B2a, B2b, and B2c). Incidences of adenocarcinoma in the control groups were similar to those previously reported (15/107 in two control groups) under identical experimental conditions in the 2-year feed study of genistein (NTP, 2008a). Fibroadenomas in the female mammary gland generally were not affected by ethinyl estradiol treatment, although there was a positive coded dose trend in F_3T21 females, which was likely due to an elevated incidence in the 10 ppb group (Table 6). The incidence of fibroadenoma in control female rats was similar to the incidence found in the 2-year feed study of genistein (64/107 in two control groups; NTP, 2008a).

Nonneoplastic lesions in the mammary gland of female rats that showed possible treatment effects included atypical focus and galactocele (Tables 6, B3a, B3b, and B3c). For atypical focus, all exposure concentrations showed significant differences from control in the F_3T21 females, but not in the F_1C or F_1T140 females. A possible contributor to this was the relatively low background incidence of atypical foci in F_3T21 control animals (23.1% versus 37.3% in the F_1C/F_1T140 control group). Atypical focus was characterized as focal hyperplasia of ductal or alveolar epithelium with cellular atypia. Atypical hyperplasia of ductal epithelium was characterized by infolding and/or stratification of the epithelium. Cells were often enlarged and had hyperchromatic nuclei and intensely staining basophilic cytoplasm. Atypical hyperplasia of alveolar epithelium consisted of foci of alveoli filled with cells or dilated alveoli in which the epithelium was stratified into several layers. Cells were larger than normal and had hyperchromatic nuclei and intensely stained eosinophilic or basophilic cytoplasm. There was a significant positive trend (P=0.04) for the incidence of galactocele in F_1C females and a marginal positive trend (P=0.08) in F_3T21 females. Galactocele is a large dilated mammary gland cyst lined by flattened epithelium and filled with mammary secretion. There was no evidence for treatment-related mammary gland alveolar hyperplasia in females.

Liver: There were treatment-related increased incidences of basophilic focus and eosinophilic focus in 50 ppb F_1C and F_1T140 males (Tables 7 and A3a). There was also an increased incidence of basophilic focus in 10 ppb F_1T140 males, but the increase is of questionable toxicological significance. In females, there was an increased incidence of eosinophilic focus in the 50 ppb F_1C group and an increased incidence of basophilic focus in the 50 ppb F_1T140 group (Tables 7, B3a, and B3b).

Clitoral gland: In F_1C females, there was a marginal positive trend for combined epithelial neoplasms (adenoma, carcinoma, squamous cell papilloma, or squamous cell carcinoma) in the clitoral gland (Tables 8 and B2a). The background incidences of combined epithelial neoplasms in the control groups of this study were slightly lower than the incidences found in the 2-year feed study of genistein (3/100 in two control groups; NTP, 2008a). There were significantly increased incidences of clitoral gland hyperplasia (combined glandular acinar cell or ductal squamous cell) in the 50 ppb groups of F_1T140 and F_3T21 females (Tables 8, B3b, and B3c). There were significant positive trends in the incidences of hyperplasia in these study arms. While there was some evidence of a treatment-related increase in the incidences of clitoral gland hyperplasia, there was no evidence of a treatment effect on the incidences of epithelial neoplasms in F_1T140 and F_3T21 females.

Table 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Male				
F_1C[a]				
Number Examined Microscopically	44	45	47	44
Alveolar Hyperplasia[b,c]	1 (1.0)[d]	4 (1.8)	6 (1.8)*	18 (2.2)***
Ductal Hyperplasia[c]	0	0	2 (1.0)	3 (1.0)*
Alveolar or Ductal Hyperplasia[c]	1 (1.0)	4 (1.8)	8 (1.6)**	21 (2.0)***
Adenocarcinoma	1	0	0	0
F_1T140[a]				
Number Examined Microscopically	44	45	47	48
Alveolar Hyperplasia[c]	1 (1.0)	2 (2.0)	6 (2.0)*	14 (1.9)***
Ductal Hyperplasia[c]	0	0	1 (2.0)	3 (2.0)*
Alveolar or Ductal Hyperplasia[c]	1 (1.0)	2 (2.0)	7 (2.0)*	17 (1.9)***
Adenoma	0	1	1	2
Adenocarcinoma	1	0	0	0
Adenoma or Adenocarcinoma				
Overall rate[e]	1/44 (2.3%)	1/45 (2.2%)	1/47 (2.1%)	2/48 (4.2%)
Adjusted rate[f]	1/39.5 (2.5%)	1/39.4 (2.5%)	1/42.2 (2.4%)	2/42.8 (4.7%)
Terminal rate[g]	1/34 (2.9%)	0/31 (0.0%)	0/33 (0.0%)	2/33 (6.1%)
First incidence (days)	761 (T)	656	746	757 (T)
Poly-3 test[h]	P=0.401/0.374	P=0.760	P=0.745N	P=0.528
F_3T21				
Number Examined Microscopically	42	42	40	45
Alveolar Hyperplasia[c]	2 (2.0)	6 (2.3)	4 (1.3)	9 (1.7)*
Ductal Hyperplasia	0	0	0	1 (2.0)
Alveolar or Ductal Hyperplasia[c]	2 (2.0)	6 (2.3)	4 (1.3)	10 (1.7)*
Adenoma	0	0	0	1
Adenocarcinoma	0	0	0	2
Adenoma or Adenocarcinoma				
Overall rate	0/42 (0.0%)	0/42 (0.0%)	0/40 (0.0%)	3/45 (6.7%)
Adjusted rate	0/37.3 (0.0%)	0/35.7 (0.0%)	0/37.3 (0.0%)	3/41.6 (7.2%)
Terminal rate	0/30 (0.0%)	0/27 (0.0%)	0/31 (0.0%)	1/35 (2.9%)
First incidence (days)	—[i]	—	—	553
Poly-3 test	P=0.011/0.021	—[j]	—	P=0.138

Table 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Female				
F_1C[a]				
Number Examined Microscopically	51	49	49	50
Atypical Focus	19 (1.5)	23 (1.8)	21 (1.3)	23 (1.6)
Galactocele[c]	2	2	4	6
Adenocarcinoma				
Overall rate	8/51 (15.7%)	10/49 (20.4%)	9/49 (18.4%)	9/50 (18.0%)
Adjusted rate	8/43.3 (18.5%)	10/40.9 (24.5%)	9/40.0 (22.5%)	9/42.4 (21.2%)
Terminal rate	4/26 (15.4%)	6/23 (26.1%)	3/19 (15.8%)	5/25 (20.0%)
First incidence (days)	675	468	538	584
Poly-3 test	P=0.567N/0.440	P=0.341	P=0.427	P=0.479
Fibroadenoma				
Overall rate	32/51 (62.7%)	28/49 (57.1%)	33/49 (67.3%)	27/50 (54.0%)
Adjusted rate	32/48.3 (66.2%)	28/42.5 (65.9%)	33/43.5 (75.9%)	27/44.2 (61.1%)
Terminal rate	14/26 (53.8%)	15/23 (65.2%)	15/19 (78.9%)	15/25 (60.0%)
First incidence (days)	529	602	555	597
Poly-3 test	P=0.260N/0.468N	P=0.576N	P=0.204	P=0.380N
F_1T140[a]				
Number Examined Microscopically	51	49	50	50
Atypical Focus	19 (1.5)	13 (2.0)	19 (1.7)	18 (1.4)
Galactocele	2	3	4	3
Adenocarcinoma				
Overall rate	8/51 (15.7%)	3/49 (6.1%)	8/50 (16.0%)	11/50 (22.0%)
Adjusted rate	8/43.3 (18.5%)	3/35.5 (8.5%)	8/41.1 (19.5%)	11/42.5 (25.9%)
Terminal rate	4/26 (15.4%)	1/16 (6.3%)	3/22 (13.6%)	3/22 (13.6%)
First incidence (days)	675	661	477	495
Poly-3 test	P=0.096/0.139	P=0.171N	P=0.564	P=0.283
Fibroadenoma				
Overall rate	32/51 (62.7%)	26/49 (53.1%)	28/50 (56.0%)	34/50 (68.0%)
Adjusted rate	32/48.3 (66.2%)	26/41.5 (62.6%)	28/43.3 (64.6%)	34/46.4 (73.3%)
Terminal rate	14/26 (53.8%)	8/16 (50.0%)	15/22 (68.2%)	16/22 (72.7%)
First incidence (days)	529	452	510	511
Poly-3 test	P=0.171/0.239	P=0.445N	P=0.526N	P=0.291

Table 6
Incidences of Neoplasms and Nonneoplastic Lesions of the Mammary Gland in Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Female (continued)				
F_3T21				
Number Examined Microscopically	52	50	50	50
Atypical Focus	12 (1.8)	21 (1.7)*	22 (1.7)*	18 (1.9)*
Galactocele	2	1	1	5
Adenocarcinoma				
Overall rate	6/52 (11.5%)	6/50 (12.0%)	9/50 (18.0%)	10/50 (20.0%)
Adjusted rate	6/42.5 (14.1%)	6/42.1 (14.3%)	9/39.8 (22.6%)	10/40.3 (24.8%)
Terminal rate	2/27 (7.4%)	4/29 (13.8%)	6/21 (28.6%)	3/24 (12.5%)
First incidence (days)	641	599	680	578
Poly-3 test	P=0.131/0.080	P=0.614	P=0.237	P=0.168
Fibroadenoma				
Overall rate	36/52 (69.2%)	34/50 (68.0%)	40/50 (80.0%)	37/50 (74.0%)
Adjusted rate	36/48.2 (74.6%)	34/46.4 (73.3%)	40/46.4 (86.2%)	37/43.5 (85.0%)
Terminal rate	17/27 (63.0%)	19/29 (65.5%)	17/21 (81.0%)	21/24 (87.5%)
First incidence (days)	504	541	490	427
Poly-3 test	P=0.122/0.048	P=0.536N	P=0.112	P=0.152

(T) Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** ($P \leq 0.01$)
***($P \leq 0.001$)
[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] Number of animals with lesion
[c] Significant trend ($P \leq 0.05$) by the Jonckheere-Terpstra test
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Number of animals with neoplasm per number of animals with mammary gland examined microscopically
[f] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the control group incidence are P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[i] Not applicable; no neoplasms in animal group
[j] Value of statistic cannot be computed.

Table 7
Incidences of Nonneoplastic Lesions of the Liver in Male and Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Male				
F_1C[a]				
Number Examined Microscopically	49	49	50	49
Basophilic Focus[b,c]	1	3	3	17***
Eosinophilic Focus[c]	3	5	8	15***
F_1T140[a]				
Number Examined Microscopically	49	50	48	49
Basophilic Focus[c]	1	3	11***	6**
Eosinophilic Focus	3	11[d]	5	10*
F_3T21				
Number Examined Microscopically	49	47	50	49
Basophilic Focus	9	7	5	7
Eosinophilic Focus	8	5	4	5
Female				
F_1C[a]				
Number Examined Microscopically	51	50	49	50
Basophilic Focus	1	1	1	1
Eosinophilic Focus[c]	1	1	1	5*
F_1T140[a]				
Number Examined Microscopically	51	50	50	50
Basophilic Focus[c]	1	1	1	6**
Eosinophilic Focus	1	2	5[d]	4
F_3T21				
Number Examined Microscopically	52	50	50	50
Basophilic Focus	1	3	0	2
Eosinophilic Focus	2	3	2	2

* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** ($P \leq 0.01$)
***($P \leq 0.001$)
[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] Number of animals with lesion
[c] Significant trend ($P \leq 0.05$) by the Jonckheere-Terpstra test
[d] Significant ($P \leq 0.05$) by the Shirley/Williams test but monotonicity fails

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Clitoral Gland in Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
F_1C[a]				
Number Examined Microscopically	50	49	48	50
Hyperplasia[b]	2 (3.0)[c]	3 (2.0)	4 (2.0)	0
Adenoma	0	0	0	2
Carcinoma	0	1	0	1
Squamous Cell Papilloma	1	0	0	0
Squamous Cell Carcinoma	0	1	0	1
Epithelial Neoplasms				
Overall rate[d]	1/50 (2.0%)	2/49 (4.1%)	0/48 (0.0%)	4/50 (8.0%)
Adjusted rate[e]	1/41.6 (2.4%)	2/39.1 (5.1%)	0/37.0 (0.0%)	4/41.9 (9.5%)
Terminal rate[f]	1/26 (3.8%)	2/23 (8.7%)	0/19 (0.0%)	1/25 (4.0%)
First incidence (days)	757 (T)	757 (T)	—[h]	653
Poly-3 test[g]	P=0.083/0.142	P=0.478	P=0.523N	P=0.179
F_1T140[a]				
Number Examined Microscopically	50	50	49	49
Hyperplasia[i]	2 (3.0)	1 (1.0)	2 (2.0)	8 (2.3)**
Adenoma	0	0	0	0
Carcinoma	0	1	0	0
Squamous Cell Papilloma	1	0	0	0
Squamous Cell Carcinoma	0	0	0	0
Epithelial Neoplasms				
Overall rate	1/50 (2.0%)	1/50 (2.0%)	0/49 (0.0%)	0/49 (0.0%)
Adjusted rate	1/41.6 (2.4%)	1/36.0 (2.8%)	0/38.4 (0.0%)	0/39.1 (0.0%)
Terminal rate	1/26 (3.8%)	0/16 (0.0%)	0/22 (0.0%)	0/21 (0.0%)
First incidence (days)	757 (T)	632	—	—
Poly-3 test	P=0.378N/0.189N	P=0.728	P=0.516N	P=0.512N
F_3T21				
Number Examined Microscopically	50	50	49	48
Hyperplasia[i]	0	1 (2.0)	2 (1.5)	3 (2.0)*
Adenoma	0	1	1	2
Carcinoma	0	0	0	0
Squamous Cell Papilloma	0	0	0	0
Squamous Cell Carcinoma	0	0	0	0
Epithelial Neoplasms				
Overall rate	0/50 (0.0%)	1/50 (2.0%)	1/49 (2.0%)	2/48 (4.2%)
Adjusted rate	0/40.2 (0.0%)	1/41.3 (2.4%)	1/38.9 (2.6%)	2/37.0 (5.4%)
Terminal rate	0/27 (0.0%)	1/29 (3.4%)	0/21 (0.0%)	0/24 (0.0%)
First incidence (days)	—	749 (T)	666	682
Poly-3 test	P=0.203/0.117	P=0.505	P=0.493	P=0.218

Table 8
Incidences of Neoplasms and Nonneoplastic Lesions of the Clitoral Gland in Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

(T) Terminal sacrifice
* Significantly different (P≤0.05) from the control group by Shirley's test.
** (P≤0.01)
[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] Number of animals with lesion
[c] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[d] Number of animals with neoplasm per number of animals with clitoral gland examined microscopically
[e] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[f] Observed incidence at terminal kill
[g] Beneath the control group incidence are P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[h] Not applicable; no neoplasms in animal group
[i] Significant trend (P≤0.05) by the Jonckheere-Terpstra test

Preputial gland: In F_3T21 males, there were significant positive dose (P=0.046) and coded dose (P=0.038) trends of combined epithelial neoplasms (squamous cell carcinoma, adenoma, or carcinoma), and a marginally increased incidence of these combined neoplasms in the 50 ppb group (Tables 9 and A3c). The majority (44/47) of preputial gland neoplasms observed in all arms of this study were squamous cell carcinomas originating from the squamous epithelium lining the ducts. The background incidences of preputial gland epithelial neoplasms in the control groups of this study were greater than the incidences found in the 2-year feed study of genistein (1/97 in two control groups; NTP, 2008a). There were increased incidences of preputial gland atrophy in the 10 and 50 ppb F_1C groups and all exposed F_1T140 groups (Tables 9, A3a, and A3b).

Kidney: Incidences of relatively uncommon renal neoplasms of various morphologies were observed in males and females, but there was no clear dose-response relationship and no statistically significant effect of ethinyl estradiol on renal neoplasms under the conditions of this study (Tables A1a, A1b, A1c, A2a, A2b, A2c, B1a, B1b, B1c, B2a, B2b, and B2c). In males the incidences did not show a positive trend as it relates to exposure or treatment regimen. There appeared to be increases in the combined incidences of renal tubule adenoma, renal tubule carcinoma, and nephroblastoma in the exposed groups of F_1C (0 ppb, 0/51; 2 ppb, 2/50; 10 ppb, 0/49; 50 ppb, 2/50; Table B1a) and F_1T140 (0/51, 1/50, 1/50, 0/50; Table B1b) females. In the 2-year feed study of genistein conducted under conditions identical to those of the current study, renal tubule adenoma occurred in 1/94 control males and was the only kidney neoplasm observed in male rats (NTP, 2008a). In the same 2-year feed study of genistein, renal tubule adenoma occurred in 1/107 control females and transitional epithelium papilloma occurred in 1/107 control females (NTP, 2008a). Similar incidences of kidney neoplasms in control male Sprague-Dawley rats from the NCTR colony have been reported by Duffy *et al*. (2004).

In 50 ppb males, there were significantly increased incidences of renal tubule mineralization in the F_1C (0 ppb, 0/46; 2 ppb, 0/49; 10 ppb, 1/48; 50 ppb, 4/49; Table A3a) and F_1T140 (0/46, 1/49, 0/47, 4/49; Table A3b) arms with mean severities of 1.5 and 1.3, respectively.

Spleen: The incidence of spleen pigmentation (hemosiderin) in 50 ppb F_1C females was significantly increased compared to the control group incidence (33/51, 31/50, 34/49, 43/50; Table B3a). Pigmentation is the result of red blood cell destruction, which may have been moderately increased by ethinyl estradiol exposure.

Table 9
Incidences of Neoplasms and Nonneoplastic Lesions of the Preputial Gland in Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
F_1C[a]				
Number Examined Microscopically	51	50	50	50
Atrophy[b,c]	0	2 (2.0)[d]	5 (1.8)**	4 (2.5)*
Adenoma	0	0	0	0
Carcinoma	0	0	0	0
Squamous Cell Carcinoma	4	5	5	2
Epithelial Neoplasms				
Overall rate[e]	4/51 (7.8%)	5/50 (10.0%)	5/50 (10.0%)	2/50 (4.0%)
Adjusted rate[f]	4/43.9 (9.1%)	5/41.9 (11.9%)	5/43.2 (11.6%)	2/43.0 (4.7%)
Terminal rate[g]	2/34 (5.9%)	1/31 (3.2%)	2/30 (6.7%)	2/32 (6.3%)
First incidence (days)	704	596	602	757 (T)
Poly-3 test[h]	P=0.190N/0.287N	P=0.470	P=0.489	P=0.347N
F_1T140[a]				
Number Examined Microscopically	51	50	50	50
Atrophy[c]	0	6 (1.7)**	3 (2.7)*	6 (2.8)*
Adenoma	0	1	0	0
Carcinoma	0	0	0	0
Squamous Cell Carcinoma	4	4	3	1
Epithelial Neoplasms				
Overall rate	4/51 (7.8%)	5/50 (10.0%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	4/43.9 (9.1%)	5/43.1 (11.6%)	3/43.2 (6.9%)	1/44.0 (2.3%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	1/33 (3.0%)	1/33 (3.0%)
First incidence (days)	704	642	677	763 (T)
Poly-3 test	P=0.093N/0.098N	P=0.488	P=0.509N	P=0.177N
F_3T21				
Number Examined Microscopically	49	49	50	49
Atrophy	1 (3.0)	4 (2.5)	3 (2.7)	2 (2.0)
Adenoma	0	0	0	0
Carcinoma	0	1	0	1
Squamous Cell Carcinoma	2	3	4	7
Epithelial Neoplasms				
Overall rate	2/49 (4.1%)	4/49 (8.2%)	4/50 (8.0%)	8/49 (16.3%)
Adjusted rate	2/41.3 (4.8%)	4/40.2 (9.9%)	4/42.7 (9.4%)	8/44.7 (17.9%)
Terminal rate	0/30 (0.0%)	1/27 (3.7%)	1/31 (3.2%)	2/35 (5.7%)
First incidence (days)	601	638	539	625
Poly-3 test	P=0.046/0.038	P=0.324	P=0.351	P=0.058

(T) Terminal sacrifice
* Significantly different ($P \leq 0.05$) from the control group by Shirley's test.
** ($P \leq 0.01$)
[a] A single group of animals served as the control group for the F_1C and F_1T140 study arms.
[b] Number of animals with lesion
[c] Significant trend ($P \leq 0.05$) by the Jonckheere-Terpstra test
[d] Average severity grade of lesions in affected animals: 1=minimal, 2=mild, 3=moderate, 4=marked
[e] Number of animals with neoplasm per number of animals with preputial gland examined microscopically
[f] Poly-3 estimated lesion incidence after adjustment for intercurrent mortality
[g] Observed incidence at terminal kill
[h] Beneath the control group incidence are P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.

DISCUSSION AND CONCLUSIONS

Ethinyl estradiol is a potent synthetic estrogen commonly used in pharmaceuticals because of its improved oral bioavailability over 17β-estradiol. The present study evaluated the chronic effects of ethinyl estradiol administered in a low phytoestrogen diet to male and female Sprague-Dawley rats. The use of the low phytoestrogen diet and relatively low doses of ethinyl estradiol are unique features of this 2-year study. Data from the short-term reproductive dose range-finding study with ethinyl estradiol (NTP, 2010) were used to select dietary exposure concentrations of 0, 2, 10, and 50 ppb for this study. The higher exposure concentrations tested in the reproductive dose range-finding study (100 and 200 ppb) were ruled out for use in the multigenerational reproductive toxicology study (NTP, 2010) due to effects on body weights and the female and male reproductive tracts.

During pregnancy in the current study, the ingested doses of the dams were approximately 0, 0.2, 0.9, or 5.8 µg ethinyl estradiol/kg body weight per day. During lactation, the dams' ingested doses were 0, 0.3, 2.0, or 10.3 µg/kg per day. The mean directly ingested ethinyl estradiol doses during the period prior to postnatal day (PND) 140 were approximately 0.2, 0.9, or 4.9 µg/kg per day for females and 0.2, 0.8, or 4.5 µg/kg per day for males. For the period between PND 140 and the end of the study, mean ingested doses were approximately 0.1, 0.6, or 3.3 µg/kg per day for females and 0.1, 0.4, or 2.1 µg/kg per day for males. Under these dosing conditions, serum levels in young adult animals exposed to the highest concentration of 50 ppb were reported to be below the limit of detection (10 pg/mL) of a sensitive liquid chromatography-mass spectrometry method (Twaddle *et al.*, 2003). This result is consistent with the low oral bioavailability of ethinyl estradiol in rats relative to humans due largely to higher first pass metabolism in rats (Dusterberg *et al.*, 1986). For example, in contrast to the low serum levels of ethinyl estradiol in the rats in the current study, van den Heuvel *et al.* (2005) reported maximum and average serum concentrations of 168 and 43.5 pg/mL, respectively, over a 21-day observation period in women taking a combined oral contraceptive containing 30 µg ethinyl estradiol (approximately 0.44 µg/kg body weight per day based on the average weight of 67.4 kg for women in the study). In addition to direct consumption of ethinyl estradiol by the animals, there is presumed transplacental and lactational exposure (Figure 1). There are limited quantitative data available on the transplacental and lactational exposure of fetuses or neonates to ethinyl estradiol administered to the mother. Slikker *et al.* (1982) demonstrated the transfer of intact ethinyl estradiol to the circulation of the fetus after intravenous administration to pregnant rhesus monkeys. In addition, multiple reports of measurable biological effects of ethinyl estradiol in pups following administration of ethinyl estradiol to pregnant rodents are consistent with transplacental transfer of the compound (NTP, 2010). Studies conducted in humans suggest that the extent of transfer of ethinyl estradiol to the newborn via milk is very limited (Nilsson *et al.*, 1978; Betrabet *et al.*, 1986). An early study that followed the appearance of radiolabeled ethinyl estradiol in nursing pups for 48 hours following administration of the compound by gavage to their dams reported less than 0.1% of the total dose in the bodies of pups at each of the 0 to 4, 0 to 24, and 24 to 48 hour time intervals examined (Cargill *et al.*, 1969). Despite the low exposure concentrations used in the current study, a number of treatment-related effects were observed that should be of interest in evaluating less potent agents with estrogenic activity.

Three exposure arms were examined in the current study: continuous exposure from conception through 2 years (designated F_1C), exposure from conception through 20 weeks (PND 140) followed by control diet to 2 years (designated F_1T140), and exposure from conception through weaning at PND 21 followed by control diet to 2 years (designated F_3T21). Survival was similar in exposed and control groups within each sex in this study and in all arms of the study. Two-year survival rates (mean of 63% with a range of 55% to 70% for males and 46% with a range of 32% to 58% for females) were somewhat lower than those in the genistein 2-year feed study that was conducted under identical conditions (means of 72% and 54% for males and females,

respectively; NTP, 2008a). The survival rate of males in the current study was similar to the results of Duffy *et al*. (2001, 2002), who previously reported that the survival rates of male NCTR CD rats consuming NIH-31 or AIN-93M diets *ad libitum* were 63% and 45%, respectively, after 104 weeks on study (age 110 weeks). For comparison, a recent compilation of approximately 30 studies using the CRL:CD (SD) rat (Giknis and Clifford, 2004) reported mean 2-year survival rates of 39% for males (range 17% to 63%) and 37% for females (range 20% to 62%). Thus, the survival rates for both sexes in the current study were higher than the averages that have been reported for 2-year studies with Sprague-Dawley rats fed *ad libitum*.

The mean body weights of the control animals in the current study were also less than those typically achieved with other stocks of Sprague-Dawley rats fed *ad libitum*. For the male NCTR Sprague Dawley rat, Duffy *et al*. (2001, 2002) reported mean terminal body weights of 657 g and 747 g in 2-year studies with NIH-31 and AIN-93M diets, respectively, fed *ad libitum*. In the current study, mean terminal body weights for the control males consuming the 5K96 diet were 683 g and 670 g for the F_1 and F_3 animals, respectively. Mean terminal body weights of control females were 482 g (F_1C/F_1T140) and 497 g (F_3T21) in the present study. These control mean terminal body weights are approximately 18% to 19% higher for both sexes than those observed in the 2-year study of genistein (average terminal body weights of 572 g and 411 g for control males and control females, respectively; NTP, 2008a).

Ethinyl estradiol had clear effects on body weight in both sexes, with a more pronounced effect in continuously exposed females (F_1C). In F_3T21 rats that were removed from exposure at weaning, ethinyl estradiol did not have an effect on body weight, indicating that at these exposure concentrations there was not a developmentally programmed effect on body weight gain. Estrogens are known to have anorectic effects and to modulate energy utilization (Wade and Schneider, 1992). Under the conditions of the present study, reduction in feed consumption did not appear to account for the lower body weights in exposure groups, but a reduction in metabolic efficiency was associated with the observed reductions in body weight gain.

In the multigenerational reproductive toxicology study conducted in conjunction with this chronic bioassay (NTP, 2010), males continuously exposed from conception to PND 140 and necropsied at that time showed increased incidences of mammary gland hyperplasia even at the lowest exposure concentration (2 ppb). Males exposed only as adults or those that had exposure stopped at weaning showed increased incidences of mammary hyperplasia at the 50 ppb exposure concentration, indicating the contribution of both developmental and continuous exposure to the effect. Similar observations on the sensitivity of the male mammary gland to genistein have previously been reported using the same test system used in the current study. Furthermore, You *et al.* (2002) have reported on the sensitivity of the male mammary gland to genistein and methoxychlor. In the current 2-study with ethinyl estradiol, alveolar hyperplasia of the male mammary gland was evident, with significant effects in the 10 and 50 ppb groups of F_1C and F_1T140 and the 50 ppb F_3T21 group. There was no evidence of neoplasms in the mammary gland of F_1C or F_1T140 males, but there was a significant positive trend for adenoma or adenocarcinoma (combined) in F_3T21 males, with three of 45 animals affected in the 50 ppb group and incidences in the other groups. Given that the combined incidences of these neoplasms in the four control groups in the 2-year feed study of genistein (NTP, 2008a) and in the present study was 1/166, this was considered an equivocal effect. The evidence does not strongly support the progression of the hyperplasia to neoplasia given that no neoplasms appeared in the groups where the hyperplastic effect was most prominent. Although the stimulation of hyperplasia of the male mammary gland was stronger with ethinyl estradiol than with genistein, supporting observations on the lack of progression of hyperplasia to neoplasia in the male mammary gland were made in the genistein chronic study (NTP, 2008a). Schardein (1980) reported an increased incidence of fibroepithelial tumors in male rats fed 1.5 ppm ethinyl estradiol (approximately 60 µg/kg body weight per day) for 105 weeks, but there was no increased incidence of fibroadenoma in males under the conditions of the current study. Nonetheless, the body of evidence from the multigenerational reproductive toxicology studies of genistein and of ethinyl estradiol (NTP, 2008b, 2010) together with other studies on genistein and methoxychlor (Delclos *et al.*, 2001; You *et al*. 2002; Wang *et al.*, 2006) and estradiol (Biegel *et al*., 1998) indicate the high sensitivity of the male mammary gland to estrogenic stimulation and lends strong support to the inclusion of the evaluation of the male mammary gland in endocrine disruptor evaluations.

In females, the mammary gland did not show clear treatment effects, although there were marginal positive

trends for adenocarcinoma in the F_1T140 and F_3T21 groups and for fibroadenoma in the F_3T21 groups. In the genistein study, increased incidences of mammary gland adenoma or adenocarcinoma (combined) were noted as treatment effects in females, while there was a strong reduction in the incidence of fibroadenoma in continuously exposed (F_1C) high exposure concentration females that was suggested to be related to the decreased body weight in that group (NTP, 2008a). In the current study, 50 ppb ethinyl estradiol had a marked effect on body weight, but mammary gland neoplasms were not affected, indicating that factors in addition to body weight reduction may be involved in the effect of genistein. Another clear difference between the genistein and ethinyl estradiol studies was the acceleration of the onset of aberrant cycles, a potential marker of early reproductive senescence, in all arms of the genistein study (NTP, 2008a); an effect not observed with ethinyl estradiol. The differential preferences of these compounds for estrogen receptors α and β (Barkhem *et al*., 1998; Casanova *et al*., 1999; Gutendorf and Westendorf, 2001) may be a significant factor in these discrepant effects.

The uterus showed the most marked effects in females under the conditions of the current study. Increased incidences of nonneoplastic lesions (hyperplasia and metaplasia) were observed in F_1C and F_3T21 females. The strong response in the F_3T21 animals indicates either that early exposure is sufficient to induce these effects or that the multigenerational exposure of the F_3 animals magnified the effect. Reasons for the lack of significant nonneoplastic uterine effects in F_1T140 females are not clear, but this could suggest that the multigenerational exposure contributed to the response in the F_3T21 females. Marginal increased incidences of uterine stromal polyps were observed in F_1T140 and F_3T21 females, with a statistically significant increased incidence in the 2 ppb F_3T21 females. Schardein (1980) also reported an increase in the incidence of uterine stromal polyps in female rats fed 0.15 ppm ethinyl estradiol, although there was no increase at a 10-fold higher dose in that same study.

Both males and females showed increased incidences of altered hepatocellular foci in the F_1C and F_1T140 arms of the study, although there were no increased incidences of liver neoplasms. The liver is a known estrogen target organ, and Schardein (1980) reported increased incidences of altered hepatocellular foci in both sexes at 0.15 and 1.5 ppm ethinyl estradiol and an increase in neoplastic nodules at 1.5 ppm at 105 weeks. Schardein (1980) also reported atrophy of the male reproductive tract (both doses), ovarian atrophy (high dose), and increased incidences of pituitary gland adenoma (high dose) in a 2-year feed study of ethinyl estradiol, but these effects were not observed under the very different conditions, in terms of exposure concentrations, diets, and test animals of the current study.

The preputial and clitoral glands in males and females, respectively, showed effects of exposure to ethinyl estradiol, with the more pronounced effects in males. Increased incidences of combined epithelial neoplasms, primarily ductal squamous cell carcinomas, were observed in F_3T21 males. Increased incidences of atrophy of the preputial gland were seen in F_1C and F_1T140 males. The apparent dose response in the F_3T21 males and the relatively high incidence (8/49) compared to the overall background incidence (7/197) in the four control groups from this study and the 2-year genistein study (NTP, 2008a), which was conducted under identical conditions with regard to diet and rat strain, suggest that this is an exposure-related response. On the other hand, the lack of increased incidences of these neoplasms in the F_1C and F_1T140 study arms and the high background incidence in the F_1 controls (4/51) weakens the strength of the association of these neoplasms with ethinyl estradiol exposure. The fact that there was a positive trend in the incidences of preputial gland squamous cell carcinomas in the F_3T21 groups (0 ppm, 0/48; 5 ppm, 0/48; 100 ppm, 5/47; 500 ppm, 3/47) but not in the F_1C or F_1T140 males in the genistein study (NTP, 2008a), suggests that multigenerational exposure may be involved in the weak neoplastic response in this gland. The clitoral gland in females showed a weaker response to ethinyl estradiol, perhaps due to the higher background of estrogen. Marginal increased incidences in epithelial neoplasms, primarily glandular acinar cell adenomas or carcinomas, in the F_1C animals and increased incidences of clitoral gland hyperplasia in the 50 ppb F_1T140 and F_3T21 females were observed. These effects indicated that ethinyl estradiol targeted the clitoral gland and supported the observed treatment-related effects in the homologous preputial gland in males.

Finally, in both sexes, relatively rare kidney neoplasms of various origins were noted by the study pathologists and pathology working group primarily in exposed groups. These neoplasms were low in number, the incidences were not statistically significant, and there was no evidence of an exposure concentration response, which makes their relationship to ethinyl estradiol exposure

questionable. In the hamster kidney tumor model of estrogen carcinogenesis, which typically uses higher doses of estrogens than were used in the current study, ethinyl estradiol shows weak activity relative to other estrogens that have lower affinity for the estrogen receptor, and it has been proposed that this results from the low production of catechols from the metabolism of ethinyl estradiol (Zhu *et al.*, 1993; Yager, 2000). The kidney does possess estrogen receptors (Couse *et al.*, 1997; Kuiper *et al.*, 1997; Lovegrove *et al.*, 2004; Wells *et al.*, 2005) and in both the multigenerational reproductive toxicology study (NTP, 2010) and the current study, the only treatment-related nonneoplastic lesion noted was mild tubular mineralization observed in the 50 ppb groups of male rats. Whether there is any biological significance to the kidney neoplasms observed in the current study is unknown and cannot be determined from the available data.

Conclusions

Under the conditions of this 2-year feed study with continuous exposure to the test compound from conception through termination (F_1C), there was *no evidence of carcinogenic activity** of ethinyl estradiol in male or female Sprague-Dawley rats exposed to 2, 10, or 50 ppb. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the uterus and liver of females.

Under the conditions of this 2-year feed study with exposure to the test compound from conception through 20 weeks followed by control feed until termination (F_1T140), there was *no evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats exposed to 2, 10, or 50 ppb. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Nonneoplastic lesions were observed in the mammary gland and liver of males and in the liver and clitoral gland of females.

Under the conditions of this study where offspring of two prior generations of animals exposed to ethinyl estradiol in feed were exposed from conception through weaning (PND 21), followed by control feed through termination (F_3T21), there was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in male Sprague-Dawley rats based on increased incidences of preputial gland epithelial neoplasms and a marginal increased incidence of mammary gland adenoma or adenocarcinoma (combined). A significantly increased incidence of male mammary gland alveolar hyperplasia was also observed. There was *equivocal evidence of carcinogenic activity* of ethinyl estradiol in female Sprague-Dawley rats based on marginally increased incidences of uterine stromal polyps. Increased incidences of squamous metaplasia and atypical hyperplasia in the uterus and hyperplasia in the clitoral gland were also observed.

* Explanation of Levels of Evidence of Carcinogenic Activity is on page 11. A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 13.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE 2-YEAR FEED STUDY OF ETHINYL ESTRADIOL

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Table A1a
Summary of the Incidence of Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	9	13	17	12
Natural deaths	7	6	3	4
Survivors				
Died last week of study	1			2
Terminal sacrifice	34	31	30	32
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(50)	(50)
Intestine large, cecum	(44)	(45)	(47)	(46)
Leukemia granulocytic		1 (2%)		
Lymphoma malignant	1 (2%)			1 (2%)
Intestine large, colon	(45)	(47)	(48)	(47)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant				2 (4%)
Intestine large, rectum	(45)	(45)	(47)	(46)
Intestine small, duodenum	(44)	(45)	(48)	(47)
Adenocarcinoma	1 (2%)			
Intestine small, ileum	(43)	(45)	(47)	(46)
Adenocarcinoma			1 (2%)	
Lymphoma malignant				1 (2%)
Intestine small, jejunum	(43)	(45)	(46)	(46)
Lymphoma malignant				1 (2%)
Sarcoma		1 (2%)		
Liver	(49)	(49)	(50)	(49)
Cholangiocarcinoma			1 (2%)	
Hepatocellular adenoma		3 (6%)	1 (2%)	
Hepatocellular carcinoma				1 (2%)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Mesentery	(1)	(1)	(2)	(1)
Fat, lymphoma malignant	1 (100%)			
Oral mucosa	(3)	(2)	(4)	(9)
Sarcoma		1 (50%)		
Squamous cell carcinoma	3 (100%)		4 (100%)	7 (78%)
Pancreas	(46)	(48)	(48)	(48)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Salivary glands	(48)	(50)	(49)	(50)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			
Stomach, forestomach	(47)	(48)	(49)	(48)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(45)	(47)	(48)	(46)
Tongue	(0)	(0)	(0)	(0)

Table A1a
Summary of the Incidence of Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Cardiovascular System				
Heart	(51)	(50)	(50)	(50)
Leukemia granulocytic		2 (4%)		
Mesothelioma benign			1 (2%)	
Schwannoma benign				1 (2%)
Endocardium, schwannoma malignant	1 (2%)	2 (4%)		
Endocrine System				
Adrenal cortex	(47)	(50)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Carcinoma			2 (4%)	
Leukemia granulocytic		2 (4%)		
Adrenal medulla	(46)	(48)	(49)	(49)
Leukemia granulocytic		1 (2%)		
Pheochromocytoma benign	2 (4%)	2 (4%)	4 (8%)	1 (2%)
Pheochromocytoma complex	1 (2%)	1 (2%)	1 (2%)	
Pheochromocytoma malignant	1 (2%)	1 (2%)	2 (4%)	
Islets, pancreatic	(46)	(48)	(49)	(49)
Adenoma				2 (4%)
Leukemia granulocytic		1 (2%)		
Parathyroid gland	(49)	(46)	(46)	(46)
Adenoma	4 (8%)		1 (2%)	1 (2%)
Leukemia granulocytic		1 (2%)		
Pituitary gland	(48)	(50)	(50)	(50)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Schwannoma malignant	1 (2%)			
Pars distalis, adenoma	25 (52%)	24 (48%)	28 (56%)	16 (32%)
Thyroid gland	(47)	(49)	(50)	(49)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant				1 (2%)
C-cell, adenoma	5 (11%)		1 (2%)	
C-cell, carcinoma		1 (2%)		
Follicular cell, adenoma			1 (2%)	
Follicular cell, carcinoma				1 (2%)
General Body System				
Tissue NOS	(1)	(0)	(1)	(0)
Lipoma			1 (100%)	
Genital System				
Coagulating gland	(46)	(49)	(49)	(49)
Leukemia granulocytic		1 (2%)		
Lymphoma malignant	1 (2%)			
Ductus deferens	(0)	(0)	(0)	(0)
Epididymis	(51)	(50)	(50)	(50)
Leukemia granulocytic		1 (2%)		
Lymphoma malignant	1 (2%)			1 (2%)
Mesothelioma benign			1 (2%)	
Penis	(0)	(1)	(0)	(0)
Preputial gland	(51)	(50)	(50)	(50)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Squamous cell carcinoma	4 (8%)	5 (10%)	5 (10%)	2 (4%)

Table A1a
Summary of the Incidence of Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Genital System (continued)				
Prostate, dorsal/lateral lobe	(49)	(50)	(50)	(50)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Prostate, ventral lobe	(49)	(50)	(50)	(50)
Lymphoma malignant	1 (2%)			1 (2%)
Rete testes	(46)	(44)	(46)	(47)
Seminal vesicle	(43)	(46)	(47)	(47)
Adenoma	1 (2%)			
Carcinoma			1 (2%)	1 (2%)
Leukemia granulocytic		1 (2%)		
Testes	(48)	(50)	(50)	(50)
Lymphoma malignant	1 (2%)			
Seminoma benign				1 (2%)
Hematopoietic System				
Bone marrow	(46)	(48)	(48)	(48)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Lymph node	(14)	(16)	(16)	(15)
Axillary, leukemia granulocytic		1 (6%)		
Axillary, lymphoma malignant	1 (7%)			1 (7%)
Deep cervical, leukemia granulocytic		1 (6%)		
Lumbar, leukemia granulocytic		1 (6%)		
Lumbar, lymphoma malignant				1 (7%)
Mediastinal, lymphoma malignant	1 (7%)			
Pancreatic, leukemia granulocytic		1 (6%)		
Pancreatic, lymphoma malignant				1 (7%)
Renal, leukemia granulocytic		1 (6%)		
Renal, lymphoma malignant	1 (7%)			1 (7%)
Lymph node, mandibular	(49)	(50)	(50)	(48)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Lymph node, mesenteric	(45)	(46)	(49)	(47)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Spleen	(48)	(49)	(49)	(49)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Thymus	(45)	(44)	(46)	(44)
Leukemia granulocytic		2 (5%)		
Lymphoma malignant	1 (2%)			1 (2%)
Sarcoma			1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(44)	(45)	(47)	(44)
Adenocarcinoma	1 (2%)			
Fibroadenoma	1 (2%)	3 (7%)	1 (2%)	2 (5%)
Fibroma	1 (2%)	3 (7%)		
Leukemia granulocytic		2 (4%)		
Lipoma	1 (2%)			
Lymphoma malignant	1 (2%)			1 (2%)

Table A1a
Summary of the Incidence of Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Integumentary System (continued)				
Skin	(51)	(50)	(50)	(50)
Basal cell adenoma	1 (2%)	1 (2%)	1 (2%)	
Fibroma	2 (4%)		1 (2%)	2 (4%)
Hemangioma			1 (2%)	
Keratoacanthoma	2 (4%)	3 (6%)	1 (2%)	2 (4%)
Lymphoma malignant				1 (2%)
Sarcoma	4 (8%)		1 (2%)	
Schwannoma malignant				1 (2%)
Squamous cell carcinoma		1 (2%)		
Squamous cell papilloma			1 (2%)	
Musculoskeletal System				
Bone	(2)	(0)	(0)	(1)
Cranium, osteosarcoma	1 (50%)			
Cranium, schwannoma malignant, metastatic, uncertain primary site	1 (50%)			
Bone, femur	(51)	(50)	(50)	(50)
Leukemia granulocytic		1 (2%)		
Nervous System				
Brain, brain stem	(49)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)			
Leukemia granulocytic		1 (2%)		
Lymphoma malignant				1 (2%)
Brain, cerebellum	(49)	(50)	(50)	(50)
Brain, cerebrum	(49)	(50)	(50)	(50)
Astrocytoma malignant	1 (2%)			
Granular cell tumor benign		1 (2%)		
Granular cell tumor malignant		1 (2%)	1 (2%)	
Lymphoma malignant	1 (2%)			
Respiratory System				
Lung	(46)	(46)	(48)	(48)
Alveolar/bronchiolar adenoma	1 (2%)			
Carcinoma, metastatic, adrenal cortex			1 (2%)	
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Sarcoma, metastatic, thymus				1 (2%)
Squamous cell carcinoma, metastatic, preputial gland		1 (2%)		
Nose	(48)	(49)	(50)	(49)
Adenoma	1 (2%)			
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Osteosarcoma		1 (2%)		
Schwannoma malignant			1 (2%)	
Trachea	(49)	(50)	(50)	(50)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			

Table A1a
Summary of the Incidence of Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Special Senses System				
Eye	(46)	(46)	(47)	(46)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Schwannoma malignant			1 (2%)	
Retrobulbus, squamous cell carcinoma, deep invasion				1 (2%)
Harderian gland	(48)	(47)	(48)	(48)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			1 (2%)
Schwannoma malignant			1 (2%)	
Lacrimal gland	(0)	(0)	(0)	(2)
Lymphoma malignant				1 (50%)
Zymbal's gland	(1)	(0)	(0)	(2)
Carcinoma	1 (100%)			1 (50%)
Squamous cell carcinoma				1 (50%)
Urinary System				
Kidney	(46)	(49)	(48)	(49)
Leukemia granulocytic		2 (4%)		
Lymphoma malignant	1 (2%)			2 (4%)
Mesenchymal tumor malignant				1 (2%)
Bilateral, renal tubule, carcinoma		1 (2%)		1 (2%)
Transitional epithelium, papilloma	1 (2%)			
Urethra	(2)	(2)	(0)	(2)
Urinary bladder	(45)	(48)	(49)	(49)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table A1b
Summary of the Incidence of Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	9	15	7	14
Natural deaths	7	4	10	3
Survivors				
Died last week of study	1			
Terminal sacrifice	34	31	33	33
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(49)	(50)
Intestine large, cecum	(44)	(48)	(43)	(46)
Lymphoma malignant	1 (2%)			
Intestine large, colon	(45)	(49)	(43)	(48)
Intestine large, rectum	(45)	(49)	(42)	(48)
Intestine small, duodenum	(44)	(49)	(42)	(47)
Adenocarcinoma	1 (2%)	2 (4%)		
Intestine small, ileum	(43)	(48)	(41)	(45)
Intestine small, jejunum	(43)	(48)	(41)	(48)
Adenocarcinoma				1 (2%)
Liver	(49)	(50)	(48)	(49)
Cholangiocarcinoma		1 (2%)		1 (2%)
Hepatocellular adenoma				1 (2%)
Hepatocellular carcinoma			1 (2%)	1 (2%)
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Mesentery	(1)	(1)	(2)	(0)
Carcinoma, metastatic, uncertain primary site			1 (50%)	
Fat, lymphoma malignant	1 (100%)			
Oral mucosa	(3)	(5)	(1)	(6)
Squamous cell carcinoma	3 (100%)	3 (60%)		4 (67%)
Pancreas	(46)	(49)	(47)	(50)
Lymphoma malignant	1 (2%)		2 (4%)	
Salivary glands	(48)	(50)	(48)	(49)
Lymphoma malignant	1 (2%)		1 (2%)	
Stomach, forestomach	(47)	(50)	(46)	(49)
Squamous cell papilloma	1 (2%)			
Stomach, glandular	(45)	(50)	(45)	(49)
Adenoma		1 (2%)		
Tongue	(0)	(0)	(0)	(1)
Cardiovascular System				
Heart	(51)	(50)	(48)	(50)
Endocardium, schwannoma malignant	1 (2%)			

Table A1b
Summary of the Incidence of Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System				
Adrenal cortex	(47)	(49)	(45)	(50)
Adenoma	2 (4%)		1 (2%)	1 (2%)
Adrenal medulla	(46)	(49)	(45)	(50)
Pheochromocytoma benign	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Pheochromocytoma complex	1 (2%)			
Pheochromocytoma malignant	1 (2%)			
Bilateral, pheochromocytoma benign		1 (2%)		
Islets, pancreatic	(46)	(49)	(47)	(50)
Adenoma			2 (4%)	
Parathyroid gland	(49)	(50)	(44)	(50)
Adenoma	4 (8%)	3 (6%)	1 (2%)	
Pituitary gland	(48)	(50)	(47)	(49)
Lymphoma malignant	1 (2%)		2 (4%)	
Schwannoma malignant	1 (2%)			
Pars distalis, adenoma	25 (52%)	29 (58%)	28 (60%)	24 (49%)
Thyroid gland	(47)	(50)	(47)	(49)
Lymphoma malignant			1 (2%)	
C-cell, adenoma	5 (11%)	3 (6%)	1 (2%)	1 (2%)
C-cell, carcinoma		2 (4%)		1 (2%)
General Body System				
Tissue NOS	(1)	(1)	(0)	(0)
Schwannoma malignant		1 (100%)		
Genital System				
Coagulating gland	(46)	(49)	(47)	(48)
Carcinoma, metastatic, prostate dorsal/lateral lobe			1 (2%)	
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Lymphoma malignant	1 (2%)			
Ductus deferens	(0)	(0)	(1)	(0)
Epididymis	(51)	(50)	(49)	(50)
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Penis	(0)	(1)	(1)	(0)
Squamous cell carcinoma, metastatic, preputial gland		1 (100%)		
Preputial gland	(51)	(50)	(50)	(50)
Adenoma		1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Squamous cell carcinoma	4 (8%)	4 (8%)	3 (6%)	1 (2%)
Prostate, dorsal/lateral lobe	(49)	(50)	(49)	(50)
Carcinoma			1 (2%)	
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Lymphoma malignant	1 (2%)			
Prostate, ventral lobe	(49)	(50)	(49)	(50)
Carcinoma			1 (2%)	
Lymphoma malignant	1 (2%)			
Rete testes	(46)	(47)	(43)	(46)

Table A1b
Summary of the Incidence of Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Genital System (continued)				
Seminal vesicle	(43)	(49)	(43)	(48)
Adenoma	1 (2%)			
Carcinoma, metastatic, prostate, dorsal/lateral lobe			1 (2%)	
Carcinoma, metastatic, uncertain primary site			1 (2%)	
Testes	(48)	(50)	(48)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
Sarcoma		1 (2%)		
Hematopoietic System				
Bone marrow	(46)	(50)	(46)	(49)
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Lymph node	(14)	(19)	(14)	(19)
Squamous cell carcinoma, metastatic, preputial gland		1 (5%)		
Axillary, lymphoma malignant	1 (7%)		1 (7%)	
Deep cervical, lymphoma malignant			1 (7%)	
Inguinal, lymphoma malignant			1 (7%)	
Lumbar, lymphoma malignant			1 (7%)	
Mediastinal, lymphoma malignant	1 (7%)		1 (7%)	
Renal, lymphoma malignant	1 (7%)		1 (7%)	
Lymph node, mandibular	(49)	(50)	(47)	(50)
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Lymph node, mesenteric	(45)	(50)	(44)	(48)
Lymphoma malignant	1 (2%)	1 (2%)	2 (5%)	
Spleen	(48)	(50)	(47)	(50)
Hemangiosarcoma				1 (2%)
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Thymus	(45)	(46)	(40)	(47)
Lymphoma malignant	1 (2%)		1 (3%)	
Thymoma benign		1 (2%)		
Integumentary System				
Mammary gland	(44)	(45)	(47)	(48)
Adenocarcinoma	1 (2%)			
Adenoma		1 (2%)	1 (2%)	2 (4%)
Fibroadenoma	1 (2%)		1 (2%)	1 (2%)
Fibroma	1 (2%)	1 (2%)		1 (2%)
Lipoma	1 (2%)			
Lymphoma malignant	1 (2%)		1 (2%)	
Skin	(51)	(50)	(49)	(50)
Basal cell adenoma	1 (2%)			2 (4%)
Fibroma	2 (4%)		1 (2%)	
Keratoacanthoma	2 (4%)	1 (2%)	2 (4%)	1 (2%)
Lipoma		1 (2%)		
Lymphoma malignant			1 (2%)	
Osteosarcoma				1 (2%)
Sarcoma	4 (8%)	1 (2%)		
Squamous cell carcinoma			1 (2%)	1 (2%)
Squamous cell papilloma		2 (4%)	1 (2%)	
Prepuce, keratoacanthoma			1 (2%)	
Prepuce, squamous cell carcinoma				1 (2%)
Sebaceous gland, adenoma		1 (2%)		

Table A1b
Summary of the Incidence of Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Musculoskeletal System				
Bone	(2)	(0)	(0)	(1)
Cranium, nerve, squamous cell carcinoma, deep invasion				1 (100%)
Cranium, osteosarcoma	1 (50%)			
Cranium, schwannoma malignant, metastatic, uncertain primary site	1 (50%)			
Bone, femur	(51)	(50)	(50)	(50)
Nervous System				
Brain, brain stem	(49)	(50)	(48)	(50)
Astrocytoma malignant	1 (2%)			1 (2%)
Brain, cerebellum	(49)	(50)	(48)	(50)
Astrocytoma malignant				1 (2%)
Brain, cerebrum	(49)	(50)	(48)	(50)
Astrocytoma malignant	1 (2%)			1 (2%)
Granular cell tumor benign				1 (2%)
Lymphoma malignant	1 (2%)			
Reticulosis malignant		1 (2%)		
Respiratory System				
Lung	(46)	(50)	(45)	(50)
Alveolar/bronchiolar adenoma	1 (2%)			
Alveolar/bronchiolar carcinoma		1 (2%)		
Lymphoma malignant	1 (2%)		2 (4%)	
Nose	(48)	(49)	(48)	(50)
Adenoma	1 (2%)			
Lymphoma malignant	1 (2%)	1 (2%)	2 (4%)	
Trachea	(49)	(50)	(46)	(50)
Lymphoma malignant	1 (2%)			
Special Senses System				
Eye	(46)	(49)	(45)	(48)
Lymphoma malignant	1 (2%)		2 (4%)	
Harderian gland	(48)	(50)	(46)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
Squamous cell carcinoma, deep invasion		1 (2%)		1 (2%)
Lacrimal gland	(0)	(0)	(1)	(1)
Zymbal's gland	(1)	(1)	(0)	(0)
Adenoma		1 (100%)		
Carcinoma	1 (100%)			

Table A1b
Summary of the Incidence of Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Urinary System				
Kidney	(46)	(49)	(47)	(49)
Lymphoma malignant	1 (2%)		2 (4%)	
Mesenchymal tumor malignant		1 (2%)		
Sarcoma				1 (2%)
Bilateral, renal tubule, adenoma		1 (2%)		2 (4%)
Renal tubule, adenoma		2 (4%)		
Renal tubule, carcinoma		1 (2%)		
Transitional epithelium, papilloma	1 (2%)			
Urethra	(2)	(1)	(5)	(1)
Urinary bladder	(45)	(49)	(45)	(49)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table A1c
Summary of the Incidence of Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	50	49	50	50
Early deaths				
Moribund	13	16	13	12
Natural deaths	7	6	6	3
Survivors				
Terminal sacrifice	30	27	31	35
Animals examined microscopically	50	49	50	50
Alimentary System				
Esophagus	(50)	(48)	(50)	(50)
Intestine large, cecum	(45)	(43)	(47)	(47)
Lymphoma malignant			1 (2%)	
Intestine large, colon	(45)	(43)	(48)	(48)
Adenoma	2 (4%)		1 (2%)	
Lymphoma malignant			1 (2%)	
Intestine large, rectum	(45)	(44)	(48)	(48)
Anus, sarcoma			1 (2%)	
Intestine small, duodenum	(44)	(43)	(47)	(47)
Adenocarcinoma	2 (5%)			
Lymphoma malignant	1 (2%)			
Intestine small, ileum	(44)	(38)	(47)	(47)
Intestine small, jejunum	(44)	(41)	(45)	(46)
Liver	(49)	(47)	(50)	(49)
Cholangiocarcinoma	2 (4%)			
Cholangioma	1 (2%)		1 (2%)	
Hepatocellular adenoma	1 (2%)			1 (2%)
Leukemia mononuclear		1 (2%)	1 (2%)	
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Mesentery	(4)	(0)	(6)	(3)
Squamous cell carcinoma, metastatic, uncertain primary stie			1 (17%)	
Oral mucosa	(3)	(8)	(4)	(6)
Squamous cell carcinoma	3 (100%)	4 (50%)	1 (25%)	5 (83%)
Pancreas	(47)	(48)	(49)	(49)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	1 (2%)			
Squamous cell carcinoma, metastatic stomach, forestomach		1 (2%)		
Salivary glands	(48)	(49)	(49)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
Stomach, forestomach	(46)	(48)	(49)	(49)
Squamous cell carcinoma		1 (2%)		
Stomach, glandular	(45)	(46)	(47)	(47)
Cardiovascular System				
Heart	(50)	(49)	(50)	(50)
Leukemia mononuclear		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Endocardium, schwannoma malignant		1 (2%)		

Table A1c
Summary of the Incidence of Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System				
Adrenal cortex	(49)	(49)	(49)	(50)
Adenoma	1 (2%)	1 (2%)	1 (2%)	
Leukemia mononuclear		1 (2%)		
Adrenal medulla	(49)	(48)	(49)	(50)
Pheochromocytoma benign	4 (8%)	4 (8%)	5 (10%)	1 (2%)
Pheochromocytoma malignant	1 (2%)		2 (4%)	1 (2%)
Bilateral, pheochromocytoma benign			1 (2%)	
Islets, pancreatic	(48)	(48)	(50)	(50)
Adenoma	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Parathyroid gland	(47)	(44)	(50)	(46)
Adenoma	1 (2%)	1 (2%)		
Pituitary gland	(49)	(48)	(49)	(50)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Pars distalis, adenoma	24 (49%)	20 (42%)	18 (37%)	26 (52%)
Pars intermedia, adenoma		1 (2%)	1 (2%)	
Thyroid gland	(48)	(49)	(50)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
C-cell, adenoma			1 (2%)	
C-cell, carcinoma	3 (6%)		3 (6%)	1 (2%)
Follicular cell, adenoma		1 (2%)		
General Body System				
Tissue NOS	(0)	(0)	(0)	(1)
Sarcoma				1 (100%)
Genital System				
Coagulating gland	(47)	(49)	(48)	(50)
Carcinoma, metastatic, uncertain primary site				1 (2%)
Schwannoma malignant				1 (2%)
Ductus deferens	(0)	(0)	(0)	(1)
Epididymis	(49)	(49)	(50)	(50)
Lymphoma malignant	1 (2%)			
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Preputial gland	(49)	(49)	(50)	(49)
Carcinoma		1 (2%)		1 (2%)
Lymphoma malignant	1 (2%)			
Squamous cell carcinoma	2 (4%)	3 (6%)	4 (8%)	7 (14%)
Prostate, dorsal/lateral lobe	(50)	(48)	(50)	(50)
Lymphoma malignant	1 (2%)			
Schwannoma malignant				1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Prostate, ventral lobe	(49)	(48)	(50)	(50)
Lymphoma malignant	1 (2%)			
Schwannoma malignant				1 (2%)
Rete testes	(46)	(45)	(43)	(44)
Seminal vesicle	(44)	(44)	(47)	(47)
Lymphoma malignant	1 (2%)		1 (2%)	

Table A1c
Summary of the Incidence of Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Genital System (continued)				
Testes	(50)	(49)	(50)	(50)
Lymphoma malignant	1 (2%)			
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Interstitial cell, adenoma		1 (2%)		1 (2%)
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Lymph node	(14)	(16)	(13)	(16)
Axillary, lymphoma malignant	1 (7%)		1 (8%)	
Inguinal, lymphoma malignant	1 (7%)			
Lumbar, lymphoma malignant			1 (8%)	
Mediastinal, lymphoma malignant	1 (7%)	1 (6%)	1 (8%)	
Renal, lymphoma malignant			1 (8%)	
Lymph node, mandibular	(46)	(48)	(50)	(49)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Lymph node, mesenteric	(44)	(46)	(49)	(48)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	1 (2%)		1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Spleen	(49)	(48)	(49)	(50)
Leukemia mononuclear		1 (2%)	1 (2%)	
Lymphoma malignant	1 (2%)	1 (2%)	1 (2%)	
Sarcoma				1 (2%)
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Thymus	(47)	(45)	(47)	(45)
Leukemia mononuclear		1 (2%)		
Lymphoma malignant	2 (4%)		1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Integumentary System				
Mammary gland	(42)	(42)	(40)	(45)
Adenocarcinoma				2 (4%)
Adenoma				1 (2%)
Fibroadenoma		2 (5%)		1 (2%)
Fibroma	1 (2%)	1 (2%)		
Skin	(50)	(49)	(50)	(50)
Basal cell adenoma	2 (4%)		1 (2%)	
Carcinoma				1 (2%)
Fibroma			1 (2%)	2 (4%)
Keratoacanthoma		2 (4%)	3 (6%)	1 (2%)
Lipoma		2 (4%)		2 (4%)
Sarcoma	1 (2%)			1 (2%)
Schwannoma malignant			1 (2%)	

Table A1c
Summary of the Incidence of Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Musculoskeletal System				
Bone	(0)	(1)	(1)	(0)
Cranium, squamous cell carcinoma, deep invasion		1 (100%)		
Skeletal muscle	(3)	(4)	(3)	(0)
Lymphoma malignant	1 (33%)			
Diaphragm, squamous cell carcinoma, metastatic, stomach, forestomach		1 (25%)		
Nervous System				
Brain	(0)	(0)	(0)	(1)
Cranial nerve, schwannoma malignant				1 (100%)
Brain, brain stem	(49)	(49)	(50)	(50)
Reticulosis malignant				1 (2%)
Brain, cerebellum	(49)	(49)	(50)	(50)
Granular cell tumor malignant			1 (2%)	
Brain, cerebrum	(49)	(49)	(50)	(50)
Granular cell tumor malignant			1 (2%)	
Osteoma	1 (2%)			
Meninges, granular cell tumor benign				1 (2%)
Respiratory System				
Lung	(47)	(48)	(48)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	
Leukemia mononuclear		1 (2%)	1 (2%)	
Lymphoma malignant	1 (2%)		1 (2%)	
Schwannoma malignant, metastatic, skin			1 (2%)	
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Squamous cell carcinoma, metastatic, uncertain primary site			1 (2%)	
Thymoma malignant, metastatic, uncertain primary site				1 (2%)
Nose	(49)	(48)	(49)	(50)
Lymphoma malignant	1 (2%)			
Osteosarcoma				1 (2%)
Trachea	(49)	(49)	(50)	(50)
Lymphoma malignant	1 (2%)			
Special Senses System				
Ear	(0)	(0)	(1)	(1)
Neural crest tumor				1 (100%)
Squamous cell papilloma			1 (100%)	
Eye	(46)	(47)	(48)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
Iris, melanoma benign				1 (2%)
Retrobulbar, squamous cell carcinoma, deep invasion	1 (2%)			
Harderian gland	(49)	(48)	(50)	(50)
Lymphoma malignant	1 (2%)		1 (2%)	
Lacrimal gland	(1)	(1)	(2)	(1)
Lymphoma malignant	1 (100%)		1 (50%)	

Table A1c
Summary of the Incidence of Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Special Senses System (continued)				
Zymbal's gland	(1)	(0)	(2)	(0)
Carcinoma	1 (100%)		1 (50%)	
Squamous cell carcinoma			1 (50%)	
Urinary System				
Kidney	(49)	(48)	(50)	(50)
Leukemia mononuclear		1 (2%)		
Liposarcoma				1 (2%)
Lymphoma malignant	1 (2%)		1 (2%)	
Sarcoma		1 (2%)		
Squamous cell carcinoma, metastatic, stomach, forestomach		1 (2%)		
Urinary bladder	(49)	(48)	(49)	(50)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table A2a
Statistical Analysis of Primary Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Adrenal Cortex: Adenoma or Carcinoma				
Overall rate[a]	2/47 (4.3%)	1/50 (2.0%)	4/50 (8.0%)	1/50 (2.0%)
Adjusted rate[b]	2/41.5 (4.8%)	1/41.0 (2.4%)	4/42.7 (9.4%)	1/43.0 (2.3%)
Terminal rate[c]	2/34 (5.9%)	1/31 (3.2%)	3/30 (10.0%)	1/32 (3.1%)
First incidence (days)	757 (T)	756 (T)	615	762 (T)
Poly-3 test[d]	P=0.394N/P=0.545N	P=0.504N	P=0.350	P=0.487N
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate	2/46 (4.3%)	2/48 (4.2%)	4/49 (8.2%)	1/49 (2.0%)
Adjusted rate	2/40.5 (4.9%)	2/39.0 (5.1%)	4/41.9 (9.5%)	1/42.3 (2.4%)
Terminal rate	2/33 (6.1%)	2/29 (6.9%)	3/30 (10.0%)	0/31 (0.0%)
First incidence (days)	757 (T)	757 (T)	709	661
Poly-3 test	P=0.308N/P=0.474N	P=0.680	P=0.352	P=0.484N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	4/46 (8.7%)	3/48 (6.3%)	7/49 (14.3%)	1/49 (2.0%)
Adjusted rate	4/40.5 (9.9%)	3/39.0 (7.7%)	7/41.9 (16.7%)	1/42.3 (2.4%)
Terminal rate	4/33 (12.1%)	3/29 (10.3%)	6/30 (20.0%)	0/31 (0.0%)
First incidence (days)	757 (T)	757 (T)	709	661
Poly-3 test	P=0.103N/P=0.278N	P=0.520N	P=0.279	P=0.164N
Liver: Hepatocellular Adenoma				
Overall rate	0/49 (0.0%)	3/49 (6.1%)	1/50 (2.0%)	0/49 (0.0%)
Adjusted rate	0/42.5 (0.0%)	3/41.7 (7.2%)	1/42.2 (2.4%)	0/42.9 (0.0%)
Terminal rate	0/34 (0.0%)	1/31 (3.2%)	1/30 (3.3%)	0/32 (0.0%)
First incidence (days)	—[e]	596	761 (T)	—
Poly-3 test	P=0.254N/P=0.409N	P=0.114	P=0.498	—[f]
Liver: Hepatocellular Adenoma or Carcinoma				
Overall rate	0/49 (0.0%)	3/49 (6.1%)	1/50 (2.0%)	1/49 (2.0%)
Adjusted rate	0/42.5 (0.0%)	3/41.7 (7.2%)	1/42.2 (2.4%)	1/42.9 (2.3%)
Terminal rate	0/34 (0.0%)	1/31 (3.2%)	1/30 (3.3%)	1/32 (3.1%)
First incidence (days)	—	596	761 (T)	757 (T)
Poly-3 test	P=0.572N/P=0.503	P=0.114	P=0.498	P=0.501
Mammary Gland: Fibroma				
Overall rate	1/44 (2.3%)	3/45 (6.7%)	0/47 (0.0%)	0/44 (0.0%)
Adjusted rate	1/39.6 (2.5%)	3/38.5 (7.8%)	0/40.3 (0.0%)	0/39.0 (0.0%)
Terminal rate	0/34 (0.0%)	3/31 (9.7%)	0/30 (0.0%)	0/31 (0.0%)
First incidence (days)	730	758 (T)	—	—
Poly-3 test	P=0.185N/P=0.132N	P=0.294	P=0.496N	P=0.502N
Mammary Gland: Fibroadenoma				
Overall rate	1/44 (2.3%)	3/45 (6.7%)	1/47 (2.1%)	2/44 (4.5%)
Adjusted rate	1/39.5 (2.5%)	3/38.5 (7.8%)	1/40.9 (2.4%)	2/39.1 (5.1%)
Terminal rate	1/34 (2.9%)	3/31 (9.7%)	0/30 (0.0%)	1/31 (3.2%)
First incidence (days)	756 (T)	761 (T)	562	737
Poly-3 test	P=0.587/P=0.500	P=0.295	P=0.752N	P=0.497
Mammary Gland: Fibroma or Fibroadenoma				
Overall rate	2/44 (4.5%)	6/45 (13.3%)	1/47 (2.1%)	2/44 (4.5%)
Adjusted rate	2/39.6 (5.1%)	6/38.5 (15.6%)	1/40.9 (2.4%)	2/39.1 (5.1%)
Terminal rate	1/34 (2.9%)	6/31 (19.4%)	0/30 (0.0%)	1/31 (3.2%)
First incidence (days)	730	758 (T)	562	737
Poly-3 test	P=0.338N/P=0.292N	P=0.121	P=0.488N	P=0.690

Table A2a
Statistical Analysis of Primary Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Mammary Gland: Fibroma, Fibroadenoma, or Adenocarcinoma				
Overall rate	3/44 (6.8%)	6/45 (13.3%)	1/47 (2.1%)	2/44 (4.5%)
Adjusted rate	3/39.6 (7.6%)	6/38.5 (15.6%)	1/40.9 (2.4%)	2/39.1 (5.1%)
Terminal rate	2/34 (5.9%)	6/31 (19.4%)	0/30 (0.0%)	1/31 (3.2%)
First incidence (days)	730	758 (T)	562	737
Poly-3 test	P=0.262N/P=0.174N	P=0.225	P=0.293N	P=0.505N
Parathyroid Gland: Adenoma				
Overall rate	4/49 (8.2%)	0/46 (0.0%)	1/46 (2.2%)	1/46 (2.2%)
Adjusted rate	4/42.3 (9.4%)	0/38.8 (0.0%)	1/39.7 (2.5%)	1/39.6 (2.5%)
Terminal rate	2/33 (6.1%)	0/31 (0.0%)	1/29 (3.4%)	0/29 (0.0%)
First incidence (days)	647	—	763 (T)	661
Poly-3 test	P=0.398N/P=0.104N	P=0.071N	P=0.197N	P=0.198N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	25/48 (52.1%)	24/50 (48.0%)	28/50 (56.0%)	16/50 (32.0%)
Adjusted rate	25/43.9 (57.0%)	24/43.1 (55.7%)	28/46.1 (60.7%)	16/44.4 (36.1%)
Terminal rate	20/34 (58.8%)	18/31 (58.1%)	18/30 (60.0%)	12/32 (37.5%)
First incidence (days)	478	596	552	500
Poly-3 test	P=0.010N/P=0.047N	P=0.538N	P=0.441	P=0.035N
Preputial Gland: Squamous Cell Carcinoma				
Overall rate	4/51 (7.8%)	5/50 (10.0%)	5/50 (10.0%)	2/50 (4.0%)
Adjusted rate	4/43.9 (9.1%)	5/41.9 (11.9%)	5/43.2 (11.6%)	2/43.0 (4.7%)
Terminal rate	2/34 (5.9%)	1/31 (3.2%)	2/30 (6.7%)	2/32 (6.3%)
First incidence (days)	704	596	602	757 (T)
Poly-3 test	P=0.190N/P=0.287N	P=0.470	P=0.489	P=0.347N
Skin: Keratoacanthoma				
Overall rate	2/51 (3.9%)	3/50 (6.0%)	1/50 (2.0%)	2/50 (4.0%)
Adjusted rate	2/43.6 (4.6%)	3/41.5 (7.2%)	1/42.3 (2.4%)	2/43.0 (4.7%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	0/30 (0.0%)	2/32 (6.3%)
First incidence (days)	757 (T)	596	737	756 (T)
Poly-3 test	P=0.582N/P=0.442N	P=0.477	P=0.510N	P=0.688
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	2/51 (3.9%)	3/50 (6.0%)	2/50 (4.0%)	2/50 (4.0%)
Adjusted rate	2/43.6 (4.6%)	3/41.5 (7.2%)	2/42.3 (4.7%)	2/43.0 (4.7%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	1/30 (3.3%)	2/32 (6.3%)
First incidence (days)	757 (T)	596	737	756 (T)
Poly-3 test	P=0.540N/P=0.505N	P=0.477	P=0.683	P=0.688
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	2/51 (3.9%)	4/50 (8.0%)	2/50 (4.0%)	2/50 (4.0%)
Adjusted rate	2/43.6 (4.6%)	4/42.0 (9.5%)	2/42.3 (4.7%)	2/43.0 (4.7%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	1/30 (3.3%)	2/32 (6.3%)
First incidence (days)	757 (T)	596	737	756 (T)
Poly-3 test	P=0.454N/P=0.449N	P=0.319	P=0.683	P=0.688
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	3/51 (5.9%)	5/50 (10.0%)	3/50 (6.0%)	2/50 (4.0%)
Adjusted rate	3/43.6 (6.9%)	5/42.0 (11.9%)	3/42.3 (7.1%)	2/43.0 (4.7%)
Terminal rate	3/34 (8.8%)	3/31 (9.7%)	2/30 (6.7%)	2/32 (6.3%)
First incidence (days)	757 (T)	596	737	756 (T)
Poly-3 test	P=0.276N/P=0.310N	P=0.335	P=0.649	P=0.506N

Table A2a
Statistical Analysis of Primary Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Skin: Sarcoma				
Overall rate	4/51 (7.8%)	0/50 (0.0%)	1/50 (2.0%)	0/50 (0.0%)
Adjusted rate	4/45.5 (8.8%)	0/41.0 (0.0%)	1/43.0 (2.3%)	0/43.0 (0.0%)
Terminal rate	1/34 (2.9%)	0/31 (0.0%)	0/30 (0.0%)	0/32 (0.0%)
First incidence (days)	383	—	494	—
Poly-3 test	P=0.130N/P=0.020N	P=0.074N	P=0.196N	P=0.067N
Skin: Fibroma or Sarcoma				
Overall rate	5/51 (9.8%)	0/50 (0.0%)	2/50 (4.0%)	2/50 (4.0%)
Adjusted rate	5/45.5 (11.0%)	0/41.0 (0.0%)	2/43.5 (4.6%)	2/43.0 (4.7%)
Terminal rate	2/34 (5.9%)	0/31 (0.0%)	0/30 (0.0%)	2/32 (6.3%)
First incidence (days)	383	—	494	756 (T)
Poly-3 test	P=0.489N/P=0.180N	P=0.040N	P=0.234N	P=0.239N
Skin: All Neoplastic Morphologies				
Overall rate	8/51 (15.7%)	5/50 (10.0%)	6/50 (12.0%)	5/50 (10.0%)
Adjusted rate	8/45.5 (17.6%)	5/42.0 (11.9%)	6/43.6 (13.8%)	5/43.3 (11.6%)
Terminal rate	5/34 (14.7%)	3/31 (9.7%)	3/30 (10.0%)	4/32 (12.5%)
First incidence (days)	383	596	494	682
Poly-3 test	P=0.371N/P=0.268N	P=0.328N	P=0.420N	P=0.308N
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/47 (10.6%)	0/49 (0.0%)	1/50 (2.0%)	0/49 (0.0%)
Adjusted rate	5/42.2 (11.9%)	0/41.0 (0.0%)	1/42.5 (2.4%)	0/42.9 (0.0%)
Terminal rate	3/34 (8.8%)	0/31 (0.0%)	0/30 (0.0%)	0/32 (0.0%)
First incidence (days)	619	—	688	—
Poly-3 test	P=0.079N/P=0.005N	P=0.032N	P=0.098N	P=0.028N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/47 (10.6%)	1/49 (2.0%)	1/50 (2.0%)	0/49 (0.0%)
Adjusted rate	5/42.2 (11.9%)	1/41.0 (2.4%)	1/42.5 (2.4%)	0/42.9 (0.0%)
Terminal rate	3/34 (8.8%)	1/31 (3.2%)	0/30 (0.0%)	0/32 (0.0%)
First incidence (days)	619	761 (T)	688	—
Poly-3 test	P=0.060N/P=0.005N	P=0.107N	P=0.098N	P=0.028N
All Organs: Benign Neoplasms				
Overall rate	36/51 (70.6%)	29/50 (58.0%)	34/50 (68.0%)	23/50 (46.0%)
Adjusted rate	36/45.7 (78.7%)	29/43.1 (67.3%)	34/46.9 (72.5%)	23/44.7 (51.4%)
Terminal rate	28/34 (82.4%)	23/31 (74.2%)	22/30 (73.3%)	18/32 (56.3%)
First incidence (days)	478	596	552	500
Poly-3 test	P=0.004N/P=0.006N	P=0.151N	P=0.319N	P=0.004N

Table A2a
Statistical Analysis of Primary Neoplasms in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
All Organs: Malignant Neoplasms				
Overall rate	19/51 (37.3%)	15/50 (30.0%)	19/50 (38.0%)	19/50 (38.0%)
Adjusted rate	19/47.4 (40.1%)	15/45.2 (33.2%)	19/45.7 (41.6%)	19/46.2 (41.1%)
Terminal rate	9/34 (26.5%)	5/31 (16.1%)	8/30 (26.7%)	7/32 (21.9%)
First incidence (days)	383	386	494	500
Poly-3 test	P=0.401/P=0.391	P=0.320N	P=0.524	P=0.543

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

Table A2b
Statistical Analysis of Primary Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	2/46 (4.3%)	4/49 (8.2%)	1/45 (2.2%)	1/50 (2.0%)
Adjusted rate[b]	2/40.5 (4.9%)	4/41.6 (9.6%)	1/39.9 (2.5%)	1/44.0 (2.3%)
Terminal rate[c]	2/33 (6.1%)	4/31 (12.9%)	1/32 (3.1%)	1/33 (3.0%)
First incidence (days)	757 (T)	756 (T)	761 (T)	763 (T)
Poly-3 test[d]	P=0.238N/P=0.193N	P=0.348	P=0.504N	P=0.470N
Adrenal Medulla: Benign, Complex, or Malignant Pheochromocytoma				
Overall rate	4/46 (8.7%)	4/49 (8.2%)	1/45 (2.2%)	1/50 (2.0%)
Adjusted rate	4/40.5 (9.9%)	4/41.6 (9.6%)	1/39.9 (2.5%)	1/44.0 (2.3%)
Terminal rate	4/33 (12.1%)	4/31 (12.9%)	1/32 (3.1%)	1/33 (3.0%)
First incidence (days)	757 (T)	756 (T)	761 (T)	763 (T)
Poly-3 test	P=0.123N/P=0.047N	P=0.629N	P=0.181N	P=0.153N
Kidney: Renal Tubule Adenoma				
Overall rate	0/46 (0.0%)	3/49 (6.1%)	0/47 (0.0%)	2/49 (4.1%)
Adjusted rate	0/41.3 (0.0%)	3/43.2 (6.9%)	0/42.0 (0.0%)	2/44.4 (4.5%)
Terminal rate	0/34 (0.0%)	1/31 (3.2%)	0/33 (0.0%)	0/33 (0.0%)
First incidence (days)	—[e]	534	—	556
Poly-3 test	P=0.439/P=0.355	P=0.126	—[f]	P=0.252
Mammary Gland: Fibroma, Fibroadenoma or Adenoma				
Overall rate	2/44 (4.5%)	2/45 (4.4%)	2/47 (4.3%)	4/48 (8.3%)
Adjusted rate	2/39.6 (5.1%)	2/39.7 (5.0%)	2/42.2 (4.7%)	4/42.9 (9.3%)
Terminal rate	1/34 (2.9%)	0/31 (0.0%)	1/33 (3.0%)	3/33 (9.1%)
First incidence (days)	730	656	746	746
Poly-3 test	P=0.247/P=0.270	P=0.693N	P=0.671N	P=0.374
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Adenocarcinoma				
Overall rate	3/44 (6.8%)	2/45 (4.4%)	2/47 (4.3%)	4/48 (8.3%)
Adjusted rate	3/39.6 (7.6%)	2/39.7 (5.0%)	2/42.2 (4.7%)	4/42.9 (9.3%)
Terminal rate	2/34 (5.9%)	0/31 (0.0%)	1/33 (3.0%)	3/33 (9.1%)
First incidence (days)	730	656	746	746
Poly-3 test	P=0.342/P=0.437	P=0.498N	P=0.471N	P=0.543
Parathyroid Gland: Adenoma				
Overall rate	4/49 (8.2%)	3/50 (6.0%)	1/44 (2.3%)	0/50 (0.0%)
Adjusted rate	4/42.3 (9.4%)	3/42.5 (7.1%)	1/36.7 (2.7%)	0/44.0 (0.0%)
Terminal rate	2/33 (6.1%)	3/31 (9.7%)	1/27 (3.7%)	0/33 (0.0%)
First incidence (days)	647	762 (T)	757 (T)	—
Poly-3 test	P=0.051N/P=0.018N	P=0.497N	P=0.223N	P=0.054N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	25/48 (52.1%)	29/50 (58.0%)	28/47 (59.6%)	24/49 (49.0%)
Adjusted rate	25/43.9 (57.0%)	29/46.8 (62.0%)	28/43.4 (64.6%)	24/46.6 (51.5%)
Terminal rate	20/34 (58.8%)	17/31 (54.8%)	19/33 (57.6%)	14/33 (42.4%)
First incidence (days)	478	534	659	556
Poly-3 test	P=0.184N/P=0.340N	P=0.392	P=0.304	P=0.376N
Preputial Gland: Squamous Cell Carcinoma				
Overall rate	4/51 (7.8%)	4/50 (8.0%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	4/43.9 (9.1%)	4/43.1 (9.3%)	3/43.2 (6.9%)	1/44.0 (2.3%)
Terminal rate	2/34 (5.9%)	1/31 (3.2%)	1/33 (3.0%)	1/33 (3.0%)
First incidence (days)	704	642	677	763 (T)
Poly-3 test	P=0.121N/P=0.114N	P=0.633	P=0.509N	P=0.177N

Table A2b
Statistical Analysis of Primary Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Preputial Gland: Epithelial Neoplasms				
Overall rate	4/51 (7.8%)	5/50 (10.0%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	4/43.9 (9.1%)	5/43.1 (11.6%)	3/43.2 (6.9%)	1/44.0 (2.3%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	1/33 (3.0%)	1/33 (3.0%)
First incidence (days)	704	642	677	763 (T)
Poly-3 test	P=0.093N/P=0.098N	P=0.488	P=0.509N	P=0.177N
Skin: Keratoacanthoma				
Overall rate	2/51 (3.9%)	1/50 (2.0%)	3/49 (6.1%)	1/50 (2.0%)
Adjusted rate	2/43.6 (4.6%)	1/43.0 (2.3%)	3/42.5 (7.1%)	1/44.0 (2.3%)
Terminal rate	2/34 (5.9%)	0/31 (0.0%)	2/33 (6.1%)	1/33 (3.0%)
First incidence (days)	757 (T)	603	746	758 (T)
Poly-3 test	P=0.441N/P=0.497N	P=0.504N	P=0.488	P=0.496N
Skin: Squamous Cell Papilloma or Keratoacanthoma				
Overall rate	2/51 (3.9%)	3/50 (6.0%)	4/49 (8.2%)	1/50 (2.0%)
Adjusted rate	2/43.6 (4.6%)	3/43.0 (7.0%)	4/42.5 (9.4%)	1/44.0 (2.3%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	3/33 (9.1%)	1/33 (3.0%)
First incidence (days)	757 (T)	603	746	758 (T)
Poly-3 test	P=0.255N/P=0.436N	P=0.493	P=0.324	P=0.496N
Skin: Squamous Cell Papilloma, Keratoacanthoma, or Squamous Cell Carcinoma				
Overall rate	2/51 (3.9%)	3/50 (6.0%)	5/49 (10.2%)	3/50 (6.0%)
Adjusted rate	2/43.6 (4.6%)	3/43.0 (7.0%)	5/42.8 (11.7%)	3/44.0 (6.8%)
Terminal rate	2/34 (5.9%)	2/31 (6.5%)	3/33 (9.1%)	3/33 (9.1%)
First incidence (days)	757 (T)	603	669	757 (T)
Poly-3 test	P=0.601/P=0.311	P=0.493	P=0.208	P=0.504
Skin: Squamous Cell Papilloma, Keratoacanthoma, Basal Cell Adenoma, or Squamous Cell Carcinoma				
Overall rate	3/51 (5.9%)	3/50 (6.0%)	5/49 (10.2%)	5/50 (10.0%)
Adjusted rate	3/43.6 (6.9%)	3/43.0 (7.0%)	5/42.8 (11.7%)	5/44.0 (11.4%)
Terminal rate	3/34 (8.8%)	2/31 (6.5%)	3/33 (9.1%)	5/33 (15.2%)
First incidence (days)	757 (T)	603	669	756 (T)
Poly-3 test	P=0.325/P=0.211	P=0.656	P=0.346	P=0.361
Skin: Sarcoma				
Overall rate	4/51 (7.8%)	1/50 (2.0%)	0/49 (0.0%)	0/50 (0.0%)
Adjusted rate	4/45.5 (8.8%)	1/43.0 (2.3%)	0/42.4 (0.0%)	0/44.0 (0.0%)
Terminal rate	1/34 (2.9%)	0/31 (0.0%)	0/33 (0.0%)	0/33 (0.0%)
First incidence (days)	383	619	—	—
Poly-3 test	P=0.101N/P=0.007N	P=0.196N	P=0.069N	P=0.064N
Skin: Fibroma or Sarcoma				
Overall rate	5/51 (9.8%)	1/50 (2.0%)	1/49 (2.0%)	0/50 (0.0%)
Adjusted rate	5/45.5 (11.0%)	1/43.0 (2.3%)	1/42.7 (2.3%)	0/44.0 (0.0%)
Terminal rate	2/34 (5.9%)	0/31 (0.0%)	0/33 (0.0%)	0/33 (0.0%)
First incidence (days)	383	619	677	—
Poly-3 test	P=0.067N/P=0.007N	P=0.114N	P=0.116N	P=0.033N
Skin: All Neoplastic Morphologies				
Overall rate	8/51 (15.7%)	5/50 (10.0%)	6/49 (12.2%)	6/50 (12.0%)
Adjusted rate	8/45.5 (17.6%)	5/43.5 (11.5%)	6/43.1 (13.9%)	6/44.5 (13.5%)
Terminal rate	5/34 (14.7%)	3/31 (9.7%)	3/33 (9.1%)	5/33 (15.2%)
First incidence (days)	383	603	669	619
Poly-3 test	P=0.505N/P=0.367N	P=0.305N	P=0.428N	P=0.403N

Table A2b
Statistical Analysis of Primary Neoplasms in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Thyroid Gland (C-Cell): Adenoma				
Overall rate	5/47 (10.6%)	3/50 (6.0%)	1/47 (2.1%)	1/49 (2.0%)
Adjusted rate	5/42.2 (11.9%)	3/42.5 (7.1%)	1/42.1 (2.4%)	1/43.1 (2.3%)
Terminal rate	3/34 (8.8%)	3/31 (9.7%)	1/33 (3.0%)	1/33 (3.0%)
First incidence (days)	619	758 (T)	758 (T)	756 (T)
Poly-3 test	P=0.126N/P=0.026N	P=0.351N	P=0.100N	P=0.095N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	5/47 (10.6%)	3/50 (6.0%)[g]	1/47 (2.1%)	2/49 (4.1%)
Adjusted rate	5/42.2 (11.9%)	3/42.5 (7.1%)	1/42.1 (2.4%)	2/43.7 (4.6%)
Terminal rate	3/34 (8.8%)	3/31 (9.7%)	1/33 (3.0%)	1/33 (3.0%)
First incidence (days)	619	758 (T)	758 (T)	556
Poly-3 test	P=0.280N/P=0.075N	P=0.351N	P=0.100N	P=0.200N
All Organs: Benign Neoplasms				
Overall rate	36/51 (70.6%)	37/50 (74.0%)	29/50 (58.0%)	29/50 (58.0%)
Adjusted rate	36/45.7 (78.7%)	37/47.5 (78.0%)	29/44.3 (65.5%)	29/47.2 (61.4%)
Terminal rate	28/34 (82.4%)	23/31 (74.2%)	20/33 (60.6%)	19/33 (57.6%)
First incidence (days)	478	534	659	556
Poly-3 test	P=0.033N/P=0.015N	P=0.569N	P=0.112N	P=0.048N
All Organs: Malignant Neoplasms				
Overall rate	19/51 (37.3%)	17/50 (34.0%)	8/50 (16.0%)	15/50 (30.0%)
Adjusted rate	19/47.4 (40.1%)	17/46.3 (36.7%)	8/46.2 (17.3%)	15/47.8 (31.4%)
Terminal rate	9/34 (26.5%)	6/31 (19.4%)	2/33 (6.1%)	6/33 (18.2%)
First incidence (days)	383	485	219	469
Poly-3 test	P=0.376N/P=0.078N	P=0.452N	P=0.012N	P=0.252N

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.
[g] Carcinoma occurred in two animals that also had adenoma.

Table A2c
Statistical Analysis of Primary Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Adrenal Medulla: Benign Pheochromocytoma				
Overall rate[a]	4/49 (8.2%)	4/48 (8.3%)	6/49 (12.2%)	1/50 (2.0%)
Adjusted rate[b]	4/40.6 (9.9%)	4/38.7 (10.3%)	6/41.3 (14.5%)	1/43.7 (2.3%)
Terminal rate[c]	4/30 (13.3%)	3/26 (11.5%)	4/31 (12.9%)	0/35 (0.0%)
First incidence (days)	745 (T)	734	686	625
Poly-3 test[d]	P=0.076N/P=0.189N	P=0.618	P=0.379	P=0.156N
Adrenal Medulla: Benign or Malignant Pheochromocytoma				
Overall rate	5/49 (10.2%)	4/48 (8.3%)	7/49 (14.3%)[e]	2/50 (4.0%)
Adjusted rate	5/40.6 (12.3%)	4/38.7 (10.3%)	7/41.3 (17.0%)	2/43.7 (4.6%)
Terminal rate	5/30 (16.7%)	3/26 (11.5%)	5/31 (16.1%)	1/35 (2.9%)
First incidence (days)	745 (T)	734	686	625
Poly-3 test	P=0.118N/P=0.234N	P=0.529N	P=0.389	P=0.186N
Liver: Cholangioma or Cholangiocarcinoma				
Overall rate	3/49 (6.1%)	0/47 (0.0%)	1/50 (2.0%)	0/49 (0.0%)
Adjusted rate	3/41.3 (7.3%)	0/38.6 (0.0%)	1/41.6 (2.4%)	0/42.8 (0.0%)
Terminal rate	2/30 (6.7%)	0/27 (0.0%)	1/31 (3.2%)	0/35 (0.0%)
First incidence (days)	521	—[f]	754 (T)	—
Poly-3 test	P=0.193N/P=0.051N	P=0.129N	P=0.301N	P=0.111N
Mammary Gland: Fibroma, Fibroadenoma or Adenoma				
Overall rate	1/42 (2.4%)	3/42 (7.1%)	0/40 (0.0%)	2/45 (4.4%)
Adjusted rate	1/37.3 (2.7%)	3/36.1 (8.3%)	0/37.3 (0.0%)	2/40.8 (4.9%)
Terminal rate	1/30 (3.3%)	2/27 (7.4%)	0/31 (0.0%)	2/35 (5.7%)
First incidence (days)	755 (T)	628	—	749 (T)
Poly-3 test	P=0.589/P=0.542N	P=0.292	P=0.500N	P=0.530
Mammary Gland: Adenoma or Adenocarcinoma				
Overall rate	0/42 (0.0%)	0/42 (0.0%)	0/40 (0.0%)	3/45 (6.7%)
Adjusted rate	0/37.3 (0.0%)	0/35.7 (0.0%)	0/37.3 (0.0%)	3/41.6 (7.2%)
Terminal rate	0/30 (0.0%)	0/27 (0.0%)	0/31 (0.0%)	1/35 (2.9%)
First incidence (days)	—	—	—	553
Poly-3 test	P=0.011/P=0.021	—[g]	—	P=0.138
Mammary Gland: Fibroma, Fibroadenoma, Adenoma, or Adenocarcinoma				
Overall rate	1/42 (2.4%)	3/42 (7.1%)	0/40 (0.0%)	4/45 (8.9%)
Adjusted rate	1/37.3 (2.7%)	3/36.1 (8.3%)	0/37.3 (0.0%)	4/41.6 (9.6%)
Terminal rate	1/30 (3.3%)	2/27 (7.4%)	0/31 (0.0%)	2/35 (5.7%)
First incidence (days)	755 (T)	628	—	553
Poly-3 test	P=0.167/P=0.242	P=0.292	P=0.500N	P=0.212
Pancreatic Islets: Adenoma				
Overall rate	2/48 (4.2%)	1/48 (2.1%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	2/40.7 (4.9%)	1/39.5 (2.5%)	3/41.6 (7.2%)	1/43.2 (2.3%)
Terminal rate	0/30 (0.0%)	0/27 (0.0%)	3/31 (9.7%)	1/35 (2.9%)
First incidence (days)	630	586	748 (T)	749 (T)
Poly-3 test	P=0.418N/P=0.468N	P=0.510N	P=0.509	P=0.479N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	24/49 (49.0%)	20/48 (41.7%)	18/49 (36.7%)	26/50 (52.0%)
Adjusted rate	24/43.1 (55.6%)	20/41.7 (47.9%)	18/43.1 (41.8%)	26/46.0 (56.5%)
Terminal rate	17/30 (56.7%)	12/27 (44.4%)	11/31 (35.5%)	19/35 (54.3%)
First incidence (days)	585	434	389	504
Poly-3 test	P=0.280/P=0.509N	P=0.307N	P=0.136N	P=0.553

Table A2c
Statistical Analysis of Primary Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Preputial Gland: Squamous Cell Carcinoma				
Overall rate	2/49 (4.1%)	3/49 (6.1%)	4/50 (8.0%)	7/49 (14.3%)
Adjusted rate	2/41.3 (4.8%)	3/40.2 (7.5%)	4/42.7 (9.4%)	7/44.7 (15.7%)
Terminal rate	0/30 (0.0%)	1/27 (3.7%)	1/31 (3.2%)	1/35 (2.9%)
First incidence (days)	601	638	539	625
Poly-3 test	P=0.066/P=0.051	P=0.486	P=0.351	P=0.098
Preputial Gland: Epithelial Neoplasms				
Overall rate	2/49 (4.1%)	4/49 (8.2%)	4/50 (8.0%)	8/49 (16.3%)
Adjusted rate	2/41.3 (4.8%)	4/40.3 (9.9%)	4/42.7 (9.4%)	8/44.7 (17.9%)
Terminal rate	0/30 (0.0%)	1/27 (3.7%)	1/31 (3.2%)	2/35 (5.7%)
First incidence (days)	601	638	539	625
Poly-3 test	P=0.046/P=0.038	P=0.324	P=0.351	P=0.058
Skin: Keratoacanthoma				
Overall rate	0/50 (0.0%)	2/49 (4.1%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	0/40.6 (0.0%)	2/39.7 (5.0%)	3/41.6 (7.2%)	1/43.2 (2.3%)
Terminal rate	0/30 (0.0%)	1/27 (3.7%)	3/31 (9.7%)	1/35 (2.9%)
First incidence (days)	—	730	748 (T)	754 (T)
Poly-3 test	P=0.555N/P=0.326	P=0.231	P=0.122	P=0.512
Skin: Keratoacanthoma, Basal Cell Adenoma, or Carcinoma				
Overall rate	2/50 (4.0%)	2/49 (4.1%)	4/50 (8.0%)	2/50 (4.0%)
Adjusted rate	2/40.6 (4.9%)	2/39.7 (5.0%)	4/41.7 (9.6%)	2/43.6 (4.6%)
Terminal rate	2/30 (6.7%)	1/27 (3.7%)	3/31 (9.7%)	1/35 (2.9%)
First incidence (days)	750 (T)	730	737	655
Poly-3 test	P=0.511N/P=0.492	P=0.687	P=0.349	P=0.668N
Skin: Fibroma or Sarcoma				
Overall rate	1/50 (2.0%)	0/49 (0.0%)	1/50 (2.0%)	3/50 (6.0%)
Adjusted rate	1/40.9 (2.4%)	0/39.6 (0.0%)	1/41.9 (2.4%)	3/43.2 (6.9%)
Terminal rate	0/30 (0.0%)	0/27 (0.0%)	0/31 (0.0%)	3/35 (8.6%)
First incidence (days)	660	—	686	752 (T)
Poly-3 test	P=0.091/P=0.121	P=0.506N	P=0.755N	P=0.324
Skin: All Neoplastic Morphologies				
Overall rate	3/50 (6.0%)	4/49 (8.2%)	6/50 (12.0%)	7/50 (14.0%)
Adjusted rate	3/40.9 (7.3%)	4/40.0 (10.0%)	6/42.2 (14.2%)	7/44.0 (15.9%)
Terminal rate	2/30 (6.7%)	2/27 (7.4%)	3/31 (9.7%)	5/35 (14.3%)
First incidence (days)	660	680	686	655
Poly-3 test	P=0.193/P=0.109	P=0.487	P=0.255	P=0.186
Thyroid Gland (C-Cell): Carcinoma				
Overall rate	3/48 (6.3%)	0/49 (0.0%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	3/40.0 (7.5%)	0/39.6 (0.0%)	3/41.8 (7.2%)	1/43.2 (2.3%)
Terminal rate	2/30 (6.7%)	0/27 (0.0%)	2/31 (6.5%)	1/35 (2.9%)
First incidence (days)	741	—	720	749 (T)
Poly-3 test	P=0.395N/P=0.329N	P=0.119N	P=0.643N	P=0.277N

Table A2c
Statistical Analysis of Primary Neoplasms in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	3/48 (6.3%)	0/49 (0.0%)	4/50 (8.0%)	1/50 (2.0%)
Adjusted rate	3/40.0 (7.5%)	0/39.6 (0.0%)	4/41.8 (9.6%)	1/43.2 (2.3%)
Terminal rate	2/30 (6.7%)	0/27 (0.0%)	3/31 (9.7%)	1/35 (2.9%)
First incidence (days)	741	—	720	749 (T)
Poly-3 test	P=0.357N/P=0.392N	P=0.119N	P=0.522	P=0.277N
All Organs: Benign Neoplasms				
Overall rate	33/50 (66.0%)	30/49 (61.2%)	25/50 (50.0%)	31/50 (62.0%)
Adjusted rate	33/45.4 (72.7%)	30/44.0 (68.2%)	25/44.2 (56.5%)	31/46.1 (67.2%)
Terminal rate	22/30 (73.3%)	17/27 (63.0%)	16/31 (51.6%)	23/35 (65.7%)
First incidence (days)	429	434	389	504
Poly-3 test	P=0.506N/P=0.204N	P=0.405N	P=0.076N	P=0.363N
All Organs: Malignant Neoplasms				
Overall rate	14/50 (28.0%)	13/49 (26.5%)	14/50 (28.0%)	19/50 (38.0%)
Adjusted rate	14/45.1 (31.1%)	13/43.9 (29.6%)	14/44.8 (31.3%)	19/47.7 (39.8%)
Terminal rate	3/30 (10.0%)	2/27 (7.4%)	5/31 (16.1%)	7/35 (20.0%)
First incidence (days)	422	347	539	504
Poly-3 test	P=0.156/P=0.194	P=0.534N	P=0.581	P=0.253

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Malignant pheochromocytoma occurred in one animal that also had benign pheochromocytoma.
[f] Not applicable; no neoplasms in animal group
[g] Value of statistic cannot be computed.

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	51		50		50		50	
Early deaths								
Moribund	9		13		17		12	
Natural deaths	7		6		3		4	
Survivors								
Died last week of study	1						2	
Terminal sacrifice	34		31		30		32	
Animals examined microscopically	51		50		50		50	
Alimentary System								
Esophagus	(51)		(50)		(50)		(50)	
Dilatation	1	(2%)						
Hyperkeratosis					3	(6%)	3	(6%)
Inflammation, suppurative	1	(2%)						
Intestine large, cecum	(44)		(45)		(47)		(46)	
Dilatation							1	(2%)
Hyperplasia, lymphoid					1	(2%)		
Inflammation, chronic active					1	(2%)		
Intestine large, colon	(45)		(47)		(48)		(47)	
Hyperplasia, lymphoid	1	(2%)						
Intestine large, rectum	(45)		(45)		(47)		(46)	
Intestine small, duodenum	(44)		(45)		(48)		(47)	
Intestine small, ileum	(43)		(45)		(47)		(46)	
Intestine small, jejunum	(43)		(45)		(46)		(46)	
Liver	(49)		(49)		(50)		(49)	
Angiectasis	3	(6%)	5	(10%)	1	(2%)	4	(8%)
Basophilic focus	1	(2%)	3	(6%)	3	(6%)	17	(35%)
Clear cell focus	1	(2%)					1	(2%)
Cyst multilocular					1	(2%)		
Degeneration, cystic	4	(8%)	3	(6%)	5	(10%)	8	(16%)
Eosinophilic focus	3	(6%)	5	(10%)	8	(16%)	15	(31%)
Focal cellular change					1	(2%)		
Hematopoietic cell proliferation	2	(4%)	1	(2%)	1	(2%)	3	(6%)
Hepatodiaphragmatic nodule	6	(12%)	1	(2%)	4	(8%)	6	(12%)
Infiltration cellular, lymphocyte			3	(6%)			1	(2%)
Inflammation, chronic					1	(2%)		
Inflammation, chronic active	13	(27%)	5	(10%)	8	(16%)	9	(18%)
Mixed cell focus					1	(2%)	1	(2%)
Necrosis			1	(2%)	2	(4%)	2	(4%)
Tension lipidosis					3	(6%)	3	(6%)
Vacuolization cytoplasmic	12	(24%)	4	(8%)	10	(20%)	13	(27%)
Bile duct, hyperplasia	2	(4%)	9	(18%)	7	(14%)		
Biliary tract, fibrosis	4	(8%)	2	(4%)	1	(2%)	1	(2%)
Left lateral lobe, developmental malformation	1	(2%)						
Oval cell, hyperplasia			1	(2%)				
Mesentery	(1)		(1)		(2)		(1)	
Fat, necrosis			1	(100%)	2	(100%)	1	(100%)
Oral mucosa	(3)		(2)		(4)		(9)	
Keratin cyst			1	(50%)			2	(22%)
Pancreas	(46)		(48)		(48)		(48)	
Basophilic focus							1	(2%)
Acinar cell, degeneration	40	(87%)	42	(88%)	40	(83%)	39	(81%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Alimentary System (continued)				
Salivary glands	(48)	(50)	(49)	(50)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active				1 (2%)
Acinus, degeneration				1 (2%)
Duct, dilatation				1 (2%)
Stomach, forestomach	(47)	(48)	(49)	(48)
Edema	1 (2%)	1 (2%)		
Keratin cyst	1 (2%)		1 (2%)	
Necrosis		1 (2%)		
Epithelium, hyperplasia	2 (4%)			
Stomach, glandular	(45)	(47)	(48)	(46)
Cyst		1 (2%)		
Edema				1 (2%)
Epithelium, hyperplasia				1 (2%)
Tongue	(0)	(0)	(0)	(0)
Cardiovascular System				
Heart	(51)	(50)	(50)	(50)
Cardiomyopathy	42 (82%)	34 (68%)	40 (80%)	37 (74%)
Inflammation, suppurative				1 (2%)
Metaplasia, osseous	1 (2%)			
Atrium, dilatation		3 (6%)		
Endocardium, hyperplasia			1 (2%)	
Endocrine System				
Adrenal cortex	(47)	(50)	(50)	(50)
Accessory adrenal cortical nodule	3 (6%)	1 (2%)	2 (4%)	1 (2%)
Angiectasis	1 (2%)	2 (4%)	3 (6%)	3 (6%)
Atrophy				1 (2%)
Cyst				1 (2%)
Degeneration, cystic	4 (9%)		4 (8%)	4 (8%)
Hyperplasia	8 (17%)	6 (12%)	7 (14%)	6 (12%)
Hypertrophy	3 (6%)	3 (6%)	1 (2%)	3 (6%)
Metaplasia, osseous		1 (2%)		
Pigmentation				1 (2%)
Vacuolization cytoplasmic	11 (23%)	9 (18%)	10 (20%)	11 (22%)
Adrenal medulla	(46)	(48)	(49)	(49)
Angiectasis		1 (2%)		
Hyperplasia	7 (15%)	4 (8%)	5 (10%)	13 (27%)
Bilateral, hyperplasia	1 (2%)			
Islets, pancreatic	(46)	(48)	(49)	(49)
Hyperplasia	12 (26%)	13 (27%)	5 (10%)	7 (14%)
Parathyroid gland	(49)	(46)	(46)	(46)
Hyperplasia	7 (14%)	5 (11%)	6 (13%)	9 (20%)
Pituitary gland	(48)	(50)	(50)	(50)
Pars distalis, cyst	3 (6%)	1 (2%)	5 (10%)	6 (12%)
Pars distalis, hyperplasia	11 (23%)	6 (12%)	12 (24%)	17 (34%)
Pars intermedia, cyst		1 (2%)		1 (2%)
Pars intermedia, hyperplasia		1 (2%)	1 (2%)	
Thyroid gland	(47)	(49)	(50)	(49)
Cyst, squamous	5 (11%)		16 (32%)	8 (16%)
C-cell, hyperplasia	6 (13%)	4 (8%)	6 (12%)	10 (20%)
Follicular cell, hyperplasia			1 (2%)	

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
General Body System								
Tissue NOS	(1)		(0)		(1)		(0)	
Degeneration, cystic	1	(100%)						
Infiltration cellular, plasma cell	1	(100%)						
Genital System								
Coagulating gland	(46)		(49)		(49)		(49)	
Atrophy			2	(4%)	2	(4%)	1	(2%)
Degeneration, cystic							1	(2%)
Developmental malformation	9	(20%)	10	(20%)	11	(22%)	14	(29%)
Inflammation, suppurative							2	(4%)
Lumen, dilatation			2	(4%)			1	(2%)
Ductus deferens	(0)		(0)		(0)		(0)	
Epididymis	(51)		(50)		(50)		(50)	
Atrophy	3	(6%)	5	(10%)	5	(10%)	2	(4%)
Granuloma sperm			1	(2%)				
Hypospermia	3	(6%)	5	(10%)	6	(12%)	3	(6%)
Penis	(0)		(1)		(0)		(0)	
Preputial gland	(51)		(50)		(50)		(50)	
Abscess	1	(2%)	1	(2%)	1	(2%)	2	(4%)
Atrophy			2	(4%)	5	(10%)	4	(8%)
Hyperplasia, basal cell					1	(2%)		
Infiltration cellular, lymphocyte	10	(20%)	17	(34%)	10	(20%)	10	(20%)
Inflammation, suppurative	20	(39%)	21	(42%)	23	(46%)	25	(50%)
Inflammation, chronic active	2	(4%)			2	(4%)	2	(4%)
Keratin cyst					1	(2%)		
Duct, dilatation	14	(27%)	9	(18%)	15	(30%)	14	(28%)
Prostate, dorsal/lateral lobe	(49)		(50)		(50)		(50)	
Atrophy			1	(2%)	1	(2%)		
Cyst	1	(2%)	1	(2%)				
Infiltration cellular, lymphocyte					1	(2%)		
Inflammation, suppurative	34	(69%)	33	(66%)	39	(78%)	38	(76%)
Inflammation, chronic active	2	(4%)	2	(4%)	4	(8%)	2	(4%)
Prostate, ventral lobe	(49)		(50)		(50)		(50)	
Atrophy			1	(2%)	1	(2%)		
Degeneration			1	(2%)				
Hyperplasia	7	(14%)	4	(8%)	3	(6%)	3	(6%)
Infiltration cellular, lymphocyte	2	(4%)	1	(2%)	4	(8%)	3	(6%)
Inflammation, suppurative	3	(6%)	3	(6%)	6	(12%)	3	(6%)
Inflammation, chronic active	1	(2%)	2	(4%)	2	(4%)	3	(6%)
Rete testes	(46)		(44)		(46)		(47)	
Dilatation	2	(4%)			2	(4%)	3	(6%)
Fibrosis	1	(2%)			2	(4%)	1	(2%)
Seminal vesicle	(43)		(46)		(47)		(47)	
Atrophy	4	(9%)	4	(9%)	4	(9%)	4	(9%)
Inflammation, suppurative			1	(2%)			1	(2%)
Epithelium, hyperplasia			1	(2%)				
Lumen, dilatation	2	(5%)	5	(11%)	2	(4%)	4	(9%)
Testes	(48)		(50)		(50)		(50)	
Malformation			1	(2%)				
Interstitial cell, hyperplasia					1	(2%)		
Seminiferous tubule, degeneration	19	(40%)	19	(38%)	19	(38%)	20	(40%)

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System								
Bone marrow	(46)		(48)		(48)		(48)	
Hypocellularity	1	(2%)			2	(4%)	1	(2%)
Erythroid cell, hyperplasia			1	(2%)	1	(2%)	2	(4%)
Myeloid cell, hyperplasia	6	(13%)	7	(15%)	8	(17%)	7	(15%)
Lymph node	(14)		(16)		(16)		(15)	
Degeneration, cystic	1	(7%)	2	(13%)				
Axillary, hyperplasia, lymphoid	1	(7%)	1	(6%)	1	(6%)	1	(7%)
Axillary, infiltration cellular, plasma cell					1	(6%)	2	(13%)
Inguinal, degeneration, cystic	1	(7%)						
Inguinal, hyperplasia, lymphoid	1	(7%)					1	(7%)
Inguinal, infiltration cellular, plasma cell	1	(7%)					2	(13%)
Lumbar, degeneration, cystic	8	(57%)	11	(69%)	8	(50%)	9	(60%)
Lumbar, hyperplasia, lymphoid	3	(21%)	2	(13%)	2	(13%)	1	(7%)
Lumbar, infiltration cellular, plasma cell	7	(50%)	5	(31%)	7	(44%)	5	(33%)
Mediastinal, infiltration cellular, plasma cell	1	(7%)	1	(6%)	1	(6%)		
Pancreatic, degeneration, cystic					1	(6%)		
Popliteal, degeneration, cystic			1	(6%)				
Popliteal, infiltration cellular, plasma cell			1	(6%)			1	(7%)
Renal, degeneration, cystic	1	(7%)	3	(19%)	3	(19%)	3	(20%)
Renal, hyperplasia, lymphoid							1	(7%)
Renal, infiltration cellular, plasma cell			1	(6%)			2	(13%)
Thoracic, degeneration, cystic					1	(6%)		
Lymph node, mandibular	(49)		(50)		(50)		(48)	
Degeneration, cystic	10	(20%)	11	(22%)	6	(12%)	11	(23%)
Hyperplasia, lymphoid	27	(55%)	26	(52%)	25	(50%)	29	(60%)
Infiltration cellular, plasma cell	38	(78%)	33	(66%)	37	(74%)	32	(67%)
Lymph node, mesenteric	(45)		(46)		(49)		(47)	
Degeneration, cystic	1	(2%)	3	(7%)	1	(2%)		
Hyperplasia, lymphoid	3	(7%)	1	(2%)	1	(2%)	3	(6%)
Infiltration cellular, plasma cell					1	(2%)		
Inflammation, granulomatous	9	(20%)	15	(33%)	14	(29%)	9	(19%)
Spleen	(48)		(49)		(49)		(49)	
Depletion lymphoid	1	(2%)	1	(2%)	1	(2%)	1	(2%)
Hematopoietic cell proliferation	29	(60%)	14	(29%)	23	(47%)	24	(49%)
Hyperplasia, lymphoid	5	(10%)	1	(2%)	2	(4%)		
Hyperplasia, stromal					1	(2%)	2	(4%)
Infiltration cellular, polymorphonuclear	1	(2%)	1	(2%)				
Inflammation, chronic active			1	(2%)				
Necrosis			2	(4%)			1	(2%)
Pigmentation	20	(42%)	21	(43%)	22	(45%)	30	(61%)
Capsule, cyst					1	(2%)		
Red pulp, hyperplasia					1	(2%)		
Thymus	(45)		(44)		(46)		(44)	
Atrophy	43	(96%)	40	(91%)	44	(96%)	41	(93%)
Cyst	1	(2%)			1	(2%)		
Epithelial cell, hyperplasia	2	(4%)	2	(5%)	2	(4%)	4	(9%)

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Integumentary System				
Mammary gland	(44)	(45)	(47)	(44)
Atypical focus		1 (2%)		
Ectasia	1 (2%)	3 (7%)	7 (15%)	4 (9%)
Lactation	3 (7%)	9 (20%)	10 (21%)	4 (9%)
Acinus, degeneration	29 (66%)	19 (42%)	24 (51%)	11 (25%)
Alveolus, hyperplasia	1 (2%)	4 (9%)	6 (13%)	18 (41%)
Duct, dilatation			1 (2%)	
Duct, ectasia			1 (2%)	
Duct, hyperplasia			2 (4%)	3 (7%)
Duct, inflammation, chronic active			1 (2%)	
Skin	(51)	(50)	(50)	(50)
Abscess			1 (2%)	
Cyst epithelial inclusion	2 (4%)	1 (2%)	2 (4%)	4 (8%)
Fibrosis	1 (2%)		1 (2%)	
Foreign body			1 (2%)	
Hyperkeratosis	1 (2%)	1 (2%)		1 (2%)
Inflammation, suppurative				2 (4%)
Inflammation, chronic active	24 (47%)	14 (28%)	21 (42%)	16 (32%)
Ulcer				1 (2%)
Epidermis, hyperplasia	1 (2%)			1 (2%)
Epidermis, inflammation, suppurative	1 (2%)			1 (2%)
Epidermis, necrosis		1 (2%)		
Musculoskeletal System				
Bone	(2)	(0)	(0)	(1)
Bone, femur	(51)	(50)	(50)	(50)
Nervous System				
Brain, brain stem	(49)	(50)	(50)	(50)
Compression	5 (10%)	7 (14%)	9 (18%)	4 (8%)
Hemorrhage			1 (2%)	
Brain, cerebellum	(49)	(50)	(50)	(50)
Angiectasis			1 (2%)	
Brain, cerebrum	(49)	(50)	(50)	(50)
Hydrocephalus	1 (2%)	3 (6%)	1 (2%)	1 (2%)
Infiltration cellular				1 (2%)
Respiratory System				
Lung	(46)	(46)	(48)	(48)
Congestion		1 (2%)		
Infiltration cellular, histiocyte	18 (39%)	18 (39%)	13 (27%)	11 (23%)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic active		1 (2%)	2 (4%)	1 (2%)
Metaplasia, osseous	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Alveolar epithelium, hyperplasia	7 (15%)	2 (4%)	4 (8%)	3 (6%)
Artery, mineralization				2 (4%)
Mediastinum, hemorrhage	1 (2%)			
Mediastinum, inflammation, suppurative	1 (2%)			
Mediastinum, necrosis	1 (2%)			

Table A3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Respiratory System (continued)				
Nose	(48)	(49)	(50)	(49)
Exudate		1 (2%)		
Foreign body				1 (2%)
Inflammation, suppurative	4 (8%)	6 (12%)	7 (14%)	7 (14%)
Inflammation, chronic active	3 (6%)		1 (2%)	
Metaplasia, squamous		1 (2%)		
Polyp, inflammatory		1 (2%)		
Mucosa, hyperkeratosis	2 (4%)			
Mucosa, keratin cyst			2 (4%)	1 (2%)
Vomeronasal organ, dilatation		3 (6%)	3 (6%)	
Trachea	(49)	(50)	(50)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Epithelium, hyperplasia				1 (2%)
Special Senses System				
Eye	(46)	(46)	(47)	(46)
Cataract	1 (2%)			1 (2%)
Hemorrhage	1 (2%)			
Inflammation, suppurative	1 (2%)			2 (4%)
Inflammation, chronic active				1 (2%)
Bilateral, retina, degeneration				1 (2%)
Bilateral, cataract	2 (4%)			
Cornea, inflammation, chronic active				1 (2%)
Harderian gland	(48)	(47)	(48)	(48)
Degeneration			1 (2%)	
Infiltration cellular, lymphocyte	3 (6%)		1 (2%)	5 (10%)
Acinus, degeneration			4 (8%)	1 (2%)
Lacrimal gland	(0)	(0)	(0)	(2)
Ectopic harderian				1 (50%)
Zymbal's gland	(1)	(0)	(0)	(2)
Urinary System				
Kidney	(46)	(49)	(48)	(49)
Cyst	20 (43%)	18 (37%)	21 (44%)	19 (39%)
Hydronephrosis			1 (2%)	
Mineralization			1 (2%)	4 (8%)
Nephropathy	42 (91%)	41 (84%)	46 (96%)	40 (82%)
Polycystic kidney			1 (2%)	
Capsule, inflammation, chronic active			1 (2%)	
Medulla, cyst	1 (2%)		1 (2%)	
Pelvis, inflammation, suppurative		2 (4%)	2 (4%)	1 (2%)
Renal tubule, hyperplasia			1 (2%)	1 (2%)
Transitional epithelium, hyperplasia	3 (7%)	2 (4%)	5 (10%)	
Urethra	(2)	(2)	(0)	(2)
Urinary bladder	(45)	(48)	(49)	(49)
Dilatation	1 (2%)			
Hemorrhage				2 (4%)
Inflammation, suppurative				2 (4%)
Transitional epithelium, hyperplasia				1 (2%)

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	9	15	7	14
Natural deaths	7	4	10	3
Survivors				
Died last week of study	1			
Terminal sacrifice	34	31	33	33
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(49)	(50)
Dilatation	1 (2%)			
Hyperkeratosis		2 (4%)		
Inflammation, suppurative	1 (2%)			
Intestine large, cecum	(44)	(48)	(43)	(46)
Dilatation		1 (2%)		
Hyperplasia, lymphoid			1 (2%)	
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active			1 (2%)	
Intestine large, colon	(45)	(49)	(43)	(48)
Dilatation		1 (2%)		
Hyperplasia, lymphoid	1 (2%)			
Polyarteritis				1 (2%)
Intestine large, rectum	(45)	(49)	(42)	(48)
Dilatation		1 (2%)		
Intestine small, duodenum	(44)	(49)	(42)	(47)
Intestine small, ileum	(43)	(48)	(41)	(45)
Dilatation		1 (2%)		
Intestine small, jejunum	(43)	(48)	(41)	(48)
Dilatation		1 (2%)		
Inflammation, chronic active			1 (2%)	
Liver	(49)	(50)	(48)	(49)
Angiectasis	3 (6%)			1 (2%)
Basophilic focus	1 (2%)	3 (6%)	11 (23%)	6 (12%)
Clear cell focus	1 (2%)			
Cyst				2 (4%)
Degeneration, cystic	4 (8%)	9 (18%)	10 (21%)	10 (20%)
Eosinophilic focus	3 (6%)	11 (22%)	5 (10%)	10 (20%)
Hematopoietic cell proliferation	2 (4%)		5 (10%)	2 (4%)
Hepatodiaphragmatic nodule	6 (12%)	2 (4%)	2 (4%)	3 (6%)
Infiltration cellular, lymphocyte		1 (2%)	1 (2%)	2 (4%)
Inflammation, suppurative			1 (2%)	
Inflammation, chronic active	13 (27%)	9 (18%)	7 (15%)	12 (24%)
Mixed cell focus		1 (2%)		
Necrosis		2 (4%)	3 (6%)	1 (2%)
Tension lipidosis			1 (2%)	2 (4%)
Vacuolization cytoplasmic	12 (24%)	8 (16%)	16 (33%)	12 (24%)
Bile duct, hyperplasia	2 (4%)	3 (6%)	7 (15%)	4 (8%)
Biliary tract, fibrosis	4 (8%)	6 (12%)	2 (4%)	
Capsule, fibrosis		1 (2%)		
Left lateral lobe, developmental malformation	1 (2%)			

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Alimentary System (continued)								
Mesentery	(1)		(1)		(2)		(0)	
Fat, necrosis			1	(100%)	1	(50%)		
Oral mucosa	(3)		(5)		(1)		(6)	
Keratin cyst			1	(20%)			1	(17%)
Gingival, inflammation, chronic active					1	(100%)		
Pancreas	(46)		(49)		(47)		(50)	
Polyarteritis							1	(2%)
Acinar cell, degeneration	40	(87%)	46	(94%)	39	(83%)	46	(92%)
Salivary glands	(48)		(50)		(48)		(49)	
Acinus, degeneration			1	(2%)				
Stomach, forestomach	(47)		(50)		(46)		(49)	
Edema	1	(2%)	1	(2%)	1	(2%)		
Hyperplasia, basal cell			1	(2%)				
Inflammation, suppurative			1	(2%)	1	(2%)		
Keratin cyst	1	(2%)						
Ulcer			1	(2%)	1	(2%)		
Epithelium, hyperplasia	2	(4%)	4	(8%)	1	(2%)	2	(4%)
Stomach, glandular	(45)		(50)		(45)		(49)	
Edema			1	(2%)				
Tongue	(0)		(0)		(0)		(1)	
Inflammation, chronic active							1	(100%)
Cardiovascular System								
Heart	(51)		(50)		(48)		(50)	
Cardiomyopathy	42	(82%)	40	(80%)	41	(85%)	41	(82%)
Inflammation, suppurative			1	(2%)				
Metaplasia, osseous	1	(2%)	1	(2%)			1	(2%)
Atrium, dilatation			2	(4%)	1	(2%)	2	(4%)
Atrium, thrombosis			1	(2%)				
Endocardium, hyperplasia					1	(2%)		
Ventricle, dilatation			1	(2%)	1	(2%)	1	(2%)
Endocrine System								
Adrenal cortex	(47)		(49)		(45)		(50)	
Accessory adrenal cortical nodule	3	(6%)	2	(4%)	2	(4%)		
Angiectasis	1	(2%)	4	(8%)	4	(9%)	2	(4%)
Atrophy			1	(2%)			1	(2%)
Degeneration, cystic	4	(9%)	6	(12%)	4	(9%)	6	(12%)
Hyperplasia	8	(17%)	1	(2%)	4	(9%)	14	(28%)
Hypertrophy	3	(6%)	4	(8%)	2	(4%)	6	(12%)
Vacuolization cytoplasmic	11	(23%)	11	(22%)	15	(33%)	15	(30%)
Adrenal medulla	(46)		(49)		(45)		(50)	
Atrophy			1	(2%)				
Cyst					1	(2%)		
Hyperplasia	7	(15%)	12	(24%)	16	(36%)	7	(14%)
Bilateral, hyperplasia	1	(2%)						
Islets, pancreatic	(46)		(49)		(47)		(50)	
Hyperplasia	12	(26%)	10	(20%)	10	(21%)	7	(14%)
Parathyroid gland	(49)		(50)		(44)		(50)	
Hyperplasia	7	(14%)	8	(16%)	7	(16%)	6	(12%)
Bilateral, hyperplasia							1	(2%)

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Endocrine System (continued)								
Pituitary gland	(48)		(50)		(47)		(49)	
Pars distalis, cyst	3	(6%)	6	(12%)	1	(2%)	1	(2%)
Pars distalis, hyperplasia	11	(23%)	8	(16%)	9	(19%)	10	(20%)
Pars intermedia, cyst			1	(2%)	1	(2%)	1	(2%)
Thyroid gland	(47)		(50)		(47)		(49)	
Cyst, squamous	5	(11%)	11	(22%)	11	(23%)	8	(16%)
Infiltration cellular, lymphocyte			1	(2%)				
C-cell, hyperplasia	6	(13%)	2	(4%)	7	(15%)	7	(14%)
General Body System								
Tissue NOS	(1)		(1)		(0)		(0)	
Degeneration, cystic	1	(100%)						
Infiltration cellular, plasma cell	1	(100%)						
Genital System								
Coagulating gland	(46)		(49)		(47)		(48)	
Atrophy			1	(2%)			2	(4%)
Developmental malformation	9	(20%)	14	(29%)	8	(17%)	8	(17%)
Hemorrhage			1	(2%)				
Inflammation, suppurative					2	(4%)	1	(2%)
Inflammation, chronic active					1	(2%)		
Lumen, dilatation							3	(6%)
Ductus deferens	(0)		(0)		(1)		(0)	
Inflammation, suppurative					1	(100%)		
Epididymis	(51)		(50)		(49)		(50)	
Atrophy	3	(6%)	4	(8%)	4	(8%)	8	(16%)
Hyperplasia			1	(2%)				
Hypospermia	3	(6%)	4	(8%)	4	(8%)	8	(16%)
Infiltration cellular, lymphocyte			1	(2%)	1	(2%)		
Polyarteritis							1	(2%)
Penis	(0)		(1)		(1)		(0)	
Dilatation					1	(100%)		
Preputial gland	(51)		(50)		(50)		(50)	
Abscess	1	(2%)			1	(2%)	1	(2%)
Atrophy			6	(12%)	3	(6%)	6	(12%)
Cyst			1	(2%)				
Infiltration cellular, lymphocyte	10	(20%)	9	(18%)	9	(18%)	8	(16%)
Inflammation, suppurative	20	(39%)	25	(50%)	28	(56%)	23	(46%)
Inflammation, chronic active	2	(4%)	2	(4%)			3	(6%)
Keratin cyst							2	(4%)
Polyarteritis							1	(2%)
Duct, dilatation	14	(27%)	12	(24%)	16	(32%)	12	(24%)
Prostate, dorsal/lateral lobe	(49)		(50)		(49)		(50)	
Atrophy							1	(2%)
Cyst	1	(2%)	1	(2%)				
Degeneration							1	(2%)
Hemorrhage			1	(2%)				
Infiltration cellular, lymphocyte					2	(4%)		
Inflammation, suppurative	34	(69%)	34	(68%)	37	(76%)	35	(70%)
Inflammation, chronic active	2	(4%)	4	(8%)	2	(4%)	3	(6%)

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Prostate, ventral lobe	(49)		(50)		(49)		(50)	
Hemorrhage			1	(2%)				
Hyperplasia	7	(14%)	7	(14%)	5	(10%)	3	(6%)
Infiltration cellular, lymphocyte	2	(4%)	2	(4%)	4	(8%)	2	(4%)
Inflammation, suppurative	3	(6%)	3	(6%)	5	(10%)	3	(6%)
Inflammation, chronic active	1	(2%)	6	(12%)	4	(8%)	4	(8%)
Rete testes	(46)		(47)		(43)		(46)	
Dilatation	2	(4%)	2	(4%)	2	(5%)	5	(11%)
Fibrosis	1	(2%)	1	(2%)	2	(5%)		
Seminal vesicle	(43)		(49)		(43)		(48)	
Atrophy	4	(9%)	1	(2%)	1	(2%)	5	(10%)
Dilatation			1	(2%)				
Hemorrhage			1	(2%)				
Inflammation, suppurative					1	(2%)	2	(4%)
Epithelium, hyperplasia							1	(2%)
Lumen, dilatation	2	(5%)	5	(10%)	5	(12%)		
Testes	(48)		(50)		(48)		(50)	
Granuloma sperm			1	(2%)				
Polyarteritis							1	(2%)
Seminiferous tubule, degeneration	19	(40%)	23	(46%)	29	(60%)	23	(46%)
Hematopoietic System								
Bone marrow	(46)		(50)		(46)		(49)	
Hypocellularity	1	(2%)	1	(2%)	1	(2%)		
Erythroid cell, hyperplasia							1	(2%)
Myeloid cell, hyperplasia	6	(13%)	6	(12%)	7	(15%)	7	(14%)
Lymph node	(14)		(19)		(14)		(19)	
Degeneration, cystic	1	(7%)						
Axillary, hyperplasia, lymphoid	1	(7%)	1	(5%)			2	(11%)
Axillary, infiltration cellular, plasma cell			2	(11%)	1	(7%)	2	(11%)
Hemal, degeneration, cystic					1	(7%)		
Hemal, inflammation, chronic active					1	(7%)		
Hemal, necrosis					1	(7%)		
Inguinal, degeneration, cystic	1	(7%)					1	(5%)
Inguinal, hyperplasia, lymphoid	1	(7%)	1	(5%)	1	(7%)	1	(5%)
Inguinal, infiltration cellular, plasma cell	1	(7%)	1	(5%)	1	(7%)		
Lumbar, degeneration, cystic	8	(57%)	9	(47%)	8	(57%)	17	(89%)
Lumbar, hyperplasia, lymphoid	3	(21%)	3	(16%)	6	(43%)	4	(21%)
Lumbar, infiltration cellular, plasma cell	7	(50%)	9	(47%)	10	(71%)	9	(47%)
Mediastinal, degeneration, cystic							1	(5%)
Mediastinal, infiltration cellular, histiocyte							1	(5%)
Mediastinal, infiltration cellular, plasma cell	1	(7%)			1	(7%)		
Pancreatic, hyperplasia, lymphoid							1	(5%)
Popliteal, infiltration cellular, plasma cell							1	(5%)
Renal, degeneration, cystic	1	(7%)	1	(5%)	3	(21%)	4	(21%)
Renal, hyperplasia, lymphoid			1	(5%)	1	(7%)		
Renal, infiltration cellular, plasma cell			2	(11%)	3	(21%)	2	(11%)
Thoracic, infiltration cellular, plasma cell							1	(5%)
Lymph node, mandibular	(49)		(50)		(47)		(50)	
Degeneration, cystic	10	(20%)	6	(12%)	4	(9%)	15	(30%)
Hyperplasia, lymphoid	27	(55%)	24	(48%)	26	(55%)	29	(58%)
Infiltration cellular, plasma cell	38	(78%)	39	(78%)	32	(68%)	33	(66%)

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System (continued)								
Lymph node, mesenteric	(45)		(50)		(44)		(48)	
Degeneration, cystic	1	(2%)	1	(2%)	1	(2%)	2	(4%)
Hyperplasia, lymphoid	3	(7%)	1	(2%)	5	(11%)	8	(17%)
Infiltration cellular, mast cell					1	(2%)		
Infiltration cellular, plasma cell					1	(2%)		
Inflammation, granulomatous	9	(20%)	14	(28%)	10	(23%)	7	(15%)
Spleen	(48)		(50)		(47)		(50)	
Depletion lymphoid	1	(2%)	2	(4%)			2	(4%)
Hematopoietic cell proliferation	29	(60%)	16	(32%)	19	(40%)	21	(42%)
Hyperplasia, lymphoid	5	(10%)	5	(10%)			2	(4%)
Hyperplasia, stromal					1	(2%)		
Infiltration cellular, polymorphonuclear	1	(2%)	1	(2%)	1	(2%)	1	(2%)
Necrosis					1	(2%)		
Pigmentation	20	(42%)	18	(36%)	18	(38%)	16	(32%)
Thymus	(45)		(46)		(40)		(47)	
Atrophy	43	(96%)	42	(91%)	39	(98%)	47	(100%)
Cyst	1	(2%)						
Cyst, squamous			1	(2%)				
Degeneration, cystic			1	(2%)				
Epithelial cell, hyperplasia	2	(4%)	2	(4%)	2	(5%)	1	(2%)
Integumentary System								
Mammary gland	(44)		(45)		(47)		(48)	
Atypical focus					1	(2%)	1	(2%)
Ectasia	1	(2%)	3	(7%)	12	(26%)	4	(8%)
Fibrosis							1	(2%)
Lactation	3	(7%)	5	(11%)	14	(30%)	9	(19%)
Acinus, degeneration	29	(66%)	24	(53%)	17	(36%)	11	(23%)
Alveolus, hyperplasia	1	(2%)	2	(4%)	6	(13%)	14	(29%)
Duct, dilatation							1	(2%)
Duct, hyperplasia					1	(2%)	3	(6%)
Skin	(51)		(50)		(49)		(50)	
Abscess					1	(2%)	1	(2%)
Cyst epithelial inclusion	2	(4%)	2	(4%)	2	(4%)	4	(8%)
Fibrosis	1	(2%)						
Hyperkeratosis	1	(2%)	2	(4%)	1	(2%)		
Inflammation, pyogranulomatous			1	(2%)				
Inflammation, suppurative			1	(2%)	1	(2%)	1	(2%)
Inflammation, chronic active	24	(47%)	28	(56%)	28	(57%)	29	(58%)
Keratin cyst							1	(2%)
Dermis, fibrosis			1	(2%)				
Dermis, inflammation, chronic active			1	(2%)	2	(4%)		
Dermis, necrosis			1	(2%)				
Epidermis, hyperplasia	1	(2%)			1	(2%)		
Epidermis, inflammation, suppurative	1	(2%)						
Hair follicle, hemorrhage					1	(2%)		
Musculoskeletal System								
Bone	(2)		(0)		(0)		(1)	
Bone, femur	(51)		(50)		(50)		(50)	

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Nervous System				
Brain, brain stem	(49)	(50)	(48)	(50)
Compression	5 (10%)	7 (14%)	10 (21%)	7 (14%)
Gliosis			1 (2%)	
Hemorrhage		1 (2%)	3 (6%)	
Brain, cerebellum	(49)	(50)	(48)	(50)
Gliosis				1 (2%)
Hemorrhage				1 (2%)
Hydrocephalus		2 (4%)		
Brain, cerebrum	(49)	(50)	(48)	(50)
Hydrocephalus	1 (2%)	3 (6%)	4 (8%)	4 (8%)
Meninges, hyperplasia				1 (2%)
Respiratory System				
Lung	(46)	(50)	(45)	(50)
Hemorrhage				1 (2%)
Infiltration cellular, histiocyte	18 (39%)	23 (46%)	17 (38%)	11 (22%)
Inflammation, chronic		2 (4%)		
Inflammation, chronic active		2 (4%)	1 (2%)	1 (2%)
Metaplasia, osseous	1 (2%)		2 (4%)	1 (2%)
Thrombosis			1 (2%)	
Alveolar epithelium, hyperplasia	7 (15%)	6 (12%)	9 (20%)	3 (6%)
Artery, mineralization		2 (4%)	3 (7%)	2 (4%)
Mediastinum, hemorrhage	1 (2%)			
Mediastinum, inflammation, suppurative	1 (2%)			
Mediastinum, necrosis	1 (2%)			
Nose	(48)	(49)	(48)	(50)
Exudate		1 (2%)		
Inflammation, suppurative	4 (8%)	6 (12%)	2 (4%)	5 (10%)
Inflammation, chronic active	3 (6%)	3 (6%)	3 (6%)	
Mucosa, hyperkeratosis	2 (4%)	2 (4%)	1 (2%)	
Mucosa, hyperplasia		1 (2%)	1 (2%)	
Mucosa, keratin cyst		1 (2%)	1 (2%)	
Vomeronasal organ, dilatation		1 (2%)		
Trachea	(49)	(50)	(46)	(50)
Special Senses System				
Eye	(46)	(49)	(45)	(48)
Cataract	1 (2%)			2 (4%)
Hemorrhage	1 (2%)			
Inflammation, suppurative	1 (2%)	1 (2%)		
Inflammation, chronic active				1 (2%)
Bilateral, cataract	2 (4%)			
Retina, degeneration			1 (2%)	1 (2%)
Harderian gland	(48)	(50)	(46)	(50)
Infiltration cellular, lymphocyte	3 (6%)	3 (6%)	1 (2%)	8 (16%)
Polyarteritis				1 (2%)
Acinus, degeneration		2 (4%)		5 (10%)
Lacrimal gland	(0)	(0)	(1)	(1)
Ectopic harderian			1 (100%)	
Zymbal's gland	(1)	(1)	(0)	(0)
Inflammation, suppurative		1 (100%)		

Table A3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Urinary System								
Kidney	(46)		(49)		(47)		(49)	
Cyst	20	(43%)	25	(51%)	24	(51%)	23	(47%)
Hydronephrosis			2	(4%)	2	(4%)		
Inflammation, suppurative			1	(2%)	1	(2%)		
Mineralization			1	(2%)			4	(8%)
Nephropathy	42	(91%)	47	(96%)	44	(94%)	46	(94%)
Polycystic kidney			1	(2%)				
Medulla, cyst	1	(2%)	1	(2%)				
Pelvis, hemorrhage			1	(2%)				
Pelvis, inflammation, suppurative			2	(4%)			1	(2%)
Renal tubule, hyperplasia			1	(2%)			1	(2%)
Transitional epithelium, hyperplasia	3	(7%)	1	(2%)			2	(4%)
Urethra	(2)		(1)		(5)		(1)	
Hemorrhage			1	(100%)				
Urinary bladder	(45)		(49)		(45)		(49)	
Dilatation	1	(2%)	2	(4%)	3	(7%)		
Hemorrhage			1	(2%)	1	(2%)		
Inflammation, suppurative			1	(2%)	1	(2%)		
Inflammation, chronic active			1	(2%)				
Transitional epithelium, hyperplasia			2	(4%)				

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	50	49	50	50
Early deaths				
Moribund	13	16	13	12
Natural deaths	7	6	6	3
Survivors				
Terminal sacrifice	30	27	31	35
Animals examined microscopically	50	49	50	50
Alimentary System				
Esophagus	(50)	(48)	(50)	(50)
Dilatation			1 (2%)	
Hyperkeratosis	3 (6%)	4 (8%)		1 (2%)
Intestine large, cecum	(45)	(43)	(47)	(47)
Dilatation			1 (2%)	
Edema		1 (2%)		
Inflammation, suppurative		1 (2%)		
Inflammation, chronic active				1 (2%)
Necrosis				1 (2%)
Polyarteritis				1 (2%)
Intestine large, colon	(45)	(43)	(48)	(48)
Intestine large, rectum	(45)	(44)	(48)	(48)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation, chronic active				1 (2%)
Necrosis				1 (2%)
Intestine small, duodenum	(44)	(43)	(47)	(47)
Intestine small, ileum	(44)	(38)	(47)	(47)
Inflammation, chronic active				1 (2%)
Necrosis				1 (2%)
Ulcer				1 (2%)
Intestine small, jejunum	(44)	(41)	(45)	(46)
Lymphoid tissue, hyperplasia			1 (2%)	
Liver	(49)	(47)	(50)	(49)
Angiectasis	3 (6%)	1 (2%)	1 (2%)	5 (10%)
Basophilic focus	9 (18%)	7 (15%)	5 (10%)	7 (14%)
Congestion			1 (2%)	
Cyst	1 (2%)	1 (2%)		
Cyst multilocular	2 (4%)			1 (2%)
Cytomegaly			1 (2%)	
Degeneration, cystic	10 (20%)	7 (15%)	6 (12%)	5 (10%)
Eosinophilic focus	8 (16%)	5 (11%)	4 (8%)	5 (10%)
Hematopoietic cell proliferation	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Hepatodiaphragmatic nodule	8 (16%)	2 (4%)	4 (8%)	7 (14%)
Hyperplasia				1 (2%)
Hyperplasia, regenerative	1 (2%)	1 (2%)		
Infiltration cellular, lymphocyte	2 (4%)	2 (4%)	4 (8%)	
Inflammation, suppurative				1 (2%)
Inflammation, chronic active	6 (12%)	7 (15%)	3 (6%)	4 (8%)
Karyomegaly			1 (2%)	
Mixed cell focus	1 (2%)			
Necrosis	2 (4%)	4 (9%)	2 (4%)	3 (6%)
Pigmentation	1 (2%)			
Tension lipidosis	3 (6%)	1 (2%)	2 (4%)	2 (4%)
Vacuolization cytoplasmic	6 (12%)	7 (15%)	6 (12%)	3 (6%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Alimentary System (continued)								
Liver (continued)	(49)		(47)		(50)		(49)	
Bile duct, hyperplasia	8	(16%)	9	(19%)	11	(22%)	5	(10%)
Biliary tract, cyst	1	(2%)			1	(2%)	1	(2%)
Biliary tract, fibrosis	7	(14%)	4	(9%)	7	(14%)	3	(6%)
Capsule, inflammation, chronic active			1	(2%)				
Left lateral lobe, developmental malformation	1	(2%)						
Oval cell, hyperplasia					1	(2%)	1	(2%)
Mesentery	(4)		(0)		(6)		(3)	
Abscess					1	(17%)		
Fat, necrosis	4	(100%)			4	(67%)	3	(100%)
Oral mucosa	(3)		(8)		(4)		(6)	
Inflammation, suppurative	1	(33%)			1	(25%)		
Keratin cyst			3	(38%)	3	(75%)	1	(17%)
Gingival, inflammation, chronic active			1	(13%)				
Pancreas	(47)		(48)		(49)		(49)	
Infiltration cellular, lymphocyte							1	(2%)
Polyarteritis	1	(2%)	1	(2%)			1	(2%)
Acinar cell, degeneration	42	(89%)	39	(81%)	36	(73%)	40	(82%)
Salivary glands	(48)		(49)		(49)		(50)	
Infiltration cellular, lymphocyte							1	(2%)
Stomach, forestomach	(46)		(48)		(49)		(49)	
Edema					2	(4%)		
Hyperkeratosis	2	(4%)	1	(2%)	1	(2%)	1	(2%)
Inflammation, chronic active					1	(2%)		
Epithelium, hyperplasia	1	(2%)	1	(2%)	2	(4%)		
Stomach, glandular	(45)		(46)		(47)		(47)	
Infiltration cellular, lymphocyte							1	(2%)
Cardiovascular System								
Heart	(50)		(49)		(50)		(50)	
Cardiomyopathy	40	(80%)	37	(76%)	38	(76%)	42	(84%)
Inflammation, suppurative							1	(2%)
Metaplasia, osseous			2	(4%)				
Polyarteritis			1	(2%)				
Atrium, dilatation	2	(4%)	2	(4%)	1	(2%)		
Endocardium, hyperplasia			1	(2%)	1	(2%)		
Ventricle, dilatation	1	(2%)	1	(2%)	1	(2%)	1	(2%)
Endocrine System								
Adrenal cortex	(49)		(49)		(49)		(50)	
Accessory adrenal cortical nodule	1	(2%)			4	(8%)	1	(2%)
Angiectasis	3	(6%)	1	(2%)	1	(2%)	2	(4%)
Degeneration, cystic	2	(4%)	4	(8%)	5	(10%)	1	(2%)
Hyperplasia	7	(14%)	7	(14%)	3	(6%)	4	(8%)
Hypertrophy	3	(6%)	5	(10%)	4	(8%)	4	(8%)
Vacuolization cytoplasmic	7	(14%)	8	(16%)	9	(18%)	4	(8%)
Bilateral, hyperplasia	1	(2%)						
Adrenal medulla	(49)		(48)		(49)		(50)	
Hyperplasia	15	(31%)	9	(19%)	8	(16%)	10	(20%)
Islets, pancreatic	(48)		(48)		(50)		(50)	
Degeneration							1	(2%)
Hyperplasia	7	(15%)	13	(27%)	8	(16%)	10	(20%)

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Endocrine System (continued)								
Parathyroid gland	(47)		(44)		(50)		(46)	
Cyst			1	(2%)				
Hyperplasia	4	(9%)	2	(5%)	7	(14%)	6	(13%)
Bilateral, hyperplasia			1	(2%)	1	(2%)		
Pituitary gland	(49)		(48)		(49)		(50)	
Inflammation, suppurative			1	(2%)				
Necrosis			1	(2%)				
Pars distalis, cyst	3	(6%)	5	(10%)	6	(12%)	6	(12%)
Pars distalis, hyperplasia	8	(16%)	18	(38%)	15	(31%)	13	(26%)
Pars intermedia, cyst			3	(6%)	2	(4%)	2	(4%)
Thyroid gland	(48)		(49)		(50)		(50)	
Cyst, squamous	12	(25%)	12	(24%)	13	(26%)	9	(18%)
Inflammation, chronic active			1	(2%)				
C-cell, hyperplasia	4	(8%)	6	(12%)	4	(8%)	3	(6%)
Follicle, cyst					1	(2%)		
Follicular cell, hyperplasia					1	(2%)		
General Body System								
Tissue NOS	(0)		(0)		(0)		(1)	
Genital System								
Coagulating gland	(47)		(49)		(48)		(50)	
Atrophy	1	(2%)	1	(2%)	1	(2%)	2	(4%)
Developmental malformation	6	(13%)	5	(10%)	6	(13%)	9	(18%)
Inflammation, suppurative							1	(2%)
Inflammation, chronic active	1	(2%)						
Lumen, dilatation			2	(4%)			1	(2%)
Ductus deferens	(0)		(0)		(0)		(1)	
Dilatation							1	(100%)
Inflammation, chronic active							1	(100%)
Epididymis	(49)		(49)		(50)		(50)	
Atrophy	4	(8%)	7	(14%)	7	(14%)	5	(10%)
Granuloma sperm							1	(2%)
Hypospermia	4	(8%)	9	(18%)	8	(16%)	7	(14%)
Infiltration cellular, lymphocyte					2	(4%)		
Polyarteritis							1	(2%)
Preputial gland	(49)		(49)		(50)		(49)	
Abscess			1	(2%)	2	(4%)	2	(4%)
Atrophy	1	(2%)	4	(8%)	3	(6%)	2	(4%)
Infiltration cellular, lymphocyte	10	(20%)	8	(16%)	9	(18%)	7	(14%)
Inflammation, suppurative	21	(43%)	19	(39%)	24	(48%)	22	(45%)
Inflammation, chronic active	1	(2%)	4	(8%)	1	(2%)	2	(4%)
Keratin cyst							2	(4%)
Duct, dilatation	12	(24%)	8	(16%)	11	(22%)	12	(24%)
Prostate, dorsal/lateral lobe	(50)		(48)		(50)		(50)	
Atrophy			1	(2%)			2	(4%)
Cyst	1	(2%)			2	(4%)		
Dilatation							1	(2%)
Hemorrhage							1	(2%)
Infiltration cellular, lymphocyte			2	(4%)				
Inflammation, suppurative	32	(64%)	30	(63%)	34	(68%)	36	(72%)
Inflammation, chronic active	11	(22%)	5	(10%)	7	(14%)	5	(10%)

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Genital System (continued)				
Prostate, ventral lobe	(49)	(48)	(50)	(50)
Atrophy		1 (2%)		2 (4%)
Dilatation				1 (2%)
Hemorrhage				1 (2%)
Hyperplasia	4 (8%)	3 (6%)	2 (4%)	3 (6%)
Infiltration cellular, lymphocyte	1 (2%)	4 (8%)	2 (4%)	3 (6%)
Inflammation, suppurative	3 (6%)	1 (2%)	4 (8%)	2 (4%)
Inflammation, chronic active	5 (10%)	7 (15%)	4 (8%)	5 (10%)
Rete testes	(46)	(45)	(43)	(44)
Dilatation	4 (9%)	3 (7%)	4 (9%)	2 (5%)
Fibrosis	1 (2%)	2 (4%)	1 (2%)	
Seminal vesicle	(44)	(44)	(47)	(47)
Atrophy	7 (16%)	5 (11%)	4 (9%)	10 (21%)
Inflammation, suppurative				1 (2%)
Inflammation, chronic active	1 (2%)			
Lumen, dilatation	2 (5%)	4 (9%)	3 (6%)	6 (13%)
Testes	(50)	(49)	(50)	(50)
Polyarteritis			1 (2%)	1 (2%)
Seminiferous tubule, degeneration	19 (38%)	20 (41%)	17 (34%)	19 (38%)
Hematopoietic System				
Bone marrow	(49)	(49)	(50)	(50)
Hypocellularity		1 (2%)		2 (4%)
Erythroid cell, hyperplasia				2 (4%)
Myeloid cell, hyperplasia	3 (6%)	6 (12%)	12 (24%)	7 (14%)
Sinusoid, dilatation			1 (2%)	
Lymph node	(14)	(16)	(13)	(16)
Axillary, degeneration, cystic				1 (6%)
Axillary, hyperplasia, lymphoid				1 (6%)
Axillary, infiltration cellular, plasma cell				1 (6%)
Hemal, degeneration, cystic				1 (6%)
Inguinal, degeneration, cystic		1 (6%)		
Inguinal, infiltration cellular, plasma cell		1 (6%)		
Lumbar, degeneration, cystic	10 (71%)	8 (50%)	7 (54%)	10 (63%)
Lumbar, hyperplasia, lymphoid	4 (29%)	4 (25%)	2 (15%)	3 (19%)
Lumbar, infiltration cellular, plasma cell	4 (29%)	8 (50%)	5 (38%)	5 (31%)
Mediastinal, degeneration, cystic			1 (8%)	
Pancreatic, hyperplasia, lymphoid				1 (6%)
Pancreatic, inflammation, granulomatous				1 (6%)
Pancreatic, necrosis				1 (6%)
Popliteal, hyperplasia, lymphoid		2 (13%)	2 (15%)	2 (13%)
Popliteal, infiltration cellular, plasma cell		2 (13%)	2 (15%)	2 (13%)
Renal, degeneration, cystic	2 (14%)	6 (38%)	3 (23%)	2 (13%)
Renal, hyperplasia, lymphoid		1 (6%)		
Renal, infiltration cellular, plasma cell	1 (7%)	3 (19%)		
Lymph node, mandibular	(46)	(48)	(50)	(49)
Degeneration, cystic	10 (22%)	11 (23%)	11 (22%)	7 (14%)
Hyperplasia, lymphoid	24 (52%)	27 (56%)	29 (58%)	28 (57%)
Infiltration cellular, plasma cell	28 (61%)	26 (54%)	30 (60%)	28 (57%)
Necrosis				1 (2%)
Lymph node, mesenteric	(44)	(46)	(49)	(48)
Hyperplasia, lymphoid	2 (5%)	1 (2%)	1 (2%)	1 (2%)
Infiltration cellular, mast cell	1 (2%)	1 (2%)		1 (2%)
Inflammation, granulomatous	11 (25%)	6 (13%)	10 (20%)	8 (17%)
Pigmentation				1 (2%)

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System (continued)								
Spleen	(49)		(48)		(49)		(50)	
Depletion lymphoid	1	(2%)			1	(2%)	1	(2%)
Hematopoietic cell proliferation	13	(27%)	13	(27%)	8	(16%)	15	(30%)
Hyperplasia, lymphoid	2	(4%)	3	(6%)	4	(8%)	2	(4%)
Hyperplasia, stromal					2	(4%)		
Infiltration cellular, plasma cell							1	(2%)
Infiltration cellular, polymorphonuclear			1	(2%)	1	(2%)	3	(6%)
Inflammation, suppurative			1	(2%)				
Necrosis			1	(2%)			2	(4%)
Pigmentation	30	(61%)	28	(58%)	29	(59%)	27	(54%)
Polyarteritis	1	(2%)	1	(2%)				
Thymus	(47)		(45)		(47)		(45)	
Atrophy	45	(96%)	41	(91%)	45	(96%)	43	(96%)
Cyst			2	(4%)	1	(2%)		
Polyarteritis			1	(2%)				
Epithelial cell, hyperplasia	8	(17%)	7	(16%)	1	(2%)	4	(9%)
Integumentary System								
Mammary gland	(42)		(42)		(40)		(45)	
Atypical focus			1	(2%)	1	(3%)		
Cyst			1	(2%)				
Ectasia	3	(7%)	4	(10%)	6	(15%)	4	(9%)
Fibrosis					1	(3%)		
Galactocele			2	(5%)			2	(4%)
Lactation	7	(17%)	6	(14%)	7	(18%)	8	(18%)
Polyarteritis			1	(2%)				
Acinus, degeneration	25	(60%)	21	(50%)	17	(43%)	26	(58%)
Alveolus, hyperplasia	2	(5%)	6	(14%)	4	(10%)	9	(20%)
Duct, hyperplasia							1	(2%)
Skin	(50)		(49)		(50)		(50)	
Abscess	1	(2%)	1	(2%)				
Cyst epithelial inclusion	4	(8%)	4	(8%)	2	(4%)	2	(4%)
Fibrosis	1	(2%)	1	(2%)				
Hyperkeratosis	1	(2%)						
Inflammation, chronic	1	(2%)						
Inflammation, chronic active	20	(40%)	17	(35%)	10	(20%)	13	(26%)
Keratin cyst							1	(2%)
Musculoskeletal System								
Bone	(0)		(1)		(1)		(0)	
Skeletal muscle	(3)		(4)		(3)		(0)	
Degeneration	1	(33%)	2	(50%)	1	(33%)		
Nervous System								
Brain	(0)		(0)		(0)		(1)	
Brain, brain stem	(49)		(49)		(50)		(50)	
Compression	7	(14%)	9	(18%)	9	(18%)	2	(4%)
Hemorrhage	1	(2%)						
Meninges, inflammation, suppurative			1	(2%)				
Brain, cerebellum	(49)		(49)		(50)		(50)	
Brain, cerebrum	(49)		(49)		(50)		(50)	
Gliosis					1	(2%)	1	(2%)
Hydrocephalus	1	(2%)	2	(4%)	1	(2%)	3	(6%)

Table A3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Respiratory System								
Lung	(47)		(48)		(48)		(50)	
Infiltration cellular, histiocyte	19	(40%)	19	(40%)	14	(29%)	14	(28%)
Infiltration cellular, lymphocyte					1	(2%)	1	(2%)
Inflammation, chronic active			2	(4%)			1	(2%)
Metaplasia, osseous	1	(2%)	1	(2%)	1	(2%)		
Polyarteritis					1	(2%)		
Alveolar epithelium, hyperplasia	3	(6%)	8	(17%)	3	(6%)	4	(8%)
Nose	(49)		(48)		(49)		(50)	
Infiltration cellular, lymphocyte							1	(2%)
Inflammation, suppurative	2	(4%)	6	(13%)	8	(16%)	3	(6%)
Inflammation, chronic active	2	(4%)	2	(4%)	1	(2%)	1	(2%)
Mucosa, keratin cyst	1	(2%)	1	(2%)	1	(2%)	1	(2%)
Vomeronasal organ, dilatation			2	(4%)				
Trachea	(49)		(49)		(50)		(50)	
Special Senses System								
Ear	(0)		(0)		(1)		(1)	
Eye	(46)		(47)		(48)		(50)	
Cataract			1	(2%)				
Inflammation, suppurative	1	(2%)	1	(2%)				
Inflammation, chronic active	1	(2%)	2	(4%)	1	(2%)		
Bilateral, cataract							3	(6%)
Cornea, hemorrhage			1	(2%)				
Retina, degeneration							1	(2%)
Harderian gland	(49)		(48)		(50)		(50)	
Infiltration cellular, lymphocyte	2	(4%)	1	(2%)	1	(2%)	4	(8%)
Inflammation, suppurative	1	(2%)	1	(2%)			1	(2%)
Acinus, degeneration	3	(6%)	3	(6%)	3	(6%)		
Lacrimal gland	(1)		(1)		(2)		(1)	
Ectopic harderian	1	(100%)	1	(100%)	2	(100%)	1	(100%)
Zymbal's gland	(1)		(0)		(2)		(0)	
Inflammation, suppurative					1	(50%)		
Urinary System								
Kidney	(49)		(48)		(50)		(50)	
Cyst	18	(37%)	19	(40%)	18	(36%)	27	(54%)
Hemorrhage							1	(2%)
Hydronephrosis	2	(4%)	3	(6%)	3	(6%)	3	(6%)
Infiltration cellular, lymphocyte							1	(2%)
Inflammation, suppurative							1	(2%)
Nephropathy	46	(94%)	44	(92%)	44	(88%)	48	(96%)
Polyarteritis	1	(2%)	1	(2%)				
Polycystic kidney							1	(2%)
Pelvis, inflammation, suppurative					1	(2%)		
Renal tubule, hyperplasia					1	(2%)		
Transitional epithelium, hyperplasia			1	(2%)	4	(8%)	1	(2%)
Urinary bladder	(49)		(48)		(49)		(50)	
Dilatation							1	(2%)
Hemorrhage					1	(2%)		
Inflammation, suppurative					1	(2%)		
Inflammation, chronic active	1	(2%)						

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE 2-YEAR FEED STUDY OF ETHINYL ESTRADIOL

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Table B1a
Summary of the Incidence of Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	24	22	27	17
Natural deaths		5	3	8
Survivors				
Died last week of study	1		1	
Terminal sacrifice	26	23	19	25
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(49)	(50)
Intestine large, cecum	(51)	(50)	(49)	(49)
Lymphoid tissue, leukemia mononuclear				1 (2%)
Intestine large, colon	(51)	(50)	(49)	(49)
Lymphoid tissue, leukemia mononuclear				1 (2%)
Intestine large, rectum	(51)	(50)	(49)	(50)
Granular cell tumor malignant, metastatic, vagina				1 (2%)
Leiomyosarcoma, metastatic, uterus		1 (2%)		
Intestine small, duodenum	(51)	(50)	(49)	(49)
Intestine small, ileum	(51)	(50)	(48)	(49)
Intestine small, jejunum	(51)	(50)	(47)	(47)
Liver	(51)	(50)	(49)	(50)
Cholangiocarcinoma	1 (2%)			
Hepatocellular adenoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Mesentery	(2)	(2)	(0)	(1)
Leiomyosarcoma, metastatic, uterus		1 (50%)		
Oral mucosa	(3)	(1)	(4)	(4)
Squamous cell carcinoma	3 (100%)	1 (100%)	1 (25%)	2 (50%)
Pancreas	(51)	(50)	(49)	(49)
Acinar cell, adenocarcinoma				1 (2%)
Acinar cell, adenoma		1 (2%)		
Salivary glands	(51)	(49)	(49)	(50)
Leukemia mononuclear				1 (2%)
Stomach, forestomach	(51)	(50)	(49)	(50)
Squamous cell papilloma	1 (2%)		1 (2%)	
Stomach, glandular	(51)	(50)	(49)	(50)
Tooth	(0)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(51)	(50)	(49)	(50)
Heart	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Endocardium, schwannoma malignant		1 (2%)		

Table B1a
Summary of the Incidence of Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System				
Adrenal cortex	(51)	(50)	(49)	(50)
Adenoma	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Leukemia mononuclear				1 (2%)
Adrenal medulla	(48)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Pheochromocytoma benign	2 (4%)			
Islets, pancreatic	(51)	(50)	(49)	(49)
Adenoma	1 (2%)	1 (2%)		1 (2%)
Carcinoma			1 (2%)	1 (2%)
Parathyroid gland	(48)	(47)	(46)	(43)
Adenoma		1 (2%)		1 (2%)
Carcinoma, metastatic, thyroid gland		1 (2%)		
Leukemia mononuclear				1 (2%)
Pituitary gland	(51)	(50)	(50)	(50)
Leukemia mononuclear				1 (2%)
Pars distalis, adenoma	38 (75%)	35 (70%)	40 (80%)	37 (74%)
Pars distalis, carcinoma		1 (2%)		1 (2%)
Pars nervosa, schwannoma malignant, metastatic, nose				1 (2%)
Thyroid gland	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Bilateral, c-cell, carcinoma		1 (2%)		
C-cell, adenoma	2 (4%)	3 (6%)	1 (2%)	1 (2%)
Follicular cell, adenoma	1 (2%)			
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Sarcoma			1 (100%)	
Genital System				
Clitoral gland	(50)	(49)	(48)	(50)
Adenoma				2 (4%)
Carcinoma		1 (2%)		1 (2%)
Leukemia mononuclear				1 (2%)
Squamous cell carcinoma		1 (2%)		1 (2%)
Squamous cell papilloma	1 (2%)			
Ovary	(51)	(50)	(49)	(50)
Granulosa cell tumor benign			1 (2%)	
Leukemia mononuclear				1 (2%)
Sertoli cell tumor benign	1 (2%)	1 (2%)	1 (2%)	
Oviduct	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Uterus	(51)	(50)	(49)	(50)
Leiomyoma		1 (2%)		
Leiomyosarcoma		1 (2%)		
Leukemia mononuclear				1 (2%)
Polyp stromal	2 (4%)	4 (8%)	5 (10%)	5 (10%)
Cervix, squamous cell carcinoma				1 (2%)
Endometrium, adenocarcinoma		1 (2%)		
Endometrium, adenoma	1 (2%)			2 (4%)
Vagina	(51)	(50)	(49)	(49)
Granular cell tumor benign			1 (2%)	
Granular cell tumor malignant				1 (2%)
Leukemia mononuclear				1 (2%)
Polyp		1 (2%)	1 (2%)	

Table B1a
Summary of the Incidence of Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Hematopoietic System				
Bone marrow	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Lymph node	(16)	(14)	(6)	(10)
Axillary, lymphoma malignant				1 (10%)
Deep cervical, lymphoma malignant				1 (10%)
Lumbar, leukemia mononuclear				1 (10%)
Lumbar, lymphoma malignant				1 (10%)
Mediastinal, lymphoma malignant				1 (10%)
Pancreatic, lymphoma malignant				1 (10%)
Popliteal, lymphoma malignant				1 (10%)
Renal, leukemia mononuclear				1 (10%)
Renal, lymphoma malignant				1 (10%)
Lymph node, mandibular	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Lymph node, mesenteric	(51)	(50)	(47)	(50)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Spleen	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Sarcoma				1 (2%)
Thymus	(51)	(44)	(45)	(48)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Integumentary System				
Mammary gland	(51)	(49)	(49)	(50)
Adenocarcinoma	5 (10%)	9 (18%)	9 (18%)	6 (12%)
Adenocarcinoma, multiple	3 (6%)	1 (2%)		3 (6%)
Fibroadenoma	18 (35%)	14 (29%)	18 (37%)	22 (44%)
Fibroadenoma, multiple	14 (27%)	14 (29%)	15 (31%)	5 (10%)
Lymphoma malignant				1 (2%)
Skin	(51)	(50)	(49)	(50)
Keratoacanthoma			1 (2%)	
Sarcoma		1 (2%)		
Squamous cell papilloma	1 (2%)			
Sebaceous gland, carcinoma	1 (2%)			
Subcutaneous tissue, leiomyosarcoma, metastatic, uterus		1 (2%)		
Musculoskeletal System				
Bone, femur	(51)	(50)	(49)	(50)
Skeletal muscle	(1)	(0)	(1)	(1)

Table B1a
Summary of the Incidence of Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Nervous System				
Brain, brain stem	(51)	(50)	(49)	(50)
Carcinoma, metastatic, pituitary gland		1 (2%)		1 (2%)
Brain, cerebellum	(51)	(50)	(49)	(50)
Brain, cerebrum	(51)	(50)	(49)	(50)
Granular cell tumor benign	1 (2%)			
Leukemia mononuclear				1 (2%)
Oligodendroglioma NOS			1 (2%)	
Respiratory System				
Lung	(51)	(50)	(49)	(50)
Alveolar/bronchiolar adenoma		1 (2%)		
Leukemia mononuclear				1 (2%)
Nose	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Schwannoma malignant				1 (2%)
Trachea	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Special Senses System				
Ear	(0)	(1)	(0)	(0)
Neural crest tumor		1 (100%)		
Eye	(51)	(49)	(48)	(49)
Bilateral, leukemia mononuclear				1 (2%)
Harderian gland	(51)	(49)	(49)	(50)
Leukemia mononuclear				1 (2%)
Zymbal's gland	(0)	(0)	(0)	(0)
Urinary System				
Kidney	(51)	(50)	(49)	(50)
Leukemia mononuclear				1 (2%)
Lymphoma malignant				1 (2%)
Bilateral, renal tubule, carcinoma		2 (4%)		1 (2%)
Renal tubule, adenoma				1 (2%)
Urinary bladder	(51)	(49)	(49)	(48)
Leukemia mononuclear				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table B1b
Summary of the Incidence of Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	24	26	27	22
Natural deaths		7	1	6
Survivors				
Died last week of study	1	1		
Terminal sacrifice	26	16	22	22
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(50)	(50)
Intestine large, cecum	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Intestine large, colon	(51)	(50)	(50)	(50)
Carcinoma		1 (2%)		
Lymphoma malignant		1 (2%)	1 (2%)	
Intestine large, rectum	(51)	(50)	(50)	(50)
Intestine small, duodenum	(51)	(50)	(50)	(50)
Leiomyoma				1 (2%)
Lymphoma malignant			1 (2%)	
Intestine small, ileum	(51)	(48)	(50)	(49)
Lymphoma malignant			1 (2%)	
Intestine small, jejunum	(51)	(48)	(50)	(50)
Leiomyoma			1 (2%)	
Lymphoma malignant			1 (2%)	
Liver	(51)	(50)	(50)	(50)
Cholangiocarcinoma	1 (2%)			
Cholangioma				1 (2%)
Hepatocellular adenoma	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Hepatocellular adenoma, multiple				1 (2%)
Lymphoma malignant		2 (4%)	1 (2%)	
Mesentery	(2)	(2)	(1)	(0)
Mesothelioma malignant			1 (100%)	
Oral mucosa	(3)	(5)	(7)	(4)
Lymphoma malignant			1 (14%)	
Squamous cell carcinoma	3 (100%)	3 (60%)	2 (29%)	1 (25%)
Pancreas	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Salivary glands	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Stomach, forestomach	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Squamous cell papilloma	1 (2%)	1 (2%)		
Stomach, glandular	(51)	(50)	(50)	(50)
Tooth	(0)	(1)	(0)	(0)
Cardiovascular System				
Blood vessel	(51)	(50)	(50)	(50)
Heart	(51)	(50)	(50)	(50)
Lymphoma malignant		1 (2%)		

Table B1b
Summary of the Incidence of Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System				
Adrenal cortex	(51)	(50)	(50)	(50)
Adenoma	1 (2%)	1 (2%)	2 (4%)	3 (6%)
Lymphoma malignant			1 (2%)	
Adrenal medulla	(48)	(49)	(48)	(49)
Pheochromocytoma benign	2 (4%)		1 (2%)	2 (4%)
Islets, pancreatic	(51)	(50)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Lymphoma malignant		2 (4%)	1 (2%)	
Parathyroid gland	(48)	(46)	(44)	(46)
Adenoma				1 (2%)
Lymphoma malignant			1 (2%)	
Pituitary gland	(51)	(50)	(50)	(49)
Lymphoma malignant			1 (2%)	
Pars distalis, adenoma	38 (75%)	37 (74%)	36 (72%)	34 (69%)
Pars distalis, carcinoma		1 (2%)	1 (2%)	1 (2%)
Thyroid gland	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
C-cell, adenoma	2 (4%)	1 (2%)	3 (6%)	
C-cell, carcinoma		1 (2%)		1 (2%)
Follicular cell, adenoma	1 (2%)	1 (2%)		
Follicular cell, carcinoma		2 (4%)		
General Body System				
Tissue NOS	(0)	(0)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(49)	(49)
Carcinoma		1 (2%)		
Lymphoma malignant		2 (4%)	1 (2%)	
Sarcoma		1 (2%)		
Squamous cell papilloma	1 (2%)			
Ovary	(51)	(50)	(50)	(50)
Carcinoma, metastatic, kidney		1 (2%)		
Granulosa cell tumor benign		1 (2%)		2 (4%)
Lymphoma malignant		1 (2%)	1 (2%)	
Sertoli cell tumor benign	1 (2%)			
Oviduct	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Uterus	(51)	(50)	(50)	(50)
Fibrous histiocytoma			1 (2%)	
Lymphoma malignant		1 (2%)	1 (2%)	
Polyp stromal	2 (4%)	5 (10%)	6 (12%)	7 (14%)
Sarcoma stromal			1 (2%)	
Endometrium, adenoma	1 (2%)	1 (2%)	2 (4%)	1 (2%)
Vagina	(51)	(50)	(48)	(50)
Lymphoma malignant		1 (2%)	1 (2%)	
Polyp		1 (2%)		

Table B1b
Summary of the Incidence of Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Hematopoietic System				
Bone marrow	(51)	(50)	(50)	(50)
Lymphoma malignant		1 (2%)	1 (2%)	
Lymph node	(16)	(15)	(12)	(13)
Carcinoma, metastatic, thyroid gland		1 (7%)		
Axillary, lymphoma malignant			1 (8%)	
Deep cervical, carcinoma, metastatic, thyroid gland		1 (7%)		
Deep cervical, lymphoma malignant			1 (8%)	
Inguinal, lymphoma malignant			1 (8%)	
Lumbar, lymphoma malignant		1 (7%)	1 (8%)	
Mediastinal, lymphoma malignant		1 (7%)	1 (8%)	
Renal, lymphoma malignant			1 (8%)	
Lymph node, mandibular	(51)	(50)	(49)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Lymph node, mesenteric	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Spleen	(51)	(50)	(50)	(50)
Lymphoma malignant		1 (2%)	1 (2%)	
Thymus	(51)	(44)	(46)	(48)
Lymphoma malignant		1 (2%)	1 (2%)	
Thymoma benign				1 (2%)
Integumentary System				
Mammary gland	(51)	(49)	(50)	(50)
Adenocarcinoma	5 (10%)	3 (6%)	8 (16%)	11 (22%)
Adenocarcinoma, multiple	3 (6%)			
Fibroadenoma	18 (35%)	16 (33%)	15 (30%)	21 (42%)
Fibroadenoma, multiple	14 (27%)	10 (20%)	13 (26%)	13 (26%)
Lymphoma malignant		2 (4%)	1 (2%)	
Skin	(51)	(50)	(50)	(50)
Basal cell carcinoma			1 (2%)	
Fibrous histiocytoma			1 (2%)	1 (2%)
Keratoacanthoma			1 (2%)	
Lymphoma malignant		2 (4%)		
Sarcoma				1 (2%)
Squamous cell papilloma	1 (2%)			
Sebaceous gland, carcinoma	1 (2%)			
Subcutaneous tissue, granular cell tumor benign				1 (2%)
Subcutaneous tissue, lipoma				1 (2%)
Musculoskeletal System				
Bone, femur	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Skeletal muscle	(1)	(0)	(4)	(1)
Lymphoma malignant			1 (25%)	

Table B1b
Summary of the Incidence of Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Nervous System				
Brain, brain stem	(51)	(50)	(50)	(50)
Carcinoma, metastatic, pituitary gland		1 (2%)	1 (2%)	1 (2%)
Lymphoma malignant			1 (2%)	
Brain, cerebellum	(51)	(50)	(50)	(50)
Brain, cerebrum	(51)	(50)	(50)	(50)
Astrocytoma NOS		1 (2%)		
Granular cell tumor benign	1 (2%)			
Respiratory System				
Lung	(51)	(50)	(50)	(50)
Alveolar/bronchiolar adenoma			1 (2%)	
Lymphoma malignant		2 (4%)	1 (2%)	
Nephroblastoma, metastatic, kidney			1 (2%)	
Nose	(51)	(50)	(50)	(50)
Lymphoma malignant		1 (2%)	1 (2%)	
Trachea	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Special Senses System				
Ear	(0)	(0)	(0)	(0)
Eye	(51)	(50)	(50)	(47)
Lymphoma malignant		2 (4%)	1 (2%)	
Squamous cell carcinoma, deep invasion		1 (2%)		
Harderian gland	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Zymbal's gland	(0)	(0)	(0)	(1)
Carcinoma				1 (100%)
Urinary System				
Kidney	(51)	(50)	(50)	(50)
Lymphoma malignant		2 (4%)	1 (2%)	
Nephroblastoma			1 (2%)	
Bilateral, renal tubule, carcinoma		1 (2%)		
Urinary bladder	(51)	(48)	(50)	(49)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table B1c
Summary of the Incidence of Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	52	50	50	50
Early deaths				
Moribund	24	19	23	21
Natural deaths	1	2	5	3
Survivors				
Died last week of study			1	2
Terminal sacrifice	27	29	21	24
Animals examined microscopically	52	50	50	50
Alimentary System				
Esophagus	(52)	(49)	(50)	(50)
Intestine large, cecum	(51)	(49)	(49)	(50)
Lymphoma malignant			1 (2%)	1 (2%)
Intestine large, colon	(52)	(50)	(50)	(50)
Lymphoma malignant				1 (2%)
Intestine large, rectum	(51)	(49)	(50)	(50)
Adenoma	1 (2%)			
Intestine small, ileum	(52)	(49)	(49)	(50)
Intestine small, jejunum	(51)	(49)	(48)	(50)
Carcinoma		1 (2%)	1 (2%)	
Leiomyoma		1 (2%)		
Liver	(52)	(50)	(50)	(50)
Adenocarcinoma, metastatic, uterus	1 (2%)			
Carcinoma, metastatic, intestine small, jejunum		1 (2%)		
Cholangiocarcinoma	1 (2%)	1 (2%)		
Cholangioma				1 (2%)
Hepatocellular adenoma	2 (4%)			
Hepatocellular carcinoma		1 (2%)		
Histiocytic sarcoma			1 (2%)	
Leukemia mononuclear, metastatic, spleen	1 (2%)			
Lymphoma malignant			1 (2%)	1 (2%)
Mesothelioma malignant			1 (2%)	
Nephroblastoma, metastatic, kidney	1 (2%)			
Mesentery	(3)	(3)	(4)	(4)
Adenocarcinoma, metastatic, uterus	1 (33%)			
Lymphoma malignant			1 (25%)	1 (25%)
Mesothelioma malignant		1 (33%)	1 (25%)	
Nephroblastoma, metastatic, kidney	1 (33%)			
Oral mucosa	(2)	(4)	(7)	(2)
Fibrous histiocytoma			1 (14%)	
Squamous cell carcinoma	2 (100%)	1 (25%)	2 (29%)	1 (50%)
Pancreas	(52)	(49)	(50)	(50)
Adenocarcinoma, metastatic, uterus	1 (2%)			
Salivary glands	(52)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Stomach, forestomach	(52)	(49)	(50)	(50)
Adenocarcinoma, metastatic, uterus	1 (2%)			
Squamous cell carcinoma				1 (2%)
Squamous cell papilloma	1 (2%)		2 (4%)	
Stomach, glandular	(52)	(49)	(50)	(50)
Leiomyosarcoma	1 (2%)			

Table B1c
Summary of the Incidence of Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Cardiovascular System				
Heart	(52)	(50)	(50)	(50)
Endocrine System				
Adrenal cortex	(52)	(50)	(50)	(50)
Adenoma	2 (4%)	2 (4%)	1 (2%)	
Adrenal medulla	(52)	(50)	(50)	(50)
Pheochromocytoma benign				1 (2%)
Islets, pancreatic	(52)	(49)	(50)	(50)
Adenoma	1 (2%)			1 (2%)
Parathyroid gland	(48)	(45)	(47)	(44)
Pituitary gland	(52)	(50)	(50)	(50)
Pars distalis, adenoma	32 (62%)	30 (60%)	36 (72%)	32 (64%)
Thyroid gland	(52)	(49)	(50)	(50)
Bilateral, c-cell, adenoma				1 (2%)
C-cell, adenoma	1 (2%)	1 (2%)	1 (2%)	1 (2%)
C-cell, carcinoma	1 (2%)		1 (2%)	
General Body System				
Tissue NOS	(0)	(0)	(1)	(1)
Alveolar/bronchiolar carcinoma, metastatic, lung				1 (100%)
Squamous cell carcinoma, deep invasion			1 (100%)	
Genital System				
Clitoral gland	(50)	(50)	(49)	(48)
Adenoma		1 (2%)	1 (2%)	2 (4%)
Lymphoma malignant			1 (2%)	
Ovary	(51)	(50)	(50)	(50)
Granulosa cell tumor benign		1 (2%)	1 (2%)	2 (4%)
Granulosa cell tumor malignant		1 (2%)		
Lymphoma malignant			1 (2%)	
Mesothelioma malignant			1 (2%)	
Sertoli cell tumor benign		2 (4%)	1 (2%)	
Uterus	(52)	(50)	(50)	(50)
Leiomyoma				1 (2%)
Lymphoma malignant			1 (2%)	
Nephroblastoma, metastatic, kidney	1 (2%)			
Polyp stromal	1 (2%)	7 (14%)	2 (4%)	5 (10%)
Endometrium, adenocarcinoma	4 (8%)	1 (2%)		
Endometrium, adenoma	1 (2%)	1 (2%)	2 (4%)	
Vagina	(51)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Nephroblastoma, metastatic, kidney	1 (2%)			
Hematopoietic System				
Bone marrow	(52)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Schwannoma malignant, metastatic, skin	1 (2%)			

Table B1c
Summary of the Incidence of Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Hematopoietic System (continued)				
Lymph node	(10)	(17)	(10)	(5)
Axillary, lymphoma malignant			1 (10%)	
Deep cervical, lymphoma malignant			1 (10%)	
Lumbar, lymphoma malignant			1 (10%)	
Mediastinal, lymphoma malignant			1 (10%)	
Renal, lymphoma malignant			1 (10%)	
Lymph node, mandibular	(51)	(50)	(50)	(50)
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Lymphoma malignant			1 (2%)	1 (2%)
Lymph node, mesenteric	(51)	(49)	(50)	(49)
Lymphoma malignant				1 (2%)
Nephroblastoma, metastatic, kidney	1 (2%)			
Spleen	(52)	(50)	(50)	(50)
Leukemia mononuclear	1 (2%)			
Lymphoma malignant			1 (2%)	1 (2%)
Mesothelioma malignant			1 (2%)	
Thymus	(50)	(45)	(46)	(49)
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant			1 (2%)	1 (2%)
Integumentary System				
Mammary gland	(52)	(50)	(50)	(50)
Adenocarcinoma	6 (12%)	4 (8%)	6 (12%)	10 (20%)
Adenocarcinoma, multiple		2 (4%)	3 (6%)	
Fibroadenoma	17 (33%)	13 (26%)	21 (42%)	13 (26%)
Fibroadenoma, multiple	19 (37%)	21 (42%)	19 (38%)	24 (48%)
Lymphoma malignant			1 (2%)	
Skin	(52)	(50)	(50)	(50)
Basal cell adenoma				1 (2%)
Fibroma		2 (4%)		1 (2%)
Fibrous histiocytoma			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Lipoma	1 (2%)			
Lymphoma malignant			1 (2%)	
Sarcoma	1 (2%)		1 (2%)	1 (2%)
Schwannoma malignant	1 (2%)			
Ear, basal cell adenoma		1 (2%)		
Musculoskeletal System				
Bone	(0)	(1)	(0)	(0)
Bone, femur	(52)	(50)	(50)	(50)
Skeletal muscle	(3)	(4)	(0)	(1)
Diaphragm, adenocarcinoma, metastatic, uterus	1 (33%)			
Diaphragm, granulosa cell tumor malignant, metastatic, ovary		1 (25%)		
Diaphragm, mesothelioma malignant, metastatic, mesentery		1 (25%)		
Diaphragm, nephroblastoma, metastatic, kidney	1 (33%)			

Table B1c
Summary of the Incidence of Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Nervous System				
Brain, brain stem	(52)	(50)	(50)	(50)
Brain, cerebellum	(51)	(50)	(50)	(50)
Astrocytoma malignant				1 (2%)
Brain, cerebrum	(52)	(50)	(50)	(50)
Meninges, lymphoma malignant			1 (2%)	
Spinal cord	(0)	(1)	(0)	(1)
Respiratory System				
Lung	(52)	(50)	(50)	(50)
Adenocarcinoma, metastatic, mammary gland			1 (2%)	1 (2%)
Alveolar/bronchiolar adenoma	1 (2%)			1 (2%)
Alveolar/bronchiolar carcinoma				1 (2%)
Carcinoma, metastatic, thyroid gland	1 (2%)		1 (2%)	
Carcinoma, metastatic, Zymbal's gland			1 (2%)	
Histiocytic sarcoma			1 (2%)	
Lymphoma malignant			1 (2%)	1 (2%)
Mesothelioma malignant, metastatic, mesentery		1 (2%)		
Nose	(52)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Trachea	(52)	(49)	(50)	(50)
Special Senses System				
Ear	(0)	(0)	(2)	(0)
Neural crest tumor			2 (100%)	
Eye	(52)	(50)	(49)	(50)
Harderian gland	(52)	(50)	(50)	(50)
Lymphoma malignant			1 (2%)	
Squamous cell carcinoma, deep invasion			1 (2%)	
Zymbal's gland	(0)	(0)	(1)	(0)
Carcinoma			1 (100%)	
Urinary System				
Kidney	(52)	(50)	(50)	(50)
Lipoma	1 (2%)			
Liposarcoma				1 (2%)
Lymphoma malignant			1 (2%)	
Nephroblastoma	1 (2%)			1 (2%)
Renal tubule, adenoma			1 (2%)	1 (2%)
Urinary bladder	(52)	(49)	(49)	(50)
Lymphoma malignant			1 (2%)	
Transitional epithelium, papilloma				1 (2%)

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm

Table B2a
Statistical Analysis of Primary Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Clitoral Gland: Adenoma or Carcinoma				
Overall rate[a]	0/50 (0.0%)	1/49 (2.0%)	0/48 (0.0%)	3/50 (6.0%)
Adjusted rate[b]	0/41.6 (0.0%)	1/39.1 (2.6%)	0/37.0 (0.0%)	3/41.9 (7.2%)
Terminal rate[c]	0/26 (0.0%)	1/23 (4.3%)	0/19 (0.0%)	0/25 (0.0%)
First incidence (days)	—[e]	757 (T)	—	653
Poly-3 test[d]	P=0.043/P=0.053	P=0.487	—[f]	P=0.119
Clitoral Gland: Epithelial Neoplasms				
Overall rate	1/50 (2.0%)	2/49 (4.1%)	0/48 (0.0%)	4/50 (8.0%)
Adjusted rate	1/41.6 (2.4%)	2/39.1 (5.1%)	0/37.0 (0.0%)	4/41.9 (9.5%)
Terminal rate	1/26 (3.8%)	2/23 (8.7%)	0/19 (0.0%)	1/25 (4.0%)
First incidence (days)	757 (T)	757 (T)	—	653
Poly-3 test	P=0.083/P=0.142	P=0.478	P=0.523N	P=0.179
Mammary Gland: Fibroadenoma				
Overall rate	32/51 (62.7%)	28/49 (57.1%)	33/49 (67.3%)	27/50 (54.0%)
Adjusted rate	32/48.3 (66.2%)	28/42.5 (65.9%)	33/43.5 (75.9%)	27/44.2 (61.1%)
Terminal rate	14/26 (53.8%)	15/23 (65.2%)	15/19 (78.9%)	15/25 (60.0%)
First incidence (days)	529	602	555	597
Poly-3 test	P=0.260N/P=0.468N	P=0.576N	P=0.204	P=0.380N
Mammary Gland: Adenocarcinoma				
Overall rate	8/51 (15.7%)	10/49 (20.4%)	9/49 (18.4%)	9/50 (18.0%)
Adjusted rate	8/43.3 (18.5%)	10/40.9 (24.5%)	9/40.0 (22.5%)	9/42.4 (21.2%)
Terminal rate	4/26 (15.4%)	6/23 (26.1%)	3/19 (15.8%)	5/25 (20.0%)
First incidence (days)	675	468	538	584
Poly-3 test	P=0.567N/P=0.440	P=0.341	P=0.427	P=0.479
Mammary Gland: Fibroadenoma or Adenocarcinoma				
Overall rate	35/51 (68.6%)	33/49 (67.3%)	38/49 (77.6%)	31/50 (62.0%)
Adjusted rate	35/48.6 (72.0%)	33/43.9 (75.2%)	38/44.9 (84.6%)	31/45.2 (68.6%)
Terminal rate	16/26 (61.5%)	18/23 (78.3%)	16/19 (84.2%)	17/25 (68.0%)
First incidence (days)	529	468	538	584
Poly-3 test	P=0.236N/P=0.525	P=0.451	P=0.098	P=0.446N
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	38/51 (74.5%)	35/50 (70.0%)	40/50 (80.0%)	37/50 (74.0%)
Adjusted rate	38/48.0 (79.2%)	35/45.5 (76.9%)	40/46.3 (86.3%)	37/46.8 (79.0%)
Terminal rate	21/26 (80.8%)	17/23 (73.9%)	16/19 (84.2%)	20/25 (80.0%)
First incidence (days)	539	499	538	516
Poly-3 test	P=0.548N/P=0.395	P=0.491N	P=0.246	P=0.596N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	38/51 (74.5%)	36/50 (72.0%)	40/50 (80.0%)	38/50 (76.0%)
Adjusted rate	38/48.0 (79.2%)	36/45.6 (79.0%)	40/46.3 (86.3%)	38/47.2 (80.5%)
Terminal rate	21/26 (80.8%)	17/23 (73.9%)	16/19 (84.2%)	20/25 (80.0%)
First incidence (days)	539	499	538	516
Poly-3 test	P=0.563/P=0.355	P=0.595N	P=0.246	P=0.541
Thyroid Gland (C-Cell): Adenoma				
Overall rate	2/51 (3.9%)	3/50 (6.0%)	1/49 (2.0%)	1/50 (2.0%)
Adjusted rate	2/42.4 (4.7%)	3/39.2 (7.7%)	1/37.9 (2.6%)	1/41.2 (2.4%)
Terminal rate	2/26 (7.7%)	3/23 (13.0%)	1/19 (5.3%)	0/25 (0.0%)
First incidence (days)	757 (T)	756 (T)	758 (T)	711
Poly-3 test	P=0.329N/P=0.265N	P=0.463	P=0.539N	P=0.510N

Table B2a
Statistical Analysis of Primary Neoplasms in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	2/51 (3.9%)	4/50 (8.0%)	1/49 (2.0%)	1/50 (2.0%)
Adjusted rate	2/42.4 (4.7%)	4/39.5 (10.1%)	1/37.9 (2.6%)	1/41.2 (2.4%)
Terminal rate	2/26 (7.7%)	3/23 (13.0%)	1/19 (5.3%)	0/25 (0.0%)
First incidence (days)	757 (T)	667	758 (T)	711
Poly-3 test	P=0.257N/P=0.230N	P=0.303	P=0.539N	P=0.510N
Uterus: Stromal Polyp				
Overall rate	2/51 (3.9%)	4/50 (8.0%)	5/49 (10.2%)	5/50 (10.0%)
Adjusted rate	2/42.4 (4.7%)	4/39.8 (10.1%)	5/38.7 (12.9%)	5/42.3 (11.8%)
Terminal rate	2/26 (7.7%)	3/23 (13.0%)	2/19 (10.5%)	0/25 (0.0%)
First incidence (days)	756 (T)	563	687	626
Poly-3 test	P=0.321/P=0.143	P=0.306	P=0.178	P=0.212
All Organs: Benign Neoplasms				
Overall rate	48/51 (94.1%)	44/50 (88.0%)	48/50 (96.0%)	47/50 (94.0%)
Adjusted rate	48/50.8 (94.5%)	44/46.9 (93.9%)	48/48.2 (99.6%)	47/48.3 (97.4%)
Terminal rate	24/26 (92.3%)	22/23 (95.7%)	19/19 (100.0%)	25/25 (100.0%)
First incidence (days)	529	499	538	516
Poly-3 test	P=0.346/P=0.129	P=0.624N	P=0.170	P=0.408
All Organs: Malignant Neoplasms				
Overall rate	11/51 (21.6%)	18/50 (36.0%)	11/50 (22.0%)	16/50 (32.0%)
Adjusted rate	11/43.6 (25.2%)	18/43.7 (41.2%)	11/41.4 (26.6%)	16/44.7 (35.8%)
Terminal rate	6/26 (23.1%)	8/23 (34.8%)	3/19 (15.8%)	7/25 (28.0%)
First incidence (days)	672	349	538	289
Poly-3 test	P=0.369/P=0.311	P=0.082	P=0.543	P=0.195

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group
[f] Value of statistic cannot be computed.

Table B2b
Statistical Analysis of Primary Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Adrenal Cortex: Adenoma				
Overall rate[a]	1/51 (2.0%)	1/50 (2.0%)	2/50 (4.0%)	3/50 (6.0%)
Adjusted rate[b]	1/42.4 (2.4%)	1/35.5 (2.8%)	2/39.2 (5.1%)	3/40.4 (7.4%)
Terminal rate[c]	1/26 (3.8%)	1/16 (6.3%)	1/22 (4.5%)	1/22 (4.5%)
First incidence (days)	758 (T)	756 (T)	738	715
Poly-3 test[d]	P=0.213/P=0.152	P=0.719	P=0.472	P=0.286
Liver: Hepatocellular Adenoma				
Overall rate	1/51 (2.0%)	1/50 (2.0%)	1/50 (2.0%)	3/50 (6.0%)
Adjusted rate	1/42.4 (2.4%)	1/35.5 (2.8%)	1/39.3 (2.5%)	3/40.6 (7.4%)
Terminal rate	1/26 (3.8%)	1/16 (6.3%)	0/22 (0.0%)	1/22 (4.5%)
First incidence (days)	758 (T)	762 (T)	714	696
Poly-3 test	P=0.165/P=0.178	P=0.719	P=0.743	P=0.288
Mammary Gland: Fibroadenoma				
Overall rate	32/51 (62.7%)	26/49 (53.1%)	28/50 (56.0%)	34/50 (68.0%)
Adjusted rate	32/48.3 (66.2%)	26/41.5 (62.6%)	28/43.3 (64.6%)	34/46.4 (73.3%)
Terminal rate	14/26 (53.8%)	8/16 (50.0%)	15/22 (68.2%)	16/22 (72.7%)
First incidence (days)	529	452	510	511
Poly-3 test	P=0.171/P=0.239	P=0.445N	P=0.526N	P=0.291
Mammary Gland: Adenocarcinoma				
Overall rate	8/51 (15.7%)	3/49 (6.1%)	8/50 (16.0%)	11/50 (22.0%)
Adjusted rate	8/43.3 (18.5%)	3/35.5 (8.5%)	8/41.1 (19.5%)	11/42.5 (25.9%)
Terminal rate	4/26 (15.4%)	1/16 (6.3%)	3/22 (13.6%)	3/22 (13.6%)
First incidence (days)	675	661	477	495
Poly-3 test	P=0.096/P=0.139	P=0.171N	P=0.564	P=0.283
Mammary Gland: Fibroadenoma or Adenocarcinoma				
Overall rate	35/51 (68.6%)	28/49 (57.1%)	33/50 (66.0%)	37/50 (74.0%)
Adjusted rate	35/48.6 (72.0%)	28/41.9 (66.9%)	33/44.9 (73.4%)	37/47.2 (78.4%)
Terminal rate	16/26 (61.5%)	9/16 (56.3%)	17/22 (77.3%)	17/22 (77.3%)
First incidence (days)	529	452	477	495
Poly-3 test	P=0.177/P=0.203	P=0.380N	P=0.529	P=0.304
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	38/51 (74.5%)	37/50 (74.0%)	36/50 (72.0%)	34/49 (69.4%)
Adjusted rate	38/48.0 (79.2%)	37/44.3 (83.6%)	36/44.8 (80.3%)	34/43.9 (77.4%)
Terminal rate	21/26 (80.8%)	13/16 (81.3%)	18/22 (81.8%)	18/22 (81.8%)
First incidence (days)	539	452	537	561
Poly-3 test	P=0.364N/P=0.410N	P=0.384	P=0.555	P=0.519N
Pituitary Gland (Pars Distalis): Adenoma or Carcinoma				
Overall rate	38/51 (74.5%)	38/50 (76.0%)	37/50 (74.0%)	35/49 (71.4%)
Adjusted rate	38/48.0 (79.2%)	38/44.6 (85.3%)	37/45.2 (81.8%)	35/44.4 (78.9%)
Terminal rate	21/26 (80.8%)	13/16 (81.3%)	18/22 (81.8%)	18/22 (81.8%)
First incidence (days)	539	452	537	561
Poly-3 test	P=0.396N/P=0.478N	P=0.298	P=0.479	P=0.591N
Skin: All Neoplastic Morphologies				
Overall rate	1/51 (2.0%)	0/50 (0.0%)	3/50 (6.0%)	4/50 (8.0%)
Adjusted rate	1/42.4 (2.4%)	0/35.5 (0.0%)	3/39.8 (7.5%)	4/41.3 (9.7%)
Terminal rate	1/26 (3.8%)	0/16 (0.0%)	0/22 (0.0%)	2/22 (9.1%)
First incidence (days)	762 (T)	—[e]	646	404
Poly-3 test	P=0.073/P=0.041	P=0.535N	P=0.281	P=0.169

Table B2b
Statistical Analysis of Primary Neoplasms in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Thyroid Gland (C-Cell): Adenoma				
Overall rate	2/51 (3.9%)	1/50 (2.0%)	3/50 (6.0%)	0/50 (0.0%)
Adjusted rate	2/42.4 (4.7%)	1/35.6 (2.8%)	3/39.8 (7.5%)	0/40.1 (0.0%)
Terminal rate	2/26 (7.7%)	0/16 (0.0%)	1/22 (4.5%)	0/22 (0.0%)
First incidence (days)	757 (T)	737	561	—
Poly-3 test	P=0.188N/P=0.298N	P=0.560N	P=0.470	P=0.249N
Thyroid Gland (C-Cell): Adenoma or Carcinoma				
Overall rate	2/51 (3.9%)	2/50 (4.0%)	3/50 (6.0%)	1/50 (2.0%)
Adjusted rate	2/42.4 (4.7%)	2/35.6 (5.6%)	3/39.8 (7.5%)	1/40.1 (2.5%)
Terminal rate	2/26 (7.7%)	1/16 (6.3%)	1/22 (4.5%)	1/22 (4.5%)
First incidence (days)	757 (T)	737	561	762 (T)
Poly-3 test	P=0.353N/P=0.454N	P=0.629	P=0.470	P=0.519N
Thyroid Gland (Follicular Cell): Adenoma or Carcinoma				
Overall rate	1/51 (2.0%)	3/50 (6.0%)	0/50 (0.0%)	0/50 (0.0%)
Adjusted rate	1/42.8 (2.3%)	3/36.6 (8.2%)	0/39.1 (0.0%)	0/40.1 (0.0%)
Terminal rate	0/26 (0.0%)	0/16 (0.0%)	0/22 (0.0%)	0/22 (0.0%)
First incidence (days)	668	597	—	—
Poly-3 test	P=0.195N/P=0.153N	P=0.251	P=0.517N	P=0.512N
Uterus: Stromal Polyp				
Overall rate	2/51 (3.9%)	5/50 (10.0%)	6/50 (12.0%)	7/50 (14.0%)
Adjusted rate	2/42.4 (4.7%)	5/36.6 (13.7%)	6/40.0 (15.0%)	7/41.2 (17.0%)
Terminal rate	2/26 (7.7%)	2/16 (12.5%)	3/22 (13.6%)	4/22 (18.2%)
First incidence (days)	756 (T)	618	641	561
Poly-3 test	P=0.152/P=0.052	P=0.158	P=0.112	P=0.069
All Organs: Benign Neoplasms				
Overall rate	48/51 (94.1%)	45/50 (90.0%)	45/50 (90.0%)	47/50 (94.0%)
Adjusted rate	48/50.8 (94.5%)	45/46.7 (96.4%)	45/46.4 (96.9%)	47/47.9 (98.1%)
Terminal rate	24/26 (92.3%)	16/16 (100.0%)	22/22 (100.0%)	22/22 (100.0%)
First incidence (days)	529	452	510	511
Poly-3 test	P=0.298/P=0.168	P=0.519	P=0.468	P=0.325
All Organs: Malignant Neoplasms				
Overall rate	11/51 (21.6%)	13/50 (26.0%)	17/50 (34.0%)	16/50 (32.0%)
Adjusted rate	11/43.6 (25.2%)	13/38.2 (34.0%)	11/43.7 (38.9%)	16/44.3 (36.1%)
Terminal rate	6/26 (23.1%)	5/16 (31.3%)	6/22 (27.3%)	4/22 (18.2%)
First incidence (days)	672	597	254	404
Poly-3 test	P=0.314/P=0.128	P=0.261	P=0.123	P=0.188

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

Table B2c
Statistical Analysis of Primary Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Mammary Gland: Fibroadenoma				
Overall rate[a]	36/52 (69.2%)	34/50 (68.0%)	40/50 (80.0%)	37/50 (74.0%)
Adjusted rate[b]	36/48.2 (74.6%)	34/46.4 (73.3%)	40/46.4 (86.2%)	37/43.5 (85.0%)
Terminal rate[c]	17/27 (63.0%)	19/29 (65.5%)	17/21 (81.0%)	21/24 (87.5%)
First incidence (days)	504	541	490	427
Poly-3 test[d]	P=0.122/P=0.048	P=0.536N	P=0.112	P=0.152
Mammary Gland: Adenocarcinoma				
Overall rate	6/52 (11.5%)	6/50 (12.0%)	9/50 (18.0%)	10/50 (20.0%)
Adjusted rate	6/42.5 (14.1%)	6/42.1 (14.3%)	9/39.8 (22.6%)	10/40.3 (24.8%)
Terminal rate	2/27 (7.4%)	4/29 (13.8%)	6/21 (28.6%)	3/24 (12.5%)
First incidence (days)	641	599	680	578
Poly-3 test	P=0.131/P=0.080	P=0.614	P=0.237	P=0.168
Mammary Gland: Fibroadenoma or Adenocarcinoma				
Overall rate	38/52 (73.1%)	36/50 (72.0%)	41/50 (82.0%)	39/50 (78.0%)
Adjusted rate	38/48.6 (78.2%)	36/47.1 (76.4%)	41/46.4 (88.4%)	39/44.5 (87.6%)
Terminal rate	18/27 (66.7%)	19/29 (65.5%)	18/21 (85.7%)	21/24 (87.5%)
First incidence (days)	504	541	490	427
Poly-3 test	P=0.123/P=0.055	P=0.513N	P=0.134	P=0.166
Pituitary Gland (Pars Distalis): Adenoma				
Overall rate	32/52 (61.5%)	30/50 (60.0%)	36/50 (72.0%)	32/50 (64.0%)
Adjusted rate	32/46.4 (68.9%)	30/45.3 (66.2%)	36/44.5 (81.0%)	32/43.2 (74.0%)
Terminal rate	17/27 (63.0%)	21/29 (72.4%)	17/21 (81.0%)	16/24 (66.7%)
First incidence (days)	548	522	490	430
Poly-3 test	P=0.339/P=0.159	P=0.477N	P=0.125	P=0.378
Skin: All Neoplastic Morphologies				
Overall rate	3/52 (5.8%)	3/50 (6.0%)	2/50 (4.0%)	3/50 (6.0%)
Adjusted rate	3/42.6 (7.0%)	3/41.8 (7.2%)	2/39.4 (5.1%)	3/38.8 (7.7%)
Terminal rate	1/27 (3.7%)	1/29 (3.4%)	1/21 (4.8%)	1/24 (4.2%)
First incidence (days)	474	683	687	597
Poly-3 test	P=0.546/P=0.550N	P=0.653	P=0.536N	P=0.618
Uterus (Endometrium): Adenocarcinoma				
Overall rate	4/52 (7.7%)	1/50 (2.0%)	0/50 (0.0%)	0/50 (0.0%)
Adjusted rate	4/41.9 (9.6%)	1/42.0 (2.4%)	0/39.1 (0.0%)	0/38.1 (0.0%)
Terminal rate	2/27 (7.4%)	0/29 (0.0%)	0/21 (0.0%)	0/24 (0.0%)
First incidence (days)	644	522	—[e]	—
Poly-3 test	P=0.116N/P=0.008N	P=0.176N	P=0.068N	P=0.072N
Uterus (Endometrium): Adenoma or Adenocarcinoma				
Overall rate	5/52 (9.6%)	2/50 (4.0%)	2/50 (4.0%)	0/50 (0.0%)
Adjusted rate	5/42.0 (11.9%)	2/42.0 (4.8%)	2/39.9 (5.0%)	0/38.1 (0.0%)
Terminal rate	2/27 (7.4%)	1/29 (3.4%)	0/21 (0.0%)	0/24 (0.0%)
First incidence (days)	644	522	608	—
Poly-3 test	P=0.065N/P=0.021N	P=0.214N	P=0.235N	P=0.038N

Table B2c
Statistical Analysis of Primary Neoplasms in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Uterus: Stromal Polyp				
Overall rate	1/52 (1.9%)	7/50 (14.0%)	2/50 (4.0%)	5/50 (10.0%)
Adjusted rate	1/41.8 (2.4%)	7/41.3 (16.9%)	2/39.1 (5.1%)	5/39.5 (12.7%)
Terminal rate	0/27 (0.0%)	7/29 (24.1%)	2/21 (9.5%)	2/24 (8.3%)
First incidence (days)	548	747 (T)	749 (T)	561
Poly-3 test	P=0.315/P=0.202	P=0.027	P=0.476	P=0.087
All Organs: Benign Neoplasms				
Overall rate	46/52 (88.5%)	47/50 (94.0%)	46/50 (92.0%)	44/50 (88.0%)
Adjusted rate	46/49.1 (93.7%)	47/48.4 (97.0%)	46/47.4 (97.1%)	44/46.3 (95.0%)
Terminal rate	25/27 (92.6%)	28/29 (96.6%)	20/21 (95.2%)	23/24 (95.8%)
First incidence (days)	504	522	490	427
Poly-3 test	P=0.590N/P=0.426	P=0.366	P=0.360	P=0.575
All Organs: Malignant Neoplasms				
Overall rate	15/52 (28.8%)	12/50 (24.0%)	16/50 (32.0%)	16/50 (32.0%)
Adjusted rate	15/45.8 (32.8%)	12/42.7 (28.1%)	16/41.7 (38.4%)	16/43.2 (37.0%)
Terminal rate	5/27 (18.5%)	9/29 (31.0%)	8/21 (38.1%)	5/24 (20.8%)
First incidence (days)	474	522	532	287
Poly-3 test	P=0.321/P=0.262	P=0.402N	P=0.370	P=0.421

(T) Terminal sacrifice
[a] Number of neoplasm-bearing animals/number of animals with tissue examined microscopically
[b] Poly-3 estimated neoplasm incidence after adjustment for intercurrent mortality
[c] Observed incidence at terminal kill
[d] Beneath the control incidence are the P values associated with the trend tests (dose trend/coded dose trend); coded dose treated the exposure concentrations as evenly spaced (0, 1, 2, and 3). Beneath the exposed group incidence are the P values corresponding to pairwise comparisons between the controls and that exposed 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 an exposed group is indicated by **N**.
[e] Not applicable; no neoplasms in animal group

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	24	22	27	17
Natural deaths		5	3	8
Survivors				
Died last week of study	1		1	
Terminal sacrifice	26	23	19	25
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(49)	(50)
Hemorrhage				1 (2%)
Inflammation		2 (4%)	1 (2%)	1 (2%)
Intestine large, cecum	(51)	(50)	(49)	(49)
Inflammation, chronic		1 (2%)		
Intestine large, colon	(51)	(50)	(49)	(49)
Developmental malformation			1 (2%)	
Inflammation		1 (2%)		
Mineralization			1 (2%)	
Lymphoid tissue, inflammation		1 (2%)		
Muscularis, inflammation, chronic			1 (2%)	
Intestine large, rectum	(51)	(50)	(49)	(50)
Inflammation, chronic		1 (2%)		
Intestine small, duodenum	(51)	(50)	(49)	(49)
Autolysis				1 (2%)
Dilatation			1 (2%)	
Intestine small, ileum	(51)	(50)	(48)	(49)
Autolysis				1 (2%)
Intestine small, jejunum	(51)	(50)	(47)	(47)
Autolysis				1 (2%)
Inflammation, chronic		1 (2%)		
Liver	(51)	(50)	(49)	(50)
Angiectasis	2 (4%)	1 (2%)	1 (2%)	1 (2%)
Basophilic focus	1 (2%)	1 (2%)	1 (2%)	1 (2%)
Cyst		1 (2%)		
Cyst multilocular	3 (6%)		1 (2%)	
Degeneration, cystic	1 (2%)			2 (4%)
Eosinophilic focus	1 (2%)	1 (2%)	1 (2%)	5 (10%)
Hematopoietic cell proliferation	2 (4%)	4 (8%)	3 (6%)	1 (2%)
Hepatodiaphragmatic nodule	1 (2%)			1 (2%)
Infiltration cellular, lymphocyte	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Inflammation, chronic active	6 (12%)	1 (2%)	6 (12%)	5 (10%)
Tension lipidosis			1 (2%)	1 (2%)
Vacuolization cytoplasmic	12 (24%)	8 (16%)	4 (8%)	2 (4%)
Bile duct, hyperplasia	13 (25%)	15 (30%)	10 (20%)	10 (20%)
Capsule, hemorrhage, focal	2 (4%)			
Centrilobular, necrosis			1 (2%)	
Periportal, inflammation, chronic	3 (6%)	7 (14%)	5 (10%)	5 (10%)
Right lateral lobe, developmental malformation		1 (2%)		
Mesentery	(2)	(2)	(0)	(1)
Fat, necrosis	2 (100%)	1 (50%)		1 (100%)
Oral mucosa	(3)	(1)	(4)	(4)
Keratin cyst			3 (75%)	2 (50%)
Gingival, inflammation				1 (25%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Alimentary System (continued)				
Pancreas	(51)	(50)	(49)	(49)
Inflammation, chronic		7 (14%)		1 (2%)
Lipomatosis	1 (2%)	1 (2%)	1 (2%)	2 (4%)
Acinar cell, degeneration	20 (39%)	24 (48%)	20 (41%)	24 (49%)
Acinar cell, hyperplasia				1 (2%)
Artery, mineralization			1 (2%)	
Salivary glands	(51)	(49)	(49)	(50)
Inflammation, chronic	1 (2%)	2 (4%)		
Artery, mineralization			1 (2%)	
Stomach, forestomach	(51)	(50)	(49)	(50)
Edema	1 (2%)		1 (2%)	2 (4%)
Hyperplasia	2 (4%)	2 (4%)		1 (2%)
Inflammation	1 (2%)	4 (8%)	1 (2%)	
Ulcer				1 (2%)
Stomach, glandular	(51)	(50)	(49)	(50)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation				1 (2%)
Mineralization			1 (2%)	
Glands, dilatation			1 (2%)	
Tooth	(0)	(0)	(0)	(0)
Cardiovascular System				
Blood vessel	(51)	(50)	(49)	(50)
Mineralization			1 (2%)	
Heart	(51)	(50)	(49)	(50)
Cardiomyopathy	23 (45%)	18 (36%)	10 (20%)	12 (24%)
Artery, mineralization			1 (2%)	
Myocardium, necrosis		1 (2%)		
Endocrine System				
Adrenal cortex	(51)	(50)	(49)	(50)
Angiectasis	2 (4%)	1 (2%)		2 (4%)
Atrophy	2 (4%)		2 (4%)	
Cyst				1 (2%)
Degeneration, cystic	46 (90%)	43 (86%)	43 (88%)	45 (90%)
Hematopoietic cell proliferation				1 (2%)
Hyperplasia	1 (2%)	3 (6%)	1 (2%)	
Hypertrophy	5 (10%)	11 (22%)	7 (14%)	7 (14%)
Metaplasia, osseous	1 (2%)			
Vacuolization cytoplasmic	1 (2%)		1 (2%)	
Adrenal medulla	(48)	(50)	(49)	(50)
Islets, pancreatic	(51)	(50)	(49)	(49)
Hyperplasia	1 (2%)	1 (2%)	3 (6%)	
Parathyroid gland	(48)	(47)	(46)	(43)
Hyperplasia		2 (4%)	1 (2%)	1 (2%)
Pituitary gland	(51)	(50)	(50)	(50)
Angiectasis	2 (4%)			
Cyst			1 (2%)	
Pars distalis, cyst	2 (4%)	2 (4%)	1 (2%)	1 (2%)
Pars distalis, hyperplasia	4 (8%)	3 (6%)	3 (6%)	2 (4%)
Pars intermedia, cyst	1 (2%)	3 (6%)		

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System (continued)				
Thyroid gland	(51)	(50)	(49)	(50)
Infiltration cellular, lymphocyte	1 (2%)			
Inflammation, chronic	1 (2%)	1 (2%)		2 (4%)
Ultimobranchial cyst	2 (4%)	4 (8%)	1 (2%)	5 (10%)
C-cell, hyperplasia	7 (14%)	4 (8%)	3 (6%)	4 (8%)
Follicular cell, hyperplasia				1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(0)
Genital System				
Clitoral gland	(50)	(49)	(48)	(50)
Atrophy		6 (12%)	4 (8%)	2 (4%)
Hyperplasia		2 (4%)	2 (4%)	
Inflammation	34 (68%)	33 (67%)	32 (67%)	28 (56%)
Duct, dilatation	11 (22%)	12 (24%)	10 (21%)	9 (18%)
Duct, hyperplasia, squamous	2 (4%)	1 (2%)	2 (4%)	
Ovary	(51)	(50)	(49)	(50)
Angiectasis		1 (2%)		
Atrophy	49 (96%)	47 (94%)	46 (94%)	46 (92%)
Cyst	15 (29%)	9 (18%)	17 (35%)	22 (44%)
Hyperplasia, sertoliform	23 (45%)	17 (34%)	13 (27%)	23 (46%)
Corpus luteum, cyst	1 (2%)	2 (4%)	1 (2%)	2 (4%)
Granulosa cell, hyperplasia				1 (2%)
Interstitial cell, hyperplasia		1 (2%)	2 (4%)	
Oviduct	(51)	(50)	(49)	(50)
Uterus	(51)	(50)	(49)	(50)
Atrophy	4 (8%)	1 (2%)	2 (4%)	
Dilatation	1 (2%)			
Hemorrhage		1 (2%)		
Hyperplasia, atypical, focal	6 (12%)	14 (28%)	16 (33%)	20 (40%)
Hypoplasia		1 (2%)		
Inflammation	2 (4%)	1 (2%)		1 (2%)
Metaplasia, squamous	2 (4%)	6 (12%)	8 (16%)	13 (26%)
Pigmentation, focal	1 (2%)			
Endometrium, hyperplasia	2 (4%)	7 (14%)	3 (6%)	12 (24%)
Endometrium, hyperplasia, cystic	15 (29%)	14 (28%)	20 (41%)	17 (34%)
Vagina	(51)	(50)	(49)	(49)
Inflammation	12 (24%)	22 (44%)	18 (37%)	17 (35%)
Mucocyte, hyperplasia	43 (84%)	33 (66%)	35 (71%)	33 (67%)
Hematopoietic System				
Bone marrow	(51)	(50)	(49)	(50)
Hyperplasia	1 (2%)	1 (2%)		
Myeloid cell, hyperplasia		1 (2%)		
Lymph node	(16)	(14)	(6)	(10)
Hyperplasia, lymphoid	1 (6%)			
Infiltration cellular, plasma cell	1 (6%)			
Axillary, degeneration, cystic	1 (6%)			
Axillary, infiltration cellular, plasma cell	2 (13%)			
Deep cervical, hyperplasia, lymphoid				1 (10%)
Deep cervical, infiltration cellular, plasma cell	1 (6%)			1 (10%)

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System (continued)								
Lymph node (continued)	(16)		(14)		(6)		(10)	
Inguinal, hyperplasia, lymphoid	1	(6%)						
Inguinal, infiltration cellular, plasma cell	1	(6%)	1	(7%)				
Lumbar, degeneration, cystic	12	(75%)	11	(79%)	3	(50%)	5	(50%)
Lumbar, hyperplasia, lymphoid	5	(31%)	10	(71%)	3	(50%)	3	(30%)
Lumbar, infiltration cellular, plasma cell	10	(63%)	12	(86%)	5	(83%)	5	(50%)
Mediastinal, hemorrhage					1	(17%)	1	(10%)
Mediastinal, hyperplasia, lymphoid	1	(6%)						
Mediastinal, pigmentation	1	(6%)					1	(10%)
Pancreatic, hemorrhage							1	(10%)
Pancreatic, pigmentation	1	(6%)					1	(10%)
Popliteal, degeneration, cystic	1	(6%)			1	(17%)	1	(10%)
Popliteal, hyperplasia, lymphoid	2	(13%)			2	(33%)		
Popliteal, infiltration cellular, plasma cell	2	(13%)	1	(7%)	2	(33%)	1	(10%)
Renal, degeneration, cystic	1	(6%)	1	(7%)				
Renal, hyperplasia, lymphoid			1	(7%)				
Renal, infiltration cellular, plasma cell	1	(6%)	1	(7%)				
Thoracic, infiltration cellular, plasma cell	1	(6%)						
Lymph node, mandibular	(51)		(50)		(49)		(50)	
Degeneration, cystic	16	(31%)	9	(18%)	8	(16%)	8	(16%)
Hemorrhage	1	(2%)						
Hyperplasia, lymphoid	4	(8%)	14	(28%)	11	(22%)	5	(10%)
Infiltration cellular, plasma cell	43	(84%)	40	(80%)	43	(88%)	39	(78%)
Inflammation, suppurative							1	(2%)
Lymph node, mesenteric	(51)		(50)		(47)		(50)	
Degeneration, cystic	1	(2%)			1	(2%)	1	(2%)
Depletion lymphoid	1	(2%)	1	(2%)			1	(2%)
Hemorrhage			1	(2%)			2	(4%)
Hyperplasia, lymphoid	5	(10%)	3	(6%)	1	(2%)	2	(4%)
Infiltration cellular, plasma cell	5	(10%)	3	(6%)	1	(2%)	1	(2%)
Pigmentation	2	(4%)	1	(2%)				
Sinus, dilatation							1	(2%)
Spleen	(51)		(50)		(49)		(50)	
Hematopoietic cell proliferation	15	(29%)	22	(44%)	10	(20%)	6	(12%)
Hyperplasia, stromal			1	(2%)				
Pigmentation	33	(65%)	31	(62%)	34	(69%)	43	(86%)
Lymphocyte, atrophy			1	(2%)				
Red pulp, atrophy	1	(2%)						
Thymus	(51)		(44)		(45)		(48)	
Cyst	17	(33%)	18	(41%)	21	(47%)	17	(35%)
Hemorrhage							1	(2%)
Inflammation, chronic			1	(2%)				
Integumentary System								
Mammary gland	(51)		(49)		(49)		(50)	
Atypical focus	19	(37%)	23	(47%)	21	(43%)	23	(46%)
Galactocele	2	(4%)	2	(4%)	4	(8%)	6	(12%)
Lactation	26	(51%)	32	(65%)	31	(63%)	30	(60%)
Alveolus, hyperplasia	42	(82%)	38	(78%)	42	(86%)	40	(80%)
Duct, dilatation	3	(6%)	5	(10%)			4	(8%)
Lobules, hyperplasia					1	(2%)		
Skin	(51)		(50)		(49)		(50)	
Abscess	1	(2%)						
Inflammation	23	(45%)	16	(32%)	9	(18%)	7	(14%)

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Musculoskeletal System								
Bone, femur	(51)		(50)		(49)		(50)	
Fibrous osteodystrophy					1	(2%)		
Skeletal muscle	(1)		(0)		(1)		(1)	
Head, hyalinization, focal					1	(100%)		
Nervous System								
Brain, brain stem	(51)		(50)		(49)		(50)	
Compression	26	(51%)	26	(52%)	23	(47%)	26	(52%)
Hemorrhage			1	(2%)	1	(2%)	1	(2%)
Hydrocephalus					1	(2%)		
Brain, cerebellum	(51)		(50)		(49)		(50)	
Hydrocephalus	2	(4%)					2	(4%)
Brain, cerebrum	(51)		(50)		(49)		(50)	
Hydrocephalus	2	(4%)	2	(4%)	6	(12%)	5	(10%)
Respiratory System								
Lung	(51)		(50)		(49)		(50)	
Edema			1	(2%)				
Hemorrhage	1	(2%)					2	(4%)
Infiltration cellular, histiocyte	11	(22%)	10	(20%)	10	(20%)	16	(32%)
Infiltration cellular, lymphocyte					1	(2%)	1	(2%)
Inflammation	3	(6%)	1	(2%)	4	(8%)	3	(6%)
Alveolar epithelium, hyperplasia	1	(2%)	1	(2%)	2	(4%)	1	(2%)
Artery, mineralization			1	(2%)			3	(6%)
Peribronchial, inflammation, chronic			1	(2%)	1	(2%)		
Perivascular, inflammation			1	(2%)	3	(6%)	1	(2%)
Nose	(51)		(50)		(49)		(50)	
Fibrous osteodystrophy					1	(2%)		
Foreign body			1	(2%)			1	(2%)
Hemorrhage			1	(2%)				
Inflammation	4	(8%)	5	(10%)	5	(10%)	2	(4%)
Goblet cell, hyperplasia	1	(2%)	2	(4%)	2	(4%)		
Nasolacrimal duct, inflammation	29	(57%)	29	(58%)	31	(63%)	30	(60%)
Olfactory epithelium, hyaline droplet	4	(8%)	5	(10%)	1	(2%)	6	(12%)
Respiratory epithelium, hyperplasia	1	(2%)						
Trachea	(51)		(50)		(49)		(50)	
Inflammation	2	(4%)			3	(6%)	3	(6%)
Special Senses System								
Ear	(0)		(1)		(0)		(0)	
Eye	(51)		(49)		(48)		(49)	
Cataract			1	(2%)	1	(2%)	1	(2%)
Bilateral, retina, degeneration	2	(4%)	5	(10%)	1	(2%)	4	(8%)
Bilateral, cataract							1	(2%)
Cornea, inflammation	1	(2%)						
Retina, degeneration	3	(6%)	1	(2%)	5	(10%)		
Harderian gland	(51)		(49)		(49)		(50)	
Atrophy, focal	2	(4%)						
Hyperplasia	3	(6%)	2	(4%)	4	(8%)	5	(10%)
Infiltration cellular, lymphocyte	3	(6%)	2	(4%)	2	(4%)	1	(2%)
Inflammation	7	(14%)	10	(20%)	10	(20%)	4	(8%)
Zymbal's gland	(0)		(0)		(0)		(0)	

Table B3a
Summary of the Incidence of Nonneoplastic Lesions in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Urinary System				
Kidney	(51)	(50)	(49)	(50)
Cyst	15 (29%)	16 (32%)	8 (16%)	7 (14%)
Degeneration, mucoid			1 (2%)	1 (2%)
Hemorrhage	1 (2%)			1 (2%)
Hydronephrosis			1 (2%)	1 (2%)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation	1 (2%)	1 (2%)		
Mineralization	31 (61%)	33 (66%)	27 (55%)	34 (68%)
Nephropathy	27 (53%)	21 (42%)	18 (37%)	17 (34%)
Polycystic kidney			1 (2%)	1 (2%)
Epithelium, pelvis, hyperplasia	1 (2%)			
Urinary bladder	(51)	(49)	(49)	(48)
Dilatation				1 (2%)
Hemorrhage				1 (2%)
Infiltration cellular, lymphocyte	2 (4%)			

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	51	50	50	50
Early deaths				
Moribund	24	26	27	22
Natural deaths		7	1	6
Survivors				
Died last week of study	1	1		
Terminal sacrifice	26	16	22	22
Animals examined microscopically	51	50	50	50
Alimentary System				
Esophagus	(51)	(50)	(50)	(50)
Inflammation		1 (2%)	2 (4%)	
Necrosis		1 (2%)		
Intestine large, cecum	(51)	(50)	(50)	(50)
Dilatation		1 (2%)		
Erosion		1 (2%)		
Inflammation, chronic		1 (2%)		
Intestine large, colon	(51)	(50)	(50)	(50)
Intestine large, rectum	(51)	(50)	(50)	(50)
Intestine small, duodenum	(51)	(50)	(50)	(50)
Intestine small, ileum	(51)	(48)	(50)	(49)
Intestine small, jejunum	(51)	(48)	(50)	(50)
Liver	(51)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)	4 (8%)	3 (6%)
Basophilic focus	1 (2%)	1 (2%)	1 (2%)	6 (12%)
Cyst		2 (4%)	2 (4%)	
Cyst multilocular	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Degeneration, cystic	1 (2%)	3 (6%)	5 (10%)	1 (2%)
Eosinophilic focus	1 (2%)	2 (4%)	5 (10%)	3 (6%)
Eosinophilic focus, multiple				1 (2%)
Hematopoietic cell proliferation	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Hepatodiaphragmatic nodule	1 (2%)	2 (4%)		1 (2%)
Infiltration cellular, lymphocyte	3 (6%)		1 (2%)	2 (4%)
Inflammation, chronic active	6 (12%)	3 (6%)	6 (12%)	4 (8%)
Mixed cell focus				1 (2%)
Necrosis		2 (4%)		
Vacuolization cytoplasmic	12 (24%)	9 (18%)	11 (22%)	13 (26%)
Bile duct, hyperplasia	13 (25%)	7 (14%)	16 (32%)	8 (16%)
Capsule, hemorrhage, focal	2 (4%)	1 (2%)		
Caudate lobe, developmental malformation				1 (2%)
Left lateral lobe, developmental malformation				1 (2%)
Median lobe, developmental malformation		1 (2%)		
Oval cell, hyperplasia		1 (2%)		
Periportal, inflammation, chronic	3 (6%)	3 (6%)	5 (10%)	2 (4%)
Mesentery	(2)	(2)	(1)	(0)
Cyst		1 (50%)		
Fat, necrosis	2 (100%)	2 (100%)		
Oral mucosa	(3)	(5)	(7)	(4)
Keratin cyst		2 (40%)	4 (57%)	3 (75%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Alimentary System (continued)				
Pancreas	(51)	(50)	(50)	(50)
Inflammation, chronic		1 (2%)		2 (4%)
Lipomatosis	1 (2%)	1 (2%)	3 (6%)	1 (2%)
Acinar cell, degeneration	20 (39%)	19 (38%)	26 (52%)	23 (46%)
Acinar cell, hyperplasia			1 (2%)	
Artery, inflammation, chronic			1 (2%)	
Salivary glands	(51)	(50)	(50)	(50)
Inflammation, chronic	1 (2%)	2 (4%)	1 (2%)	1 (2%)
Stomach, forestomach	(51)	(50)	(50)	(50)
Edema	1 (2%)	3 (6%)		1 (2%)
Hyperplasia	2 (4%)	4 (8%)		
Inflammation	1 (2%)	2 (4%)		1 (2%)
Keratin cyst		1 (2%)		1 (2%)
Ulcer			1 (2%)	
Stomach, glandular	(51)	(50)	(50)	(50)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation		1 (2%)		
Tooth	(0)	(1)	(0)	(0)
Dysplasia		1 (100%)		
Cardiovascular System				
Blood vessel	(51)	(50)	(50)	(50)
Mineralization		1 (2%)		
Heart	(51)	(50)	(50)	(50)
Cardiomyopathy	23 (45%)	22 (44%)	16 (32%)	26 (52%)
Endocardium, hyperplasia				1 (2%)
Endocrine System				
Adrenal cortex	(51)	(50)	(50)	(50)
Angiectasis	2 (4%)	1 (2%)	3 (6%)	1 (2%)
Atrophy	2 (4%)	1 (2%)		1 (2%)
Degeneration, cystic	46 (90%)	43 (86%)	44 (88%)	42 (84%)
Hematopoietic cell proliferation			1 (2%)	
Hyperplasia	1 (2%)			1 (2%)
Hypertrophy	5 (10%)	2 (4%)	2 (4%)	5 (10%)
Metaplasia, osseous	1 (2%)			
Vacuolization cytoplasmic	1 (2%)			
Adrenal medulla	(48)	(49)	(48)	(49)
Hyperplasia		1 (2%)	1 (2%)	2 (4%)
Islets, pancreatic	(51)	(50)	(50)	(50)
Hyperplasia	1 (2%)	1 (2%)		
Parathyroid gland	(48)	(46)	(44)	(46)
Hyperplasia		1 (2%)		
Pituitary gland	(51)	(50)	(50)	(49)
Angiectasis	2 (4%)		2 (4%)	1 (2%)
Pigmentation			1 (2%)	
Pars distalis, cyst	2 (4%)	1 (2%)	1 (2%)	2 (4%)
Pars distalis, hyperplasia	4 (8%)	2 (4%)	4 (8%)	1 (2%)
Pars intermedia, cyst	1 (2%)	1 (2%)		1 (2%)

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Endocrine System (continued)				
Thyroid gland	(51)	(50)	(50)	(50)
Infiltration cellular, lymphocyte	1 (2%)	1 (2%)		
Inflammation, chronic	1 (2%)		1 (2%)	1 (2%)
Ultimobranchial cyst	2 (4%)	1 (2%)	2 (4%)	2 (4%)
C-cell, hyperplasia	7 (14%)		3 (6%)	4 (8%)
Follicle, cyst			1 (2%)	
Follicular cell, hyperplasia		1 (2%)	2 (4%)	
General Body System				
Tissue NOS	(0)	(0)	(0)	(0)
Genital System				
Clitoral gland	(50)	(50)	(49)	(49)
Atrophy		3 (6%)	1 (2%)	2 (4%)
Hyperplasia			1 (2%)	3 (6%)
Infiltration cellular, lymphocyte		1 (2%)		
Inflammation	34 (68%)	32 (64%)	29 (59%)	34 (69%)
Keratin cyst				2 (4%)
Duct, dilatation	11 (22%)	17 (34%)	9 (18%)	9 (18%)
Duct, hyperplasia, squamous	2 (4%)	1 (2%)	1 (2%)	5 (10%)
Ovary	(51)	(50)	(50)	(50)
Atrophy	49 (96%)	46 (92%)	45 (90%)	42 (84%)
Cyst	15 (29%)	12 (24%)	15 (30%)	17 (34%)
Hyperplasia, sertoliform	23 (45%)	20 (40%)	19 (38%)	28 (56%)
Corpus luteum, cyst	1 (2%)	1 (2%)		2 (4%)
Interstitial cell, hyperplasia		2 (4%)	2 (4%)	
Oviduct	(51)	(50)	(50)	(50)
Pigmentation		1 (2%)		
Uterus	(51)	(50)	(50)	(50)
Atrophy	4 (8%)	1 (2%)	2 (4%)	
Dilatation	1 (2%)			
Hyperplasia, atypical, focal	6 (12%)	10 (20%)	7 (14%)	9 (18%)
Hypoplasia		1 (2%)		
Inflammation	2 (4%)	3 (6%)	2 (4%)	3 (6%)
Metaplasia, squamous	2 (4%)	6 (12%)	6 (12%)	1 (2%)
Pigmentation, focal	1 (2%)			
Cervix, muscularis, hypertrophy				1 (2%)
Cervix, hyperplasia			1 (2%)	
Endometrium, hyperplasia	2 (4%)	2 (4%)	5 (10%)	4 (8%)
Endometrium, hyperplasia, cystic	15 (29%)	23 (46%)	12 (24%)	22 (44%)
Vagina	(51)	(50)	(48)	(50)
Hemorrhage		1 (2%)		1 (2%)
Inflammation	12 (24%)	17 (34%)	15 (31%)	18 (36%)
Mucocyte, hyperplasia	43 (84%)	33 (66%)	33 (69%)	39 (78%)

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Hematopoietic System				
Bone marrow	(51)	(50)	(50)	(50)
Hyperplasia	1 (2%)		1 (2%)	1 (2%)
Myeloid cell, hyperplasia		2 (4%)		1 (2%)
Lymph node	(16)	(15)	(12)	(13)
Degeneration, cystic		1 (7%)		
Hyperplasia, lymphoid	1 (6%)			
Infiltration cellular, plasma cell	1 (6%)	3 (20%)		
Axillary, degeneration, cystic	1 (6%)			
Axillary, hyperplasia, lymphoid			1 (8%)	
Axillary, infiltration cellular, plasma cell	2 (13%)		1 (8%)	
Deep cervical, infiltration cellular, plasma cell	1 (6%)			
Inguinal, degeneration, cystic				2 (15%)
Inguinal, hyperplasia, lymphoid	1 (6%)		1 (8%)	2 (15%)
Inguinal, infiltration cellular, plasma cell	1 (6%)	1 (7%)	1 (8%)	2 (15%)
Lumbar, degeneration, cystic	12 (75%)	7 (47%)	9 (75%)	5 (38%)
Lumbar, hemorrhage		1 (7%)		
Lumbar, hyperplasia, lymphoid	5 (31%)	4 (27%)	7 (58%)	7 (54%)
Lumbar, infiltration cellular, plasma cell	10 (63%)	7 (47%)	10 (83%)	9 (69%)
Mediastinal, sinus, dilatation			1 (8%)	
Mediastinal, hemorrhage			1 (8%)	1 (8%)
Mediastinal, hyperplasia, lymphoid	1 (6%)		1 (8%)	
Mediastinal, infiltration cellular, plasma cell				2 (15%)
Mediastinal, pigmentation	1 (6%)			
Pancreatic, hyperplasia, lymphoid		1 (7%)		
Pancreatic, pigmentation	1 (6%)			
Popliteal, degeneration, cystic	1 (6%)			
Popliteal, hyperplasia, lymphoid	2 (13%)			
Popliteal, infiltration cellular, plasma cell	2 (13%)			1 (8%)
Renal, degeneration, cystic	1 (6%)			3 (23%)
Renal, hyperplasia, lymphoid		1 (7%)		2 (15%)
Renal, infiltration cellular, plasma cell	1 (6%)			4 (31%)
Thoracic, infiltration cellular, plasma cell	1 (6%)			
Lymph node, mandibular	(51)	(50)	(49)	(50)
Degeneration, cystic	16 (31%)	8 (16%)	6 (12%)	12 (24%)
Hemorrhage	1 (2%)			1 (2%)
Hyperplasia, lymphoid	4 (8%)	10 (20%)	11 (22%)	5 (10%)
Infiltration cellular, plasma cell	43 (84%)	40 (80%)	41 (84%)	41 (82%)
Inflammation, suppurative		1 (2%)		
Lymph node, mesenteric	(51)	(50)	(50)	(50)
Degeneration, cystic	1 (2%)	1 (2%)		5 (10%)
Depletion lymphoid	1 (2%)	1 (2%)		1 (2%)
Hemorrhage		1 (2%)	1 (2%)	
Hyperplasia, lymphoid	5 (10%)	3 (6%)	4 (8%)	7 (14%)
Infiltration cellular, plasma cell	5 (10%)	1 (2%)	1 (2%)	5 (10%)
Pigmentation	2 (4%)			
Spleen	(51)	(50)	(50)	(50)
Hematopoietic cell proliferation	15 (29%)	13 (26%)	17 (34%)	10 (20%)
Hyperplasia, lymphoid			1 (2%)	
Pigmentation	33 (65%)	28 (56%)	25 (50%)	30 (60%)
Red pulp, atrophy	1 (2%)			
Thymus	(51)	(44)	(46)	(48)
Cyst	17 (33%)	13 (30%)	22 (48%)	17 (35%)
Hemorrhage		1 (2%)		
Hyperplasia, lymphoid		1 (2%)		
Inflammation, chronic		1 (2%)		

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Integumentary System								
Mammary gland	(51)		(49)		(50)		(50)	
Atypical focus	19	(37%)	13	(27%)	19	(38%)	18	(36%)
Fibrosis							1	(2%)
Galactocele	2	(4%)	3	(6%)	4	(8%)	3	(6%)
Lactation	26	(51%)	29	(59%)	29	(58%)	20	(40%)
Alveolus, hyperplasia	42	(82%)	36	(73%)	43	(86%)	45	(90%)
Duct, dilatation	3	(6%)			1	(2%)	4	(8%)
Duct, inflammation							1	(2%)
Skin	(51)		(50)		(50)		(50)	
Abscess	1	(2%)						
Cyst epithelial inclusion			1	(2%)				
Hyperkeratosis			1	(2%)				
Inflammation	23	(45%)	23	(46%)	21	(42%)	29	(58%)
Musculoskeletal System								
Bone, femur	(51)		(50)		(50)		(50)	
Osteopetrosis					1	(2%)		
Skeletal muscle	(1)		(0)		(4)		(1)	
Nervous System								
Brain, brain stem	(51)		(50)		(50)		(50)	
Compression	26	(51%)	23	(46%)	18	(36%)	19	(38%)
Hemorrhage			2	(4%)	1	(2%)		
Hydrocephalus							1	(2%)
Brain, cerebellum	(51)		(50)		(50)		(50)	
Hemorrhage			1	(2%)			1	(2%)
Hydrocephalus	2	(4%)	3	(6%)			2	(4%)
Brain, cerebrum	(51)		(50)		(50)		(50)	
Hemorrhage			1	(2%)				
Hydrocephalus	2	(4%)	3	(6%)	2	(4%)	3	(6%)
Respiratory System								
Lung	(51)		(50)		(50)		(50)	
Autolysis			1	(2%)				
Edema							1	(2%)
Hemorrhage	1	(2%)	1	(2%)			1	(2%)
Infiltration cellular, histiocyte	11	(22%)	7	(14%)	4	(8%)	8	(16%)
Infiltration cellular, lymphocyte							1	(2%)
Inflammation	3	(6%)	3	(6%)	2	(4%)	3	(6%)
Alveolar epithelium, hyperplasia	1	(2%)	1	(2%)	1	(2%)		
Artery, mineralization			3	(6%)	1	(2%)	1	(2%)
Peribronchial, inflammation, chronic			1	(2%)				
Perivascular, inflammation			1	(2%)				
Nose	(51)		(50)		(50)		(50)	
Foreign body					1	(2%)	1	(2%)
Inflammation	4	(8%)	9	(18%)	4	(8%)	5	(10%)
Osteopetrosis					1	(2%)		
Goblet cell, hyperplasia	1	(2%)	1	(2%)	1	(2%)		
Nasolacrimal duct, inflammation	29	(57%)	25	(50%)	29	(58%)	33	(66%)
Olfactory epithelium, hyaline droplet	4	(8%)			3	(6%)	2	(4%)
Respiratory epithelium, hyaline droplet			1	(2%)				
Respiratory epithelium, hyperplasia	1	(2%)	1	(2%)				
Trachea	(51)		(50)		(50)		(50)	
Inflammation	2	(4%)	1	(2%)	1	(2%)	6	(12%)

Table B3b
Summary of the Incidence of Nonneoplastic Lesions in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Special Senses System				
Ear	(0)	(0)	(0)	(0)
Eye	(51)	(50)	(50)	(47)
Cataract		2 (4%)	2 (4%)	
Bilateral, retina, degeneration	2 (4%)	1 (2%)	6 (12%)	3 (6%)
Bilateral, cataract		2 (4%)		
Cornea, inflammation	1 (2%)			2 (4%)
Retina, autolysis		2 (4%)		
Retina, degeneration	3 (6%)	2 (4%)	3 (6%)	2 (4%)
Harderian gland	(51)	(50)	(50)	(50)
Atrophy, focal	2 (4%)			
Hyperplasia	3 (6%)	2 (4%)	1 (2%)	4 (8%)
Infiltration cellular, lymphocyte	3 (6%)	2 (4%)	1 (2%)	1 (2%)
Inflammation	7 (14%)	7 (14%)	9 (18%)	8 (16%)
Epithelium, degeneration		1 (2%)		
Zymbal's gland	(0)	(0)	(0)	(1)
Urinary System				
Kidney	(51)	(50)	(50)	(50)
Cyst	15 (29%)	11 (22%)	16 (32%)	9 (18%)
Degeneration, mucoid		1 (2%)	1 (2%)	
Hemorrhage	1 (2%)			
Hydronephrosis			1 (2%)	
Infiltration cellular, lymphocyte				1 (2%)
Inflammation	1 (2%)	1 (2%)		
Mineralization	31 (61%)	30 (60%)	36 (72%)	35 (70%)
Nephropathy	27 (53%)	19 (38%)	19 (38%)	20 (40%)
Polycystic kidney				1 (2%)
Bilateral, hydronephrosis			1 (2%)	
Epithelium, pelvis, hyperplasia	1 (2%)		1 (2%)	
Renal tubule, accumulation, hyaline droplet			1 (2%)	
Renal tubule, hyperplasia, focal				1 (2%)
Urinary bladder	(51)	(48)	(50)	(49)
Infiltration cellular, lymphocyte	2 (4%)			
Inflammation			1 (2%)	

Table B3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	52	50	50	50
Early deaths				
Moribund	24	19	23	21
Natural deaths	1	2	5	3
Survivors				
Died last week of study			1	2
Terminal sacrifice	27	29	21	24
Animals examined microscopically	52	50	50	50
Alimentary System				
Esophagus	(52)	(49)	(50)	(50)
Inflammation				1 (2%)
Intestine large, cecum	(51)	(49)	(49)	(50)
Dilatation			1 (2%)	
Inflammation, chronic			1 (2%)	
Intestine large, colon	(52)	(50)	(50)	(50)
Dilatation			1 (2%)	
Intestine large, rectum	(51)	(49)	(50)	(50)
Inflammation, chronic			1 (2%)	
Intestine small, ileum	(52)	(49)	(49)	(50)
Lymphoid tissue, inflammation, chronic active			1 (2%)	
Intestine small, jejunum	(51)	(49)	(48)	(50)
Inflammation, chronic			1 (2%)	
Liver	(52)	(50)	(50)	(50)
Angiectasis	8 (15%)	5 (10%)	2 (4%)	5 (10%)
Basophilic focus	1 (2%)	3 (6%)		2 (4%)
Cyst		1 (2%)		
Cyst multilocular	2 (4%)		1 (2%)	1 (2%)
Degeneration, cystic		3 (6%)	1 (2%)	1 (2%)
Eosinophilic focus	2 (4%)	3 (6%)	2 (4%)	2 (4%)
Hematopoietic cell proliferation	1 (2%)	3 (6%)	2 (4%)	4 (8%)
Hepatodiaphragmatic nodule	4 (8%)	4 (8%)	1 (2%)	1 (2%)
Infiltration cellular, lymphocyte	2 (4%)		1 (2%)	
Inflammation, chronic active	10 (19%)	5 (10%)	5 (10%)	4 (8%)
Necrosis	1 (2%)	3 (6%)		5 (10%)
Tension lipidosis	2 (4%)	3 (6%)	1 (2%)	
Vacuolization cytoplasmic	16 (31%)	11 (22%)	10 (20%)	12 (24%)
Bile duct, hyperplasia	12 (23%)	16 (32%)	14 (28%)	6 (12%)
Centrilobular, necrosis		1 (2%)		
Hepatocyte, periportal, hypertrophy				1 (2%)
Left lateral lobe, developmental malformation		1 (2%)		2 (4%)
Median lobe, developmental malformation		1 (2%)		
Periportal, inflammation, chronic	3 (6%)	3 (6%)	6 (12%)	5 (10%)
Mesentery	(3)	(3)	(4)	(4)
Fat, necrosis	1 (33%)	2 (67%)	3 (75%)	3 (75%)
Oral mucosa	(2)	(4)	(7)	(2)
Abscess			1 (14%)	
Keratin cyst		2 (50%)	1 (14%)	1 (50%)
Gingival, inflammation		1 (25%)	1 (14%)	
Pancreas	(52)	(49)	(50)	(50)
Accessory spleen				1 (2%)
Inflammation, chronic	1 (2%)	4 (8%)	2 (4%)	1 (2%)
Lipomatosis		1 (2%)	1 (2%)	
Acinar cell, degeneration	23 (44%)	33 (67%)	21 (42%)	21 (42%)

[a] Number of animals examined microscopically at the site and the number of animals with lesion

Table B3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Alimentary System (continued)				
Salivary glands	(52)	(50)	(50)	(50)
Infiltration cellular, lymphocyte				1 (2%)
Inflammation, chronic			1 (2%)	
Parotid gland, degeneration	1 (2%)			
Stomach, forestomach	(52)	(49)	(50)	(50)
Edema	2 (4%)		2 (4%)	
Hyperplasia	2 (4%)		1 (2%)	1 (2%)
Inflammation	3 (6%)		2 (4%)	
Keratin cyst	1 (2%)		1 (2%)	
Ulcer				2 (4%)
Stomach, glandular	(52)	(49)	(50)	(50)
Edema				1 (2%)
Infiltration cellular, lymphocyte			1 (2%)	
Cardiovascular System				
Heart	(52)	(50)	(50)	(50)
Cardiomyopathy	33 (63%)	28 (56%)	24 (48%)	21 (42%)
Endocrine System				
Adrenal cortex	(52)	(50)	(50)	(50)
Accessory adrenal cortical nodule				1 (2%)
Angiectasis		1 (2%)	3 (6%)	2 (4%)
Atrophy	1 (2%)		1 (2%)	
Degeneration, cystic	48 (92%)	42 (84%)	38 (76%)	41 (82%)
Hematopoietic cell proliferation				2 (4%)
Hypertrophy	3 (6%)	9 (18%)	4 (8%)	7 (14%)
Infarct		1 (2%)		1 (2%)
Adrenal medulla	(52)	(50)	(50)	(50)
Atrophy		1 (2%)		
Islets, pancreatic	(52)	(49)	(50)	(50)
Hyperplasia		1 (2%)		1 (2%)
Parathyroid gland	(48)	(45)	(47)	(44)
Hyperplasia		1 (2%)		1 (2%)
Inflammation			1 (2%)	
Thrombosis		1 (2%)		
Pituitary gland	(52)	(50)	(50)	(50)
Angiectasis	2 (4%)	2 (4%)		1 (2%)
Degeneration, cystic	1 (2%)			
Hypertrophy, focal	1 (2%)			
Necrosis	1 (2%)			
Pars distalis, cyst	2 (4%)	1 (2%)		1 (2%)
Pars distalis, hyperplasia	6 (12%)	3 (6%)	4 (8%)	5 (10%)
Pars intermedia, cyst		1 (2%)		
Thyroid gland	(52)	(49)	(50)	(50)
Cyst			1 (2%)	
Infiltration cellular, lymphocyte	1 (2%)			1 (2%)
Inflammation, chronic			1 (2%)	
Ultimobranchial cyst	2 (4%)	5 (10%)	3 (6%)	2 (4%)
C-cell, hyperplasia	3 (6%)	3 (6%)	3 (6%)	1 (2%)
Follicular cell, hyperplasia				1 (2%)
General Body System				
Tissue NOS	(0)	(0)	(1)	(1)

Table B3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System								
Clitoral gland	(50)		(50)		(49)		(48)	
Atrophy	6	(12%)	5	(10%)	6	(12%)	6	(13%)
Hyperplasia					1	(2%)	3	(6%)
Infiltration cellular, mast cell			1	(2%)				
Inflammation	26	(52%)	26	(52%)	24	(49%)	20	(42%)
Inflammation, suppurative	1	(2%)						
Keratin cyst			1	(2%)	1	(2%)	1	(2%)
Duct, dilatation	9	(18%)	13	(26%)	7	(14%)	9	(19%)
Duct, hyperplasia, squamous			1	(2%)	1	(2%)		
Duct, inflammation					1	(2%)		
Ovary	(51)		(50)		(50)		(50)	
Atrophy	47	(92%)	46	(92%)	45	(90%)	49	(98%)
Cyst	13	(25%)	12	(24%)	15	(30%)	13	(26%)
Hyperplasia, sertoliform	22	(43%)	20	(40%)	26	(52%)	27	(54%)
Corpus luteum, cyst	1	(2%)	2	(4%)	2	(4%)		
Interstitial cell, hyperplasia	4	(8%)	3	(6%)				
Rete ovarii, cyst	2	(4%)			2	(4%)		
Uterus	(52)		(50)		(50)		(50)	
Adenomyosis	1	(2%)	1	(2%)	1	(2%)		
Atrophy			1	(2%)	2	(4%)	2	(4%)
Dilatation	1	(2%)	1	(2%)			1	(2%)
Hyperplasia, atypical, focal	6	(12%)	16	(32%)	15	(30%)	21	(42%)
Inflammation	2	(4%)			2	(4%)	1	(2%)
Metaplasia, squamous	1	(2%)	4	(8%)	3	(6%)	11	(22%)
Pigmentation, focal							1	(2%)
Endometrium, degeneration							1	(2%)
Endometrium, hyperplasia	6	(12%)	6	(12%)	8	(16%)	5	(10%)
Endometrium, hyperplasia, cystic	15	(29%)	9	(18%)	9	(18%)	18	(36%)
Vagina	(51)		(50)		(50)		(50)	
Hemorrhage			1	(2%)				
Inflammation	14	(27%)	16	(32%)	20	(40%)	12	(24%)
Epithelium, atrophy					1	(2%)	2	(4%)
Mucocyte, hyperplasia	39	(76%)	39	(78%)	36	(72%)	35	(70%)
Hematopoietic System								
Bone marrow	(52)		(50)		(50)		(50)	
Hyperplasia	1	(2%)					2	(4%)
Myeloid cell, hyperplasia					1	(2%)		
Lymph node	(10)		(17)		(10)		(5)	
Axillary, hyperplasia, lymphoid	1	(10%)						
Axillary, infiltration cellular, plasma cell	2	(20%)	1	(6%)				
Lumbar, degeneration, cystic	8	(80%)	14	(82%)	7	(70%)	4	(80%)
Lumbar, hemorrhage			1	(6%)			1	(20%)
Lumbar, hyperplasia, lymphoid	6	(60%)	11	(65%)	6	(60%)	3	(60%)
Lumbar, infiltration cellular, plasma cell	9	(90%)	15	(88%)	6	(60%)	5	(100%)
Mediastinal, hemorrhage					1	(10%)		
Popliteal, degeneration, cystic	1	(10%)	3	(18%)	1	(10%)		
Popliteal, hyperplasia, lymphoid	1	(10%)	5	(29%)	1	(10%)	1	(20%)
Popliteal, infiltration cellular, plasma cell	2	(20%)	7	(41%)	1	(10%)	1	(20%)
Renal, degeneration, cystic	1	(10%)	2	(12%)				
Renal, hemorrhage					1	(10%)		
Renal, hyperplasia, lymphoid	1	(10%)	1	(6%)				
Renal, infiltration cellular, plasma cell	1	(10%)	2	(12%)	1	(10%)		

Table B3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System (continued)								
Lymph node, mandibular	(51)		(50)		(50)		(50)	
Degeneration, cystic	6	(12%)	9	(18%)	8	(16%)	7	(14%)
Hemorrhage					1	(2%)		
Hyperplasia, lymphoid	12	(24%)	9	(18%)	9	(18%)	8	(16%)
Infiltration cellular, plasma cell	42	(82%)	38	(76%)	40	(80%)	37	(74%)
Lymph node, mesenteric	(51)		(49)		(50)		(49)	
Degeneration, cystic	1	(2%)			1	(2%)	1	(2%)
Hyperplasia, lymphoid	5	(10%)	2	(4%)	5	(10%)	1	(2%)
Infiltration cellular, plasma cell	2	(4%)			3	(6%)	2	(4%)
Inflammation, chronic	1	(2%)						
Pigmentation					1	(2%)		
Spleen	(52)		(50)		(50)		(50)	
Hematopoietic cell proliferation	14	(27%)	15	(30%)	12	(24%)	16	(32%)
Hyperplasia, histiocytic, focal					1	(2%)		
Hyperplasia, lymphoid			2	(4%)				
Pigmentation	27	(52%)	26	(52%)	24	(48%)	29	(58%)
Thymus	(50)		(45)		(46)		(49)	
Atrophy							1	(2%)
Cyst	16	(32%)	19	(42%)	17	(37%)	20	(41%)
Integumentary System								
Mammary gland	(52)		(50)		(50)		(50)	
Atypical focus	12	(23%)	21	(42%)	22	(44%)	18	(36%)
Galactocele	2	(4%)	1	(2%)	1	(2%)	5	(10%)
Inflammation					1	(2%)		
Lactation	19	(37%)	6	(12%)	17	(34%)	20	(40%)
Alveolus, hyperplasia	35	(67%)	36	(72%)	40	(80%)	39	(78%)
Duct, dilatation					3	(6%)	3	(6%)
Skin	(52)		(50)		(50)		(50)	
Abscess	1	(2%)	1	(2%)				
Cyst epithelial inclusion	1	(2%)					1	(2%)
Inflammation	15	(29%)	21	(42%)	8	(16%)	12	(24%)
Epidermis, necrosis					1	(2%)		
Musculoskeletal System								
Bone	(0)		(1)		(0)		(0)	
Cartilage, sternum, degeneration			1	(100%)				
Bone, femur	(52)		(50)		(50)		(50)	
Hyperplasia					1	(2%)		
Osteopetrosis							1	(2%)
Skeletal muscle	(3)		(4)		(0)		(1)	
Inflammation, chronic active	1	(33%)						
Head, hyalinization, focal			1	(25%)				
Nervous System								
Brain, brain stem	(52)		(50)		(50)		(50)	
Compression	13	(25%)	11	(22%)	19	(38%)	20	(40%)
Hemorrhage			1	(2%)	1	(2%)	1	(2%)
Brain, cerebellum	(51)		(50)		(50)		(50)	
Hydrocephalus	1	(2%)			4	(8%)	3	(6%)
Brain, cerebrum	(52)		(50)		(50)		(50)	
Hydrocephalus	1	(2%)			1	(2%)	3	(6%)
Spinal cord	(0)		(1)		(0)		(1)	
Necrosis							1	(100%)

Table B3c
Summary of the Incidence of Nonneoplastic Lesions in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Respiratory System				
Lung	(52)	(50)	(50)	(50)
Edema			3 (6%)	
Hemorrhage	2 (4%)			1 (2%)
Infiltration cellular, histiocyte	10 (19%)	14 (28%)	11 (22%)	7 (14%)
Infiltration cellular, lymphocyte			1 (2%)	
Inflammation	1 (2%)	4 (8%)	1 (2%)	2 (4%)
Mineralization		1 (2%)		
Alveolar epithelium, hyperplasia	1 (2%)	4 (8%)	1 (2%)	1 (2%)
Artery, mineralization	1 (2%)			
Peribronchial, inflammation, chronic			1 (2%)	
Perivascular, inflammation			1 (2%)	
Nose	(52)	(50)	(50)	(50)
Inflammation	8 (15%)	2 (4%)	3 (6%)	6 (12%)
Osteopetrosis				1 (2%)
Goblet cell, hyperplasia	3 (6%)	3 (6%)	1 (2%)	3 (6%)
Nasolacrimal duct, inflammation	26 (50%)	34 (68%)	26 (52%)	26 (52%)
Nasolacrimal duct, keratin cyst				1 (2%)
Olfactory epithelium, hyaline droplet	15 (29%)	8 (16%)	5 (10%)	9 (18%)
Respiratory epithelium, hyperplasia	1 (2%)	1 (2%)		1 (2%)
Trachea	(52)	(49)	(50)	(50)
Inflammation	2 (4%)	2 (4%)	2 (4%)	3 (6%)
Special Senses System				
Ear	(0)	(0)	(2)	(0)
Eye	(52)	(50)	(49)	(50)
Cataract		1 (2%)	2 (4%)	1 (2%)
Inflammation	1 (2%)		1 (2%)	
Phthisis bulbi			1 (2%)	1 (2%)
Bilateral, retina, degeneration	3 (6%)	2 (4%)	3 (6%)	2 (4%)
Bilateral, cataract		1 (2%)		
Retina, degeneration	2 (4%)	2 (4%)	1 (2%)	3 (6%)
Harderian gland	(52)	(50)	(50)	(50)
Hyperplasia	3 (6%)	4 (8%)	5 (10%)	
Infiltration cellular, lymphocyte	1 (2%)	2 (4%)	3 (6%)	4 (8%)
Inflammation	8 (15%)	8 (16%)	12 (24%)	14 (28%)
Zymbal's gland	(0)	(0)	(1)	(0)
Urinary System				
Kidney	(52)	(50)	(50)	(50)
Cyst	10 (19%)	12 (24%)	17 (34%)	7 (14%)
Hydronephrosis	1 (2%)	1 (2%)		1 (2%)
Infiltration cellular, lymphocyte		2 (4%)		
Inflammation	3 (6%)	5 (10%)	2 (4%)	2 (4%)
Mineralization	22 (42%)	14 (28%)	21 (42%)	20 (40%)
Nephropathy	26 (50%)	23 (46%)	19 (38%)	22 (44%)
Epithelium, pelvis, hyperplasia		1 (2%)		
Renal tubule, accumulation, hyaline droplet			2 (4%)	
Urinary bladder	(52)	(49)	(49)	(50)
Hemorrhage				1 (2%)
Inflammation				1 (2%)

APPENDIX C
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

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CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

Procurement and Characterization of Ethinyl Estradiol

Ethinyl estradiol was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in one lot (57H1178). Identity and purity analyses were conducted by the study laboratory at the National Center for Toxicological Research (NCTR; Jefferson, AR). Reports on analyses performed in support of the ethinyl estradiol study are on file at the NCTR.

Lot 57H1178 of the chemical, a white crystalline solid, was identified as ethinyl estradiol by ^{1}H- and ^{13}C-nuclear magnetic resonance (NMR) spectroscopy and by gas chomatography-electron impact mass spectrometry (GC-EI MS). A nuclear Overhauser effect experiment was performed to distinguish between the α and β isomers of ethinyl estradiol; results confirmed that the chemical was the α isomer. Carbon-13 chemical shift data were in agreement with those that have been reported for 17α-derivatives of estradiol (Dionne and Poirier, 1995). Spectra were consistent with the structure of ethinyl estradiol, the spectra of a standard mixture containing estrone, estradiol, and ethinyl estradiol, and/or literature spectra (NIST, 1998). Representative ^{1}H- and ^{13}C-NMR and MS spectra are presented in Figures C1, C2, and C3, respectively.

Before, during, and after the study, the purity of lot 57H1178 was determined using ^{1}H-NMR (based on –CH groups), GC-EI MS, and GC with flame ionization detection (FID). ^{1}H-NMR consistently indicated a purity of 98.5%. GC-EI MS by systems A or B (Table C1) gave somewhat inconsistent values for purity ranging from 95.3% to greater than 99% due to thermal and solvent decomposition of the test material, but measurements at the end of the study indicated a purity of 99%. GC-FID by system C indicated a purity of 99.7%. The overall purity of lot 57H1178 was determined to be greater than 98.5%, and no identifiable impurities were detected.

To ensure stability, the bulk chemical was stored in amber glass bottles at room temperature. The stability of the bulk chemical was monitored during the study by the study laboratory using ^{1}H-NMR and GC-EI MS by system B; no degradation of the bulk chemical was detected.

Background Isoflavone Content of Base Diet

The base diet used for the current study was an irradiated soy- and alfalfa-free rodent feed, designated 5K96, obtained from Purina Mills, Inc. (Richmond, IN), in an attempt to maintain consistently low background exposure to phytoestrogens. This feed maintains the nutritional specifications of the NIH-31 feed and contains casein in place of soy and alfalfa meals. The control feed was routinely assayed for total isoflavone content (that is, genistein and daidzein) after acid hydrolysis by the study laboratory. Prior to the current study, native isoflavone content was determined for several lots of 5K96 feed using HPLC-electrospray MS methods; methodological details and the data from these studies have been published elsewhere (Doerge *et al*., 2000). During and following the current study, an additional 27 consecutive lots of 5K96 feed were analyzed by two HPLC MS techniques. System 1 consisted of a Hewlett-Packard HPLC (Hewlett-Packard, Palo Alto, CA) coupled to a Hewlett-Packard mass spectrometer operated in electrospray ionization mode with a Prodigy ODS(3) column (Phenomenex). The column parameters were 250 mm × 2.0 mm, 5 µm particle size, 100 Å. The mobile phase (flow rate of 0.2 mL per minute) consisted of A) acetonitrile and B) 3 mM ammonium formate, changing linearly from 20% A:80% B in 40 minutes, then held for 20 minutes. The first quadrupole of this system was operated in specific ion monitoring mode using m/z 253 for daidzein and m/z 269 for genistein. HPLC MS system 2 consisted of a Hewlett-Packard HPLC coupled to a ThermoFinnigan tandem quadrupole mass spectrometer (ThermoFinnigan, San Jose, CA) operated in electrospray ionization mode with a Polaris (MetaChem, Torrance, CA) C18-A or a Prodigy ODS(3) column. The column parameters were 250 mm × 2.0 mm, 5 µm particle size, 100 Å. The mobile phase (flow rate

of 0.2 mL per minute) consisted of A) acetonitrile and B) 0.1% formic acid, changing linearly (after a 1 minute initial hold) from either 5% A:95% B or 10% A:90% B to 95% A:5% B in 30 minutes, then held for 9 minutes. The first quadrupole HPLC/MS System 2 was scanned from m/z 140 to m/z 450 in 1 second. The results for analyses of 5K96 feed showed the concentrations of genistein and daidzein (mean ± standard error) to be 0.32 ± 0.26 ppm and 0.19 ± 0.15 ppm, respectively.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared at least every 9 weeks by mixing ethinyl estradiol with feed (Table C2). For the 0, 10, and 50 ppb dose formulations, intermediate solutions of ethinyl estradiol in 95% ethanol were prepared and directly injected into Purina 5K96 feed in a Patterson-Kelley twin-shell blender; mixing was conducted for 60 minutes with the intensifier bar, vacuum, and heater (95° C) on for the entire time. Using additional 5K96 feed, the 2 ppb dose formulation was prepared by a 1:5 dry dilution of the 10 ppb dose formulation previously prepared. Formulations were stored in stainless steel cans with lids secured with tie-downs at 4° ± 2° C for up to 9 weeks.

The study laboratory performed a series of homogeneity studies: 1 and 5 ppb dose formulations were analyzed using GC-EI MS by system A (Table C1), the 10 ppb dose formulation was analyzed using GC with electron capture (EC) detection by system D, and a 200 ppb dose formulation was analyzed by HPLC-fluorescence. HPLC-fluorescence was performed on a Waters instrument and used a Spherisorb™ CN (250 mm × 2 mm, 5 µm) column (Waters Corporation), a solvent system of hexanes/3.5% isopropyl alcohol flowing at 0.5 mL/minute for 17 minutes and then 1.5 mL/minute from 17 to 30 minutes, and a fluorescence detector (excitation 281 mm; emission 304 nm). Stability studies of a 5 ppb dose formulation were also performed by the study laboratory using GC-EI MS by system A. Homogeneity was confirmed, and stability was confirmed for at least 24 weeks for dose formulations stored in stainless steel cans at 2° to 8° C and for up to 16 days under simulated animal room conditions.

Periodic analyses of the dose formulations of ethinyl estradiol were performed by the study laboratory during the 2-year study using GC-EC by system D. Because of the very low exposure concentrations used in this study, the technical difficulties associated with measurements of such concentrations in the complex diet matrix were recognized, and a somewhat higher degree of variability than would be seen in studies with higher exposure concentrations was anticipated and accepted prior to the start of the study. Specifications for the dose formulations for the 2-year feed study were set as being within 30% of the target concentrations with a coefficient of variation of ± 20%. All 82 of the dose formulations analyzed met the study specifications (Table C3). Animal room samples of these dose formulations were also analyzed; all 22 of the samples were within 50% of the target concentrations (Table C4).

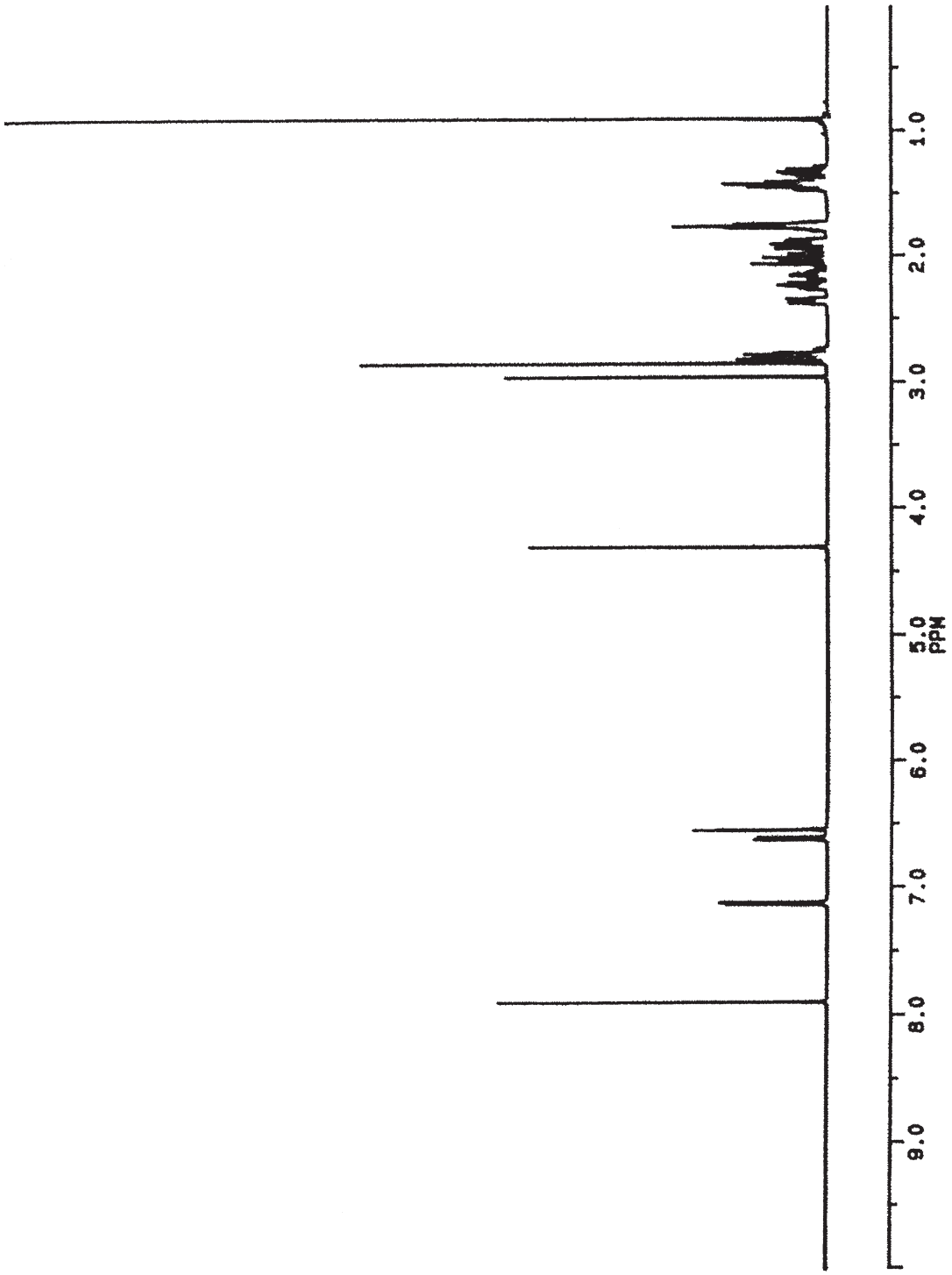

Figure C1
^{1}H-Nuclear Magnetic Resonance Spectrum of Ethinyl Estradiol

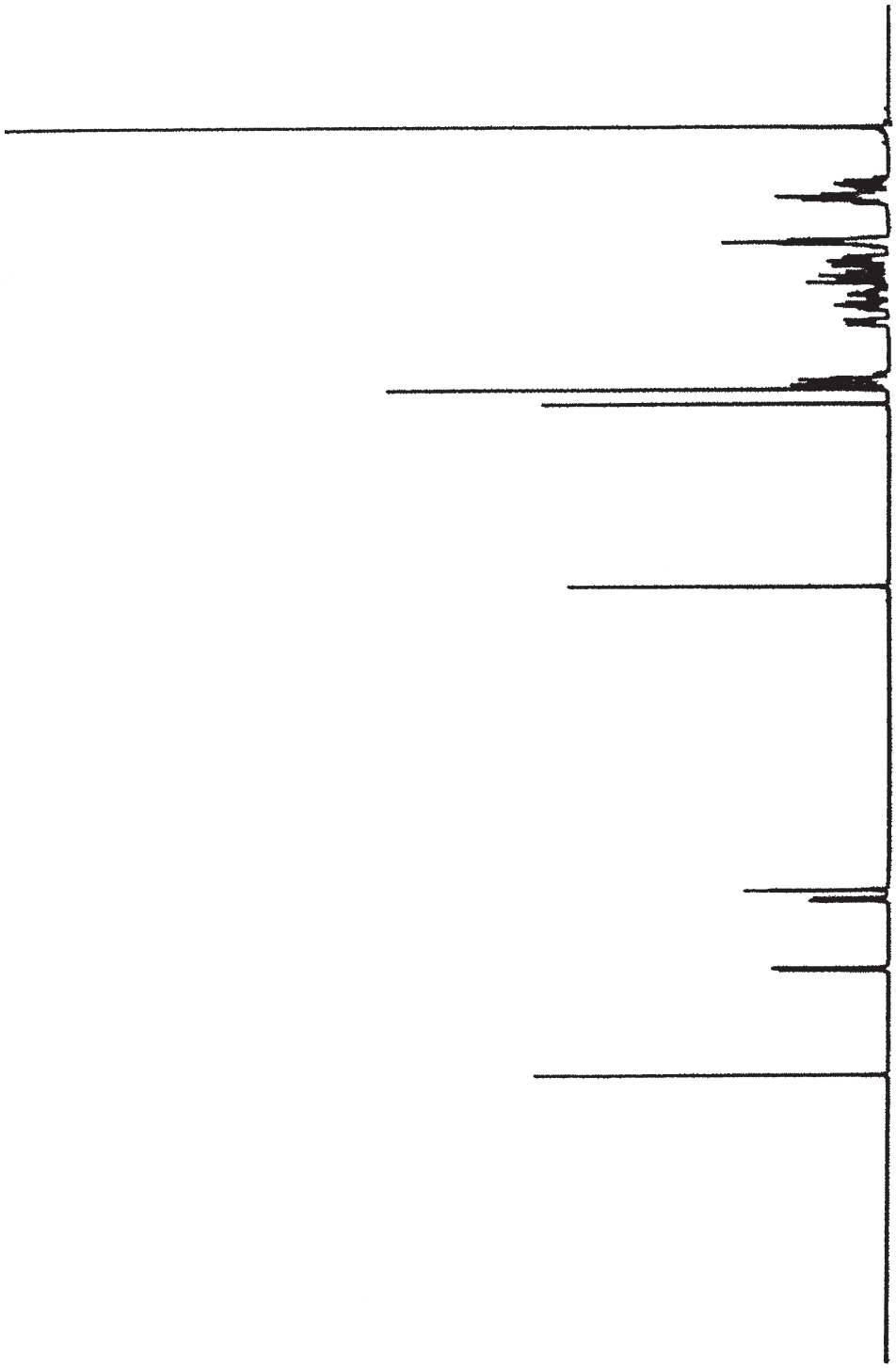

Figure C2
^{13}C- Nuclear Magnetic Resonance Spectrum of Ethinyl Estradiol

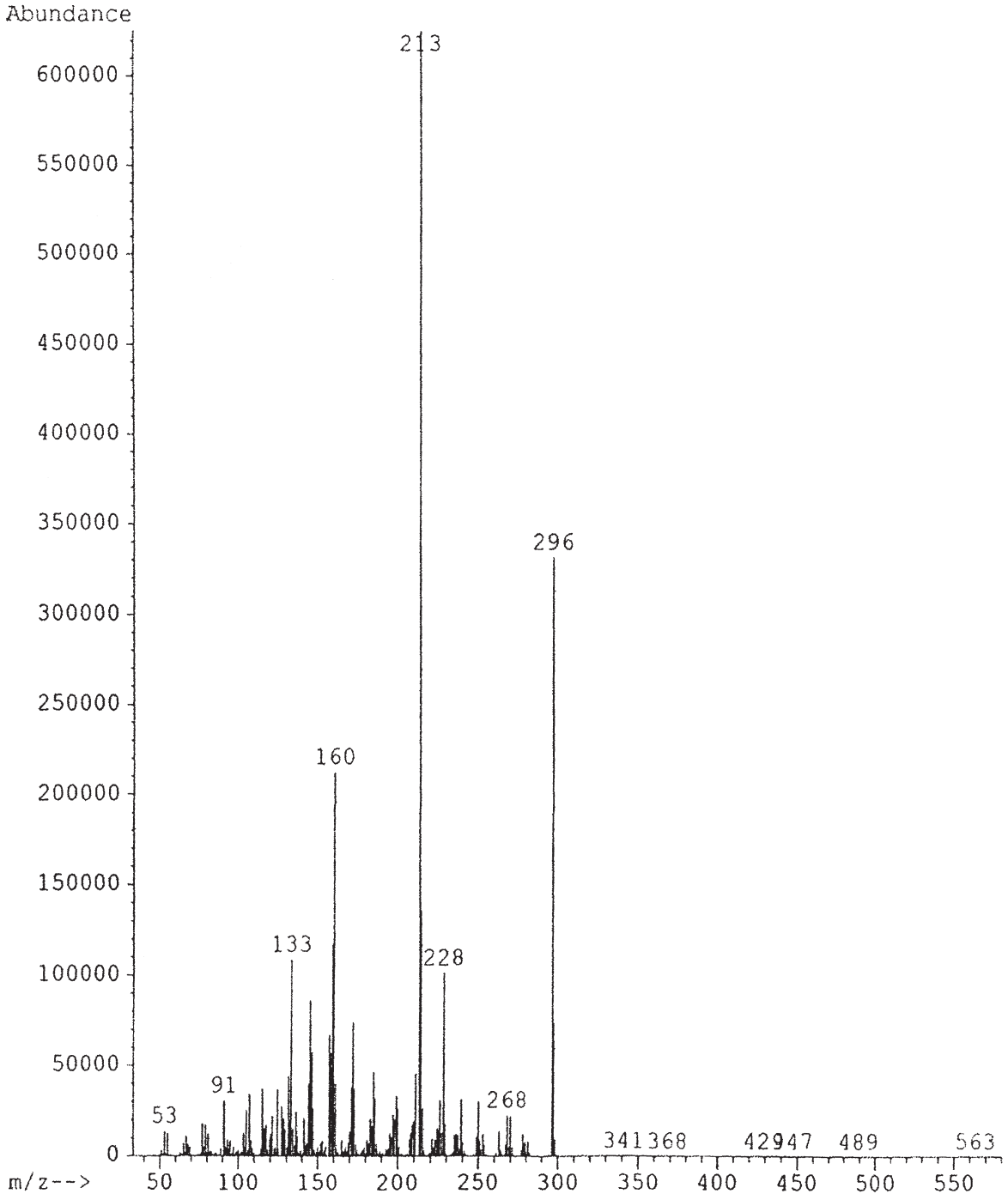

FIGURE C3
Mass Spectrum of Ethinyl Estradiol

Table C1
Gas Chromatography Systems Used in the 2-Year Feed Study of Ethinyl Estradiol[a]

Detection System	Column	Carrier Gas	Oven Temperature Program
System A			
Mass spectrometry with electron impact ionization (50 to 600 amu)	MDN-5S, ~ 60 m × 0.25 mm, 0.25-µm film (Supelco, Bellefonte, PA)	Helium at 19.2 psi	55° to 300° C at 20° C/minute, held for 18 minutes
System B			
Mass spectrometry with electron impact ionization (50 to 450 amu)	DB-1701, 30 m x 0.25 mm, 0.25-µm film, (J&W Scientific, Folsom, CA)	Helium at 1 mL/minute	90° C for 1 minute, then 15° C /minute to 280° C , held for 17 minutes
System C			
Flame ionization	HP-5, 30 m × 0.32 mm, 0.25-µm film (Hewlett-Packard, Palo Alto, CA)	Helium at 1 mL/minute	50° to 250° C at 30° C/minute, held for 18 minutes
System D			
Electron capture	DB-5, 30 m × 0.25 mm, 0.25-µm film (J&W Scientific)	Helium at 0.6 mL/minute	235° C for 23 minutes, then 40° C/minute to 300° C, held for 15 minutes

[a] All gas chromatographs were manufactured by Hewlett-Packard; the mass spectrometers were manufactured by Hewlett-Packard (System A) and ThermoFinnigan Corporation (San Jose, CA) (System B)

Table C2
Preparation and Storage of Dose Formulations in the 2-Year Feed Study of Ethinyl Estradiol

Preparation
Intermediate solutions were prepared by weighing the appropriate amounts of ethinyl estradiol and blending with 95% ethanol for the 0, 10, and 50 ppb dose formulations. The intermediate solutions of ethinyl estradiol were mixed with Purina 5K96 feed in a Patterson-Kelley blender for 60 minutes with the intensifier bar, vacuum, and heater (95° C) on for the entire mixing time. To prepare the 2 ppb dose formulation, a 1:5 dry dilution was made by adding the appropriate amounts of 10 ppb diet blend and Purina 5K96 feed to the blender and mixing for 60 minutes with the intensifier bar on. The dose formulations were prepared at least every 9 weeks.

Chemical Lot Number
57H1178

Maximum Storage Time
9 weeks

Storage Conditions
Stainless steel cans with lids secured with tie-downs at 4° ± 2° C

Study Laboratory
National Center for Toxicological Research (Jefferson, AR)

Table C3
Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
November 27-28, 2000	10	10.6 ± 0.8	+6
	10	11.6 ± 0.4	+16
	10	12.6 ± 1.8	+26
	50	58.1 ± 4.6	+16
December 19-20, 2000	10	9.7 ± 0.3	–3
	10	11.6 ± 1.5	+16
	50	48.0 ± 1.9	–4
January 9, 2001	10	9.3 ± 0.1	–7
	10	8.6 ± 0.7	–14
	10	9.5 ± 1.2	–5
	50	45.7 ± 3.6	–9
January 22-23, 2001	10	9.5 ± 1.2	–5
	10	9.5 ± 1.1	–5
	10	9.4 ± 0.7	–6
	50	49.4 ± 9.3	–1
February 5, 2001	10	8.8 ± 1.0	–12
	10	9.3 ± 0.8	–7
February 22, 2001	10	7.6 ± 1.1	–24
	10	8.5 ± 0.6	–15
	10	8.9 ± 0.5	–11
	50	44.7 ± 1.9	–11
March 5-6, 2001	10	9.3 ± 0.8	–7
	10	8.7 ± 1.3	–13
	50	41.0 ± 7.8	–18
March 21, 2001	10	7.6 ± 0.2	–24
	10	8.2 ± 1.6	–18
	50	46.4 ± 4.9	–7
March 28, 2001	10	7.6 ± 0.7	–24
	10	7.1 ± 0.4	–29
April 3-4, 2001	10	11.0 ± 1.7	+10
	10	10.8 ± 1.0	+8
	50	45.7 ± 1.6	–9
April 24-25, 2001	10	10.1 ± 1.0	+1
	10	10.0 ± 0.3	0
	10	9.9 ± 1.2	–1
May 11, 2001	10	8.5 ± 0.3	–15
	50	39.1 ± 2.3	–22

Table C3
Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
June 6, 2001	10	9.8 ± 0.6	–2
	10	10.6 ± 0.1	+6
June 11, 2001	10	8.8 ± 0.6	–12
July 3, 2001	10	10.2 ± 0.7	+2
	10	12.1 ± 0.2	+21
August 15, 2001	50	44.9 ± 5.3	–10
August 21, 2001	10	8.45 ± 0.1	–16
	10	7.10 ± 0.4	–29
	10	8.83 ± 1.0	–12
October 10, 2001	50	54.95 ± 7.4	+10
October 31, 2001	10	7.98 ± 0.6	–20
	10	9.38 ± 0.9	–6
December 11, 2001	10	9.54 ± 0.7	–5
	10	9.57 ± 1.4	–4
	10	12.10 ± 0.1	+21
January 8, 2002	50	46.7 ± 1.7	–7
January 15, 2002	10	9.22 ± 0.6	–8
	10	9.03 ± 0.2	–10
February 12-13, 2002	10	11.2 ± 0.9	+12
	10	9.30 ± 0.8	–7
	10	7.66 ± 1.3	–23
	50	55.8 ± 3.2	+12
April 2-3, 2002	10	9.26 ± 0.3	–7
	10	8.98 ± 1.3	–10
	10	9.90 ± 0.9	–1
April 16, 2002	50	46.5 ± 0.4	–7
April 30, 2002	10	10.0 ± 0.9	0
	10	9.49 ± 1.3	–5
May 14, 2002	10	8.91 ± 1.3	–11
	10	7.99 ± 0.8	–20
	50	42.3 ± 4.6	–15
June 27, 2002	10	9.64 ± 0.8	–4
	10	9.91 ± 0.7	–1
August 19, 2002	10	7.81 ± 0.2	–22
	10	8.00 ± 0.5	–20

Table C3
Results of Analyses of Dose Formulations Administered to Rats in the 2-Year Feed Studies of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
August 27, 2002	10	8.55 ± 0.9	–15
September 5-8, 2002	10	8.24 ± 1.4	–18
	10	8.16 ± 0.3	–18
	10	8.11 ± 0.8	–19
	10	8.44 ± 0.2	–16
	50	48.1 ± 5.9	–4
October 22, 2002	10	9.25 ± 0.8	–8
	10	10.1 ± 0.6	+1
	10	10.3 ± 0.3	+3
	50	47.5 ± 1.6	–5

[a] Results of triplicate analyses (mean ± standard deviation)

Table C4
Results of Analyses of Animal Room Samples of Dose Formulations Administered to Rats in the 2-Year Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
March 27-29, 2001	10	10.29 ± 0.33	+3
	50	50.2 ± 4.0	0
May 21-24, 2001	10	6.99 ± 0.63	–30
	50	40.9 ± 1.2	–18
July 16-19, 2001	10	12.5 ± 1.14	+25
	50	66.7 ± 4.2	+33
September 10-13, 2001	10	10.6 ± 0.9	+6
	50	47.0 ± 4.2	–6
November 7-8, 2001	10	10.8 ± 1.1	+8
	50	42.4 ± 3.3	–15
January 3 and February 3, 2002	10	7.3 ± 0.7	–27
	50	35.4 ± 2.0	–29
February 25-28, 2002	10	6.6 ± 0.7	–34
	50	36.4 ± 0.7	–27
April 22-24, 2002	10	8.9 ± 0.6	–11
	50	44.0 ± 1.7	–12
June 20, 2002	10	8.7 ± 1.3	–13
	50	42.6 ± 2.9	–15
October 8-10, 2002	10	11.7 ± 0.6	+17
	50	39.5 ± 4.9	–21
December 2-5, 2002	10	8.7 ± 0.2	–13
	50	36.9 ± 3.8	–26

[a] Results of quadruplicate analyses (mean ± standard deviation)

APPENDIX D
BODY WEIGHTS

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Table D1
Mean Body Weights of F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
1	15.6 ± 0.3 (51)	15.3 ± 0.3 (50)	15.2 ± 0.2 (50)	14.6 ± 0.3* (50)	*/#	-
4	77.1 ± 1.1 (51)	75.2 ± 1.3 (50)	78.8 ± 1.3 (50)	76.4 ± 1.5 (50)	-	-
8	267.7 ± 2.8 (51)	255.4 ± 3.2* (50)	269.1 ± 2.8 (50)	255.3 ± 3.5* (50)	*	-
12	393.6 ± 4.2 (51)	372.5 ± 4.5** (50)	387.7 ± 3.8 (50)	365.9 ± 4.6*** (50)	***/###	-
16	459.2 ± 5.1 (51)	421.8 ± 5.4*** (50)	441.0 ± 5.1* (50)	422.8 ± 4.9*** (50)	**/###	-
20	503.1 ± 5.8 (51)	480.4 ± 5.8* (48)	491.8 ± 5.6 (50)	468.2 ± 5.6*** (49)	***/###	-
24	535.5 ± 5.6 (51)	511.6 ± 5.9** (49)	524.0 ± 5.1 (50)	487.9 ± 5.5*** (50)	***/###	-
28	560.1 ± 5.6 (51)	535.4 ± 5.9** (48)	548.8 ± 5.6 (50)	508.9 ± 5.9*** (50)	***/###	-
32	574.1 ± 6.3 (51)	555.3 ± 6.3 (48)	567.9 ± 5.8 (50)	522.9 ± 6.6*** (49)	***/###	#
36	597.7 ± 6.4 (51)	572.3 ± 7.0* (48)	597.6 ± 5.7 (50)	548.4 ± 7.6*** (47)	***/###	##
40	603.5 ± 6.3 (51)	570.0 ± 6.5*** (48)	609.3 ± 6.3 (50)	554.6 ± 7.4*** (48)	***/###	**/##
44	617.6 ± 6.8 (51)	579.1 ± 6.4*** (48)	612.0 ± 6.4 (50)	564.6 ± 7.2*** (48)	***/###	-
48	630.3 ± 6.9 (51)	583.6 ± 6.4*** (48)	622.1 ± 6.7 (50)	572.0 ± 7.7*** (48)	***/###	-
52	636.6 ± 7.0 (50)	597.1 ± 6.6*** (47)	637.9 ± 6.9 (50)	583.2 ± 8.0*** (48)	***/###	*/#
56	643.1 ± 7.8 (49)	603.0 ± 6.5*** (47)	643.5 ± 7.2 (50)	590.7 ± 7.8*** (48)	***/###	*
60	642.9 ± 7.7 (48)	603.5 ± 7.2*** (47)	641.7 ± 7.3 (50)	595.6 ± 8.3*** (47)	***/##	-

TABLE D1
Mean Body Weights of F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
64	643.5 ± 7.5 (48)	598.2 ± 6.7*** (47)	650.3 ± 7.5 (49)	600.8 ± 9.0*** (47)	**	*
68	639.7 ± 7.9 (47)	592.6 ± 7.5*** (46)	654.7 ± 7.2 (48)	608.4 ± 9.5** (46)	-	**
72	653.8 ± 8.2 (46)	601.2 ± 7.4*** (46)	667.2 ± 7.8 (48)	622.4 ± 9.6** (46)	-	**
76	658.7 ± 8.7 (46)	605.1 ± 8.2*** (45)	673.2 ± 8.4	622.4 ± 9.9** (46)	-	**
80	672.1 ± 8.8 (45)	617.3 ± 8.6*** (45)	673.3 ± 8.5 (46)	626.8 ± 10.3*** (46)	*	-
84	665.0 ± 9.6 (44)	613.2 ± 9.1*** (41)	667.6 ± 9.3 (43)	627.1 ± 11.0** (45)	-	-
88	681.0 ± 10.3 (42)	618.6 ± 12.5*** (40)	689.1 ± 9.9 (39)	628.8 ± 11.0*** (44)	-	-
92	700.5 ± 10.5 (41)	636.5 ± 10.4*** (37)	702.6 ± 10.0 (38)	635.4 ± 11.9*** (41)	**/ #	-
96	697.1 ± 11.2 (40)	614.4 ± 11.0*** (36)	694.6 ± 11.3 (36)	639.3 ± 11.5*** (38)	*	-
100	716.5 ± 12.2 (38)	634.7 ± 11.8*** (33)	704.2 ± 11.4 (34)	650.2 ± 12.6*** (36)	*/ #	-
104	688.5 ± 11.6 (35)	635.1 ± 12.0*** (31)	679.5 ± 14.2 (30)	630.1 ± 14.1*** (34)	**/ #	-

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$

[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.

[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$; ##, $P \leq 0.01$; ###, $P \leq 0.001$. Dashes indicate no significant difference.

Table D2
Mean Body Weights of F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
1	**14.5 ± 0.3 (51)**	**14.0 ± 0.3 (50)**	**14.0 ± 0.2 (50)**	**14.0 ± 1.5 (49)**	-	-
4	**75.3 ± 2.1 (51)**	**70.7 ± 2.1 (50)**	66.1 ± 1.0*** (50)	68.5 ± 1.0* (50)	# #	***/ # #
8	**193.3 ± 2.4 (51)**	184.0 ± 2.9* (50)	182.9 ± 2.4** (50)	178.6 ± 1.7*** (50)	***/ # # #	-
12	**246.0 ± 3.0 (51)**	236.0 ± 3.0** (49)	232.5 ± 2.8*** (50)	222.4 ± 1.9*** (50)	***/ # # #	*
16	**279.5 ± 3.5 (51)**	264.5 ± 3.2*** (49)	259.0 ± 2.9*** (50)	247.3 ± 2.2*** (50)	***/ # # #	**
20	**306.5 ± 4.4 (36)**	282.6 ± 3.2*** (39)	278.0 ± 3.4*** (49)	262.8 ± 2.6*** (47)	***/ # # #	***
24	**320.6 ± 4.3 (51)**	300.1 ± 4.1*** (49)	289.1 ± 3.8*** (50)	270.1 ± 2.7*** (50)	***/ # # #	***
28	**333.3 ± 4.7 (51)**	313.4 ± 4.3*** (49)	299.2 ± 4.1*** (50)	276.6 ± 2.8*** (50)	***/ # # #	***
32	**349.3 ± 5.2 (51)**	327.8 ± 4.8*** (49)	311.4 ± 4.5*** (50)	284.2 ± 3.2*** (50)	***/ # # #	***
36	**360.1 ± 5.2 (51)**	337.1 ± 5.1*** (49)	321.2 ± 5.1*** (50)	289.7 ± 3.4*** (50)	***/ # # #	***
40	**375.2 ± 5.9 (51)**	344.7 ± 5.4*** (49)	331.9 ± 5.2*** (49)	295.2 ± 3.8*** (49)	***/ # # #	***
44	**383.0 ± 6.4 (41)**	339.2 ± 5.6*** (39)	328.1 ± 5.1*** (40)	300.1 ± 4.7*** (39)	***/ # # #	***
48	**391.0 ± 6.7 (51)**	356.1 ± 6.3*** (48)	341.4 ± 6.0*** (49)	302.7 ± 4.3*** (49)	***/ # # #	***
52	**399.8 ± 7.3 (51)**	362.1 ± 7.0*** (48)	348.5 ± 6.8*** (49)	306.5 ± 4.4*** (49)	***/ # # #	***
56	**405.8 ± 7.2 (51)**	366.3 ± 7.1*** (48)	352.2 ± 6.8*** (48)	311.5 ± 4.5*** (49)	***/ # # #	***
60	**412.0 ± 8.6 (51)**	368.7 ± 7.7*** (48)	354.6 ± 7.2*** (48)	316.9 ± 5.2*** (49)	***/ # # #	***

TABLE D2
Mean Body Weights of F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
64	**423.1 ± 8.7 (51)**	378.6 ± 7.1*** (47)	364.8 ± 7.8*** (47)	322.9 ± 5.4*** (49)	***/ # # #	***
68	**423.0 ± 9.1 (51)**	373.1 ± 8.4*** (47)	367.6 ± 7.9*** (48)	325.1 ± 5.1*** (49)	***/ # # #	**
72	**432.0 ± 9.2 (51)**	378.5 ± 7.2*** (45)	368.2 ± 8.1*** (48)	326.1 ± 4.7*** (48)	***/ # # #	***
76	**435.4 ± 9.2 (49)**	381.8 ± 7.3*** (45)	375.4 ± 8.7*** (46)	329.0 ± 5.3*** (48)	***/ # # #	***
80	**448.2 ± 9.7 (48)**	392.3 ± 7.8*** (44)	386.3 ± 9.8*** (43)	337.9 ± 5.9*** (48)	***/ # # #	**
84	**460.1 ± 10.4 (45)**	392.1 ± 8.4*** (41)	403.1 ± 11.6*** (40)	335.9 ± 4.6*** (45)	***/ # # #	*
88	**461.0 ± 10.6 (36)**	414.5 ± 9.8*** (27)	413.0 ± 12.9*** (29)	346.9 ± 7.2*** (33)	***/ # # #	*
92	**472.2 ± 13.6 (40)**	402.9 ± 10.8*** (36)	407.6 ± 12.0*** (35)	347.7 ± 7.5*** (36)	***/ # # #	-
96	**494.9 ± 13.3 (32)**	405.4 ± 11.6*** (31)	424.9 ± 13.3*** (29)	358.1 ± 7.0*** (32)	***/ # # #	*
100	**499.1 ± 15.1 (27)**	416.0 ± 13.8*** (28)	437.2 ± 16.2*** (25)	369.0 ± 8.7*** (28)	***/ # # #	*
104	**495.4 ± 17.2 (24)**	426.5 ± 14.9*** (19)	450.6 ± 24.9*** (14)	355.1 ± 8.7*** (18)	***/ # # #	-

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: ##, P ≤ 0.01; ###, P ≤ 0.001. Dashes indicate no significant difference.

Table D3
Mean Body Weights of F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
1	15.6 ± 0.3 (51)	15.4 ± 0.3 (50)	15.5 ± 0.2 (50)	14.6 ± 0.3* (50)	**/ #	-
4	77.1 ± 1.1 (51)	75.8 ± 1.4 (50)	80.0 ± 1.1 (50)	79.8 ± 1.4 (50)	#	-
8	267.7 ± 2.8 (51)	255.8 ± 3.0* (50)	268.7 ± 2.9 (50)	262.4 ± 3.0 (50)	-	-
12	393.6 ± 4.2 (51)	373.0 ± 4.5*** (50)	387.8 ± 3.9 (50)	372.5 ± 3.9*** (50)	*/ #	-
16	459.2 ± 5.1 (51)	428.2 ± 5.9*** (50)	441.9 ± 5.1* (50)	431.2 ± 4.6*** (50)	*/ #	-
20	503.1 ± 5.8 (51)	482.5 ± 6.4* (49)	496.6 ± 5.2 (50)	475.0 ± 5.8** (49)	**/ #	-
24	535.5 ± 5.6 (51)	520.8 ± 6.6 (50)	527.6 ± 5.3 (50)	512.6 ± 5.6* (50)	*/ #	-
28	560.1 ± 5.6 (51)	554.6 ± 6.3 (50)	558.0 ± 6.2 (50)	537.6 ± 6.4* (50)	**/ #	-
32	574.1 ± 6.3 (51)	567.9 ± 6.6 (50)	573.9 ± 5.8 (48)	551.1 ± 6.9* (50)	**/ #	-
36	597.7 ± 6.4 (51)	589.8 ± 6.2 (50)	594.0 ± 6.2 (48)	579.0 ± 7.0 (50)	-	-
40	603.5 ± 6.3 (51)	596.3 ± 6.7 (50)	602.3 ± 6.4 (48)	586.9 ± 7.2 (50)	-	-
44	617.6 ± 6.8 (51)	607.2 ± 6.4 (50)	612.8 ± 6.7 (48)	599.0 ± 7.6 (50)	-	-
48	630.3 ± 6.9 (51)	619.7 ± 5.9 (50)	626.8 ± 6.9 (48)	616.6 ± 7.3 (50)	-	-
52	636.6 ± 7.0 (50)	632.1 ± 6.1 (50)	642.0 ± 7.0 (47)	630.5 ± 7.7 (50)	-	-
56	643.1 ± 7.8 (49)	636.4± 5.8 (50)	648.7 ± 7.7 (47)	633.1 ± 8.1 (50)	-	-
60	642.9 ± 7.7 (48)	642.4 ± 6.7 (50)	654.3 ± 7.8 (47)	642.3 ± 8.3 (50)	-	-

TABLE D3
Mean Body Weights of F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
64	643.5 ± 7.5 (48)	637.5 ± 6.7 (50)	654.2 ± 8.1 (46)	634.6 ± 8.8 (49)	-	-
68	639.7 ± 7.9 (47)	639.6 ± 7.1 (49)	649.4 ± 8.2 (46)	636.8 ± 9.2 (49)	-	-
72	653.8 ± 8.2 (46)	658.8 ± 6.6 (48)	676.3 ± 8.4 (44)	651.8 ± 8.6 (49)	-	-
76	658.7 ± 8.7 (46)	662.2 ± 6.9 (46)	684.2 ± 8.2 (44)	654.4 ± 8.0 (49)	-	*/ #
80	672.1 ± 8.8 (45)	673.4 ± 7.5 (45)	697.4 ± 8.5 (43)	665.8 ± 8.1 (47)	-	*/ #
84	665.0 ± 9.6 (44)	665.6 ± 9.4 (44)	697.3 ± 9.5 (43)	661.4 ± 9.1 (44)	-	*/ #
88	681.0 ± 10.3 (42)	676.1 ± 7.8 (41)	714.7 ± 9.4 (43)	674.9 ± 10.6 (43)	-	**/ #
92	700.5 ± 10.5 (41)	686.7 ± 8.7 (38)	725.4 ± 9.4 (42)	697.4 ± 10.3 (40)	-	*
96	697.1 ± 11.2 (40)	673.8 ± 10.0 (37)	723.6 ± 9.3 (40)	689.4 ± 11.1 (39)	-	**/ #
100	716.5 ± 12.2 (38)	693.6 ± 10.7 (35)	734.2 ± 9.5 (40)	711.2 ± 12.5 (38)	-	-
104	688.5 ± 11.6 (35)	680.8 ± 9.8 (31)	717.6 ± 11.5 (33)	693.3 ± 13.0 (33)	-	*

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

Table D4
Mean Body Weights of F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
1	**14.5 ± 0.3 (51)**	13.5 ± 0.3* (50)	**14.4 ± 0.3 (50)**	**14.0 ± 0.2 (49)**	-	-
4	**75.3 ± 2.1 (51)**	69.4 ± 2.2* (50)	68.5 ± 1.0* (50)	67.7 ± 1.1** (50)	*/ # #	*
8	**193.3 ± 2.4 (51)**	180.1 ± 2.7*** (50)	**186.4 ± 1.7 (50)**	178.9 ± 1.7*** (50)	***/ # # #	-
12	**246.0 ± 3.0 (51)**	230.7 ± 3.1*** (49)	237.0 ± 2.1* (50)	224.3 ± 2.0*** (50)	***/ # # #	-
16	**279.5 ± 3.5 (51)**	259.1 ± 2.9*** (49)	263.0 ± 2.2*** (50)	249.2 ± 2.3*** (50)	***/ # # #	-
20	**306.5 ± 4.4 (36)**	275.7 ± 3.7*** (39)	281.7 ± 2.6*** (49)	263.2 ± 2.7*** (49)	***/ # # #	*
24	**320.6 ± 4.3 (51)**	296.1 ± 3.5*** (49)	301.8 ± 3.4*** (50)	287.7 ± 3.0*** (50)	***/ # # #	-
28	**333.3 ± 4.7 (51)**	311.0 ± 3.7*** (49)	316.4 ± 3.9** (50)	304.2 ± 3.6*** (50)	***/ # # #	-
32	**349.3 ± 5.2 (51)**	325.5 ± 4.3*** (49)	332.6 ± 4.5* (50)	318.1 ± 4.1*** (50)	***/ # # #	-
36	**360.1 ± 5.2 (51)**	335.6 ± 4.5*** (49)	341.3 ± 4.9* (49)	328.5 ± 4.5*** (50)	***/ # # #	-
40	**375.2 ± 5.9 (51)**	349.8 ± 5.1** (49)	355.3 ± 5.3* (49)	342.7 ± 5.2*** (50)	**/ # # #	-
44	**383.0 ± 6.4 (41)**	352.6 ± 6.0** (40)	353.6 ± 5.3* (39)	346.1 ± 6.6*** (35)	**/ # # #	-
48	**391.0 ± 6.7 (51)**	362.1 ± 5.8** (49)	369.2 ± 5.9* (49)	356.6 ± 6.0*** (50)	**/ # #	-
52	**399.8 ± 7.3 (51)**	372.4 ± 6.2** (49)	378.1 ± 6.4 (49)	365.6 ± 6.4*** (50)	**/ # #	-
56	**405.8 ± 7.2 (51)**	378.0 ± 6.3** (48)	383.7 ± 6.5 (49)	370.6 ± 6.9*** (49)	**/ # #	-
60	**412.0 ± 8.6 (51)**	385.3 ± 7.1* (48)	388.3 ± 7.1 (49)	377.8 ± 7.7** (49)	*/ # #	-

TABLE D4
Mean Body Weights of F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
64	423.1 ± 8.7 (51)	391.0 ± 7.1* (47)	401.1 ± 7.1 (49)	388.5 ± 7.8 (49)	*/ #	-
68	423.0 ± 9.1 (51)	387.4 ± 7.7* (47)	404.6 ± 8.1 (47)	389.9 ± 8.8* (48)	-	-
72	432.0 ± 9.2 (51)	392.3 ± 8.3** (43)	408.3 ± 8.7 (45)	390.0 ± 8.1** (47)	*/ #	-
76	435.4 ± 9.2 (49)	393.5 ± 8.2** (40)	412.8 ± 9.5 (43)	394.4 ± 7.8** (47)	*/ #	-
80	448.2 ± 9.7 (48)	409.6 ± 8.9** (39)	432.0 ± 9.8 (41)	411.0 ± 8.9** (45)	#	-
84	460.1 ± 10.4 (45)	412.2 ± 9.4** (37)	444.6 ± 10.9 (41)	425.6 ± 10.1* (43)	-	-
88	461.0 ± 10.6 (36)	423.2 ± 14.0** (23)	450.5 ± 12.8 (29)	434.3 ± 13.5* (33)	-	-
92	472.2 ± 13.6 (40)	435.5 ± 11.7* (29)	468.1 ± 11.6 (36)	441.4 ± 12.3 (35)	-	-
96	494.9 ± 13.3 (32)	448.7 ± 13.3* (24)	478.9 ± 12.6 (33)	451.7 ± 14.8 (33)	-	-
100	499.1 ± 15.1 (27)	446.5 ± 14.3* (24)	490.2 ± 14.7 (28)	446.4 ± 16.9 (27)	-	-
104	495.4 ± 17.2 (24)	415.0 ± 26.4** (12)	515.9 ± 18.8 (15)	448.0 ± 18.6 (17)	-	-

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, P ≤ 0.05; ##, P ≤ 0.01; ###, P ≤ 0.001. Dashes indicate no significant difference.

Table D5
Mean Body Weights of F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	84.8 ± 1.8 (50)	82.8 ± 1.6 (49)	83.5 ± 2.0 (50)	84.2 ± 1.3 (50)	-	-
8	299.2 ± 4.0 (50)	295.5 ± 4.1 (49)	292.8 ± 3.9 (50)	300.3 ± 3.7 (50)	-	-
12	422.4 ± 5.0 (50)	424.3 ± 5.6 (49)	422.1 ± 4.9 (50)	433.1 ± 4.5 (49)	-	-
16	493.6 ± 5.5 (50)	495.5 ± 6.4 (49)	500.3 ± 5.5 (50)	512.1 ± 5.1 (49)	*/ #	-
20	538.8 ± 6.0 (48)	540.4 ± 7.0 (49)	542.0 ± 6.2 (49)	554.0 ± 5.8 (48)	-	-
24	573.8 ± 6.5 (48)	563.8 ± 7.5 (49)	569.7 ± 7.8 (49)	583.1 ± 5.6 (48)	-	-
28	595.4 ± 7.0 (48)	594.2 ± 7.9 (49)	597.0 ± 6.7 (48)	603.6 ± 6.0 (48)	-	-
32	609.7 ± 6.8 (48)	607.8 ± 8.3 (49)	614.9 ± 7.1 (48)	618.1 ± 5.9 (48)	-	-
36	630.6 ± 8.6 (48)	623.8 ± 8.6 (49)	630.5 ± 7.4 (48)	630.7 ± 7.0 (48)	-	-
40	637.2 ± 7.5 (48)	631.8 ± 9.0 (49)	642.8 ± 7.7 (47)	645.7 ± 6.9 (48)	-	-
44	652.1 ± 7.8 (48)	642.7 ± 9.9 (48)	657.6 ± 8.1 (47)	659.6 ± 6.9 (48)	-	-
48	664.8 ± 7.6 (48)	650.2 ± 10.4 (47)	670.1 ± 8.1 (47)	668.5 ± 7.2 (48)	-	-
52	675.4 ± 8.0 (48)	665.3 ± 10.9 (46)	678.2 ± 8.3 (46)	678.3 ± 7.1 (48)	-	-
56	682.4 ± 7.9 (48)	667.8 ± 10.9 (47)	680.3 ± 8.1 (46)	684.8 ± 7.7 (48)	-	-
60	687.9 ± 8.2 (46)	670.2 ± 11.7 (46)	688.3 ± 8.7 (45)	691.5 ± 7.7 (48)	-	-
64	701.0 ± 8.7 (46)	682.6 ± 12.2 (46)	701.9 ± 8.8 (45)	705.3 ± 7.5 (48)	-	-

TABLE D5
Mean Body Weights of F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	709.8 ± 9.0 (46)	686.3 ± 12.8 (46)	708.9 ± 9.4 (45)	711.9 ± 7.7 (47)	-	-
72	718.6 ± 9.4 (44)	697.1 ± 13.0 (45)	714.4 ± 9.0 (45)	718.2 ± 9.3 (47)	-	-
76	715.0 ± 9.9 (43)	699.6 ± 13.0 (45)	715.3 ± 9.1 (44)	718.0 ± 10.1 (46)	-	-
80	708.8 ± 9.7 (41)	692.3 ± 13.3 (44)	716.8 ± 9.6 (41)	717.7 ± 10.2 (45)	-	-
84	701.8 ± 10.6 (40)	677.3 ± 13.6 (42)	709.8 ± 9.3 (42)	704.8 ± 10.3 (44)	-	-
88	701.4 ± 11.5 (39)	677.5 ± 12.6 (40)	703.9 ± 9.5 (42)	698.0 ± 10.7 (43)	-	-
92	694.1 ± 12.4 (36)	677.1 ± 13.8 (34)	696.5 ± 10.1 (41)	695.5 ± 10.9 (41)	-	-
96	682.1 ± 13.8 (35)	671.9 ± 13.5 (31)	694.3 ± 9.2 (38)	680.1 ± 11.8 (40)	-	-
100	686.9 ± 12.5 (32)	660.6 ± 14.8 (29)	686.1 ± 9.0 (34)	675.8 ± 12.8 (36)	-	-

* $P \leq 0.05$

[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.

[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

Table D6
Mean Body Weights of F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	84.2 ± 1.8 (52)	85.1 ± 1.7 (50)	92.8 ± 1.8** (50)	80.9 ± 1.9 (50)	*	***/ # # #
8	201.0 ± 2.5 (52)	199.1 ± 2.6 (50)	210.8 ± 2.8* (50)	205.3 ± 2.8 (50)	#	**
12	260.8 ± 2.8 (52)	259.8 ± 3.1 (50)	267.9 ± 3.2 (50)	267.1 ± 3.6 (50)	#	-
16	293.6 ± 3.4 (52)	289.2 ± 3.5 (50)	299.9 ± 4.1 (49)	297.4 ± 3.9 (50)	-	-
20	319.3 ± 4.2 (51)	313.9 ± 3.6 (48)	323.6 ± 5.0 (48)	321.9 ± 4.4 (50)	-	-
24	337.5 ± 4.6 (51)	331.8 ± 4.0 (48)	339.8 ± 5.9 (47)	338.5 ± 5.0 (50)	-	-
28	356.4 ±5.4 (51)	348.8 ± 4.5 (48)	358.7 ± 6.6 (47)	359.4 ± 5.7 (50)	-	-
32	367.5 ± 6.0 (51)	358.7 ± 4.7 (48)	369.9 ± 7.2 (47)	367.8 ± 6.0 (50)	-	-
36	377.8 ± 6.8 (51)	369.7 ± 5.2 (48)	380.2 ± 7.6 (47)	377.8 ± 6.4 (50)	-	-
40	389.5 ± 7.2 (51)	380.4 ± 5.6 (48)	393.1 ± 8.4 (47)	389.4 ± 7.0 (49)	-	-
44	400.2 ± 7.9 (51)	390.5 ± 6.1 (48)	401.4 ± 8.7 (47)	396.5 ± 7.5 (48)	-	-
48	403.9 ± 8.3 (51)	394.8 ± 6.6 (48)	410.4 ± 9.7 (47)	401.6 ± 7.9 (48)	-	-
52	418.8 ± 8.6 (51)	406.0 ± 6.7 (48)	425.3 ± 10.3 (47)	415.5 ± 8.2 (48)	-	-
56	434.1 ± 9.1 (51)	419.7 ± 7.2 (47)	439.7 ± 10.9 (47)	430.9 ± 9.4 (47)	-	-
60	449.9 ± 9.8 (51)	433.3 ± 7.5 (47)	454.7 ± 11.4 (47)	440.7 ± 9.4 (45)	-	-
64	462.7 ± 10.0 (51)	443.5 ± 7.5 (47)	464.8 ± 12.0 (47)	450.8 ± 10.3 (45)	-	-

TABLE D6
Mean Body Weights of F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	468.5 ± 10.5 (49)	453.6 ± 8.0 (46)	473.4 ± 12.5 (46)	463.6 ± 10.9 (44)	-	-
72	478.5 ± 11.1 (48)	465.1 ± 8.4 (45)	487.1 ± 13.5 (46)	475.9 ± 11.0 (44)	-	-
76	487.1 ± 11.4 (47)	477.2 ± 9.3 (42)	499.8 ± 14.1 (43)	484.6 ± 11.2 (42)	-	-
80	499.0 ± 12.0 (45)	486.5 ± 9.4 (42)	505.4 ± 14.4 (43)	495.3 ± 11.9 (41)	-	-
84	508.3 ± 19.7 (22)	481.5 ± 17.3 (21)	513.0 ± 17.1 (33)	496.5 ± 18.2 (16)	-	-
88	500.0 ± 13.6 (43)	480.2 ± 10.1 (39)	494.7 ± 12.4 (39)	494.2 ± 13.3 (36)	-	-
92	505.5 ± 13.0 (37)	488.7 ± 10.4 (39)	504.0 ± 13.5 (36)	500.7 ± 13.0 (36)	-	-
96	513.2 ± 13.2 (33)	480.3 ± 10.8 (36)	504.3 ± 16.0 (30)	496.4 ± 16.8 (31)	-	-
100	518.7 ± 15.3 (27)	483.2 ± 12.5 (30)	510.6 ± 18.9 (25)	513.5 ± 17.6 (27)	-	-

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
[a] Mean body weight in grams ± standard error at each indicated time point. Numbers in parentheses are the number of animals. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$; ###, $P \leq 0.001$. Dashes indicate no significant difference.

APPENDIX E
FEED CONSUMPTION

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Table E1
Feed Consumption by F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	14.0 ± 0.5 (57)	11.3 ± 0.3* (50)	15.6 ± 0.7 (50)	16.3 ± 0.8* (49)	***/ # # #	*
8	24.4 ± 0.4 (58)	20.9 ± 0.4*** (50)	23.6 ± 0.4 (50)	22.9 ± 0.4 (50)	-	# #
12	25.7 ± 0.4 (58)	22.2 ± 0.4*** (50)	24.5 ± 0.4 (50)	24.2 ± 0.4 (50)	-	# # #
16	23.9 ± 0.5 (58)	19.2 ± 0.7*** (50)	21.1 ± 0.6* (50)	22.3 ± 0.7 (50)	-	# # #
20	26.4 ± 0.7 (58)	25.6 ± 0.7 (48)	26.7 ± 0.8 (50)	26.2 ± 0.7 (49)	-	-
24	25.7 ± 0.4 (58)	24.5 ± 0.6 (49)	26.3 ± 0.8 (50)	24.5 ± 0.6 (50)	-	-
28	27.0 ± 0.6 (58)	25.3 ± 0.7 (47)	25.3 ± 0.5 (50)	23.8 ± 0.5*** (50)	**/ # # #	-
32	24.2 ± 0.6 (56)	23.6 ± 0.4 (48)	24.4 ± 0.5 (50)	22.6 ± 0.7 (49)	*	-
36	25.1 ± 0.7 (56)	22.6 ± 0.8 (48)	26.8 ± 0.7 (50)	25.4 ± 0.8 (47)	-	*
40	25.2 ± 0.6 (56)	21.5 ± 0.6 (48)	25.7 ± 0.7 (50)	23.0 ± 0.6 (48)	-	*
44	25.1 ± 0.5 (56)	22.3 ± 0.6 (48)	23.6 ± 0.6 (50)	23.2 ± 0.6 (48)	-	-
48	24.9 ± 0.4 (55)	21.4 ± 0.4*** (48)	23.7 ± 0.5 (50)	22.5 ± 0.5** (48)	#	-
52	25.2 ± 0.5 (55)	22.7 ± 0.6** (48)	25.0 ± 0.4 (50)	23.6 ± 0.5 (48)	-	-
56	27.8 ± 0.7 (51)	23.6 ± 0.7*** (46)	26.1 ± 0.4 (48)	26.7 ± 0.6 (47)	-	# # #
60	24.2 ± 0.5 (52)	21.8 ± 0.7* (46)	25.3 ± 0.7 (50)	22.8 ± 0.5 (47)	-	**
64	24.6 ± 0.5 (51)	22.3 ± 0.5* (47)	27.2 ± 0.6** (49)	24.0 ± 0.7 (47)	-	***

Table E1
Feed Consumption by F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	23.7 ± 0.5 (50)	20.4 ± 0.7** (46)	24.2 ± 0.7 (49)	23.9 ± 0.7 (46)	*	-
72	26.8 ± 0.7 (50)	22.3 ± 0.4*** (46)	25.6 ± 0.5 (48)	25.9 ± 0.8 (45)	-	# #
76	26.7 ± 0.7 (48)	21.8 ± 0.6*** (45)	26.1 ± 0.8 (46)	22.9 ± 0.6*** (45)	*/ #	-
80	28.7 ± 0.8 (48)	25.8 ± 0.8* (44)	27.5 ± 0.6 (46)	26.0 ± 0.6* (45)	-	-
84	24.6 ± 0.8 (44)	21.9 ± 0.9 (40)	23.2 ± 0.8 (43)	24.4 ± 0.7 (43)	-	#
88	30.0 ± 0.9 (42)	24.4 ± 0.9*** (38)	26.4 ± 0.9* (39)	24.9 ± 0.8*** (43)	*/ # #	#
92	33.7 ± 0.9 (40)	26.5 ± 0.7*** (36)	30.0 ± 0.8** (39)	27.6 ± 0.8*** (40)	**/ # # #	# #
96	29.7 ± 1.0 (40)	21.6 ± 0.8*** (35)	25.2 ± 1.0** (36)	26.3 ± 0.9* (36)	-	# # #
100	33.9 ± 0.9 (39)	24.9 ± 1.3*** (30)	28.2 ± 0.9*** (34)	29.0 ± 0.8** (35)	#	*/ # # #
104	25.1 ± 0.9 (36)	24.5 ± 1.3 (30)	22.5 ± 1.0 (30)	24.8 ± 1.1 (33)	-	-

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001

[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.

[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, P ≤ 0.05; ##, P ≤ 0.01; ###, P ≤ 0.001. Dashes indicate no significant difference.

Table E2
Feed Consumption by F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	13.8 ± 0.6 (57)	11.7 ± 0.5 (48)	13.8 ± 0.9 (49)	13.8 ± 0.6 (50)	-	-
8	18.5 ± 0.2 (58)	17.2 ± 0.3* (50)	18.4 ± 0.3 (50)	18.4 ± 0.4 (50)	-	-
12	18.4 ± 0.3 (58)	17.2 ± 0.3 (49)	18.0 ± 0.4 (50)	18.3 ± 0.4 (50)	-	-
16	18.3 ± 0.3 (58)	16.9 ± 0.4* (49)	18.2 ± 0.3 (49)	19.4 ± 0.4 (50)	***/ # #	# #
20	19.5 ± 0.5 (43)	17.7 ± 0.4 (39)	18.6 ± 0.5 (48)	20.0 ± 0.8 (47)	-	#
24	20.5 ± 0.5 (57)	18.8 ± 0.5 (49)	19.2 ± 0.4 (50)	20.5 ± 0.7 (50)	-	#
28	20.7 ± 0.5 (57)	18.9 ± 0.5 (49)	20.1 ± 0.8 (50)	18.5 ± 0.4* (49)	*/ #	-
32	21.0 ± 0.8 (56)	18.7 ± 0.5* (49)	19.8 ± 0.5 (48)	19.2 ± 0.5 (49)	-	-
36	21.4 ± 0.6 (56)	19.7 ± 0.6 (49)	21.0 ± 0.6 (50)	20.6 ± 0.6 (49)	-	-
40	20.9 ± 0.5 (55)	17.3 ± 0.4*** (49)	20.4 ± 0.6 (49)	19.2 ± 0.6* (49)	-	-
44	20.2 ± 0.5 (56)	18.5 ± 0.7 (48)	19.9 ± 0.6 (49)	20.7 ± 0.6 (49)	-	-
48	21.7 ± 0.6 (56)	19.7 ± 0.7 (48)	21.3 ± 0.6 (49)	18.6 ± 0.5*** (49)	***/ # #	-
52	21.6 ± 0.5 (56)	17.9 ± 0.4*** (48)	20.5 ± 0.5 (49)	20.6 ± 1.0 (49)	-	#
56	21.7 ± 0.6 (54)	19.0 ± 0.4** (47)	19.5 ± 0.5* (48)	20.9 ± 0.6 (49)	-	*/ # # #
60	21.6 ± 0.6 (54)	18.3 ± 0.4*** (48)	20.5 ± 0.5 (48)	19.4 ± 0.5** (49)	-	-
64	20.5 ± 0.5 (54)	19.2 ± 0.6 (48)	22.0 ± 0.5 (47)	20.7 ± 0.7 (49)	-	*

TABLE E2
Feed Consumption by F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	21.0 ± 0.6 (54)	19.5 ± 0.8 (46)	21.3 ± 0.7 (48)	22.1 ± 0.9 (49)	-	-
72	20.5 ± 0.4 (54)	18.7 ± 0.6* (45)	19.8 ± 0.5 (48)	19.5 ± 0.4 (48)	-	-
76	21.8 ± 0.8 (37)	18.3 ± 0.7*** (34)	19.7 ± 0.4* (46)	18.1 ± 0.4 (45)	**/ # # #	-
80	24.3 ± 0.6 (36)	20.9 ± 0.9** (34)	21.6 ± 0.6* (42)	20.7 ± 0.5*** (45)	*/ # # #	-
84	20.3 ± 0.6 (33)	16.5 ± 0.5*** (32)	19.4 ± 0.6 (38)	17.3 ± 0.5*** (42)	*/ #	-
88	24.6 ± 0.5 (32)	20.7 ± 0.9** (31)	21.1 ± 0.9** (36)	21.3 ± 0.7** (40)	# #	*/ #
92	27.1 ± 1.2 (28)	20.1 ± 1.1*** (29)	22.9 ± 1.0* (35)	20.4 ± 0.7*** (35)	**/ # # #	#
96	29.5 ± 1.2 (25)	24.8 ± 0.7*** (24)	24.3 ± 0.8*** (29)	25.8 ± 0.7** (29)	# #	***/ # # #
100	30.2 ± 1.4 (21)	24.0 ± 1.1** (23)	26.9 ± 1.0 (23)	26.4 ± 1.3 (26)	-	-
104	25.3 ± 1.3 (17)	25.6 ± 1.7 (16)	23.3 ± 1.7 (13)	25.7 ± 1.1 (15)	-	-

* P ≤ 0.05
** P ≤ 0.01
*** P ≤ 0.001
[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, P ≤ 0.05; ##, P ≤ 0.01; ###, P ≤ 0.001. Dashes indicate no significant difference.

Table E3
Feed Consumption by F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	14.0 ± 0.5 (57)	12.0 ± 0.4* (50)	14.3 ± 0.5 (50)	14.7 ± 0.7 (49)	*/ #	-
8	24.4 ± 0.4 (58)	21.5 ± 0.3*** (50)	24.1 ± 0.5 (49)	23.8 ± 0.4 (50)	-	#
12	25.7 ± 0.4 (58)	22.4 ± 0.4*** (50)	24.7 ± 0.5 (50)	24.2 ± 0.3* (50)	-	#
16	23.9 ± 0.5 (58)	19.8 ± 0.8*** (49)	20.6 ± 0.6** (50)	22.6 ± 0.8 (50)	-	**/ # # #
20	26.4 ± 0.7 (58)	25.5 ± 0.6 (49)	26.9 ± 0.6 (50)	25.9 ± 0.6 (49)	-	-
24	25.7 ± 0.4 (58)	25.2 ± 0.6 (49)	25.5 ± 0.7 (50)	25.2 ± 0.4 (50)	-	-
28	27.0 ± 0.6 (58)	26.7 ± 0.4 (50)	26.8 ± 0.5 (50)	26.2 ± 0.6 (50)	-	-
32	24.2 ± 0.6 (56)	24.3 ± 0.5 (50)	23.6 ± 0.5 (48)	23.1 ± 0.5 (50)	-	-
36	25.1 ± 0.7 (56)	25.3 ± 0.6 (50)	25.1 ± 0.7 (48)	24.9 ± 0.6 (49)	-	-
40	25.2 ± 0.6 (56)	24.2 ± 0.5 (50)	25.1 ± 0.7 (48)	24.5 ± 0.6 (50)	-	-
44	25.1 ± 0.5 (56)	25.2 ± 0.6 (50)	24.5 ± 0.7 (48)	25.0 ± 0.8 (50)	-	-
48	24.9 ± 0.4 (55)	24.5 ± 0.4 (50)	24.9 ± 0.5 (48)	24.8 ± 0.4 (50)	-	-
52	25.2 ± 0.5 (55)	24.5 ± 0.5 (50)	25.0 ± 0.7 (48)	25.5 ± 0.6 (50)	-	-
56	27.8 ± 0.7 (51)	27.6 ± 0.6 (48)	28.2 ± 0.7 (44)	26.4 ± 0.4 (48)	*	-
60	24.2 ± 0.5 (52)	24.2 ± 0.8 (50)	24.4 ± 0.4 (47)	24.3 ± 0.6 (50)	-	-
64	24.6 ± 0.5 (51)	24.0 ± 0.5 (50)	24.8 ± 0.6 (46)	23.9 ± 0.6 (49)	-	-

Table E3
Feed Consumption by F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	23.7 ± 0.5 (50)	23.5 ± 0.5 (49)	23.3 ± 0.5 (46)	24.1 ± 0.7 (49)	-	-
72	26.8 ± 0.7 (50)	26.2 ± 0.5 (48)	26.5 ± 0.7 (44)	25.6 ± 0.6 (49)	-	-
76	26.7 ± 0.7 (48)	26.3 ± 0.5 (45)	26.8 ± 0.6 (43)	25.5 ± 0.8 (48)	-	-
80	28.7 ± 0.8 (48)	30.2 ± 0.6 (44)	30.3 ± 0.7 (41)	29.1 ± 0.6 (46)	-	#
84	24.6 ± 0.8 (44)	24.9 ± 0.7 (43)	26.2 ± 0.9 (43)	25.0 ± 0.9 (42)	-	-
88	30.0 ± 0.9 (42)	30.1 ± 0.8 (38)	31.6 ± 0.9 (41)	29.8 ± 0.7 (41)	-	-
92	33.7 ± 0.9 (40)	33.8 ± 1.0 (37)	34.1 ± 1.0 (42)	34.8 ± 1.0 (37)	-	-
96	29.7 ± 1.0 (40)	27.0 ± 1.1 (36)	28.6 ± 0.9 (39)	27.6 ± 0.8 (38)	-	-
100	33.9 ± 0.9 (39)	32.1 ± 0.8 (34)	33.0 ± 0.9 (40)	32.4 ± 0.7 (36)	-	-
104	25.1 ± 0.9 (36)	25.8 ± 0.9 (30)	27.0 ± 1.0 (33)	27.0 ± 1.2 (34)	-	-

* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$; ###, $P \leq 0.001$. Dashes indicate no significant difference.

Table E4
Feed Consumption by F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	13.8 ± 0.6 (57)	12.0 ± 0.4 (49)	13.8 ± 0.6 (50)	14.4 ± 0.6 (50)	*	-
8	18.5 ± 0.2 (58)	17.4 ± 0.4 (50)	18.7 ± 0.3 (50)	18.5 ± 0.4 (50)	-	-
12	18.4 ± 0.3 (58)	17.0 ± 0.4* (49)	18.4 ± 0.4 (50)	18.1 ± 0.3 (50)	-	-
16	18.3 ± 0.3 (58)	17.1 ± 0.5 (48)	18.5 ± 0.5 (50)	19.4 ± 0.6 (50)	**/ #	-
20	19.5 ± 0.5 (43)	16.9 ± 0.3** (39)	18.9 ± 0.4 (49)	18.9 ± 0.5 (49)	-	#
24	20.5 ± 0.5 (57)	20.4 ± 0.4 (49)	21.6 ± 0.5 (50)	21.7 ± 0.6 (50)	#	-
28	20.7 ± 0.5 (57)	20.7 ± 0.5 (49)	22.1 ± 0.8 (50)	22.2 ± 0.7 (50)	#	-
32	21.0 ± 0.8 (56)	19.5 ± 0.5 (49)	20.9 ± 0.7 (50)	21.6 ± 0.6 (50)	-	-
36	21.4 ± 0.6 (56)	21.4 ± 0.7 (49)	21.6 ± 0.5 (49)	23.2 ± 1.0 (50)	*	-
40	20.9 ± 0.5 (55)	20.6 ± 0.4 (49)	20.8 ± 0.6 (49)	21.4 ± 0.5 (50)	-	-
44	20.2 ± 0.5 (56)	20.3 ± 0.5 (49)	20.4 ± 0.5 (49)	20.9 ± 0.6 (50)	-	-
48	21.7 ± 0.6 (56)	21.3 ± 0.5 (49)	22.1 ± 0.6 (49)	21.8 ± 0.7 (50)	-	-
52	21.6 ± 0.5 (56)	22.0 ± 0.7 (49)	22.1 ± 0.6 (49)	21.7 ± 0.5 (50)	-	-
56	21.7 ± 0.6 (54)	21.4 ± 0.5 (48)	21.3 ± 0.5 (49)	20.8 ± 0.6 (49)	-	-
60	21.6 ± 0.6 (54)	21.7 ± 0.6 (48)	21.7 ± 0.6 (48)	21.8 ± 0.6 (49)	-	-
64	20.5 ± 0.5 (54)	20.2 ± 0.5 (47)	21.1 ± 0.8 (48)	21.1 ± 0.7 (49)	-	-

Table E4
Feed Consumption by F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	21.0 ± 0.6 (54)	20.3 ± 0.6 (47)	22.2 ± 0.7 (47)	20.9 ± 0.7 (49)	-	-
72	20.5 ± 0.4 (54)	19.9 ± 0.5 (43)	20.6 ± 0.6 (45)	21.5 ± 0.5 (46)	*	-
76	21.8 ± 0.8 (37)	20.5 ± 0.6 (32)	20.7 ± 0.6 (42)	20.3 ± 0.5 (46)	-	-
80	24.3 ± 0.6 (36)	22.4 ± 0.9 (31)	24.0 ± 0.7 (40)	23.6 ± 0.6 (44)	-	-
84	20.3 ± 0.6 (33)	20.2 ± 1.0 (29)	20.9 ± 0.7 (40)	20.3 ± 0.7 (42)	-	-
88	24.6 ± 0.5 (32)	26.0 ± 1.2 (24)	24.5 ± 0.8 (38)	25.4 ± 0.8 (38)	-	-
92	27.1 ± 1.2 (28)	23.8 ± 1.1 (23)	26.7 ± 0.8 (35)	24.0 ± 0.7* (35)	-	-
96	29.5 ± 1.2 (25)	31.2 ± 1.3 (19)	30.5 ± 0.9 (32)	28.9 ± 1.3 (34)	-	-
100	30.2 ± 1.4 (21)	30.4 ± 1.7 (19)	29.4 ± 1.1 (28)	30.7 ± 1.3 (28)	-	-
104	25.3 ± 1.3 (17)	27.6 ± 2.0 (7)	27.3 ± 1.4 (15)	27.9 ± 1.7 (16)	-	-

* $P \leq 0.05$
** $P \leq 0.01$
[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

Table E5
Feed Consumption by F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	11.3 ± 0.4 (62)	10.7 ± 0.2 (49)	11.0 ± 0.4 (48)	10.6 ± 0.2 (50)	-	-
8	25.8 ± 0.3 (62)	25.4 ± 0.4 (49)	24.8 ± 0.4 (50)	25.9 ± 0.3 (50)	-	*/#
12	26.8 ± 0.4 (62)	26.8 ± 0.5 (49)	26.2 ± 0.4 (50)	27.0 ± 0.4 (49)	-	-
16	27.1 ± 0.4 (62)	28.0 ± 0.6 (49)	27.7 ± 0.5 (50)	27.8 ± 0.5 (49)	-	-
20	25.6 ± 0.4 (60)	26.1 ± 0.4 (49)	25.6 ± 0.5 (49)	26.4 ± 0.4 (48)	-	-
24	25.3 ± 0.7 (57)	24.9 ± 0.7 (49)	24.8 ± 0.8 (50)	25.0 ± 0.5 (48))	-	-
28	23.9 ± 0.5 (57)	23.8 ± 0.3 (49)	23.3 ± 0.5 (48)	23.0 ± 0.5 (48)	-	-
32	23.8 ± 0.4 (57)	23.5 ± 0.4 (49)	23.6 ± 0.4 (47)	23.0 ± 0.4 (48)	-	-
36	25.3 ± 0.3 (56)	24.3 ± 0.6 (49)	24.8 ± 0.7 (46)	24.9 ± 0.6 (48)	-	-
40	23.4 ± 0.4 (57)	23.5 ± 0.4 (49)	23.0 ± 0.5 (48)	23.4 ± 0.5 (48)	-	-
44	24.8 ± 0.4 (57)	24.3 ± 0.5 (48)	24.6 ± 0.4 (47)	25.1 ± 0.4 (48)	-	-
48	27.1 ± 0.3 (57)	26.3 ± 0.4 (47)	27.1 ± 0.4 (47)	27.0 ± 0.5 (48)	-	-
52	24.6 ± 0.4 (57)	25.0 ± 0.4 (46)	23.9 ± 0.5 (47)	25.1 ± 0.4 (48)	-	-
56	25.4 ± 0.4 (54)	24.5 ± 0.4 (47)	24.7 ± 0.5 (46)	25.4 ± 0.4 (48)	-	-
60	23.1 ± 0.5 (52)	22.6 ± 0.5 (46)	23.4 ± 0.5 (45)	23.5 ± 0.5 (48)	-	-
64	26.3 ± 0.5 (52)	26.1 ± 0.4 (46)	26.7 ± 0.5 (45)	26.3 ± 0.4 (48)	-	-

Table E5
Feed Consumption by F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	25.2 ± 0.5 (52)	23.9 ± 0.5 (46)	24.6 ± 0.5 (45)	24.6 ± 0.5 (48)	-	-
72	26.2 ± 0.7 (50)	25.6 ± 0.6 (45)	25.3 ± 0.6 (45)	25.4 ± 0.7 (47)	-	-
76	25.8 ± 0.7 (50)	26.9 ± 0.5 (45)	25.6 ± 0.7 (44)	27.2 ± 0.7 (46)	-	-
80	22.7 ± 0.6 (48)	22.3 ± 0.6 (45)	23.2 ± 0.6 (41)	22.4 ± 0.6 (45)	-	-
84	22.5 ± 0.6 (42)	21.8 ± 0.5 (43)	23.3 ± 0.6 (41)	22.7 ± 0.7 (44)	-	-
88	22.7 ± 0.8 (41)	22.0 ± 0.8 (39)	23.2 ± 0.8 (41)	23.6 ± 0.6 (42)	-	-
92	23.6 ± 0.7 (38)	25.1 ± 0.8 (34)	24.3 ± 0.6 (40)	23.9 ± 0.6 (41)	-	-
96	24.3 ± 1.0 (37)	24.3 ± 0.6 (31)	24.8 ± 0.8 (37)	23.7 ± 0.9 (40)	-	-
100	24.9 ± 0.7 (34)	24.9 ± 0.8 (29)	24.7 ± 0.9 (32)	25.6 ± 0.8 (35)	-	-

* $P \leq 0.05$

[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.

[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

Table E6
Feed Consumption by F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
4	12.4 ± 0.4 (60)	13.1 ± 0.5 (49)	14.0 ± 0.3* (49)	12.1 ± 0.5 (50)	-	**/ # # #
8	19.3 ± 0.3 (61)	19.7 ± 0.5 (50)	19.2 ± 0.4 (50)	19.3 ± 0.3 (50)	-	-
12	19.3 ± 0.4 (61)	18.9 ± 0.4 (50)	18.9 ± 0.4 (50)	19.0 ± 0.3 (50)	-	-
16	19.8 ± 0.3 (61)	19.5 ± 0.4 (50)	20.1 ± 0.4 (50)	20.5 ± 0.5 (50)	-	-
20	18.9 ± 0.3 (59)	19.4 ± 0.3 (48)	18.9 ± 0.3 (48)	19.7 ± 0.4 (50)	-	-
24	19.9 ± 0.4 (59)	20.2 ± 0.5 (48)	20.1 ± 0.4 (47)	20.0 ± 0.4 (50)	-	-
28	19.7 ± 0.5 (56)	20.1 ± 0.4 (48)	19.8 ± 0.4 (47)	19.5 ± 0.3 (50)	-	-
32	19.3 ± 0.4 (56)	20.4 ± 0.4 (48)	19.5 ± 0.3 (47)	20.2 ± 0.3 (50)	-	-
36	19.6 ± 0.4 (56)	19.9 ± 0.4 (48)	19.8 ± 0.4 (47)	19.8 ± 0.3 (50)	-	-
40	20.2 ± 0.4 (56)	20.6 ± 0.4 (48)	20.9 ± 0.4 (47)	21.3 ± 0.4 (49)	-	-
44	21.0 ± 0.3 (55)	21.4 ± 0.4 (48)	21.6 ± 0.3 (47)	21.1 ± 0.4 (48)	-	-
48	22.1 ± 0.5 (56)	23.0 ± 0.4 (48)	23.0 ± 0.5 (47)	23.5 ± 0.5 (48)	-	-
52	21.2 ± 0.4 (56)	21.1 ± 0.3 (48)	22.2 ± 0.4 (47)	21.5 ± 0.4 (48)	-	-
56	22.4 ± 0.3 (53)	23.0 ± 0.5 (48)	22.7 ± 0.4 (47)	23.1 ± 0.5 (47)	-	-
60	21.9 ± 0.5 (53)	21.8 ± 0.4 (47)	22.4 ± 0.5 (47)	21.9 ± 0.4 (45)	-	-
64	23.3 ± 0.4 (53)	22.9 ± 0.4 (47)	23.0 ± 0.4 (47)	23.0 ± 0.5 (45)	-	-

Table E6
Feed Consumption by F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Weeks	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
68	22.3 ± 0.5 (51)	22.0 ± 0.4 (45)	21.6 ± 0.5 (46)	21.2 ± 0.3 (44)	-	-
72	23.4 ± 0.4 (50)	23.5 ± 0.4 (45)	23.9 ± 0.6 (46)	23.5 ± 0.4 (44)	-	-
76	25.1 ± 0.7 (49)	24.9 ± 0.5 (43)	25.6 ± 0.6 (43)	25.0 ± 0.6 (43)	-	-
80	22.8 ± 0.5 (47)	23.4 ± 0.6 (42)	22.9 ± 0.6 (43)	23.4 ± 0.6 (41)	-	-
84	21.2 ± 0.5 (22)	21.0 ± 0.7 (20)	21.3 ± 0.6 (31)	19.6 ± 0.9 (17)	*	-
88	23.1 ± 1.1 (22)	22.9 ± 0.8 (19)	25.5 ± 0.8 (30)	24.8 ± 0.7 (15)	-	*
92	21.9 ± 0.8 (17)	21.8 ± 0.7 (19)	22.4 ± 0.6 (29)	21.8 ± 0.6 (17)	-	-
96	25.7 ± 1.3 (17)	24.7 ± 1.0 (18)	25.2 ± 0.7 (22)	25.9 ± 1.0 (14)	-	-
100	23.2 ± 1.2 (14)	22.2 ± 0.9 (17)	23.5 ± 1.0 (17)	23.8 ± 0.9 (13)	-	-
104	24.3 ± 1.2 (10)	25.5 ± 1.1 (13)	25.0 ± 2.0 (10)	25.2 ± 1.3 (9)	-	-

* P ≤ 0.05
** P ≤ 0.01
[a] Mean daily feed consumption in grams/day ± standard error at each indicated time point. Numbers in parentheses are the number of samples. Shaded cells in an exposed group column indicate that the value is significantly different from the control value in the same measurement week by Dunnett's (1955) test.
[b] Results of linear and quadratic exposure concentration trend tests for each measurement week. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: ###, P ≤ 0.001. Dashes indicate no significant difference.

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ONSET OF ABERRANT ESTROUS CYCLES

Methods and Results

During the 2-year feed study of ethinyl estradiol, data were collected for the initial time of cessation of normal estrous cycling in the F_1C, F_1T140, and F_3T21 treatment arms of the study. Beginning at month 5, swabbing of monitored female rats was conducted on 5 consecutive days each month (a run). The swabs were analyzed to determine the current estrous stage (estrus, diestrus, or proestrus) for each day within the run. To determine the start of cessation of normal cycling, an abnormal cycle was deemed to be a run with 4 or more consecutive diestrus days or a run with 3 or more consecutive estrus days. Onset of aberrant estrous cycles was deemed to have begun at the first of 2 consecutive months with abnormal cycling.

Because estrous cycle data collection began at month 5, many animals had already ceased normal cycling by the time the observations began. These animals were left censored; although they had ceased normal cycling by month 5, the time at which they had ceased is unknown. Every animal that lived to the end of the study ceased normal cycling; however, some animals died before any clear "cessation" event. These animals were right censored. Finally, the full month between observations yields a very grainy picture in which the actual "cessation" age may have been at any point between the last month and the current month. Accordingly, the study also exhibits interval censoring.

The presence of all three classical types of censoring in this study presents a statistical problem. Moreover, of the three types of censoring, the least important in this study is the right censoring, the type most commonly modeled in practice. Most animals ceased to cycle normally prior to becoming lost to follow-up, so right censoring was somewhat rare. On the other hand, a great many animals ceased normal cycling before data collection at 5 months, making left censoring relatively common. Data for all animals were interval censored. These factors make it imperative that the censoring be accommodated by the chosen statistical model. Because of the simplicity of accommodating data showing all types of censoring, an accelerated failure time Kaplan-Meier model was used.

A generalized gamma model was used as the distributional form for the analysis of normal cycling. The model was parameterized so that comparisons to the controls were effectively generated by the fits. The analyses were run in groups by treatment arm and exposure regimen. No significant overall exposure concentration effect ("Overall" P-value) was found for any treatment arm, and comparisons found no significant differences between exposed and control groups (Table F1). Kaplan-Meier curves for the three treatment arms are presented in Figures F1, F2, and F3.

Table F1
Analysis of Time to Onset of Aberrant Estrous Cycles in Monitored Female Rats in the 2-Year Feed Study of Ethinyl Estradiol[a]

Comparison	Generalized Gamma Survival Distribution		
	F_1C	F_1T140	F_3T21
Overall	0.590	0.397	0.390
2 ppb vs control	0.566	0.425	0.508
10 ppb vs control	0.649	0.108	0.341
50 ppb vs control	0.424	0.824	0.563

[a] P values by Wald chi-square test are unadjusted for multiple comparisons.

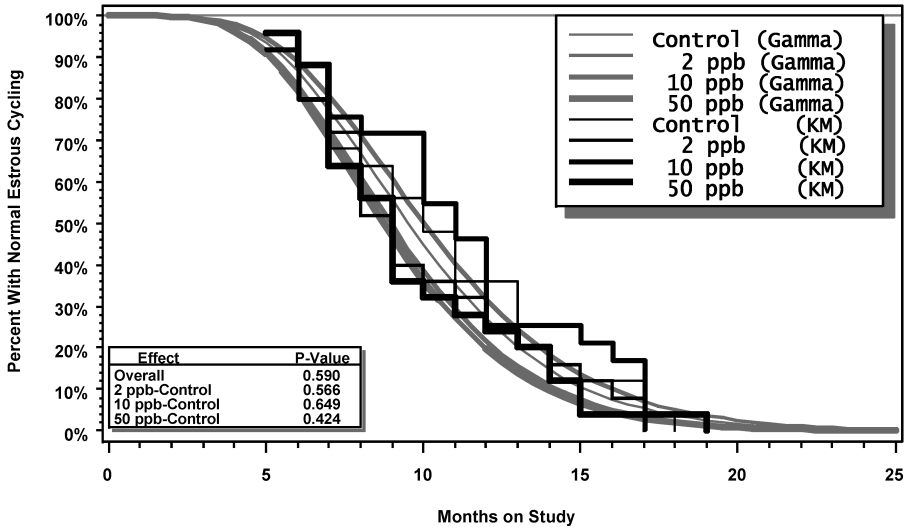

Figure F1
Kaplan-Meier Curves with Generalized Gamma Distribution Indicating Time to Onset of Aberrant Estrous Cycles in F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol
No data were collected before month 5, causing the curves to be discontinuous. Smooth lines show the curves fitted for the generalized gamma distribution. Inset shows the overall P value (Wald chi-square test) as well as comparisons of each exposed group curve to the control group curve.

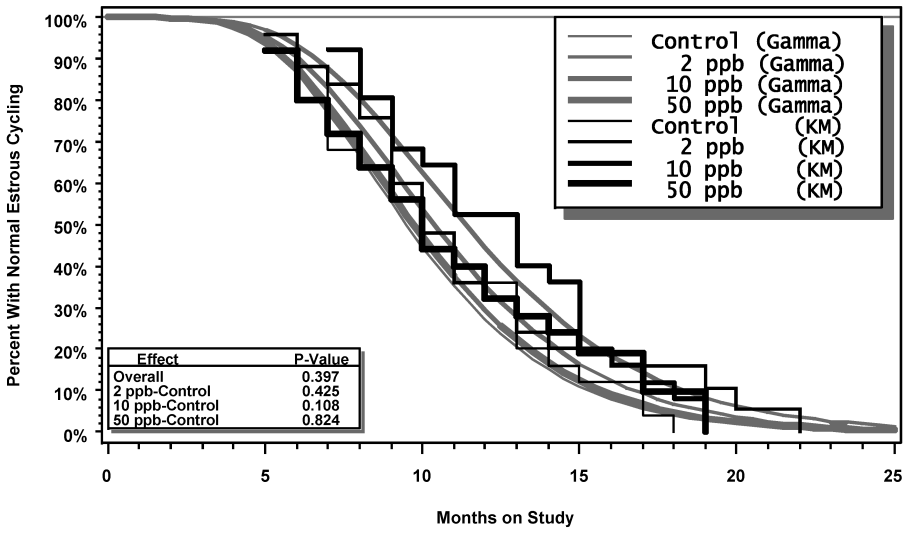

FIGURE F2
Kaplan-Meier Curves with Generalized Gamma Distribution Indicating Time to Onset of Aberrant Estrous Cycles in F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol
No data were collected before month 5, causing the curves to be discontinuous. Smooth lines show the curves fitted for the generalized gamma distribution. Inset shows the overall P value (Wald chi-square test) as well as comparisons of each exposed group curve to the control group curve.

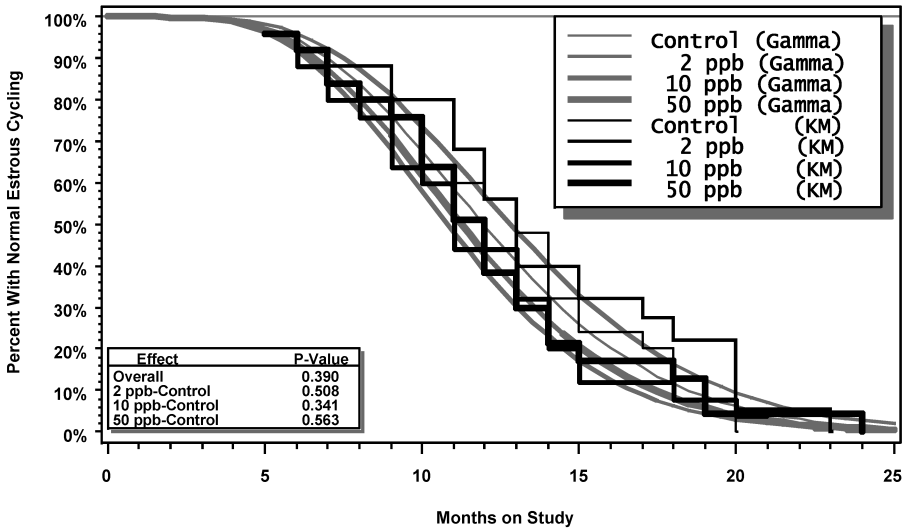

FIGURE F3
Kaplan-Meier Curves with Generalized Gamma Distribution Indicating Time to Onset of Aberrant Estrous Cycles in F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol
No data were collected before month 5, causing the curves to be discontinuous. Smooth lines show the curves fitted for the generalized gamma distribution. Inset shows the overall P value (Wald chi-square test) as well as comparisons of each exposed group curve to the control group curve.

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

Table G1 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol 190

Table G2 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol 193

Table G3 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol 195

Table G4 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol 198

Table G5 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol 200

Table G6 Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol 203

Table G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	682.7 ± 11.8 (34)	633.3 ± 11.3* (31)	679.4 ± 13.7 (30)	628.3 ± 14.0** (32)	*/ #	-
Adrenal Glands						
Absolute	0.082 ± 0.011 (33)	0.076 ± 0.004 (31)	0.113 ± 0.029 (30)	0.063 ± 0.003 (32)	-	-
Relative (mg/g)	0.125 ± 0.019 (33)	0.120 ± 0.006 (31)	0.177 ± 0.053 (30)	0.102 ± 0.007 (32)	-	-
ANCOVA[c]	-	-	-	-	-	*
Brain						
Absolute	2.220 ± 0.022 (34)	2.252 ± 0.033 (31)	2.255 ± 0.024 (30)	2.220 ± 0.029 (31)	-	-
Relative	3.285 ± 0.067 (34)	3.594 ± 0.087* (31)	3.360 ± 0.079 (30)	3.569 ± 0.091* (31)	-	-
ANCOVA	-	-	-	-	-	-
Dorsal Prostate Gland						
Absolute	0.256 ± 0.012 (34)	0.273 ± 0.017 (30)	0.252 ± 0.012 (29)	0.264 ± 0.017 (30)	-	-
Relative	0.379 ± 0.019 (34)	0.431 ± 0.027 (30)	0.374 ± 0.020 (29)	0.431 ± 0.034 (30)	-	-
ANCOVA	-	-	-	-	-	-
Epididymis						
Absolute	1.254 ± 0.033 (34)	1.248 ± 0.033 (31)	1.249 ± 0.036 (30)	1.241 ± 0.025 (32)	-	-
Relative	1.856 ± 0.061 (34)	1.990 ± 0.061 (31)	1.861 ± 0.066 (30)	2.003 ± 0.056 (32)	-	-
ANCOVA	-	-	-	-	-	-

Table G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Kidney						
Absolute	4.043 ± 0.071 (34)	4.053 ± 0.074 (31)	4.045 ± 0.098 (30)	4.167 ± 0.347 (32)	-	-
Relative	5.948 ± 0.100 (34)	6.442 ± 0.138 (31)	5.991 ± 0.150 (30)	6.617 ± 0.481 (32)	-	-
ANCOVA	-	-	-	-	-	-
Lateral Prostate Gland						
Absolute	0.278 ± 0.013 (34)	0.303 ± 0.019 (31)	0.296 ± 0.020 (30)	0.291 ± 0.014 (30)	-	-
Relative	0.413 ± 0.022 (34)	0.478 ± 0.030 (31)	0.438 ± 0.030 (30)	0.468 ± 0.027 (30)	-	-
ANCOVA	-	-	-	-	-	-
Liver						
Absolute	16.179 ± 0.342 (33)	15.845 ± 0.422 (31)	16.362 ± 0.411 (30)	15.965 ± 0.407 (31)	-	-
Relative	23.789 ± 0.419 (33)	25.034 ± 0.520 (31)	24.209 ± 0.601 (30)	25.452 ± 0.534 (31)	-	-
ANCOVA	-	-	-	-	-	-
Pituitary Gland						
Absolute	0.023 ± 0.005 (34)	0.031 ± 0.006 (31)	0.018 ± 0.001 (30)	0.024 ± 0.004 (30)	-	-
Relative	0.035 ± 0.008 (34)	0.048 ± 0.009 (31)	0.026 ± 0.001 (30)	0.039 ± 0.006 (30)	-	-
ANCOVA	-	-	-	-	-	-
Seminal Vesicle/ Coagulating Gland						
Absolute	1.270 ± 0.078 (33)	1.379 ± 0.069 (30)	1.200 ± 0.056 (29)	1.268 ± 0.072 (30)	-	-
Relative	1.893 ± 0.125 (33)	2.189 ± 0.112 (30)	1.766 ± 0.079 (29)	2.054 ± 0.140 (30)	-	-
ANCOVA	-	-	-	-	-	-

Table G1
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Spleen						
Absolute	1.132 ± 0.034 (34)	1.240 ± 0.244 (31)	1.144 ± 0.030 (30)	1.086 ± 0.032 (31)	-	-
Relative	1.665 ± 0.050 (34)	1.976 ± 0.394 (31)	1.692 ± 0.040 (30)	1.742 ± 0.047 (31)	-	-
ANCOVA	-	-	-	-	-	-
Testes						
Absolute	3.230 ± 0.112 (34)	3.229 ± 0.103 (31)	3.222 ± 0.115 (30)	3.190 ± 0.080 (32)	-	-
Relative	4.762 ± 0.184 (34)	5.154 ± 0.188 (31)	4.700 ± 0.199 (30)	5.130 ± 0.138 (32)	-	-
ANCOVA	-	-	-	-	-	-
Thymus						
Absolute	0.309 ± 0.020 (34)	0.302 ± 0.025 (31)	0.335 ± 0.026 (30)	0.273 ± 0.019 (32)	-	-
Relative	0.457 ± 0.03 (34)	0.482 ± 0.044 (31)	0.491 ± 0.036 (30)	0.437 ± 0.031 (32)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.047 ± 0.001 (34)	0.046 ± 0.002 (31)	0.046 ± 0.002 (30)	0.051 ± 0.003 (30)	-	-
Relative	0.069 ± 0.002 (34)	0.073 ± 0.003 (31)	0.068 ± 0.003 (30)	0.081 ± 0.004 (30)	*	-
ANCOVA	-	-	-	-	*	-
Ventral Prostate Gland						
Absolute	0.500 ± 0.023 (33)	0.560 ± 0.031 (30)	0.517 ± 0.027 (29)	0.572 ± 0.032 (29)	-	-
Relative	0.742 ± 0.035 (33)	0.886 ± 0.047 (30)	0.765 ± 0.041 (29)	0.915 ± 0.059* (29)	*	-
ANCOVA	-	-	-	-	-	-

* $P \leq 0.05$
** $P \leq 0.01$
[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.
[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.
[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference.

TABLE G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	482.5 ± 15.2 (26)	413.6 ± 14.2** (23)	432.0 ± 20.3* (19)	345.3 ± 9.0*** (25)	***/ # # #	-
Adrenal Glands						
Absolute	0.100 ± 0.006 (26)	0.108 ± 0.015 (23)	0.078 ± 0.005 (18)	0.080 ± 0.005 (25)	*/ #	-
Relative	0.212 ± 0.013 (26)	0.281 ± 0.051 (23)	0.189 ± 0.016 (18)	0.239 ± 0.019 (25)	-	-
ANCOVA[c]	-	-	-	*	**/ # #	*
Brain						
Absolute	2.017 ± 0.023 (26)	2.008 ± 0.026 (23)	1.994 ± 0.033 (19)	2.010 ± 0.020 (25)	-	-
Relative	4.267 ± 0.122 (26)	4.978 ± 0.176** (23)	4.748 ± 0.174 (19)	5.903 ± 0.144*** (25)	***/ # # #	-
ANCOVA	-	-	-	-	-	-
Kidney						
Absolute	2.692 ± 0.047 (26)	2.543 ± 0.067 (23)	2.307 ± 0.066*** (19)	2.149 ± 0.051*** (25)	***/ # # #	***
Relative	5.681 ± 0.171 (26)	6.289 ± 0.255 (23)	5.442 ± 0.169 (19)	6.308 ± 0.208 (25)	-	-
ANCOVA	-	-	***	***	***/ # # #	***
Liver						
Absolute	12.746 ± 0.503 (26)	11.876 ± 0.579 (23)	11.643 ± 0.741 (19)	9.404 ± 0.429*** (25)	***/ # # #	-
Relative	26.685 ± 1.069 (26)	28.801 ± 1.084 (23)	27.173 ± 1.474 (19)	27.142 ± 0.807 (25)	-	-
ANCOVA	-	-	-	-	-	-
Ovaries (both)						
Absolute	0.182 ± 0.011 (26)	0.158 ± 0.009 (23)	0.163 ± 0.012 (19)	0.252 ± 0.088 (25)	-	-
Relative	0.388 ± 0.029 (26)	0.395 ± 0.027 (23)	0.386 ± 0.029 (19)	0.747 ± 0.268 (25)	*	-
ANCOVA	-	-	-	-	-	-
Pituitary Gland						
Absolute	0.064 ± 0.010 (26)	0.099 ± 0.021 (23)	0.080 ± 0.021 (19)	0.078 ± 0.014 (24)	-	-
Relative	0.139 ± 0.023 (26)	0.253 ± 0.057 (23)	0.196 ± 0.052 (19)	0.232 ± 0.043 (24)	-	-
ANCOVA	-	-	-	-	-	-

Table G2
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1C Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Spleen						
Absolute	0.859 ± 0.035 (26)	0.778 ± 0.036 (23)	0.801 ± 0.049 (19)	1.312 ± 0.651 (25)	-	-
Relative	1.803 ± 0.078 (26)	1.898 ± 0.077 (23)	1.852 ± 0.065 (19)	3.490 ± 1.545 (25)	-	-
ANCOVA	-	-	-	-	-	-
Thymus						
Absolute	0.311 ± 0.032 (25)	0.240 ± 0.017 (22)	0.251 ± 0.026 (19)	0.187 ± 0.017** (25)	**/ # #	-
Relative	0.634 ± 0.057 (25)	0.587 ± 0.038 (22)	0.585 ± 0.064 (19)	0.534 ± 0.038 (25)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.045 ± 0.003 (26)	0.043 ± 0.002 (23)	0.044 ± 0.003 (19)	0.038 ± 0.002* (25)	*	-
Relative	0.095 ± 0.005 (26	0.107 ± 0.004 (23)	0.104 ± 0.006 (19)	0.110 ± 0.005 (25)	-	-
ANCOVA	-	-	-	-	-	-
Uterus						
Absolute	0.925 ± 0.094 (25)	1.070 ± 0.099 (22)	0.885 ± 0.092 (19)	1.166 ± 0.077 (25)	*	-
Relative	2.015 ± 0.231 (25)	2.737 ± 0.324 (22)	2.179 ± 0.268 (19)	3.478 ± 0.266***	***/# #	-
ANCOVA	-	-	-	-	-	-

* P ≤ 0.05
** P ≤ 0.01
***P ≤ 0.001
[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.
[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, P ≤ 0.05; ##, P ≤ 0.01; ###, P ≤ 0.001. Dashes indicate no significant difference.
[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference. Asterisks in shaded cells indicate significant differences from controls.

TABLE G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	682.7 ± 11.8 (34)	674.4 ± 9.1 (31)	710.8 ± 11.1 (33)	689.3 ± 13.4 (33)	-	-
Adrenal Glands						
Absolute	0.082 ± 0.011 (33)	0.067 ± 0.003 (31)	0.072 ± 0.003 (33)	0.068 ± 0.002 (33)	-	-
Relative (mg/g)	0.125 ± 0.019 (33)	0.099 ± 0.004 (31)	0.101 ± 0.004 (33)	0.098 ± 0.003 (33)	-	-
ANCOVA[c]	-	-	-	-	-	-
Brain						
Absolute	2.220 ± 0.022 (34)	2.274 ± 0.020 (31)	2.255 ± 0.025 (33)	2.255 ± 0.027 (33)	-	-
Relative	3.285 ± 0.067 (34)	3.388 ± 0.048 (31)	3.194 ± 0.056 (33)	3.312 ± 0.076 (33)	-	-
ANCOVA	-	-	-	-	-	-
Dorsal Prostate Gland						
Absolute	0.256 ± 0.012 (34)	0.252 ± 0.013 (31)	0.276 ± 0.015 (32)	0.232 ± 0.013 (33)	-	-
Relative	0.379 ± 0.019 (34)	0.375 ± 0.020 (31)	0.388 ± 0.020 (32)	0.337 ± 0.018 (33)	-	-
ANCOVA	-	-	-	-	-	-
Epididymis						
Absolute	1.254 ± 0.033 (34)	1.248 ± 0.034 (31)	1.241 ± 0.038 (33)	1.444 ± 0.158 (33)	*	-
Relative	1.856 ± 0.061 (34)	1.856 ± 0.052 (31)	1.759 ± 0.058 (33)	2.099 ± 0.218 (33)	-	-
ANCOVA	-	-	-	-	-	-
Kidney						
Absolute	4.043 ± 0.071 (34)	5.187 ± 1.169 (31)	4.119 ± 0.089 (33)	4.182 ± 0.091 (33)	-	-
Relative	5.948 ± 0.100 (34)	7.817 ± 1.845 (31)	5.831 ± 0.146 (33)	6.105 ± 0.134 (33)	-	#
ANCOVA	-	-	-	-	-	-

Table G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Lateral Prostate Gland						
Absolute	0.278 ± 0.013 (34)	0.277 ± 0.015 (31)	0.331 ± 0.019* (31)	0.305 ± 0.016 (33)	#	*
Relative	0.413 ± 0.022 (34)	0.410 ± 0.022 (31)	0.469 ± 0.028 (31)	0.445 ± 0.024 (33)	-	-
ANCOVA	-	-	-	-	#	*
Liver						
Absolute	16.179 ± 0.342 (33)	15.849 ± 0.369 (31)	17.141 ± 0.424 (33)	16.803 ± 0.442 (31)	#	*
Relative	23.789 ± 0.419 (33)	23.560 ± 0.532 (31)	24.120 ± 0.470 (33)	24.455 ± 0.445 (31)	-	-
ANCOVA	-	-	-	-	-	-
Pituitary Gland						
Absolute	0.023 ± 0.005 (34)	0.023 ± 0.004 (31)	0.031 ± 0.007 (32)	0.022 ± 0.002 (33)	-	-
Relative	0.035 ± 0.008 (34)	0.034 ± 0.006 (31)	0.044 ± 0.010 (32)	0.032 ± 0.003 (33)	-	-
ANCOVA	-	-	-	-	-	-
Seminal Vesicle/ Coagulating Gland						
Absolute	1.270 ± 0.078 (33)	1.286 ± 0.074 (31)	1.408 ± 0.077 (32)	1.190 ± 0.055 (32)	-	-
Relative	1.893 ± 0.125 (33)	1.910 ± 0.109 (31)	1.993 ± 0.114 (32)	1.727 ± 0.081 (32)	-	-
ANCOVA	-	-	-	-	-	-
Spleen						
Absolute	1.132 ± 0.034 (34)	1.137 ± 0.037 (31)	1.184 ± 0.049 (33)	1.227 ± 0.047 (33)	-	-
Relative	1.665 ± 0.050 (34)	1.686 ± 0.052 (31)	1.676 ± 0.074 (33)	1.801 ± 0.079 (33)	-	-
ANCOVA	-	-	-	-	-	-

Table G3
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Testes						
Absolute	3.230 ± 0.112 (34)	3.288 ± 0.086 (31)	3.301 ± 0.094 (33)	3.202 ± 0.092 (33)	-	-
Relative	4.762 ± 0.184 (34)	4.890 ± 0.135 (31)	4.679 ± 0.146 (33)	4.694 ± 0.157 (33)	-	-
ANCOVA	-	-	-	-	-	-
Thymus						
Absolute	0.309 ± 0.020 (34)	0.303 ± 0.025 (31)	0.317 ± 0.023 (33)	0.342 ± 0.027 (33)	-	-
Relative	0.457 ± 0.030 (34)	0.450 ± 0.037 (31)	0.444 ± 0.031 (33)	0.493 ± 0.037 (33)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.047 ± 0.001 (34)	0.049 ± 0.002 (31)	0.050 ± 0.002 (32)	0.050 ± 0.002 (33)	-	-
Relative	0.069 ± 0.002 (34)	0.073 ± 0.003 (31)	0.070 ± 0.003 (32)	0.073 ± 0.003 (33)	-	-
ANCOVA	-	-	-	-	-	-
Ventral Prostate Gland						
Absolute	0.500 ± 0.023 (33)	0.526 ± 0.030 (31)	0.526 ± 0.019 (32)	0.540 ± 0.027 (33)	-	-
Relative	0.742 ± 0.035 (33)	0.781 ± 0.044 (31)	0.743 ± 0.029 (32)	0.789 ± 0.041 (33)	-	-
ANCOVA	-	-	-	-	-	-

* $P \leq 0.05$

[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.

[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference.

Table G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	482.5 ± 15.2 (26)	425.9 ± 19.3 (16)	493.4 ± 17.3 (22)	449.0 ± 18.5 (22)	-	-
Adrenal Glands						
Absolute	0.100 ± 0.006 (26)	0.108 ± 0.010 (16)	0.098 ± 0.006 (22)	0.089 ± 0.004 (22)	-	-
Relative	0.212 ± 0.013 (26)	0.259 ± 0.024 (16)	0.204 ± 0.014 (22)	0.204 ± 0.011 (22)	-	-
ANCOVA[c]	-	-	-	-	-	-
Brain						
Absolute	2.017 ± 0.023 (26)	2.058 ± 0.037 (16)	2.049 ± 0.028 (22)	2.001 ± 0.033 (22)	-	-
Relative	4.267 ± 0.122 (26)	5.052 ± 0.352* (16)	4.258 ± 0.155 (22)	4.610 ± 0.187 (22)	-	-
ANCOVA	-	-	-	-	-	-
Kidney						
Absolute	2.692 ± 0.047 (26)	2.592 ± 0.119 (16)	2.662 ± 0.056 (22)	2.445 ± 0.064* (22)	*/ #	-
Relative	5.681 ± 0.171 (26)	6.174 ± 0.253 (16)	5.492 ± 0.168 (22)	5.569 ± 0.188 (22)	-	-
ANCOVA	-	-	-	-	*/ #	-
Liver						
Absolute	12.746 ± 0.503 (26)	11.079 ± 0.646 (16)	13.531 ± 0.872 (22)	12.512 ± 0.712 (22)	-	-
Relative	26.685 ± 1.069 (26)	26.078 ± 1.148 (16)	27.343 ± 1.356 (22)	27.711 ± 0.797 (22)	-	-
ANCOVA	-	-	-	-	-	-
Ovaries (both)						
Absolute	0.182 ± 0.011 (26)	0.152 ± 0.012 (16)	0.159 ± 0.007 (22)	0.180 ± 0.010 (22)	-	#
Relative	0.388 ± 0.029 (26)	0.379 ± 0.047 (16)	0.329 ± 0.019 (22)	0.407 ± 0.021 (22)	-	*
ANCOVA	-	-	-	-		#
Pituitary Gland						
Absolute	0.064 ± 0.010 (26)	0.077 ± 0.016 (16)	0.060 ± 0.011 (22)	0.071 ± 0.020 (22)	-	-
Relative	0.139 ± 0.023 (26)	0.181 ± 0.037 (16)	0.128 ± 0.024 (22)	0.167 ± 0.050 (22)	-	-
ANCOVA	-	-	-	-	-	-

Table G4
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_1T140 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Spleen						
Absolute	0.859 ± 0.035 (26)	0.729 ± 0.043* (16)	0.850 ± 0.037 (22)	0.834 ± 0.042 (22)	-	-
Relative	1.803 ± 0.078 (26)	1.718 ± 0.071 (16)	1.734 ± 0.064 (22)	1.904 ± 0.114 (22)	-	-
ANCOVA	-	-	-	-	-	-
Thymus						
Absolute	0.311 ± 0.032 (25)	0.279 ± 0.022 (14)	0.300 ± 0.022 (22)	0.298 ± 0.030 (22)	-	-
Relative	0.634 ± 0.057 (25)	0.635 ± 0.049 (14)	0.623 ± 0.051 (22)	0.657 ± 0.057 (22)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.045 ± 0.003 (26)	0.040 ± 0.003 (15)	0.045 ± 0.003 (22)	0.041 ± 0.002 (22)	-	-
Relative	0.095 ± 0.005 (26)	0.092 ± 0.006 (15)	0.092 ± 0.005 (22)	0.092 ± 0.005 (22)	-	-
ANCOVA	-	-	-	-	-	-
Uterus						
Absolute	0.925 ± 0.094 (25)	0.940 ± 0.071 (16)	1.004 ± 0.110 (22)	1.069 ± 0.172 (22)	-	-
Relative	2.015 ± 0.231 (25)	2.267 ± 0.199 (16)	2.069 ± 0.218 (22)	2.600 ± 0.516 (22)	-	-
ANCOVA	-	-	-	-	-	-

* $P \leq 0.05$

[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.

[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, $P \leq 0.05$. Dashes indicate no significant difference.

[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference.

Table G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	669.6 ± 13.2 (30)	631.0 ± 13.6 (27)	667.6 ± 9.0 (31)	656.6 ± 12.8 (35)	-	-
Adrenal Glands						
Absolute	0.091 ± 0.020 (30)	0.082 ± 0.009 (27)	0.092 ± 0.021 (31)	0.067 ± 0.003 (35)	-	-
Relative (mg/g)	0.138 ± 0.030 (30)	0.130 ± 0.014 (27)	0.137 ± 0.031 (31)	0.103 ± 0.005 (35)	-	-
ANCOVA[c]	-	-	-	-	-	-
Brain						
Absolute	2.253 ± 0.026 (30)	2.265 ± 0.021 (27)	2.275 ± 0.021 (31)	2.272 ± 0.024 (35)	-	-
Relative	3.419 ± 0.102 (30)	3.623 ± 0.069 (27)	3.422 ± 0.047 (31)	3.521 ± 0.099 (35)	-	-
ANCOVA	-	-	-	-	-	-
Dorsal Prostate Gland						
Absolute	0.245 ± 0.011 (28)	0.270 ± 0.016 (26)	0.284 ± 0.009 (31)	0.283 ± 0.013 (33)	#	-
Relative	0.369 ± 0.017 (28)	0.431 ± 0.026 (26)	0.427 ± 0.015 (31)	0.436 ± 0.021* (33)	#	
ANCOVA	-	-	-	*	#	-
Epididymis						
Absolute	1.162 ± 0.030 (30)	1.102 ± 0.036 (27)	1.174 ± 0.023 (31)	1.120 ± 0.031 (35)	-	-
Relative	1.756 ± 0.057 (30)	1.771 ± 0.070 (27)	1.766 ± 0.039 (31)	1.724 ± 0.052 (35)	-	-
ANCOVA	-	-	-	-	-	-
Kidney						
Absolute	4.153 ± 0.123 (30)	4.177 ± 0.228 (27)	4.075 ± 0.082 (31)	4.201 ± 0.125 (35)	-	-
Relative	6.217 ± 0.171 (30)	6.649 ± 0.350 (27)	6.109 ± 0.107 (31)	6.509 ± 0.259 (35)	-	-
ANCOVA	-	-	-	-	-	-

Table G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Lateral Prostate Gland						
Absolute	0.288 ± 0.021 (30)	0.306 ± 0.023 (27)	0.338 ± 0.018 (30)	0.329 ± 0.017 (33)	#	*
Relative	0.431 ± 0.030 (30)	0.490 ± 0.038 (27)	0.512 ± 0.028 (30)	0.499 ± 0.025 (33)	-	-
ANCOVA	-	-	-	-	#	-
Liver						
Absolute	16.508 ± 0.490 (29)	15.219 ± 0.584 (26)	16.078 ± 0.355 (31)	15.522 ± 0.251 (35)	-	-
Relative	24.740 ± 0.599 (29)	24.118 ± 0.840 (26)	24.099 ± 0.435 (31)	23.840 ± 0.441 (35)	-	-
ANCOVA	-	-	-	-	-	-
Pituitary Gland						
Absolute	0.025 ± 0.004 (30)	0.038 ± 0.011 (26)	0.031 ± 0.009 (31)	0.021 ± 0.002 (34)	-	-
Relative	0.037 ± 0.005 (30)	0.060 ± 0.017 (26)	0.047 ± 0.014 (31)	0.033 ± 0.003 (34)	-	-
ANCOVA	-	-	-	-	-	-
Seminal Vesicle/ Coagulating Gland						
Absolute	1.108 ± 0.053 (29)	1.220 ± 0.081 (26)	1.368 ± 0.066** (31)	1.298 ± 0.060* (33)	# #	***
Relative	1.651 ± 0.077 (29)	1.958 ± 0.135 (26)	2.056 ± 0.100** (31)	1.970 ± 0.082* (33)	# #	**/ #
ANCOVA	-	-	-	-	# #	**
Spleen						
Absolute	1.165 ± 0.050 (30)	1.078 ± 0.032 (27)	1.237 ± 0.122 (31)	1.405 ± 0.338 (35)	-	-
Relative	1.756 ± 0.079 (30)	1.724 ± 0.057 (27)	1.863 ± 0.180 (31)	2.176 ± 0.540 (35)	-	-
ANCOVA	-	-	-	-	-	-

Table G5
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Male Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Testes						
Absolute	3.343 ± 0.085 (30)	3.375 ± 0.120 (27)	3.234 ± 0.110 (31)	3.271 ± 0.120 (35)	-	-
Relative	5.052 ± 0.159 (30)	5.425 ± 0.230 (27)	4.840 ± 0.156 (31)	5.048 ± 0.204 (35)	-	-
ANCOVA	-	-	-	-	-	-
Thymus						
Absolute	0.187 ± 0.011 (30)	0.188 ± 0.016 (27)	0.203 ± 0.014 (31)	0.192 ± 0.012 (35)	-	-
Relative	0.287 ± 0.021 (30)	0.298 ± 0.024 (27)	0.304 ± 0.020 (31)	0.291 ± 0.016 (35)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.050 ± 0.002 (30)	0.051 ± 0.002 (27)	0.052 ± 0.002 (31)	0.219 ± 0.165 (34)	-	-
Relative	0.075 ± 0.004 (30)	0.081 ± 0.003 (27)	0.078 ± 0.003 (31)	0.346 ± 0.263 (34)	-	-
ANCOVA	-	-	-	-	-	-
Ventral Prostate Gland						
Absolute	0.539 ± 0.027 (30)	0.500 ± 0.034 (27)	0.602 ± 0.034 (31)	0.613 ± 0.031 (32)	-	-
Relative	0.804 ± 0.035 (30)	0.800 ± 0.055 (27)	0.907 ± 0.053 (31)	0.926 ± 0.044 (32)	#	-
ANCOVA	-	-	-	-	*/ #	-

* P ≤ 0.05
** P ≤ 0.01
***P ≤ 0.001

[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.

[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: #, P ≤ 0.05; ##, P ≤ 0.01. Dashes indicate no significant difference.

[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference. Asterisks in shaded cells indicate significant differences from controls.

TABLE G6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)[a]				Trends[b]	
	0	2	10	50	Linear	Quad
Terminal Body Weight (g)	497.0 ± 15.4 (27)	461.9 ± 13.8 (29)	496.8 ± 20.5 (21)	495.0 ± 19.1 (24)	-	-
Adrenal Glands						
Absolute	0.106 ± 0.010 (27)	0.097 ± 0.007 (29)	0.097 ± 0.006 (21)	0.099 ± 0.009 (24)	-	-
Relative	0.215 ± 0.018 (27)	0.213 ± 0.015 (29)	0.205 ± 0.018 (21)	0.213 ± 0.025 (24)	-	-
ANCOVA[c]	-	-	-	-	-	-
Brain						
Absolute	2.017 ± 0.017 (26)	2.102 ± 0.021* (28)	2.080 ± 0.030 (21)	2.019 ± 0.023 (24)	-	# #
Relative	4.087 ± 0.105 (26)	4.708 ± 0.141** (28)	4.309 ± 0.164 (21)	4.213 ± 0.162 (24)	-	# #
ANCOVA	-	*	-	-	-	# # #
Kidney						
Absolute	2.652 ± 0.071 (27)	2.610 ± 0.109 (29)	2.659 ± 0.054 (21)	2.684 ± 0.128 (24)	-	-
Relative	5.407 ± 0.150 (27)	5.762 ± 0.308 (29)	5.495 ± 0.213 (21)	5.605 ± 0.371 (24)	-	-
ANCOVA	-	-	-	-	-	-
Liver						
Absolute	13.475 ± 0.758 (27)	12.623 ± 0.642 (29)	13.632 ± 0.738 (21)	13.831 ± 0.788 (24)	-	-
Relative	26.873 ± 1.085 (27)	27.270 ± 1.012 (29)	27.532 ± 1.055 (21)	28.174 ± 1.344 (24)	-	-
ANCOVA	-	-	-	-	-	-
Ovaries (both)						
Absolute	0.224 ± 0.049 (27)	0.337 ± 0.157 (29)	0.163 ± 0.010 (21)	0.162 ± 0.011 (24)	-	-
Relative	0.464 ± 0.108 (27)	0.743 ± 0.331 (29)	0.333 ± 0.021 (21)	0.340 ± 0.027 (24)	-	-
ANCOVA	-	-	-	-	-	-
Pituitary Gland						
Absolute	0.053 ± 0.011 (27)	0.057 ± 0.012 (29)	0.052 ± 0.011 (21)	0.063 ± 0.014 (24)	-	-
Relative	0.115 ± 0.026 (27)	0.130 ± 0.029 (29)	0.113 ± 0.028 (21)	0.145 ± 0.038 (24)	-	-
ANCOVA	-	-	-	-	-	-

Table G6
Organ Weights and Organ-Weight-to-Body-Weight Ratios for F_3T21 Female Rats in the 2-Year Feed Study of Ethinyl Estradiol

Organ	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Spleen						
Absolute	0.817 ± 0.030 (26)	0.931 ± 0.040* (29)	0.943 ± 0.034* (20)	0.854 ± 0.027 (24)	-	*/ # #
Relative	1.684 ± 0.074 (26)	2.032 ± 0.073** (29)	1.935 ± 0.110 (20)	1.758 ± 0.062 (24)	-	# # #
ANCOVA	-	**	*	-	-	*/ # # #
Thymus						
Absolute	0.209 ± 0.017 (27)	0.196 ± 0.016 (29)	0.206 ± 0.020 (21)	0.174 ± 0.013 (24)	-	-
Relative	0.434 ± 0.041 (27)	0.430 ± 0.033 (29)	0.422 ± 0.042 (21)	0.352 ± 0.021 (24)	-	-
ANCOVA	-	-	-	-	-	-
Thyroid Gland						
Absolute	0.053 ± 0.008 (27)	0.043 ± 0.002 (29)	0.053 ± 0.003 (21)	0.045 ± 0.002 (24)	-	-
Relative	0.113 ± 0.021 (27)	0.095 ± 0.004 (29)	0.110 ± 0.006 (21)	0.092 ± 0.004 (24)	-	-
ANCOVA	-	-	-	-	-	-
Uterus						
Absolute	0.785 ± 0.081 (27)	0.846 ± 0.090 (28)	0.784 ± 0.060 (21)	0.928 ± 0.094 (23)	-	-
Relative	1.650 ± 0.194 (27)	1.922 ± 0.263 (28)	1.662 ± 0.169 (21)	2.022 ± 0.237 (23)	-	-
ANCOVA	-	-	-	-	-	-

* $P \leq 0.05$
** $P \leq 0.01$
[a] Mean organ weights ± standard error. Absolute organ weights are given in grams; relative weights are in g/kg body weight unless otherwise noted. Numbers in parentheses are the numbers of animals or organs included in the calculations. Outliers were excluded from the analyses. Asterisks in shaded cells indicate values that are significantly different from control values by Dunnett's test.
[b] Results of linear and quadratic exposure concentration trend tests. Because of the unequal spacing of exposure concentrations, trends were also determined for a scale using the natural logarithm of the dose + 1. The "log dose" trends are indicated as follows: ##, $P \leq 0.01$; ###, $P \leq 0.001$. Dashes indicate no significant difference.
[c] Results of Dunnett's tests from an analysis of covariance (ANCOVA) with body weight as the covariate are in the exposed group columns. Dashes indicate no significant difference. Asterisks in shaded cells indicate significant differences from controls.

APPENDIX H
INGREDIENTS, NUTRIENT COMPOSITION, AND CONTAMINANT LEVELS IN PURINA 5K96 RAT RATION

Ingredients of Purina 5K96 Rat Ration 206
Table H1 Vitamins and Minerals in Purina 5K96 Rat Ration 206
Table H2 Nutrient Composition of Purina 5K96 Rat Ration 207
Table H3 Contaminant Levels in Purina 5K96 Rat Ration 207

Ingredients of Purina 5K96 Rat Ration

Ground wheat, ground corn, wheat middlings, ground oats, fish meal, casein, corn gluten meal, corn oil, dicalcium phosphate, brewers dried yeast, calcium carbonate, and salt.

Table H1
Vitamins and Minerals in Purina 5K96 Rat Ration

Vitamins	Amount	Source
Carotene	1.6 ppm	multiple sources
Vitamin K	7.1 ppm	menadione sodium bisulfate
Thiamin Hydrochloride	26 ppm	thiamine mononitrate
Riboflavin	8.6 ppm	riboflavin
Niacin	91 ppm	nicotinic acid
Pantothenic acid	29 ppm	calcium pantothenate
Choline chloride	1800 ppm	choline chloride
Folic acid	2.7 ppm	folic acid
Pyridoxine	10 ppm	pyridoxine hydrochloride
Biotin	0.3 ppm	
Vitamin B_{12}	44 mcg/gm	cyanocobalamin
Vitamin A	25 IU/gm	vitamin A acetate
Vitamin E	93 IU/gm	dl-alpha tocopheryl acetate
Minerals	**Amount**	**Source**
Magnesium	0.20 %	magnesium oxide
Manganese	130 ppm	manganese oxide
Iron	170 ppm	ferrous carbonate
Zinc	85 ppm	zinc sulfate
Copper	10 ppm	copper sulfate
Iodine	0.88 ppm	calcium iodate
Cobalt	0.28 ppm	cobalt carbonate
Selenium	0.28 ppm	multiple sources
Ash	5.8 %	multiple sources
Calcium	1.15 %	multiple sources
Phosphorus	0.89 %	dicalcium phosphate
Potassium	0.44 %	multiple sources
Sulfur	0.17 %	multiple sources
Sodium	0.28 %	salt
Chlorine	0.49 %	salt
Fluorine	14 ppm	multiple sources
Chromium	1.01 ppm	multiple sources

TABLE H2
Nutrient Composition of Purina 5K96 Rat Ration

Nutrient	Mean ± Standard Deviation	Number of Lots
Total Protein, %	19.13 ± 1.23	31
Total Fat, %	5.12 ± 0.96	31
Volatiles, %	7.05 ± 1.86	31
Vitamin A, ppm	7.72 ± 1.64	31
Vitamin B_1, mg/gm	0.028 ± 0.005	31
Vitamin E, ppm	83.64 ± 21.41	31
Selenium, ppm	0.47 ± 0.15	31

TABLE H3
Contaminant Levels in Purina 5K96 Rat Ration

Contaminant	Mean ± Standard Deviation	# Lots / # Lots positive
Arsenic, ppm	0.18 ± 0.13	30 / 30
Cadmium, ppb	0.29 ± 0.26	30 / 2
Lead, ppm	0.57 ± 0.22	31 / 31
Fumonisin B_1, ppb	< MDL	31 / 2
Total Fumonisin, ppb	295.68 ± 373.09	31 / 31
Aflatoxin B_1, ppb	< MDL	31 / 31
Aflatoxin B_2, ppb	< MDL	31 / 31
Aflatoxin G_1, ppb	< MDL	31 / 31
Aflatoxin G_2, ppb	< MDL	31 / 31

APPENDIX I
SENTINEL ANIMAL PROGRAM

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Results 210

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 randomly selected rats during the 2-year study. Blood from each animal was collected and allowed to clot, and the serum was separated. Samples were processed appropriately and shipped to the Research Animal Diagnostic Laboratory, University of Missouri (Colombia, MO) for determination of antibody titers. The laboratory serology methods and viral agents for which testing was performed are tabulated below; the times at which blood was collected during the study are also listed. All sentinel animals were examined for ectoparasites, endoparasites, and bacterial pathogens.

Method and Test	Time of Analysis[a]
ELISA	
H-1 (Toolan's H-1 virus)	37, 59, 63, 85, 89, 113, and 136 weeks
KRV (Kilham Rat Virus)	37, 59, 63, 85, 89, 113, and 136 weeks
Mycoplasma arthritidis	37, 59, 63, 85, 89, 113, and 136 weeks
Mycoplasma pulmonis	37, 59, 63, 85, 89, 113, and 136 weeks
PVM (pneumonia virus of mice)	37, 59, 63, 85, 89, 113, and 136 weeks
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	37, 59, 63, 85, 89, 113, and 136 weeks
Sendai	37, 59, 63, 85, 89, 113, and 136 weeks

RESULTS

The bacterial pathogen *Pasteurella pneumotropica* was isolated from 28 of 33 sentinel animals. All serology tests were negative.

[a] Time of analysis represents weeks from the first day F_0 animals were placed on study.